Fetal Growth and Development

The Generation R Study

Bero O. Verburg

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Contents

Manuscripts based on this thesis

Chapter 3.1
Verburg BO, Steegers EAP, de Ridder MAJ, Snijders RJM, Hofman A, Smith E, Moll HA, Jaddoe VWV, Witteman JCM. New charts for ultrasound dating of pregnancy and assessment of fetal growth, longitudinal data from a population-based cohort study. In press: Ultrasound in Obstetrics and Gynecology, July 2007.

Chapter 3.2
Verburg BO, Mulder PGH, Hofman A, Jaddoe VWV, Witteman JCM, Steegers EAP.
Intra- and interobserver reproducibility study of early fetal growth parameters. Revision: Prenatal Diagnosis, June 2007.

Chapter 3.3
de Ridder MAJ, Verburg BO, Steegers EAP, Hofman A, Witteman JCM, Jaddoe VWV. Individually customised fetal weight curves derived from ultrasound measurements in a large population-based cohort study. The Generation R Study. Submitted

Chapter 3.4
Jaddoe VWV, Verburg BO, de Ridder MAJ, Hofman A, Mackenbach JP, Moll HA, Steegers EAP, Witteman JCM. Maternal smoking and fetal growth characteristics in different periods of pregnancy: the Generation R Study. Am J Epidemiol 2007; 165: 1207-1215.

Chapter 4.1
Verburg BO, Jaddoe VWV, Wladimiroff JW, Hofman A, Witteman JCM, Steegers EAP.
Fetal haemodynamic adaptive changes related to intrauterine growth. The Generation R Study. In press: Circulation, September 2007.

Chapter 4.2
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Chapter 4.3

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Chapter 4.4

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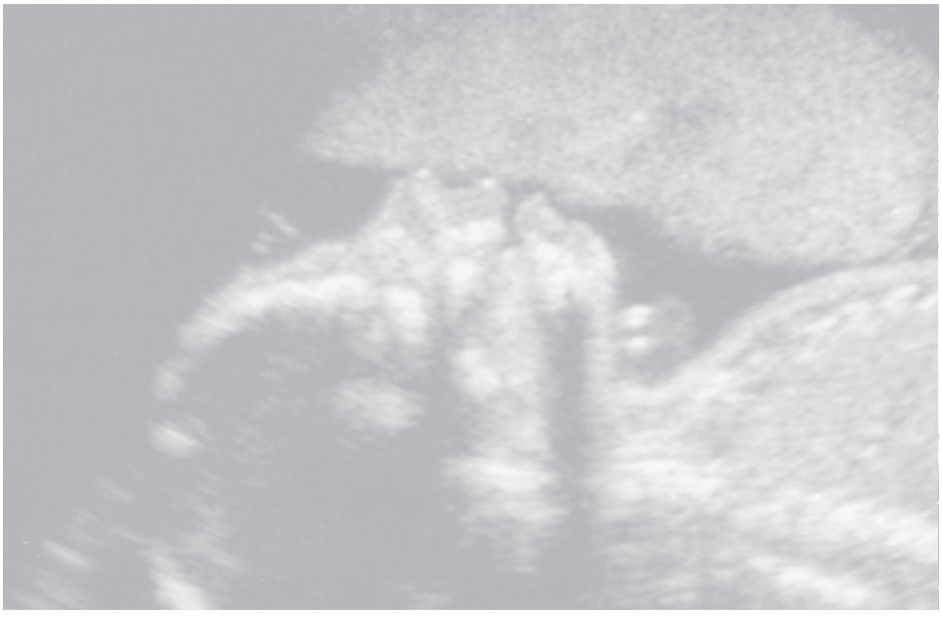
List of abbreviations

AC	Abdominal circumference
ADHD	Attention deficit hyperactivity disorder
A-wave	Active atrial contraction filling peak velocity
BPD	Biparietal diameter
CI	Confidence interval
CRL	Crown-rump length
CV	Coefficient of variation
EFW	Estimated fetal weight
E-wave	Passive early ventricular filling peak velocity
FL	Femur length
GA	Gestational age
HC	Head circumference
ICC	Intraclass correlation coefficient
LMP	Last menstrual period
OFD	Occipito-frontal diameter
PI	Pulsatility index
PIV	Venous pulsatility index
PSV	Peak systolic velocity
RI	Resistance index
SD	Standard deviation
SDS	Standard deviation score (Z-score)
TCD	Transverse cerebellar diameter
TVI	Time velocity integral

Chapter 1

Introduction



Introduction

The importance of the female womb and the mysterious processes that take place in the uterus before birth caused it to be a sacred symbol from ancient history onwards [1].

Despite this ancient general acknowledgement of the importance of fetal growth and development for human existence on earth, the only thing, however, that was generally known about pregnancy was that it lasted circa 9 lunar months. -A concept that hasn't changed considerably though- It wasn't until the 20th century that science enabled unravelling some of the mysteries of pregnancy. In 1968, still little was known about human fetal development. Kloosterman, a Dutch professor in obstetrics, described knowledge of that time in the following way: "How intrauterine growth processes during a normal pregnancy evolve and what regulates these processes in humans is practically unknown. This especially involves humans for the developing human conceptus is hardly accessible for observation. Fact is that fetal growth velocity differs and that it is malpractice to use birth weight as a measure for maturity at birth. An early born giant and a late born dwarf with a similar birth weight can differ largely in maturity and development."

In the last decades, the introduction of ultrasound made closer observation of the human fetus possible. Improved ultrasound resolution enhanced detailed observation of fetal growth and structural fetal development. Accurate fetal growth curves are needed to describe normal fetal growth and to identify fetuses with growth anomalies. We now realise that fetal environment and conditions shape the fetus. Unfavourable fetal environment may disrupt normal developmental processes with lifelong consequences. In the past two decades, epidemiological studies have demonstrated associations of fetal growth restriction and low birth weight with cardiovascular disease in later life. The fetal origins hypothesis postulates that an adverse fetal environment leads to developmental adaptations that permanently program the fetus' structure, physiology and metabolism. This programming is in favour of short-term survival and leads to fetal growth restriction and low birth weight and eventually to increased susceptibility for adult diseases. Long-term effects of this programming will be detrimental and lead to several health problems in adulthood including cardiovascular disease and diabetes. Unravelling the mechanisms underlying the associations of fetal growth restriction and low birth weight with adult

1. In prehistoric times the womb was regarded as the source of all life, the focus of the Mother's cosmic energy; her menses time was known as the flower; her breasts, belly, and yonic entrance were revered to as sacred. Its link to fertility, birth, feminine sexuality and the natural force of women was acknowledged also by the Celts, as well as pagan cultures throughout northern Europe and Latin America. The ankh ☥ symbol is hypothesized to represent in Egyptian culture a stylised womb, symbolizing life and immortality, growth and renewal, bringing health and happiness. A similar symbol (♀) was used to represent the Roman goddess Venus. This symbol, is much more associated with a representation of the female womb. The same symbol is used in biology to identify the female sex.

disease may eventually lead to new strategies for identification of groups at risk and prevention.

All studies in this thesis are embedded in the Generation R study. This recently started cohort study is designed to study growth, development and health from early fetal life until young adulthood.

The main objectives of this thesis are:

1. To accurately describe fetal growth and development as well as to determine gestational age by ultrasound.
2. To validate ultrasound measurements of fetal growth.
3. To determine the influence of different variables on fetal growth.
4. To study specific fetal organ development and fetal cardiovascular performance in relation to fetal growth.

Outline of this thesis:

In Chapter 2 the Generation R Study is described.

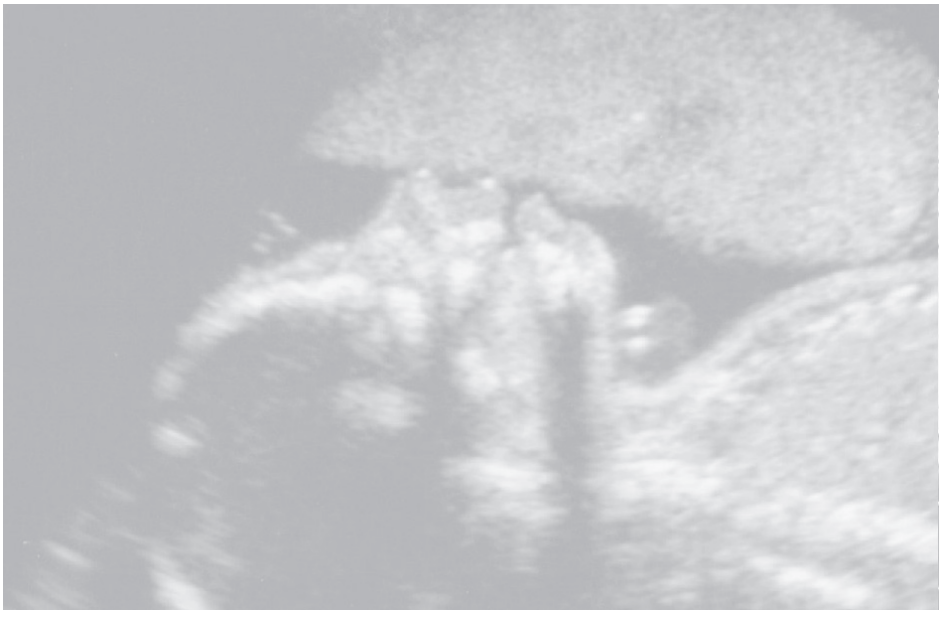
In Chapter 3 normal and abnormal fetal growth and development is studied. Lucent methods to determine gestational age are indispensable to assess fetal growth and were developed. Additionally, the reproducibility of fetal growth measurements is discussed. Further studies in this chapter deal with variables that influence fetal growth like smoking, ethnicity, fetal gender and parental size.

In Chapter 4, the fetal origins hypothesis was the main point of departure, the studies presented focus specifically on the development of fetal organs during pregnancy. Brain, kidney, the fetal circulation and heart development are studied in relation to fetal growth. Special emphasis has been put on fetal growth restriction and suboptimal fetal organ development in utero.

Chapter 5 provides a more general discussion of the main findings, considers general methodological issues and gives suggestions for further research.

Chapter 2

The Generation R Study



Abstract

The Generation R Study is a population-based prospective cohort study from fetal life until young adulthood. The study is designed to identify early environmental and genetic causes of normal and abnormal growth, development and health from fetal life until young adulthood. The study focuses on four primary areas of research: 1) growth and physical development; 2) behavioural and cognitive development; 3) diseases in childhood; and 4) health and healthcare for pregnant women and children. In total, 9,778 mothers with a delivery date from April 2002 until January 2006 were enrolled in the study. Of all eligible children at birth, 61% participate in the study. Data collection in the prenatal phase included physical examinations, questionnaires, fetal ultrasound examinations and biological samples. In addition, more detailed assessments are conducted in a subgroup of 1,232 pregnant women and their children. The children form a prenatally recruited birth-cohort that will be followed until young adulthood. Eventually, results forthcoming from the Generation R Study have to 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 specific research projects of the study have been described in detail previously (1). This chapter focuses on the study design and cohort profile in the first phase of the study.

Scope of research

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. The study focuses on four primary areas of research: 1) growth and physical development; 2) behavioural and cognitive development; 3) diseases in childhood; and 4) health and healthcare for pregnant women and children. The main outcomes and determinants are presented in Tables 1 and 2. The general aims of the study are:

1) To describe normal and abnormal growth, development and health from fetal life until young adulthood;
2) To identify biological, environmental and social determinants of normal and abnormal

Table 1. Main outcomes per research area

Growth and physical development
Fetal growth patterns and organ development
Pregnancy complications
Postnatal growth patterns
Obesity
Risk factors for development of cardiovascular disease
Risk factors for type 2 diabetes
Behaviour and cognitive development
Maternal and paternal psychopathology
Fetal and postnatal brain development
Behaviour, psychopathology and cognition
Neuromotor development
Chronic pain
Attachment
Stress reactivity
Diseases in childhood
Infectious diseases in childhood
Development of the immune system
Asthma and asthma related symptoms
Paroxysmal neurological disorders
Health and healthcare
Quality of life
Health care utilization
Effectiveness of screening programs

Table 2. Main determinants

Biological determinants
Parental anthropometrics and blood pressure
Fetal and postnatal growth characteristics
Endocrine and immunological factors
Genetic variants
Environmental determinants
Maternal and childhood diet
Parental lifestyle habits (including smoking, alcohol consumption)
Housing conditions
Social determinants
Parental education, employment status and household income
Parental marital status
Ethnicity

growth, development and health from fetal life until young adulthood;

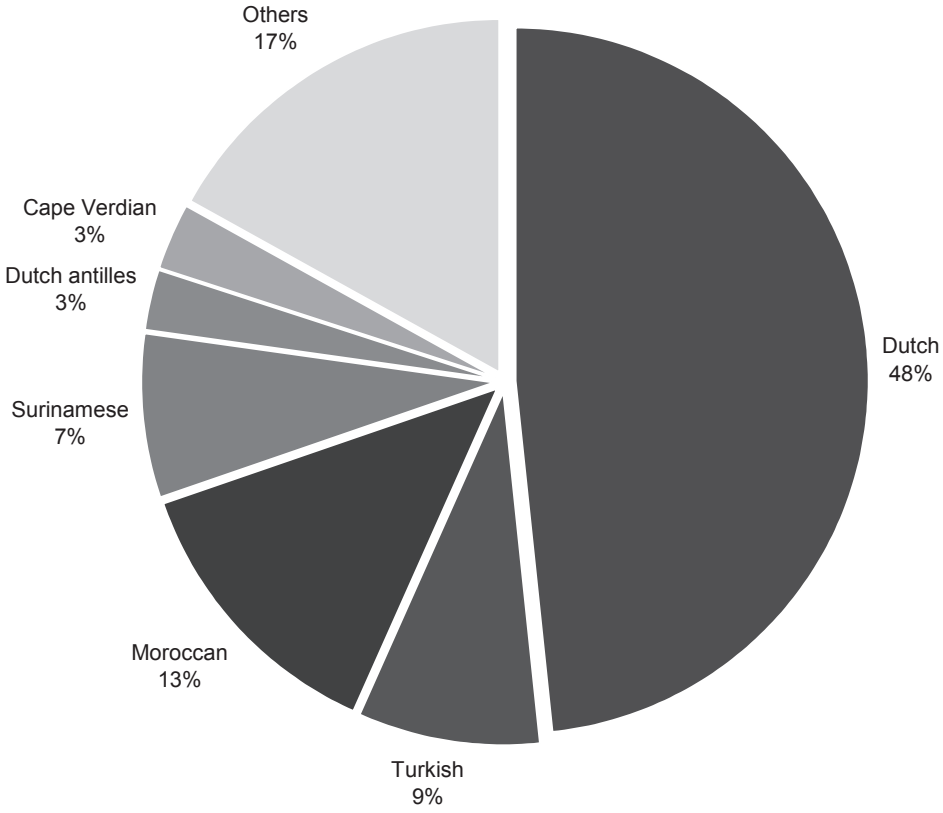
3) To examine the effectiveness of current strategies for prevention and early identification of groups at risk.

Eventually, results from the Generation R Study will contribute to the development of strategies for optimizing health and healthcare for pregnant women and children.

Study Area

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, almost 80 kilometres south of Amsterdam, the capital of the Netherlands. The total population consists of about 600,000 inhabitants of almost 150 different ethnicities. The study area is well defined by postal codes and covers more than half of the cities 350,000 inhabitants. The largest ethnic groups in this population are the Dutch (56%), Surinamese (9%), Turkish (7%), Moroccan (6%), Dutch Antillean (3%) and Cape Verdian (3%) groups (2). The percentages of the non-Dutch groups are higher in younger age groups (2). The number of children born in this study area is about 4,300 per year (2). The ethnic distribution of the newborns is shown in Figure 1. Measurements, including ultrasound assessment of fetal growth, are conducted in two well-equipped research centres in the study area. Intensive collaboration was established with all eight midwifery practices, three hospitals and sixteen child health care centres located in this area.

Figure 1. Ethnic distribution of newborns in Rotterdam



Based on children born in 2003 and 2004 (www.cos.rotterdam.nl)

Study design

Overview

The Generation R Study is a population-based prospective cohort study from fetal life until young adulthood. Mothers with a delivery date between April 2002 and January 2006 were eligible. Extensive assessments were carried out in mothers and their partners in pregnancy and are currently performed in their children (Table 3). Assessments in pregnancy were planned in early pregnancy (gestational age < 18 weeks), mid-pregnancy (gestational age 18 - 25 weeks) and late pregnancy (gestational age ≥ 25 weeks). The individual time scheme of these assessments depended on the specific gestational age at enrolment (Tables 4 and 5). The partners were assessed once in pregnancy. The children form a prenatally recruited birth-cohort that will be followed until young adulthood. Additionally, more detailed assessments of fetal and postnatal growth and development are conducted in a randomly selected subgroup of Dutch children and their parents, referred to as the Generation R Focus Cohort. This subgroup is ethnic homogeneous to exclude possible confounding or

Table 3. Assessments in the prenatal phase

Assessments in mothers
Physical examinations: height, weight, blood pressure
Questionnaires: socio-economic status, ethnicity, housing, living conditions, diet, medical history, family history, drug use, lifestyle habits, use of medical services
Interviews: expectations of parents to be (only in focus cohort)
Biological samples: blood and urine samples (storage, DNA)
Fetal ultrasounds: gestational age, fetal growth and in the focus cohort fetal brain, heart and kidney development, fetal blood flow distribution and placental function

Assessments in partners
Physical examinations: height, weight, blood pressure
Questionnaires: socio-economic status, ethnicity, housing, living conditions, medical history, family history, drug use, lifestyle habits, use of medical services
I*nterviews*: expectations of parents to be (only in focus cohort)
Biological samples: blood samples (storage, DNA)

Assessments in newborns at birth
Physical examinations: weight, cord blood sample (storage, DNA)

Table 4. Assessments in mothers, their partners and their children in the prenatal phase

	Early pregnancy	Mid-pregnancy	Late pregnancy	Birth
Mother				
Physical examination	+	+	+	
Questionnaire	+	+	+	
Interview			F	
Fetal ultrasound examination	+	+	+	
Additional detailed fetal ultrasound			F	
Blood sample	+	+		
Urine sample	+	+	+	
Partner				
Physical examination	+			
Questionnaire		+		
Interview			F	
Blood sample	+			
Child				
Physical examination				+
Cord blood sample				+

(+ =assessment in whole cohort; F = assessment only in focus cohort)
Early pregnancy: gestational age < 18 weeks; mid-pregnancy: gestational age 18 - 25 weeks; late pregnancy: gestational age ≥ 25 weeks.

effect modification by ethnicity. Studies conducted in this subgroup examine etiological associations with more in-depth methods that cannot be applied in the whole cohort

Table 5. Planned time scheme of prenatal measurements in mothers (weeks)

Gestational age at enrolment	Postal questionnaires (see text)				Visits for measurements		
	Mother 1	Mother 2	Mother 3	Mother 4	Early pregnancy	Mid-pregnancy	Late pregnancy
<12 weeks	12	15	20	30	12	20	30
13 weeks	13	15	20	30	13	20	30
14 weeks	14	16	20	30	14	20	30
15 weeks	15	17	20	30	15	20	30
16 weeks	16	18	22	30	16	22	30
17 weeks	17	19	22	30	17	22	30
18 weeks	18	20	24	30	-	18	30
19 weeks	19	21	24	30	-	19	30
20 weeks	20	22	24	30	-	20	30
21 weeks	21	23	25	30	-	21	30
22 weeks	22	24	26	30	-	22	30
23 weeks	23	25	27	30	-	23	30
24 weeks	24	-	-	30	-	24	30
≥ 25 weeks	≥ 25	-	-	≥ 30	-	-	≥ 25
Postnatal	Postnatal	-	-	-	-	-	-

Early pregnancy: gestational age < 18 weeks; mid-pregnancy: gestational age 18 - 25 weeks; late pregnancy: gestational age ≥ 25 weeks.

due to time, financial or logistical constraints. The Generation R Study is approved by the Medical Ethical Committee of the Erasmus Medical Center, Rotterdam. Mothers and their partners received written and oral information about the study. Participants are asked for their written informed consent.

Eligibility and enrolment

Eligible mothers were those who were resident in the study area at their delivery date and had a delivery date from April 2002 until January 2006. We aimed to enrol mothers in early pregnancy (gestational age < 18 weeks) but enrolment was possible until birth of their child. Midwives and obstetricians informed eligible mothers about the study at their first prenatal visit in routine care, handed out the information package and asked these mothers to make an appointment for their first ultrasound examination. The study staff contacted these mothers by phone for additional information about the study and in person at the ultrasound examination to obtain informed consent. Based on the pilot phase, it was estimated that it was possible to contact about 80% of all eligible mothers in pregnancy and 70% of these mothers would be willing to participate in the study. Mothers who were not approached in pregnancy, were approached and enrolled in the first months after birth of their child when newborns visit the routine child health centres (3). The partners were not approached directly by the study staff but the mothers were

informed about the importance of involvement of their partners in the study. There was no specific definition of being a partner but information was obtained about the biological relation of the partner with the (unborn) child.

Eligibility criteria for enrolment in the Generation R Focus Study were enrolment before a gestational age of 25 weeks in the Generation R Study, Dutch ethnicity, defined as two parents and four grandparents born in the Netherlands and a delivery date between February 2003 and August 2005. The study staff contacted these eligible mothers in pregnancy by phone to inform them about this sub-study and to invite them for the first additional measurements at a gestational age of 30 weeks.

Pilot phase

The Generation R Study entered its pilot phase in December 2001 with the recruitment of pregnant women from the whole city of Rotterdam. The main aim of this pilot phase was to test the logistics of the enrolment process. Based on the logistic results from this pilot phase, the definite study area was limited to postal codes covering the largest part but not the whole city.

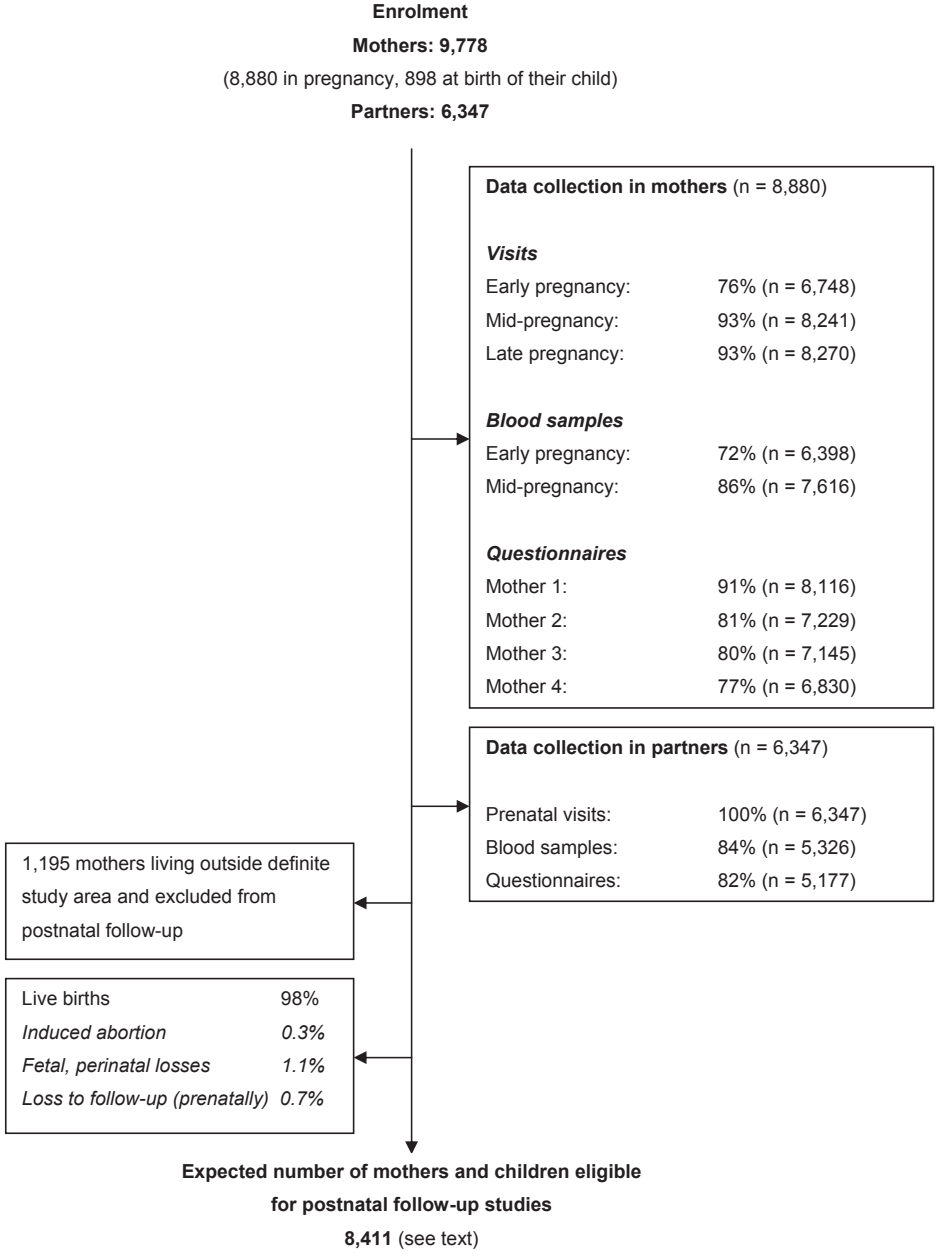
In the pilot phase, enrolment of mothers was restricted to a maximal gestational age of 24 weeks at enrolment. Since it turned out to be not feasible to approach all mothers in early or mid-pregnancy, the enrolment period for mothers was subsequently extended to the whole pregnancy until birth of their child. Full participant recruitment in the definite study area was established for mothers with a delivery date from January 2003. Mothers enrolled in the pilot phase and living outside the definite study area at their delivery date were completely followed until birth of their child. The children of these mothers do not participate in postnatal follow-up studies. Until the end of pregnancy, data collection and quality in these mothers are similar to data collected in the other participants. Therefore, these mothers are part of the total cohort for research projects studying outcomes in pregnancy. All enrolled mothers living in the definite study area at delivery with a delivery date from April 2002 and January 2006 are included as participants in the cohort for both prenatal and postnatal follow-up studies. Separate pilot studies to assess the intra- and inter-observer reproducibility were conducted in subjects outside the cohort and were, if necessary, repeated in study participants.

Study cohort

Pregnant women and their partners

In total, 9,778 mothers were enrolled in the study (Figure 2). Of these mothers, 91% (n = 8,880) was enrolled in pregnancy. Only partners from mothers enrolled in pregnancy were invited to participate. In total, 71% (n = 6,347) of all partners were enrolled. The general

Figure 2. Participant enrolment and measurements in the first phase



characteristics of the mothers and their partners are presented in Table 6. Of all participating mothers, enrolment was in early pregnancy in 69% (n = 6,748), in mid-pregnancy in 19% (n = 1,857), in late pregnancy in 3% (n = 275) and at birth of their child in 9% (n = 898). Of all mothers enrolled in pregnancy, 94% (n = 8,356), 6% (n = 516) and 0.1% (n = 8) were

Table 6. General characteristics of mothers and their partners

	Mothers (N = 9,778)	**Partners** (N = 6,347)
Gestational age at enrolment (%)		
Early pregnancy	69	-
Mid-pregnancy	19	-
Late pregnancy	3	-
Birth	9	-
Pregnancy number in study (%)		
1st pregnancy	94	-
2nd pregnancy	6	-
3rd pregnancy	0.1	-
Age (years)*	29.7 (5.3)	32.7 (5.8)
Parity (%)		
0	55	-
1	31	-
≥ 2	14	-
Ethnicity (%)		
Dutch, other-European	58	70
Surinamese	9	6
Moroccan	7	4
Turkish	9	6
Dutch Antilles	4	3
Cape verdian	4	2
Others	9	9
Education (vocational training) (%)		
Lower	13	8
Intermediate	45	41
Higher	42	51
Household income per month (%)		
<€ 800	9	-
€ 800-2200	36	-
>€ 2200	55	-

Values are percentages. *Mean (standard deviation)
All subject characteristics, except gestational age at enrolment, are based on valid data in mothers who were enrolled in pregnancy.

first, second and third pregnancies in the study, respectively. Ethnicity of participating mothers and partners was defined according the classification of Statistics Netherlands (4). This means for one specific person that: 1) if both parents are born in the Netherlands, the ethnicity is Dutch; 2) if one of the parents is born in another country than the Netherlands, that country counts; 3) if both parents are born in the same country other than

the Netherlands, that country counts; 4) if the parents are born in the different countries other than the Netherlands, the country of mothers counts; and 5) if that person and both parents are born in different countries other than the Netherlands, the country of birth of that specific person counts. As expected, the largest ethnic groups were the Dutch, Surinamese, Turkish and Moroccan groups. The ethnic distribution differed only moderately from that of the population in the study area (2). Mean household income in Rotterdam is about € 1,600 per month and the percentage subjects with a secondary or higher education level in Rotterdam is 56% (2). The educational level of participating mothers and their partners was classified in groups according to the classification of Statistics Netherlands (5). Both household income and highest followed educational level in mothers and their partners in the study cohort suggest a selection towards a higher socio-economic status. However, differences between the population and cohort characteristics may also be due to selective missing values of ethnicity and socio-economic status in the questionnaires. Additional efforts are currently made to complete the information on ethnicity, household income and educational level in the total cohort. The mean age of mothers in our study at enrolment was similar to the age of all pregnant women in the study area (29.6 years in 2003) (2).

Generation R Focus Study

Additional detailed assessment of fetal and postnatal growth and development were conducted in a subgroup of 1,232 mothers and their children. This subgroup study is called the Generation R Focus Study. The focus group consisted of Dutch participants only to create an ethnic homogeneous group, to exclude the confounding and effect modifying influence of ethnicity. All eligible parents whose children were expected to be born from April 2003 to April 2004 were invited to participate. Inclusion was before late pregnancy. Additional detailed examination during pregnancy included extended ultrasound assessments at the scan in late pregnancy. Echocardiography was performed to have an indication about cardiac performance and output and blood flow profiles during pregnancy. Extended brain ultrasounds were performed to have an indication about brain development and signs of fetal redistribution. Kidneys were measured to have insight in the development of organs during pregnancy. Furthermore placental perfusion flow velocity waveforms were acquired to assess placental function and resistance. An overview of all the prenatal measurements performed in the focus study is given in Table 7.

Children at birth

Characteristics of the live born children at birth are presented in Table 8. Among these live births, 51% were male and 49% female. These percentages are similar to the population figures in the Netherlands and in Rotterdam (6). Ethnicity of the children was defined using the strategy as described for the mothers and partners. The differences in ethnic

Table 7. Generation R Focus cohort measurements

Fetal biometry
Biparietal diameter (BPD)
Head circumference (HC)
Transverse cerebellar diameter (TCD)
Abdominal circumference (AC)
Femur length (FL)
Placental perfusion
Umbilical artery: pulsatility index, resistance index, positive or negative end diastolic velocity
Uterine artery left and right: pulsatility index, resistance index, uni- or bilateral notch
Umbilical vein: diameter, time velocity integral, volume flow
Fetal brain measurements
Occipito-frontal diameter (OFD)
Hemispheral width
Atrial width of lateral ventricle, cisterna magna diameter
Middle cerebral artery, peak systolic velocity, pulsatility index, resistance index
Anterior cerebral artery, peak systolic velocity, pulsatility index, resistance index
Fetal heart measurements
Aorta ascendens: peak systolic velocity, time velocity integral, heart rate, diameter, volume flow
Pulmonary artery: peak systolic velocity, time velocity integral, heart rate, diameter, volume flow
Left and right cardiac output, Combined cardiac output, Relative cardiac output.
Mitral valve: E-wave, A-wave, peak velocity, E/A ratio
Tricuspid valve: E-wave, A-wave, peak velocity, E/A ratio
Ductus venosus: end diastolic velocity, venous pulsatility index
Fetal kidney measurements
Kidney length
Kidney width, transverse diameter
Kidney depth, antero- posterior diameter
Kidney volume in cm^3

Table 8. Characteristics of newborns

Male (%)	51
Birth weight (grams)*	3412 (561)
Gestational age (weeks)**	40 (35.4-42.1)
Ethnicity (%)	
Dutch, other-European	62
Surinamese	8
Moroccan	7
Turkish	8
Dutch Antilles	4
Cape verdian	3
Other	8

Values are percentages.
**Mean (standard deviation)*
*** Median (95% range)*
Data are based on living born children.

distributions between all newborns in the study area and the newborns participating in the study are similar to the differences found in the mothers.

Overall response

Estimation of the precise number of eligible pregnant women in the study area is difficult since there is no satisfactory registry of pregnancies. Therefore, it was not attempted to identify overall response rates of pregnant women. The children form a prenatally recruited birth-cohort, thus the overall response of the study can be calculated at birth. Full participant recruitment in the definite study area was established for mothers with a delivery date from January 2003 until January 2006. The overall response in this period represents the number of children born to mothers living in the study area at their delivery date and participating in the study as percentage of the total number of children born to mothers in the same area and period. This gives an estimated response rate of 61% at birth.

Postnatal follow-up

Of all 9,778 mothers, 1,195 mothers were participants in the pilot phase since they lived outside the definite study area at their delivery date. Their children are not approached for postnatal follow-up studies. Of the remaining 8,583 mothers, it is expected that 98% (n = 8,411) have pregnancies resulting in living born children that could be approached for postnatal follow-up studies (Figure 2). These mothers and their children are eligible for postnatal follow-up studies. Based on figures from 2003 and 2004, it is estimated that about 92% of these eligible mothers are willing to continue to participate in the postnatal phase with their children.

Data collection in the prenatal phase

Physical examinations

Physical examinations were planned at each visit in early pregnancy, mid-pregnancy and late pregnancy and included height, weight and blood pressure of both parents. Overall response rates for these specific measurements in mothers and their partners are similar to the visit percentages presented in Figure 2. Since there was a wide range of gestational age at each visit, the physical examinations will be used in the analyses as gestational age adjusted measurements in early pregnancy, mid-pregnancy and late pregnancy.

Questionnaires

Mothers received four postal questionnaires and their partner received one postal questionnaire in the prenatal phase (Table 4). Each questionnaire comprises about 25 pages. Topics in these questionnaires were:

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

Overall response rates for these questionnaires varied from 77% to 91% (Figure 2). However, the response rates of specific questions may be lower due to missing values within questionnaires.

Fetal ultrasound examinations

Fetal ultrasound examinations were performed at each prenatal visit. Overall response rates for these ultrasound examinations were in general similar to the visit percentages given in Figure 2. These ultrasound examinations were used for both establishing gestational age and assessing fetal growth patterns. Establishing gestational age by using the first day of the last menstrual period is not reliable for a variety of reasons including the large number of women who do not know their exact date, have irregular menstrual cycles or amenorrhea, use oral contraceptive pills or bleed in early pregnancy (7). Establishing gestational age with fetal ultrasound examinations seems to overcome most of these problems. The major disadvantage of using measurements of the ultrasound examinations for establishing gestational age is that it does not allow growth studies of these measurements since no growth variability between subjects is assumed (8) at the time of the ultrasound procedure to date the pregnancy. Charts for ultrasound dating of pregnancy were derived in a sub-sample of the cohort including subjects with complete data on both the first day of the last menstrual period and crown-rump length or biparietal diameter. Subsequently, gestational age at prenatal enrolment and, as a consequence, at delivery was retrospectively established by crown-rump length or biparietal diameter measured in early pregnancy or mid-pregnancy. Subsequently, longitudinal curves of all fetal growth measurements (head circumference, biparietal diameter, transverse cerebellar diameter, abdominal circumference and femur length) were created resulting in standard deviation scores for all of these specific growth measurements. Methods to examine fetal growth patterns will be developed especially focused on fetal growth retardation in different periods of pregnancy and on identifying differences between groups.

Pregnancy complications and outcomes

The obstetric records of all mothers are looked up in the hospitals and midwifery practices. Specialists in the relevant field code items in these records. The major pregnancy

outcomes, including live births, induced abortion and fetal or perinatal loss, are known in 99% of all enrolled mothers. In all children known to be born alive, information about gender, birth weight and gestational age is available.

Data collection in the postnatal phase

Postnatal assessments from birth until the age of 4 years are currently conducted and include:

- Physical examinations: length (height), weight, head circumference, neurological development and in the focus cohort blood pressure and body composition;
- Questionnaires: diet, behaviour, cognition, living conditions, drug use, diseases, use of medical services;
- Routine health care: screening assessments performed in the routine child health centres;
- Ultrasound examinations: in the focus cohort brain, heart and kidney development;
- Biological samples: in the focus cohort blood and saliva samples.

The details and time scheme of these measurements have been described in detail previously (1). Current plans for measurements from the age of 4 years are focused on regular hands-on assessments in 2 to 3 hours sessions in the whole cohort and on postal questionnaires for the children and their parents.

Data management

Data preparation

Data collected by measurements in the research centres were directly entered onto written forms and into the electronic database. Ultrasound measurements were directly transferred from the ultrasound machine into the database to prevent mistakes from manual entry. Data collected by questionnaires were scanned and manually entered into an electronic database by a commercial bureau. Random samples of all questionnaires were double checked by study staff members to monitor the quality of this manual data entry process. All measurements were centrally checked by examination of the data including their ranges, distributions, means, standard deviations, outliers and logical errors. Data outliers and missing values were checked on the original forms. The data of one specific measurement were only distributed for analyses after data collection and preparation was completed for that measurement for the whole cohort and the data cleaning process was completed.

Privacy protection

Datasets needed for answering specific research questions are centrally built from different databases. All information in these datasets that enables identification of a particular participant (including identification number used for the logistics of the study, names and dates) is excluded before distribution to the researchers. The datasets for researchers include subject unique identification numbers that enable feedback about one subject to the data manager but do not enable identification of that particular subject.

Strengths and limitations

Strengths

The design of the Generation R Study is optimal for identification of determinants in the earliest phase of life, unbiased data collection and controlling for potential confounders.

Results from existing cohort studies in children suggest the importance of early life for various health conditions in later life and have initiated several birth-cohort studies (9-11). Compared to other planned or recently started prospective birth-cohort studies, the size of the Generation R Study cohort is not larger but the measurements are more detailed (12-16). The cohort is large enough to detect small effects of early environmental and genetic determinants on a variety of outcomes. The detailed longitudinal fetal growth examinations enable studies into both various environmental and genetic determinants and into postnatal consequences of fetal growth and development patterns. Currently, plans are being made to carry out similar detailed regular measurements in children in the whole cohort from the age of 5 years onwards.

The study cohort is rather unique since it comprises contemporary urban children including about 50% from ethnic minorities. The largest ethnic minority groups in this population are the Surinamese, Turkish, Moroccan, Dutch Antillean and Cape Verdian groups. Knowledge about the growth, development and health of these groups is lacking. Other birth-cohort studies do not provide information about these ethnic groups. The Generation R Study enables studies into determinants of ethnic specific health problems and health care utilization habits and may contribute to the development of ethnic specific strategies for pregnant women and children.

Limitations

Of all eligible children at birth, 61% participate in the study. National and regional registries do not have subject characteristics in all eligible children and their parents that enable detailed non-response analyses. However, the percentages of mothers from ethnic minorities and lower socio-economic status and the percentages of mothers or children with medical complications are lower among the participants than expected from the

population figures in Rotterdam (2). This selection towards a more affluent and healthy study population may be related to some determinants and outcomes separately, affecting the frequency rates and, as a consequence, the statistical power and generalizibility of the results. The prevalence and incidence rates found in the study should therefore carefully be interpreted considering the role of potential selection mechanisms. This selection leads only to bias in etiological association studies if the selection mechanisms are related to both the determinant and outcome. Although we do not expect that this is generally the case, the potential for selection bias should be considered in each analysis. Since the main aim of the study is to examine etiological associations of early determinants with outcomes in later life instead of disease frequency rates per se, major efforts are made to keep the follow-up rates as high as possible and to prevent selective loss to follow-up.

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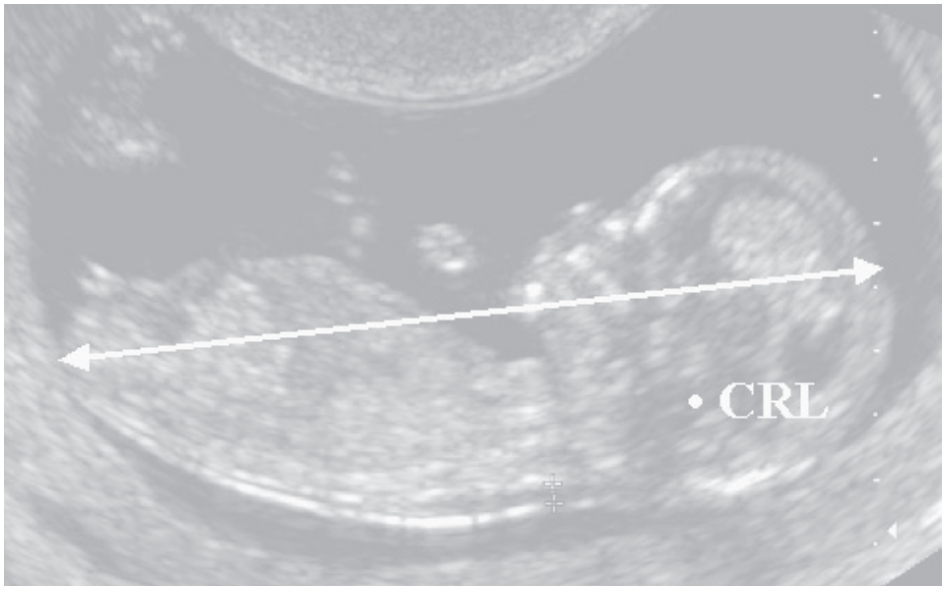
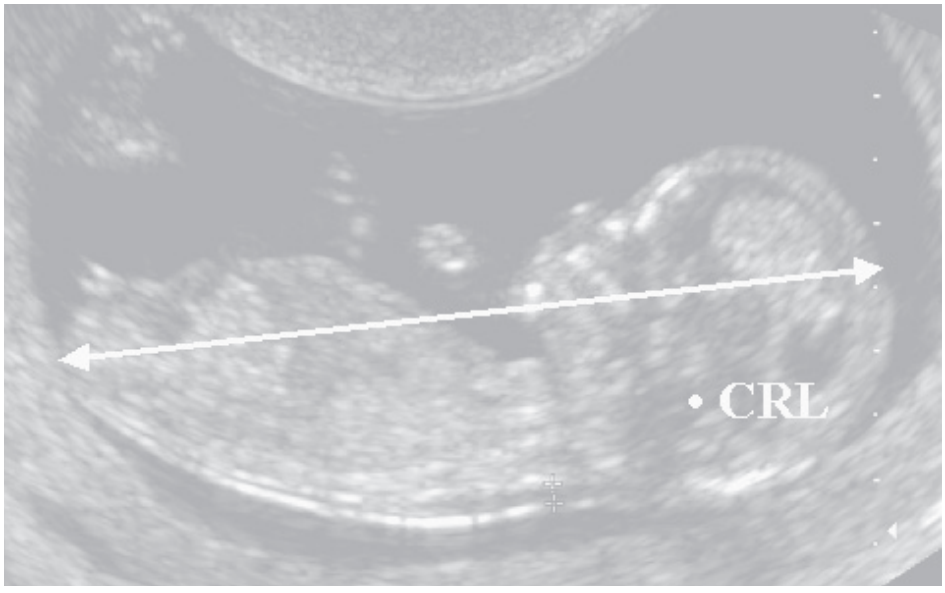
Chapter 3

Fetal growth and development



Chapter 3.1

New charts for ultrasound dating of pregnancy and assessment of fetal growth: longitudinal data from a population-based cohort study



Abstract

Objectives: Firstly, to develop charts for ultrasound dating of pregnancy based on crown-rump length and biparietal diameter. Secondly, to derive reference curves for normal fetal growth: biparietal diameter, head circumference, transverse cerebellar diameter, abdominal circumference and femur length from 10 weeks of gestational age onwards.

Methods: In a population-based prospective cohort study from early fetal life, a total of 8313 pregnant women were included for analysis. All women had repeated ultrasound assessments to examine fetal growth.

Results: Charts for ultrasound dating of pregnancy based on crown-rump length and biparietal diameter, were derived. Internal validation with the actual date of delivery showed that ultrasound provided reliable gestational age estimates. Up to 92% of deliveries took place within 37 to 42 weeks of gestation if gestational age was derived from ultrasound data, compared to 87% based on a reliable last menstrual period. The earlier the ultrasound assessment the more accurate was the prediction of the date of delivery. After 24 weeks of gestational age a reliable last menstrual period provided better estimates of gestational age. Reference curves for normal fetal growth from 10 weeks of gestational age onwards were derived.

Conclusions: Charts for ultrasound dating of pregnancy and reference curves for fetal biometry are presented. The results indicate that pregnancy dating by ultrasound provides a better prediction of the date of delivery than last menstrual period up to 20 weeks of pregnancy. The earlier the ultrasound assessment in pregnancy, preferably between 10-12 weeks, the better is the estimation of gestational age. Correct assessment of gestational age and fetal growth is essential for optimal obstetric management.

Introduction

Reliable information on gestational age is important for assessment of fetal size and fetal growth. Early detection of fetal growth restriction or macrosomia may help to reduce associated morbidity and mortality (1,2). Accurate information on gestational age is also important to avoid unnecessary obstetric interventions at the time of delivery (3).

Gestational age is historically derived on the basis of the first day of the last menstrual period (LMP) (4). However, in about 40% of pregnancies the LMP is not known or information is not reliable (5). It has been established that embryos follow the same growth pattern in early pregnancy (6). Therefore, more accurate information on gestational age can be provided by ultrasound assessment and this is widely recognised to be the method of choice (7,8). Ultrasound dating of pregnancy is usually based on crown-rump length (CRL) or biparietal diameter (BPD) (9,10). Controversy remains on the measurement of choice and the optimal gestational age for assessment (11).

Numerous studies have been conducted to derive reference charts for fetal size. Many, however, had a suboptimal design, using a hospital-based population or did not have an appropriate sample size. Additionally, substantial differences in reference charts exist depending on the population and the method of pregnancy dating (12,13). Reference charts are often based on measurements taken from 12 or more weeks of gestation onwards (12,14,15). Nowadays high-resolution ultrasound makes assessment of fetal biometry possible at an even earlier stage of pregnancy, so reference charts from earlier pregnancy onwards could have an additional value. Finally, growth charts might need an update, due to improved ultrasound equipment with higher resolution and changing health status. There is a clear need for lucent dating methods and new growth charts derived from a large population-based cohort study, using serial measurements (16).

The first aim of this population-based study was to develop charts for ultrasound dating of pregnancy based on CRL and BPD. The second aim was to construct new reference charts for fetal growth parameters, including biparietal diameter (BPD), head circumference (HC), transverse cerebellar diameter (TCD), abdominal circumference (AC), and femur length (FL), from 10 weeks of gestational age onwards.

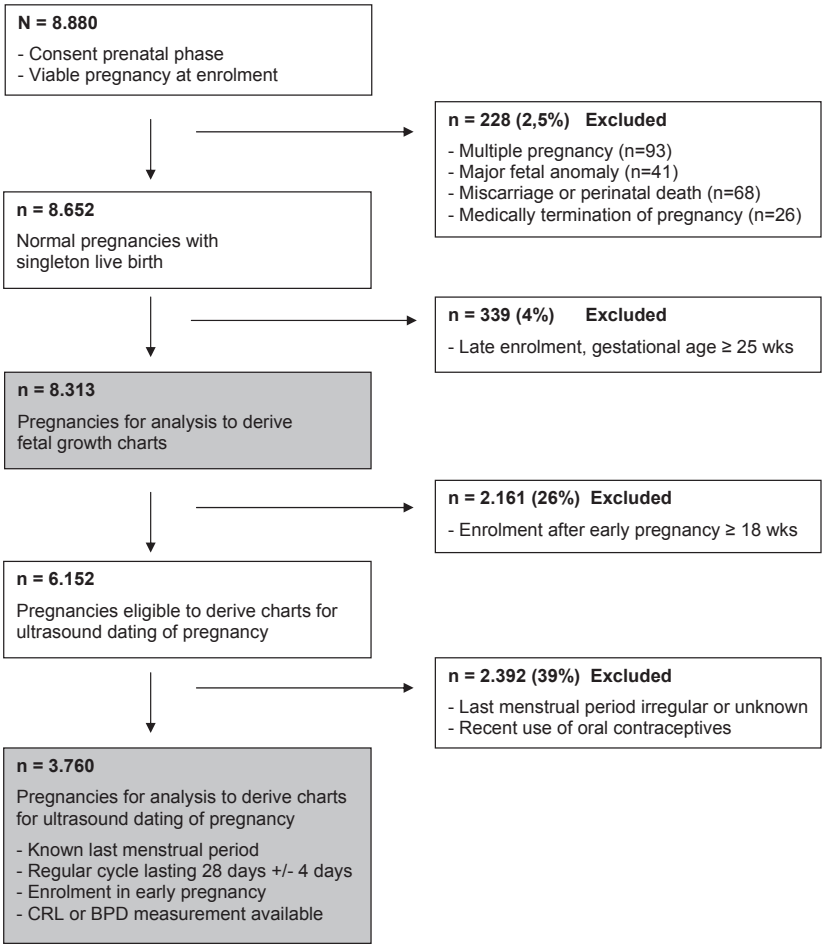
Subjects and methods

Study design

The Generation R study is a population-based, prospective cohort study from early fetal life until young adulthood. The study is designed to identify early environmental and genetic determinants of growth, development and health (17). All pregnant women in a previously defined area in Rotterdam, the Netherlands, were approached, either by

community midwife or hospital based Generation R staff, at their first antenatal visit. They received written and oral information about the study. Women who gave written consent and had a viable pregnancy at the first ultrasound visit were included. In total, 8,880 pregnant women with a delivery date between April 2002 and January 2006 were enrolled in the prenatal part of the study. During the prenatal period, data were collected longitudinally from questionnaires, physical exams and fetal ultrasound assessments. Gestational age was established by ultrasound during the first visit. Women were examined three times during pregnancy, in early (gestational age ≤ 17 weeks), mid- (gestational age 18 - 24 weeks) and late pregnancy (gestational age ≥ 25 weeks) in a research setting. The individual time scheme of these assessments depended on the specific gestational age at enrolment as described previously (18). Of 8,880 women, 76% (n = 6748)

Figure 1. Flow chart of participants in the study. The Generation R Study.



enrolled in early pregnancy, 21% (n = 1857) in mid pregnancy and 3% (n = 275) in late pregnancy. Six percent (n = 516) of women participated with two subsequent pregnancies and in 0.1% (n = 8) women participated three times. The study design is described in detail elsewhere (18). The Medical Ethical Committee of the Erasmus MC, Rotterdam, approved the Generation R study.

Exclusion criteria

Pregnant women were excluded from analysis if the ultrasound examination demonstrated a multiple pregnancy (n=93) or major fetal anomaly (n=41), if parents opted to terminate the pregnancy due to medical reasons (n=26) or if the pregnancy resulted in miscarriage or perinatal death (n=68). Pregnant women were also excluded from analysis if they joined the study after the 24th week of pregnancy, because reliable dating of pregnancy is more difficult as pregnancy proceeds (n=339), leaving data from 8313 pregnancies for analysis to derive reference charts for fetal growth. (Figure1)

Further restrictions were applied to derive charts for ultrasound dating of pregnancy. Pregnant women were included only when the first day of the last menstrual period was known and the cycle was reported to be regular, lasting 28 days +/- 4 days, and a CRL or BPD was measured in early pregnancy. For this analysis, data from 3760 pregnant women were available to derive charts for ultrasound dating of pregnancy. (Figure 1)

Data collection

The first day of the last menstrual period was obtained from the referring letter from the community midwife or hospital. The date was confirmed with the pregnant woman at the first ultrasound visit and additional information on regularity and duration of the cycle was collected.

Ultrasound exams were performed using an Aloka® Model SSD-1700 (Tokyo, Japan) or the ATL-Philips® Model HDI 5000 (Seattle, Washington, USA). Fetal biometry consisting of biparietal diameter (BPD) (outer-outer), head circumference (HC), transverse cerebellar diameter (TCD), abdominal circumference (AC), and femur length (FL) was measured during each ultrasound examination. Crown-rump length (CRL) was measured in early pregnancy if feasible. Standard ultrasound planes for fetal measurements were used as described previously (10,19-21). Appendix I shows images of the standard fetal biometry measurements. Briefly, crown-rump length was measured in a true mid-sagittal plane with the genital tubercle and the fetal spine longitudinally in view. The maximum length from cranium to the caudal rump was measured as a straight line. BPD and HC were measured in a transverse section of the head with a central mid-line echo, interrupted in the anterior third by the cavity of the septum pellucidum with the anterior and posterior horns of the lateral ventricles in view. For BPD the outer-outer diameter was measured perpendicular to the midline, for HC an ellipse was drawn around the outline of the skull. For the TCD

measurement the transducer was rotated from the transverse plane for measurement of the BPD towards the cerebellum in the back of the head whilst keeping the cavity of the septum pellucidum in view. The optimal plane was reached when the peduncles were sighted with a symmetrical shaped cerebellum. The callipers were placed on the outer, lateral edges of the cerebellum. AC was measured in a symmetrical, transverse, round section through the abdomen, with visualization of the vertebrae on a lateral position in alignment with the ribs. The measurement was taken in a plane with the stomach and the bifurcation of the umbilical and hepatic veins using an ellipse around the abdomen. FL was measured with the full length of the bone in view perpendicular to the ultrasound beam. Vaginal scanning was performed in case of limited visibility by abdominal scanning in early pregnancy.

Of the population for analysis, 74% had an ultrasound assessment in early pregnancy, 99% had an ultrasound procedure in mid gestation, and 96% had an ultrasound visit in late pregnancy. Most ultrasound exams (88%) took place in a research setting at a regional health facility in the centre of Rotterdam. The remaining exams were carried out in one of five hospitals in the vicinity under guidance of Generation R staff. All sonographers were experienced and underwent additional training according to guidelines from the Fetal Medicine Foundation to achieve optimal reproducibility. Quality checks were carried out frequently to assess the correctness of the ultrasound sections used for biometry measurements and placements of the callipers. When needed feedback was provided to optimise individual performance.

Since experience in early pregnancy is limited, intra- and interobserver reproducibility of fetal ultrasound measurements from 9 to 14 weeks of gestation was assessed in 21 pregnancies. The intraclass correlation coefficients (ICC) and coefficients of variations (CV) were calculated (22). The ICC was higher than 0.98 and the corresponding CV lower than 6% for all fetal biometry parameters. Bland and Altman plots to test agreement of measurements for fetal biometry, demonstrated normal distributions, the mean difference was around zero and 95% of measurements fell within 2 SD of the mean. The 95% limits of agreement for the differences of fetal biometry between and among operators in proportions fell within 10% difference from the mean of the measurements, indicating good reproducibility (22). So we can reliably construct reference curves for fetal size from early pregnancy onwards for clinical purposes.

Pregnancy outcome and information about labour and birth were obtained from the midwife or physician who attended the delivery. The information was collected on a specially constructed Generation R report form. For all participants, it is known whether the pregnancy resulted in live birth, miscarriage, perinatal loss, neonatal death or elective termination. For 99% of pregnancies that resulted in a live birth, information on infant gender, date of delivery and birth weight is available.

Statistical analyses

-Ultrasound dating of pregnancy-

Data were analysed as recommended by Altman and Royston (13,16). For pregnancy dating curves, gestational age based on a reliable LMP was set out against CRL and BPD. Even though strict inclusion criteria were applied by selecting participants with a reliable LMP, there were still outliers that appeared to have a LMP that seemed unlikely (5). We used previously published relations to identify the unlikely data points. For CRL, this was the relation used by Robinson (10). For BPD, this was the relation used by Chitty (9). Data points more than two standard deviation (SD) from the regression line, fitted on our data, were considered to be not realistic and therefore removed. For derivation of charts for ultrasound dating, gestational age was log-transformed to stabilize variance (13,16). The best fitting curve was determined using second-degree fractional polynomials (23). The curve was fitted using repeated measurement analyses, taking into account the dependency in the data by specifying a constant covariance between measurements of the same subject. Subsequently, in pregnancies where both CRL and BPD were measured, the mean and the SD of estimated gestational age were compared to derive the optimal cut-off point for ultrasound dating of pregnancy. Internal validation was carried out, comparing the actual date of delivery with the estimated date of delivery; both for ultrasound and LMP based pregnancy dating, to study if pregnancy dating by ultrasound is indeed superior to the use of LMP. Furthermore we studied during which time period in pregnancy estimation of gestational age is most accurate.

-Reference curves fetal biometry-

To derive growth reference curves, gestational age was established based on CRL or BPD at enrolment using the equations derived in this study. Subsequently curves were established to describe the association between gestational age and respectively BPD, HC, TCD, AC and FL. Royston has shown how to apply a particular type of statistical model to longitudinal data to produce growth centiles and the same model may also be used to calculate valid size centiles (16,24). This approach is multilevel modelling and was used in the Generation R Study. A maximum of three ultrasound visits was used for each pregnancy, one in each of the periods described in the study design, to prevent selection bias if more than 3 ultrasounds were performed we selected the ones that were performed at the initial scheduled visits. For the BPD curve, measurements, which were used for pregnancy dating, were excluded. The best fitting fractional polynomial curves were chosen by comparing the deviances and by visually checking the goodness of fit. The curves were fitted using repeated measurement analysis. Next, regression lines were fitted for the dependency of the residual SD on gestational age (25). Subsequently, plotting the SD scores against gestational age was used to assess correctness of the model. Finally, centiles were derived

and the curves were plotted on the data. Fetal growth reference curves for BPD, HC and AC were calculated for a gestational age from 10 to 40 weeks. The curve for FL was established from 12 to 40 weeks to ensure sufficient bone mineralization for reliable measurement. The TCD curve was derived from 16 until 36 weeks of gestational age. Statistical Package of Social Sciences version 11.0 for Windows (SPSS Inc, Chicago, IL, USA) and SAS 8.02 (SAS Institute Inc., Cary, NC, USA) were used to analyse the data.

Results

-Ultrasound dating of pregnancy-

Characteristics of pregnant women and their newborns are shown in Table 1. For the ultrasound dating of pregnancy charts 2079 serial CRL and 10470 serial BPD measurements were obtained during pregnancy. Formulas describing the association between CRL and BPD (outer to outer) measurements and gestational age are given in Table 2. Tables for medians, 5th and 95th centiles and plots of individual measurements with the fitted centiles of gestational age are provided in Tables 3 and 4 and Figures 2 and 3.

Table 1. Characteristics of pregnant women and their newborns

Maternal characteristics	**All women (n = 8,313)**
Age (years) mean	30.4 (19.2 to 39.6)
Parity	
0	55%
1	31%
≥ 2	14%
Ethnicity	
Dutch, other-European	58%
Surinamese	9%
Moroccan	7%
Turkish	9%
Dutch Antilles	4%
Others	9%
Education	
Lower	13%
Intermediate	45%
Higher	42%
Newborn characteristics	**All children (n=8,313)**
Male	50,7% (n=4215)
Birth weight (grams)	3420 (2135 to 4490)
Gestational age at delivery (wks)	40+1 (35+2 to 42+2)

Values are medians (95% range)

Table 2. Ultrasound dating of pregnancy: equations for the estimated median of gestational age in relation to biparietal diameter (BPD) and crown-rump length (CRL) measurements in mm

Fetal biometry	Gestational age	Regression equations fetal measurement in mm
CRL	Median GA	exp(1.4653+0.001737*CRL +0.2313*log(CRL))
	SD log (GA)	0.04590
BPD	Median GA	exp(1.4768+0.008757*BPD+0.2803*log(BPD))
	SD log(GA)	0.04238

CRL, crown-rump length (mm); BPD, biparietal diameter (mm); SD, standard deviation; GA, gestational age in exact weeks

Table 3. Crown-rump length (CRL) in relation to gestational age in weeks + days

CRL (mm)	Gestational age in weeks + days		
	5th centile	Median	95th centile
5	5 + 6	6 + 2	6 + 6
10	7 + 0	7 + 4	8 + 1
15	7 + 5	8 + 2	9 + 0
20	8 + 2	9 + 0	9 + 5
25	8 + 6	9 + 4	10 + 2
30	9 + 2	10 + 0	10 + 6
35	9 + 5	10 + 3	11 + 2
40	10 + 1	10 + 6	11 + 5
45	10 + 3	11 + 2	12 + 1
50	10 + 6	11 + 5	12 + 4
55	11 + 1	12 + 0	13 + 0
60	11 + 3	12 + 3	13 + 3
65	11 + 6	12 + 5	13 + 5
70	12 + 1	13 + 0	14 + 0
75	12 + 3	13 + 3	14 + 3
80	12 + 5	13 + 5	14 + 5
85	13 + 0	14 + 0	15 + 1
90	13 + 2	14 + 2	15 + 3
95	13 + 4	14 + 4	15 + 5
100	13 + 6	15 + 0	16 + 1

Pregnancy dating curves of CRL and BPD derived in this study were compared and demonstrated that up to a CRL of 65 mm and a BPD of 23 mm both parameters provided a similar, equally good gestational age estimate. This corresponds with a gestational age of 12 weeks and 5 days. A BPD > 23 mm provided a more accurate gestational age estimate than CRL mainly because the SD for CRL increased compared to BPD. Internal validation with this method of dating our pregnancies on ultrasound in different periods of gestation

Table 4. Biparietal diameter (BPD) outer-outer in relation to gestational age in weeks + days

BPD (mm) outer-outer	Gestational age in weeks + days		
	5th centile	Median	95th centile
10	8 + 4	9 + 1	9 + 5
15	10 + 0	10 + 5	11 + 3
20	11 + 2	12 + 1	13 + 0
25	12 + 4	13 + 3	14 + 3
30	13 + 5	14 + 5	15 + 6
35	15 + 0	16 + 1	17 + 2
40	16 + 2	17 + 3	18 + 5
45	17 + 4	18 + 6	20 + 2
50	19 + 0	20 + 2	21 + 5
55	20 + 2	21 + 6	23 + 3
60	21 + 5	23 + 2	25 + 0
65	23 + 2	24 + 0	26 + 5
70	24 + 6	26 + 4	28 + 4
75	26 + 3	28 + 2	36 + 3
80	28 + 1	30 + 1	32 + 2
85	29 + 6	32 + 0	34 + 2
90	31 + 5	34 + 0	36 + 3
95	33 + 4	36 + 0	38 + 5
100	35 + 5	38 + 2	41 + 0
105	37 + 5	40 + 3	43 + 3
110	40 + 0	42 + 6	45 + 6

Figure 2. Crown-rump length (CRL) measurements in mm in relation to gestational age in weeks with 5th and 95th fitted centiles

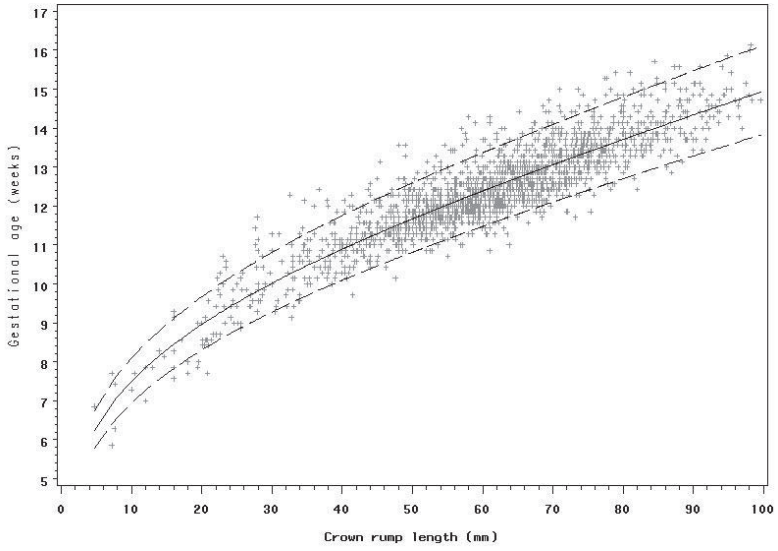
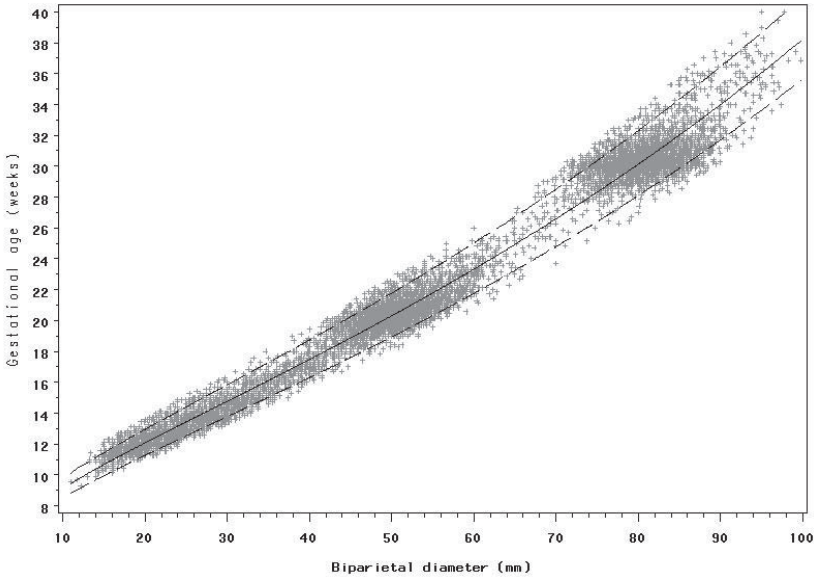


Figure 3. Biparietal diameter (BPD) outer-outer measurements in mm in relation to gestational age in weeks with 5th and 95th fitted centiles



was carried out using the actual date of delivery compared to LMP; the results are shown in Table 5. Ultrasound provided a better estimation of the date of delivery than a reliable LMP before 20 weeks of gestational age. Additionally the earlier in pregnancy ultrasound measurements were performed to establish gestational age, the more accurate the prediction of the date of delivery, with an optimum at 10-12 weeks. Reliable LMP provided a better estimation of the date of delivery than ultrasound after 24 weeks of gestational age.

Table 5. Proportion of deliveries between 37-42 weeks, comparison of gestational age based on last menstrual period or ultrasound

Gestational age based on ultrasound or LMP	Proportions of deliveries within normal range (37- 42 weeks)	Median gestational age at birth (weeks)	Standard deviation (weeks)
Reliable LMP	87 %	40.1	2.0
US < 10 weeks of GA	91 %	40.1	1.8
US 10-12 weeks of GA	92 %	40.1	1.7
US 12-14 weeks of GA	91 %	40.1	1.8
US 14-16 weeks of GA	88 %	40.1	1.9
US 16-20 weeks of GA	89 %	40.1	1.9
US 20-24 weeks of GA	87 %	40.1	2.0
US > 24 weeks of GA	82 %	40.1	2.4

LMP, last menstrual period; US, ultrasound; GA, gestational age

Between 20-24 weeks of gestational age ultrasound and a reliable LMP performed equally well (87%). (Table 5)

-Reference curves fetal biometry-

To derive reference curves for fetal growth, all pregnancies in this study (n=8313) were dated based on the CRL (n=2656) or the BPD (n=5657). CRL was used if the measurement fell in the range of 20 to 65 mm and BPD was used from 23 mm onwards. Of all participants, 6678 pregnancies (80%) were dated in early pregnancy, and 1635 (20%) in mid pregnancy. Available data to construct reference charts for fetal growth are shown in Table 6. Formulas

Table 6. Number of measurements per parameter per period (all women n=8313)

Visit in pregnancy / Characteristics	Early pregnancy (≤17 wk)	Mid pregnancy (18-24 wk)	Late pregnancy (≥25 wk)
Median gestational age at visit weeks	13.0 (10.1 - 17.6)	20.6 (18.4 - 23.8)	30.4 (28.0 - 34.2)
Total number of visits per period	6123	8236	8018
Biparietal Diameter (BPD)	6075 (99.2%)	8214 (99.7%)	7982 (99.6%)
Head circumference (HC)	5738 (93.7%)	8177 (99.2%)	7949 (99.1%)
Transverse cerebellar diameter (TCD)	330 (5.4%)	7052 (85.6%)	6662 (83.1%)
Abdominal circumference (AC)	4104 (67.0%)	8189 (99.4%)	7984 (99.6%)
Femur length (FL)	4765 (77.8%)	8196 (99.5%)	8006 (99.9%)

Values are medians (95% range)
Success rate of the measurement in % of total number of visits per period

Table 7. Reference curves for fetal biometry: equations for the mean of the measurement for fetal biometry and standard deviation

Fetal Biometry	Measurement (mm)	Regression equations Gestational age in exact weeks
BPD	Mean	$-28.2805 + 4.0352 * GA - 0.0005024 * GA^3$
	SD	$0.2388 + 0.0940 * GA$
HC	Mean	$-36.9589 + 1.7628 * GA^2 - 0.4143 * GA^2 * \log(GA)$
	SD	$-0.3106 + 0.3138 * GA$
TCD	Mean	$6.9519 + 0.03327 * GA^2$
	SD	$-0.5177 + 0.0772 * GA$
AC	Mean	$-33.2888 + 1.4251 * GA^2 - 0.3233 * GA^2 * \log(GA)$
	SD	$-1.8030 + 0.4845 * GA$
FL	Mean	$-37.4948 + 3.7089 * GA - 0.0006325 * GA^3$
	SD	$0.8778 + 0.0465 * GA$

BPD, biparietal diameter; HC, head circumference; TCD, transverse cerebellar diameter; AC, abdominal circumference; FL, femur length, SD; standard deviation; GA, gestational age in exact weeks

for growth reference curves describing the mean with the corresponding SD are given in Table 7. SD increased linearly with gestational age, higher terms did not improve the model.

In Appendix II, Scatterplots of individual measurements and fitted centiles are given in Figures 4-8. In Appendix III, Tables 8-12, the medians, 3rd, 10th, 90th and 97th centiles for BPD, HC, TCD, AC and FL against gestational age are provided.

Discussion

Pregnancy dating curves and reference curves for fetal growth were derived based on longitudinally collected ultrasound data using a large population-based cohort. The results from this study support the hypothesis that ultrasound measurements taken at an early stage of pregnancy provide more reliable information on gestational age than the first day of the last menstrual period. This study established normal ranges of fetal growth for various fetal biometry measurements from 10 weeks of gestational age onwards.

-Ultrasound dating of pregnancy-

The chart derived for pregnancy dating based on BPD corresponds well with the previously published chart from Chitty (9). In contrast, the CRL measurements observed before 11 weeks of gestation were smaller than those reported by Robinson (10). This might be due to improved high-resolution ultrasound equipment and standardisation of technique. This suggest that gestational age may be underestimated if pregnancies are dated according to the CRL curve by Robinson, before 11 weeks of gestational age. The magnitude of this error is up to 4 days with a CRL of 10 mm, this may be clinically important.

Early ultrasound is commonly recognized to provide a more valid estimation of gestational age than LMP dating, which is consistent with the results from our study (7, 8). In a substantial proportion of pregnancies, LMP cannot be used since the date is incorrect or not known, women have only recently stopped the use of oral contraceptives, or report to have irregular or prolonged menstrual cycles. Even when the LMP is known and the cycle was reported to be regular, there may be subtle variations in gestational age due to early or delayed ovulation, fertilisation or nidation, early pregnancy bleeding can be misinterpreted for LMP as well (5). In our study 39% had an unknown LMP or irregular cycle (Figure 1). A disadvantage of dating based on ultrasound measurements is that biological variation in early fetal growth is reduced to zero. Embryological studies have observed uniform development of the human embryo with small differences in size and age at different stages and support the use of ultrasound alone in preference to menstrual history for pregnancy dating (6). However, disparities in growth may occur at an early stage of pregnancy due to chromosomal or structural abnormalities, early placental maladaptation or environmental

factors including nutrition (26). Consistent with this hypothesis are the relative smaller CRL in fetuses with triploidy and trisomy (18,5). In clinical practice, substantial differences between gestational age based on ultrasound measurements and LMP, if reliable, should be considered as an indicator of possible pathology and an increased risk of fetal growth restriction (27).

Accurate pregnancy dating is important to establish gestational age for evaluation of fetal growth and prediction of the date of delivery. The increasing variation in fetal size as pregnancy proceeds implies increasing uncertainty in prediction. We found that early ultrasound assessment, preferably between 10-12 weeks, provides a better prediction of gestational age, which has important implications for the timing of the first antenatal visit. An additional advantage is that some major structural defects can be detected by ultrasound in early pregnancy after 10 weeks of gestational age (28). Increasing fetal size and variability with gestation makes ultrasound estimates of gestational age less accurate in later pregnancy. The finding that ultrasound does not improve estimates after 24 weeks of gestational age implies that a LMP, if reliable, may be used in clinical practice after 24 weeks of gestational age. (Table 5) The results show that initial dating is always more reliable than in later pregnancy, so the estimated date of delivery should not be changed during the course of pregnancy.

In clinical practice when a reliable LMP and ultrasound do not differ much in individual cases there would be no need to adjust the gestational age estimate based on LMP. Measurement error in ultrasound also contributes to errors in gestational age estimates. LMP based gestational age could be used if fetal biometry measurements fall within 2SD (which is normal variation) of the ultrasound estimate. Although the estimated date of delivery is in general more accurate if ultrasound based gestational age is used.

-Reference curves for fetal biometry-

Reference curves for fetal growth for BPD, HC, AC and FL demonstrated a similar pattern of increase with gestation and no large inconsistencies with other frequently used curves (15, 29-31). The distributions and SD of the growth characteristics are similar compared to those of the curves developed by Chitty (29-31). Snijders found a higher increase in SD and different distributions of SD for BPD, HC and AC as pregnancy proceeded compared to our study (15). Before 16 weeks of gestational age, the mean of BPD, HC, AC and FL was significantly smaller in our study than found by Snijders (15). These differences are likely to arise from different statistical methods and the way pregnancy was dated. Another possibility may be different population characteristics. Improving ultrasound resolution and standardisation of technique in our study might have some influence as well. TCD measurements are markedly larger in late pregnancy compared to those previously described by Goldstein and Snijders (15, 32). TCD measurement is not yet commonly established and difficult to obtain, especially in later pregnancy, this makes it less reliable when evaluated

retrospectively. Furthermore, different ultrasound planes and techniques may explain differences in TCD curves. We measured TCD as part of our protocol with a well-described ultrasound plane.

The size of our study exceeds most studies for fetal growth. Our study is a large, population-based study with longitudinal fetal growth measurements, allowing normal ranges for fetal measurements with gestational age to be established. Pregnancies were essentially normal, resulting in a healthy singleton birth. Fetuses were followed from early fetal life onwards, which enabled us to create fetal growth curves from 10 weeks of gestational age until birth: an important advantage compared to previous published charts. The use of reference curves covering the whole range of gestational age, as in our study, prevents confusion or inconsistency that may arise from the use of different reference curves in clinical practice.

A few limitations of the study need to be discussed. Of all eligible pregnancies in our study area, 61% participated in the study. The percentages of mothers from ethnic minorities and lower socio-economic status were slightly lower than expected from population statistics in Rotterdam. This selection resulted in a possibly more healthy study population, which may have affected the generalizibility of the results.

Variables like ethnicity, fetal gender, parity and diabetes or preeclampsia may influence fetal growth. Previous studies found no effects of these factors in the first trimester (33, 34), but they have a known influence in later pregnancy (35). Evaluation of these factors in our study showed individual differences but this did not change fetal biometry growth curves significantly.

The gestational age distribution in our participants is uneven due to the design of the study. The medians of ultrasound assessment in mid and late gestation were 20.6 and 30.4 weeks respectively and the visits are clustered around these time points, this might influence the variation and accuracy of the curves. When comparing our curves with other published curves, however, no inconsistencies in mid- and late gestation could be demonstrated.

Our study was conducted in an urban, multi-ethnic, non-hospital based population. This setting likely makes the results generalizable to normal fetal development in industrialised countries.

Conclusions

This study provides new charts for ultrasound dating of pregnancy based on CRL and BPD. The earlier the ultrasound assessment in pregnancy, preferably between 10 and 12 weeks, the better is the prediction of gestational age. Pregnancy dating could be optimised using

CRL from 20 until 65 mm and BPD from 23 mm onwards. Our results suggest that in case of a reliable LMP this is the preferred method for dating of pregnancies from 24 weeks onwards. Reference curves for normal fetal growth were developed from 10 weeks of gestational age onwards for BPD, HC, AC, and FL from 12 weeks onwards. The TCD curve was derived from 16-36 weeks of gestational age. Early ultrasound dating of pregnancy and the use of reliable growth curves can improve obstetric management in pregnancy.

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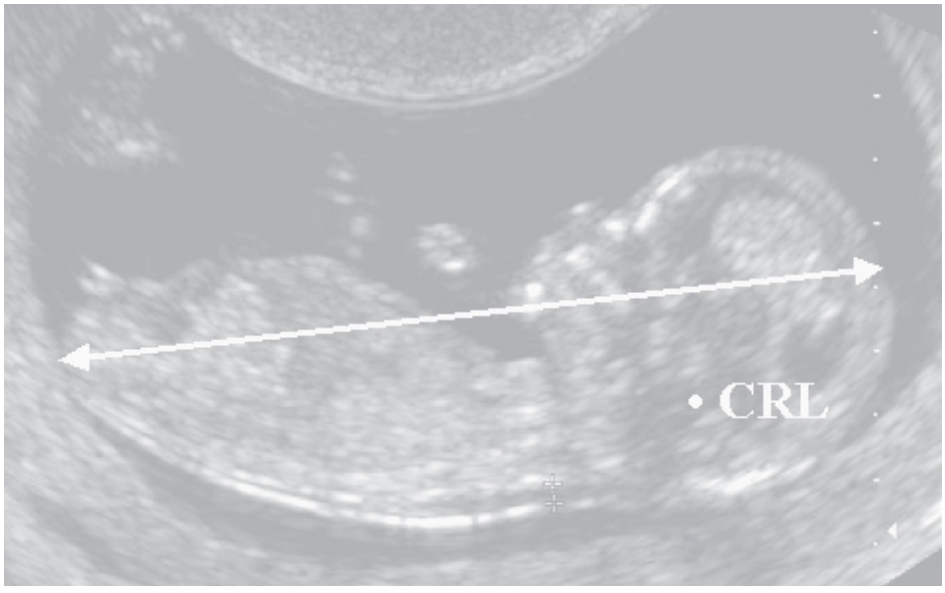
Chapter 3.2

Intra- and interobserver reproducibility study of early fetal growth measurements



Abstract

Objective: To assess the intra- and interobserver reproducibility of fetal biometry measurements by transabdominal ultrasound in early pregnancy.

Methods: The study consisted of 21 singleton pregnancies with a gestational age between 9 and 14 weeks. Intra- and interobserver agreement and reproducibility of crown-rump length (CRL), biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), and femur length (FL) measurements were assessed. One observer scanned fetal biometry randomly, subsequently the other observer did the same, and the first examiner repeated the process. Intraclass correlation coefficients (ICC) and coefficients of variation (CV) were calculated. Bland and Altman plots were computed to analyse agreement for measurements between and among observers. Limits of agreement +/- 2 SD for the differences in fetal biometry measurements in proportions of the mean of the measurements were calculated.

Results: High intra- and interobserver ICCs were found, ranging from 0.998 (CRL) to 0.982 (FL), and CVs ranging from 1.4% (CRL) to 5.9% (FL), indicating a strong relation for different fetal biometry measurements between and among observers. Limits of agreement in the Bland and Altman plots ranged from –2.7% to 2.3% (CRL) difference from the mean to –13% to 23% (FL) difference. CRL measurements showed the highest agreement followed by BPD, HC, and AC. For FL the limits of agreement were surpassed caused by differences in measurements before 12 weeks of gestational age, indicating poor reproducibility. Agreement for fetal biometry increased with fetal size.

Conclusions: This study demonstrated good reproducibility of most measurements of fetal biometry in early pregnancy by abdominal ultrasound. CRL and biparietal diameter showed high reproducibility and agreement, and head circumference to a lesser extend, from 9 weeks of gestational age onwards. Abdominal circumference is only reliable from circa 11 weeks onwards. FL has a poor reproducibility before 14 weeks of gestational age.

Introduction

Ultrasound equipment is ever improving, resulting in increasing resolution. This makes assessment of fetal growth parameters possible at an early stage of pregnancy. Early ultrasound is widely recognized to be the method of choice for pregnancy dating (1, 2). Although crown-rump length (CRL) and biparietal diameter (BPD) are used to establish gestational age in early pregnancy, other fetal biometry parameters could have an additional role in the estimation of gestational age as well. Furthermore, growth reference curves for fetal biometry in early pregnancy may facilitate early detection of fetal growth restriction or anomalies and could help to reduce associated morbidity (3, 4). Reproducibility of fetal biometry in later pregnancy is well established (5). Even though early pregnancy ultrasound is widely used for clinical purposes, validity analyses are scarce and have never been conducted properly. Before reference charts of fetal growth are used for clinical practice in early pregnancy, one should analyse the intra- and interobserver reproducibility and agreement to test reliability of the measurements.

The aim of the present study was to assess intraobserver and interobserver reproducibility of fetal biometry measurements in early pregnancy by transabdominal ultrasound in 21 pregnancies with a gestational age from 9 to 14 weeks.

Material and Methods

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood. In total 21 subjects with a gestational age between 9 and 14 weeks were asked to take part in this study, to validate measurements as performed in the Generation R Study in early pregnancy. All pregnant women had a healthy singleton gestation and visited a regional health facility for a regular ultrasound procedure in pregnancy.

Two experienced sonographers performed all examinations at the same visit. One observer scanned fetal biometry randomly, subsequently the other observer did the same, and the first examiner repeated the process. Sonographers left the ultrasound room during each other's assessment. The time interval for the first observer to rescan the patient depended on the time the second observer took for the measurements, generally around 10 minutes. Measurement results were blinded to both observers on the screen and printouts, and saved on hard disc for later analyses.

Ultrasound measurements

Fetal biometry including crown-rump length (CRL), head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC), and femur length (FL) was measured during each ultrasound examination using a transabdominal probe. Standard ultrasound planes for fetal measurements were used as described previously (6-9). Briefly, CRL was measured in a true midsagittal plane with the fetus in a neutral position and the genital tubercle and spine longitudinally in view. The callipers were placed from crown to caudal rump in a straight line. For BPD and HC a transverse section of the head was used with both lateral ventricles symmetrical in view with a horizontal midline. The BPD measurement was made perpendicular to the midline outer to outer at the widest point, for the HC measurements an ellipse was placed around the outline of the skull. AC was measured using an ellipse in a transverse, round section through the abdomen with the stomach visible. FL was measured with the whole length of the femur horizontally visible. Ultrasound exams were performed using an ATL-Philips® Model HDI 5000 (Seattle, Washington, USA) equipped with a 5.0 MHz, high frequency curved array transducer.

Statistical analyses

Statistical analysis carefully followed the steps by Bland and Altman who describe a method to compare observers in detail (10, 11).

The first step was to plot the data and draw the line of equality. This visualises the degree of agreement (10). Secondly, the consensus between and among observers was analysed using the Intraclass Correlation Coefficient (ICC) for all fetal biometry measurements (10, 12). The ICC is defined as the ratio of the variance between subjects to total variance. ICC measures the strength of the agreement of the variables, independent of the dimension of the variable considered. Additionally, the coefficient of variation (CV) was calculated for all fetal measurements, which, expressed as a percentage, is the ratio of the standard deviation (SD) of the measurement error and the overall mean (13). The within measurement SD as an index of measurement error should be independent of the subject mean. The SD was roughly proportional to the mean therefore a log transformation was indicated.

Finally a measurement of agreement was tested to investigate intra- and inter observer differences and reproducibility (10). To this end we plotted the differences of all the measurements against their mean with the 2 SD of the mean, to see the distribution and to find any possible differences from the mean within or between the observers. These graphs are the so-called Bland and Altman plots (10). A histogram of the differences was plotted to check for normal distribution and to examine if it was not skewed (11). If the differences are Gaussian distributed, 95% of the differences will lie between the mean +/- 2 SD limits. These are the limits of agreement, and the measures between and among observers can be assumed to be interchangeable within these limits. The differences increased in absolute size proportionally to the size of the measurement. This was resolved to find the

95% limits of agreement for the difference of the measurements as a proportion of the average. Log transformation could have been used as well, but we decided to use the proportion to have an indication of the average variation and thus measurement error of fetal measurements among and between observers in proportions(13). How small the limits of agreement should be is a clinical, not a statistical decision that should be made in advance of the analysis. We decided that we could confidently say that fetal biometry in early trimester would be reproducible and valid in case of ICC over 0.80, a CV under 10% and the limits of agreement from proportions of measurement error to be within 10% from the mean. Statistical analysis was performed using Statistical Package of Social Sciences version 11.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Table 1 gives the descriptive statistics for CRL, BPD, HC, AC and FL assessed in this study. Not all measurements were successfully performed in all fetuses. The lack of visibility of the landmarks or suboptimal positioning of the fetus caused measurements to be incomplete in 10 fetuses. FL, AC, HC and CRL were missing in respectively 10,6,2 and 1 fetuses. Mean

Table 1. Descriptive statistics for CRL, crown-rump length; BPD, biparietal diameter; HC, head circumference; AC, abdominal circumference and FL, femur length

Fetal biometry	Number	Mean (cm)	Minimum (cm)	Maximum (cm)	Gestational age (mean)	Gestational age (range)
CRL 1a	20	4.71	2.11	8.79	11+3	9+0, 14+1
CRL 1b	20	4.71	2.13	8.58	11+3	9+0, 14+0
CRL 2a	18	4.43	2.19	8.85	11+2	9+1, 14+1
BPD 1a	21	1.76	0.84	2.93	11+3	9+0, 14+1
BPD 1b	21	1.75	0.89	2.95	11+3	9+0, 14+0
BPD 2a	20	1.73	0.87	2.98	11+2	9+1, 14+1
HC 1a	19	6.42	3.14	10.52	11+4	9+0, 14+1
HC 1b	19	6.37	2.86	10.43	11+4	9+0, 14+0
HC 2a	18	6.25	3.22	10.34	11+3	9+1, 14+1
AC 1a	15	5.86	3.82	8.18	12+1	10+2, 14+1
AC 1b	15	5.84	3.52	8.23	12+1	10+2, 14+0
AC 2a	14	5.77	3.76	8.38	12+0	10+3, 14+1
FL 1a	12	0.82	0.39	1.44	12+4	11+1, 14+1
FL 1b	12	0.79	0.34	1.43	12+4	11+1, 14+0
FL 2a	11	0.81	0.40	1.45	12+2	11+0, 14+1

1a first measurement observer 1, 1b second measurement observer 1, 2a first measurement observer 2
Gestational age in weeks and days, Range (min, max)

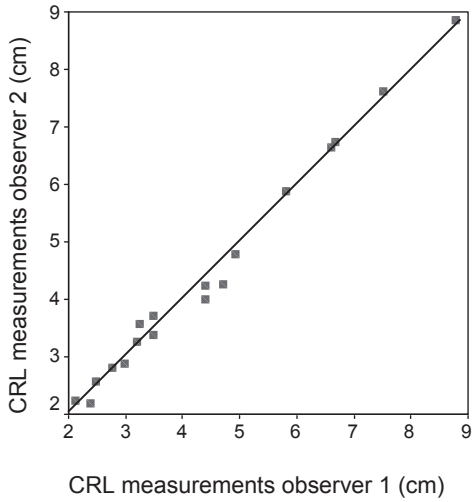


Figure 1. CRL measurements of observers with the line of equality

gestational age based on CRL was 11 weeks and 3 days (min. 9 weeks and 0 days to max.14 weeks and 1 day). FL could not be measured before 11 weeks of gestational age, whereas the AC plane could not be clearly identified before 10 weeks and 2 days of gestational age.

Figure 1 shows a plot of the CRL measurements between observers against the line of equality. All the points seem to lie randomly around this line. The plots for other fetal measurements showed similar patterns. Therefore there seems to be no bias. The variables are close to the line of equality as well, indicating good agreement and seemingly little between observer differences.

Table 2 shows the results of intraclass correlation coefficients (ICC) and coefficients of variation (CV). Intraobserver ICC ranged from 0,998 (CRL) to 0,982 (FL) and interobserver ICC ranged from 0,995 (CRL) to 0,980 (AC). Because of a wide range in fetal size and a relatively small distribution of differences between measurements within or among

Table 2. Intra- and Interobserver Intraclass Correlation Coefficient (ICC) and coefficient of variation (CV) for fetal biometry in early pregnancy

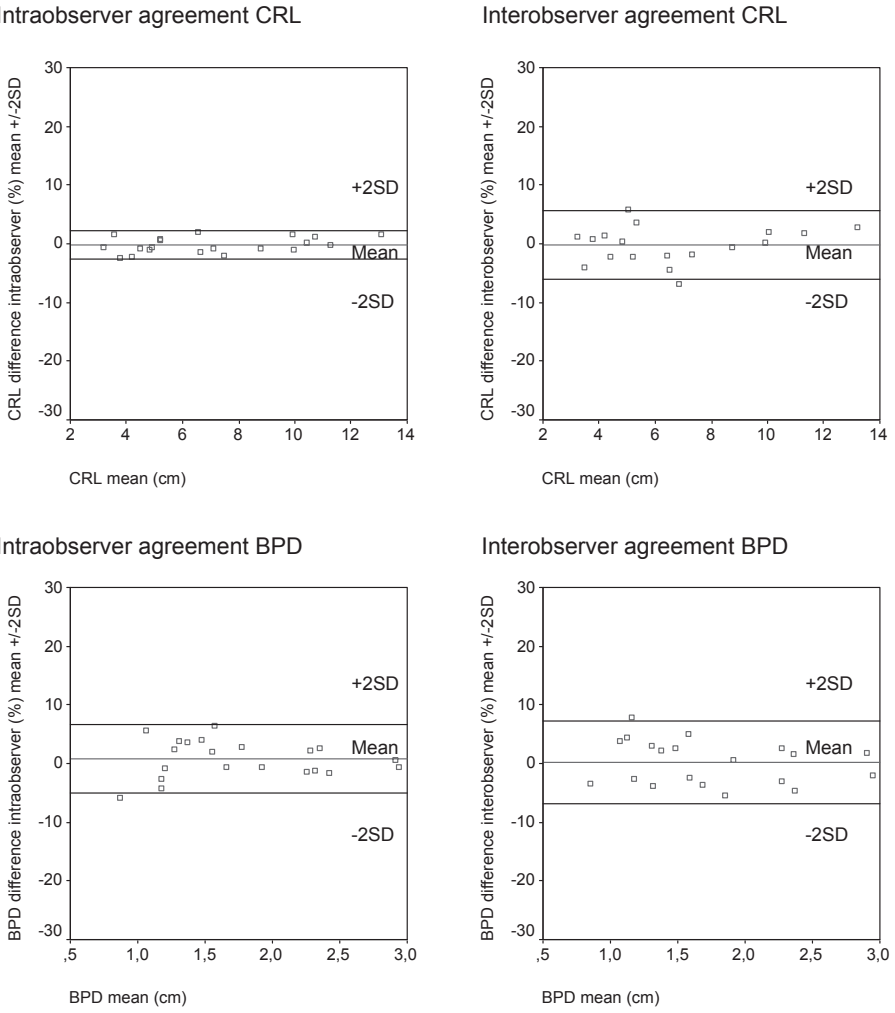
Fetal biometry	Intraobserver ICC	Intraobserver CV	Interobserver ICC	Interobserver CV
CRL	0.998	1.4 %	0.995	3.1 %
BPD	0.997	1.8 %	0.995	2.4 %
HC	0.995	2.2 %	0.988	3.8 %
AC	0.985	3.0 %	0.982	3.6 %
FL	0.982	5.9 %	0.988	2.9 %

CRL. crown-rump length; BPD. biparietal diameter; HC. head circumference;
AC. abdominal circumference; FL. femur length; ICC. intraclass correlation coefficient; CV. coefficient of variation.

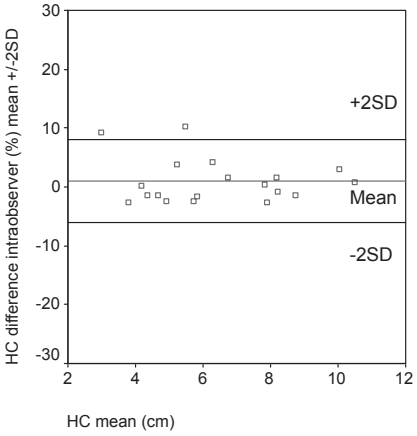
the observers, high correlation coefficients were found. The coefficient of variation (CV) ranged from 1.4% (CRL) to 5.9% (FL). For most fetal biometry measurements, intraobserver ICC was slightly higher and CV slightly lower. Thus agreement of measurements within one observer is better than between observers.

Figures 2-11 show Bland and Altman plots for the differences in fetal biometry measurements in proportions against the mean. The limits of agreement (-2SD, +2SD) are plotted in the figures. Table 3 provides the fetal biometry with the mean of the measurements

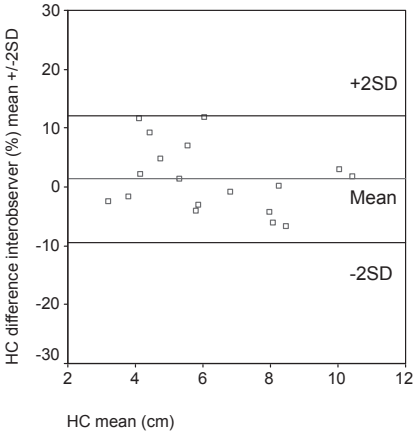
Figure 2-11. Intra- and interobserver Bland and Altman plots of variation in measurements between and among observers in proportions of the mean +/-2SD
CRL, crown-rump length; BPD, biparietal diameter; HC, head circumference; AC. abdominal circumference and FL, femur length.



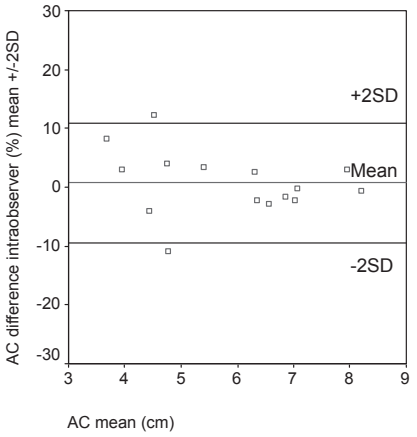
Intraobserver agreement HC



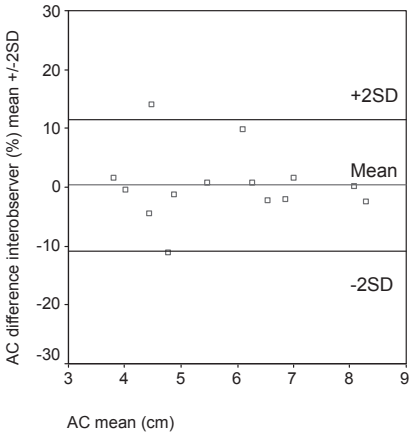
Interobserver agreement HC



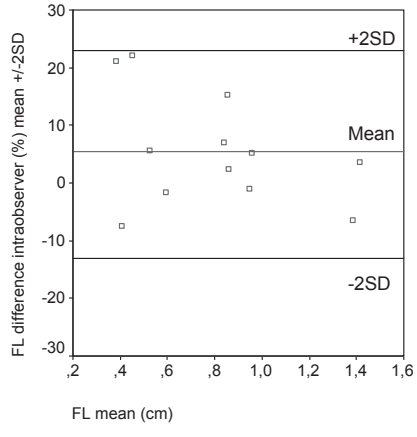
Intraobserver agreement AC



Interobserver agreement AC



Intraobserver agreement FL



Interobserver agreement FL

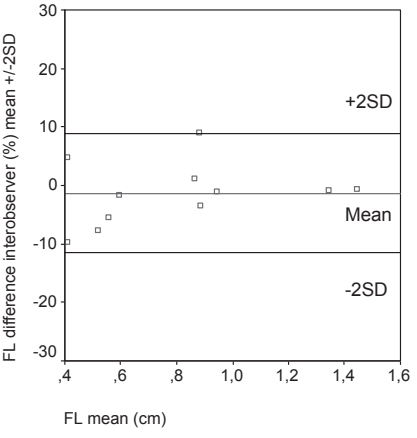


Table 3. Intra- and interobserver measurement variation in proportions of the mean with 95% limits of agreement

Fetal biometry	Intraobserver Mean (%)	Intraobserver 95% limits of agreement (%)	Interobserver Mean (%)	Interobserver 95% limits of agreement (%)
CRL	-0.2 %	-2.7 to 2.3	-0.2 %	-6.1 to 5.7
BPD	0.8 %	-5.1 to 6.7	0.2 %	-6.8 to 7.2
HC	1.0 %	-6.0 to 8.0	1.3 %	-9.4 to 12
AC	0.8 %	-9.4 to 11	0.3 %	-10.9 to 11.5
FL	5.5 %	-13 to 23	-1.4 %	-11.4 to 8.8

CRL, crown-rump length; BPD, biparietal diameter; HC, head circumference; AC. abdominal circumference and FL, femur length.

and the 95% limits of agreement for the proportions of the differences of the measurement from the mean among and between observers. The observed fetal biometry mean ranged from –0.2% (CRL) and 0.2% (BPD) to 5.5% (FL). The limits of agreement ranged from –2.7% to 2.3% (CRL), to the widest limit from –13% to 23% (FL). CRL measurements of fetal biometry showed the highest agreement, followed by BPD, HC, AC and FL. The distribution in the Bland and Altman plots suggested that (differences in) measurements in smaller fetuses caused a proportionally wider range for the limits of agreement, so reproducibility increased with the size of the fetus. Figure 12 shows the histogram for the distribution of the CRL differences between observers around the mean with the normal distribution. The histograms of other measurements showed similar distributions.

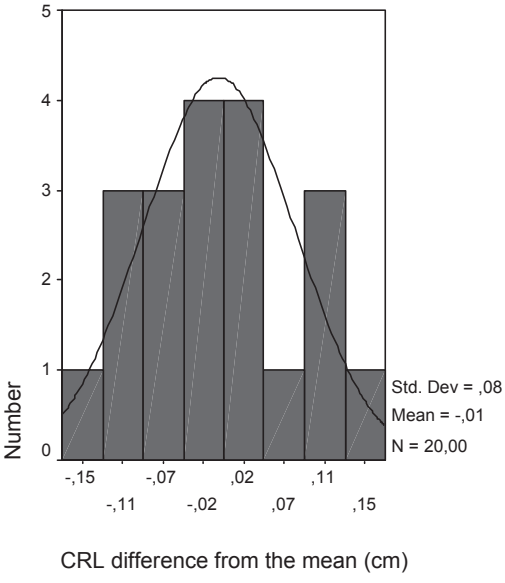


Figure 12. Histogram of distribution of measurement differences from the mean between observers with the normal distribution.

Discussion

Fetal ultrasound is an important tool for the clinician to assess gestational age and monitor fetal growth. Reference curves of fetal biometry in early pregnancy could enhance detection of early fetal growth restriction as evident in anomalies like triploidy or trisomy 18 (14). Furthermore, early ultrasound measures of fetal biometry could improve the accuracy of gestational age estimation. Therefore, it is important to have reliable information about reproducibility of fetal biometry measurements in early pregnancy. The goal of the present study was to assess the reproducibility of CRL, BPD, HC, AC and FL in early gestation by abdominal ultrasound.

Various statistical methods were used in this study to assess the reproducibility and agreement of fetal growth measurements. A simple plot of the results of one observer against the other showed that like expected the data points are clustered near the line of equality and it will be difficult to assess between observer differences. The variables are close to the line of equality, indicating good agreement and seemingly little between observer differences.

Problems concerning ICC are that it depends on the range and distribution of the variables. Since we compared the set of measurements over a whole range of values, for example CRL ranging from 2 to10, a high correlation is almost guaranteed. For a high reliability a good ICC should be accompanied by a low CV value. This study shows high ICC with low CV indicating a high degree of similarity among and between the observers for different fetal biometry measurements thus good agreement. For all fetal biometry intraobserver ICC were slightly higher than interobserver ICC. This is expected for observers measure differently, the ICC is lower among observers caused by interobserver variability. So the strength of associations within one observer is better than between observers.

A plot of the difference between the observers against their mean is more informative. This allows us to investigate any possible relationship between the measurement error and the true value. The true value is not known, and the mean of the two measurements is the best estimate we have. If there is no consistent bias, i.e. differences between the observers the mean should be close to zero, as is the case in this study. As checked by the histograms (Figure 12) a random normally distributed sample is correct, so the measures between and among observers can be assumed to be interchangeable within the limits of agreement. The Bland and Altman plots showed good agreement among and between the observers. Proportional measurement error became smaller with increasing fetal size, so reliability and reproducibility is better if the fetus is larger. The measurement for femur length surpassed our preset limits of agreement. In the lower ranges of mean FL in measurements performed before circa 12 weeks of gestational age, we saw proportional differences up to 23% indicating poor reproducibility. For FL this is probably due to lack of bone mineralization, which only becomes clearly visible by ultrasound after 12 weeks of gestational age.

The measurement of FL was not possible due to the same problem before 11 weeks of gestational age. Femur length measurements in early pregnancy are different than in later pregnancy, because ossification starts at the diathesis, only in later pregnancy the whole length of the femur can be measured. The end points of the femur are hard to identify, gain settings and side-lobe artefacts may have a considerable effect on the measurement and thus produce measurement error. FL is clearly not reliable and reproducible in early pregnancy. Some measurements of AC and HC performed before 12 weeks of gestational age caused limits of agreement to pass in some cases over 10% of measurement error. This might be due to landmarks that are not clearly visible like ribs, spine and stomach, making the exact round transverse section of the abdomen less recognizable. Vaginal ultrasound might provide better images in early pregnancy, which can improve the recognition of the landmarks and thus results in more accurate measurements.

A limitation of the study is that only 21 subjects were enrolled what makes the limits of agreements more susceptible to outliers. The strength of our study is an in-depth statistical analysis, which gives a useful indication about the reliability of fetal biometry measurements in early pregnancy.

Conclusion

In conclusion, we demonstrated good reproducibility of most measurements of fetal biometry in early pregnancy by abdominal ultrasound. Narrow limits of agreement give ascertainment that we can reliably construct charts for fetal size in early pregnancy for clinical use. These limits of agreement give enough confidence that the influence of measurement errors would not considerably influence gestational age estimates. CRL and BPD can reliably be used from circa 9 weeks of gestational age onwards and to a lesser extend HC as well. AC can only be used from circa 10 weeks onwards, provided landmarks are clearly visible in the ultrasound planes to ensure accurate measurements. Prudence should be taken into account however, when using FL before 14 weeks of gestational age for clinical purposes.

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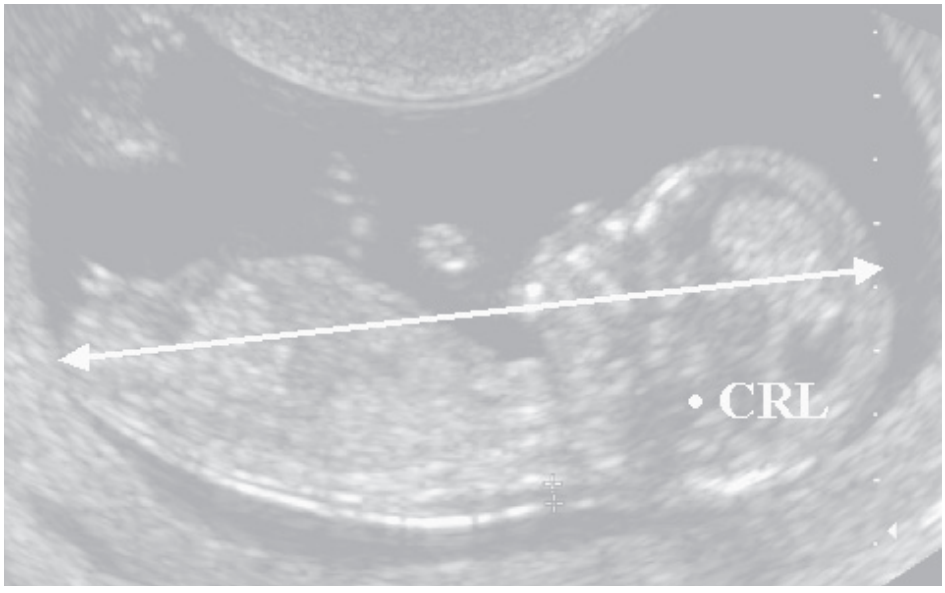
Chapter 3.3

Individually customised fetal weight curves derived from ultrasound measurements in a population-based cohort. The Generation R Study



Abstract

Objectives: To obtain a model for individually customised growth charts for estimated fetal weight (EFW), which take account for physiological fetal and maternal determinants.
Design: The Generation R Study, a prospective, population-based cohort study from early pregnancy onwards.
Setting: Rotterdam, the Netherlands.
Participants: From the 8,880 mothers enrolled during pregnancy, we included 8,162 singleton pregnancies with the first ultrasound examination in early pregnancy, having excluded those with major congenital anomalies, termination of pregnancy or perinatal mortality. Of these, 5,473 had complete data on all determinants in our final model.
Results: The final model for EFW included the following fetal and maternal determinants: gestational age, fetal gender, parity, ethnicity, maternal age, height, weight and smoking. At a gestational age of 20 weeks, the effects of the determinants were small and not significant, but at 28 and 36 weeks the effects were significant. Entering fetal and maternal characteristics into the model equation provides individually customised growth charts. In our study, 1.5% of the fetuses were classified as growth restricted (below the P10) when fetal weight was evaluated using the unadjusted reference chart, while they were classified in the normal range when individually customised growth charts were used. On the other hand, 2.6% were classified as normal using the unadjusted reference but as growth restricted when using customised growth charts.
Conclusions: We developed a model to construct individually customised growth charts, adjusted for physiological determinants that are fixed at the start of pregnancy. This is the first study using ultrasound measurements in a large population-based study to fit such a model. The use of customised growth charts may improve fetal growth monitoring and prenatal care.

Introduction

Early and accurate detection of fetal growth restriction or macrosomia is important for prenatal and early postnatal care (1, 2). In clinical practice, size and weight of fetuses are evaluated using standard reference tables for fetal biometry measurements. These references do not take into account individual characteristics of the fetus. However, it is shown that gender, parity, ethnicity, maternal weight, maternal height, paternal height and maternal age are important determinants of fetal growth (3-9). Using standard reference growth curves neglects normal variation in fetal growth due to these characteristics, which hampers the identification of fetuses with growth abnormalities. Customisation of fetal growth curves attempts to adjust for physiological characteristics and so to estimate optimal fetal growth or growth potential for an individual. It is shown that the use of individually customised growth charts improves the distinction between normal and abnormal weight and reduces the false-positive rate for the diagnosis of growth restriction (10-14).

Gardosi et al. developed a method to construct individually customised fetal growth charts (15), based on a regression model for birth weight, using the determinants gender, parity, maternal height, maternal weight at booking and ethnic origin. The growth curve for estimated fetal weight (EFW) for an individual is derived from the estimated optimal birth weight. An important principle of this method is the relation between birth weight and fetal weight during pregnancy, derived from a formula for EFW depending on gestational age (16). Furthermore, it is assumed that the effects of all determinants on fetal weight are proportional throughout pregnancy.

To overcome these limitations, we estimated the influences of physiological characteristics on fetal growth directly, using ultrasound measurements from a large population-based cohort study. We modelled EFW, obtained by an equation using abdominal circumference, head circumference and femur length (17), because this is the best overall measure of fetal size. In clinical practice EFW is mostly used to describe growth anomalies (18). We identified which determinants are relevant to be included in a multiple regression model, considering statistical and clinical significance and availability of the characteristics. Subsequently, using this model, we constructed the individually customised growth charts for the participants in our study. We evaluated how their fetal growth is assessed using these customised growth charts, compared to assessment using an unadjusted chart.

Subjects and methods

Design

The Generation R Study is a population-based prospective cohort study, designed to study growth, development and health from early fetal life until young adulthood (19, 20). Eligible mothers were resident in Rotterdam, the Netherlands, at their delivery date (between April 2002 and January 2006). In total, 9,778 mothers were enrolled, of which 8,880 during pregnancy. The response rate in the study, calculated at birth, was 61% (20). The mean maternal age and other characteristics at enrolment were similar to that of all pregnant women in the study area (21).

During the prenatal period, data were collected from physical examinations, questionnaires and fetal ultrasound assessments. The first visit usually took place before the 18th week of the pregnancy as part of routine care. Further ultrasound assessments took place in mid- (gestational age 18-25 weeks) and late-pregnancy (gestational age ≥ 25 weeks) in a research setting. Pregnancy outcome was obtained from the midwife or physician who attended the delivery.

The Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam, approved the Generation R Study. Pregnant women and their partners received written and oral information about the study and gave written consent for use of the data.

Ultrasound measurements

Ultrasound examinations were carried out in a research setting at a regional health facility in early, mid and late pregnancy. Fetal biometry including biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), and femur length (FL) was measured during each ultrasound examination using a transabdominal probe. Crown-rump length (CRL) was measured in early pregnancy if feasible.

Dating of the pregnancy was performed using the first ultrasound measurement of CRL (in case of a CRL measurement <65 mm, corresponding to 13 weeks of gestation) or BPD, using dating curves derived from this cohort. Establishing gestational age with fetal ultrasound examinations is the most accurate method for pregnancy dating (22-24).

Standardized ultrasound planes for HC, AC, and FL are described elsewhere (25-27).

EFW was calculated using the formula of Hadlock with parameters AC, HC and FL (in cm): EFW = 10**(1.326 - 0.00326*AC*FL + 0.0107*HC + 0.0438*AC + 0.158*FL) (17). The time period was restricted to gestational age of 18 weeks (earliest reliable EFW) to 36 weeks. Visits after 36 weeks were excluded since they were probably performed because of suspected pathology.

Ultrasound exams were performed using an Aloka® Model SSD-1700 (Tokyo, Japan) or the ATL-Philips® Model HDI 5000 (Seattle, Washington, USA) equipped with a 5.0 MHz, high frequency curved array transducer.

Determinants

As determinants of fetal growth, we considered physiological factors that are fixed at the start of pregnancy: fetal gender (coded as male = 1, female = -1), gravidity (primigravida = -1, other = 1), parity (nullipara = -1, other = 1), ethnicity, maternal age (age groups: ≤ 27 yr, 28 to 32 yr, ≥ 33 yr), height and pre-pregnancy weight as well as paternal height. The pathological determinant maternal smoking was used because it has a substantial effect on fetal growth (28-32). For the multivariate model, we excluded paternal height, because of its relatively low availability (in our study 73%). Because of the high correlation between gravidity and parity, only the one with the largest effect was used as potential determinant in the multivariate model.

Information about previous pregnancies, pre-pregnancy weight of the mother, smoking habits before pregnancy and ethnicity was collected by a questionnaire at enrolment. The response rate for this questionnaire was 91%. In questionnaires in mid- and late pregnancy mothers were asked whether they smoked in the past two months. Maternal smoking was coded as: smoking in mid or late pregnancy = 1, other = 0. Ethnicity of mothers was defined according to the classification of Statistics Netherlands (33), using country of birth of her parents. Mothers with Moroccan or Surinamese background were asked about their ethnic origin and further classified as Surinamese-Hindustani, Surinamese-Creole, Moroccan-Arabic or Moroccan-Berber. Height of mother and partner was examined at the first prenatal visit.

Population eligible for analysis

Pregnancies were excluded in case of multiple pregnancy (n = 93), major fetal anomalies (n = 41), termination of the pregnancy (n = 26) or perinatal mortality (n = 68). Because of difficulty in pregnancy dating, women who joined the study after the 24th week of pregnancy were excluded as well (n = 339). In 151 pregnancies ultrasound observations were only limited, making calculation of EFW impossible. This resulted in 8,162 pregnancies eligible for analysis.

Statistics

An unadjusted reference curve for EFW was constructed by modelling the relation between gestational age and EFW, using repeated measurement analysis and fractional polynomials (34). Next, the effect of each determinant was estimated separately by adding the main term and its interaction with gestational age to the model. If the interaction term was significant ($p < 0.05$) it was tested whether adding the interaction with square of gestational age was significant. If the interaction term was not significant it was removed from the model.

For the multivariate model, we started with including the potential determinants together with maternal smoking, to get estimated effects adjusted for maternal smoking.

For the construction of a customised growth chart, the term for smoking should be set to zero, whether the pregnant woman smokes or not. This provides that non-smoking is used as reference. As in the univariate models, interactions of the determinants with gestational age were included. Non-significant terms ($p < 0.05$) were removed using backward selection. Subsequently, we tested whether an additional interaction of a determinant and gestational age squared was significant.

Because of the increasing effects of the determinants during pregnancy, we computed, from the univariate models as well as from the multivariate model, estimated differences in EFW at gestational ages of 20, 28 and 36 weeks (begin, mid and end of the range used). For comparison, we also computed estimated differences in birth weight, obtained from models using the data of the neonates born after a gestation of 36 weeks or more. These models included a linear term for gestational age at birth.

Using the multivariate model, we virtually constructed customised growth charts for the participants in our study, and assessed their third trimester observation of EFW, using the unadjusted growth reference, derived from our data, as well as using the individually customised growth chart.

Results

Data from 8,162 pregnancies were used, with 16,018 EFW observations (at most 3 observations per fetus). Table 1 depicts descriptives of the study population. Table 2 provides estimated mean, SD, 5th and 95th percentile and width of the 90% reference interval for EFW at gestational ages of 20, 28 and 36 weeks. For comparison, the same descriptives are given for birth weight at 40 weeks.

In the univariate models, all considered determinants had significant influence and all effects were significantly increasing with advancing gestational age. In Table 3, the estimated differences in EFW between categories of the determinants are given for three time points during gestation. At 20 weeks gestation, the differences were small and only significant for maternal weight. At 28 and 36 weeks, almost all differences were significant.

There were 5,473 subjects with complete data on all candidate determinants for the multivariate model (fetal gender, parity, ethnicity, maternal age, maternal height, prepregnancy weight and smoking habits during pregnancy). The difference between the unadjusted curve for EFW in this group of complete cases and the unadjusted curve for the total 8,162 subjects was negligible (over the whole range less than 4 grams). All determinants had a significant contribution to the model and all interactions with gestational age were significant. For parity and maternal weight, the interaction with gestational age squared was significant. The coefficients of the multivariate model are given in the Appendix (This chapter). In Table 4, the estimated differences in EFW are presented, derived from

Table 1. Characteristics of the study population

Characteristic	Median (P5; P95) or percentage
Male fetus	50.4%
Primigravida	43.4%
Nullipara	56.6%
Ethnicity	
Dutch	59.4%
Other European	5.9%
Dutch Antilles	2.4%
Cape Verdian	3.5%
Moroccan-Arabic	1.9%
Moroccan-Berber	3.7%
Surinamese-Creole	3.2%
Surinamese-Hindustani	3.4%
Turkish	8.0%
Others	8.6%
Maternal age (year)	30.3 (20.4; 37.8)
Maternal height (cm)	167 (155; 180)
Pre-pregnancy weight (kg)	64 (50; 91)
Paternal height (cm)	182 (169; 195)
Maternal smoking during pregnancy	17.0%

Table 2. Descriptives of the distribution of estimated fetal weight (EFW) and birth weight

				90% Reference Interval	
	GA (weeks)	Mean (gr)	SD (gr)	(P5; P95)	Width
EFW	20	326	29	(277; 374)	97
	28	1201	124	(998; 1405)	406
	36	2568	291	(2091; 3046)	955
Birth weight	40	3443	447	(2710; 4176)	1466

The unadjusted reference for EFW is described by: mean EFW = 13735 -5.434·10^7*GA + 4.297·10^7* GA^{-2}·log(GA) -0.889·10^7*GA^{-2}·$(log(GA))^2$ and SD EFW = -24.659 + 0.00677*GA^3.
The unadjusted equation for birth weight, 36 < GA < 44 weeks, is: mean birth weight = 3443 + 178*(GA - 40) and SD birth weight = 447.
GA = Gestational age, P5 = 5th percentile, P95 = 95th percentile

this model. Because of the correlations between many of the determinants, most of the differences are smaller than the univariate differences. It appears that for a Moroccan Berber or a Turkish mother the expected EFW is larger than for a Dutch or other European mother with all other characteristics equal. Maternal height and weight had significant influence on EFW, independent of each other at 28 and at 36 weeks.

Filling in the individual maternal and fetal characteristics in the regression equation given in the Appendix will provide an individually customised growth chart. The expected

Table 3. Estimated differences for mean estimated fetal weight and birth weight between categories of the determinants, using univariate[a] models

		Differences (gr) in estimated fetal weight									**Differences (gr) in birth weight**		
		Gestational age (weeks)									Gestational age (weeks)		
		20			28			36			40		
Determinant	n of cases	Difference	95% CI	P-value	Difference	95% CI	P-value	Difference	95% CI	P-value	Difference	95% CI	P-value
Gender fetus (Male – Female)	8111	1	(-5; 6)	0.84	10	(5; 14)	<0.0001	19	(10; 27)	<0.0001	108	(88; 127)	<0.0001
Parity (Para 1 or more – Para 0)	7817	3	(-3; 9)	0.33	13	(6; 21)	0.0007	82	(53; 110)	<0.0001	177	(157; 197)	<0.0001
Gravidity (Multi – Primi)	7817	1	(-5; 7)	0.68	4	(-4; 12)	0.30	67	(38; 96)	<0.0001	126	(106; 146)	
Ethnicity (reference: Dutch and other European)	6768												
Dutch Antilles		3	(-17; 22)	0.79	-25	(-41; -8)	0.003	-52	(-82; -22)	0.0006	-132	(-202; -61)	0.0003
CapeVerdian		-7	(-23; 10)	0.44	-59	(-72; -45)	<0.0001	-111	(-135; -86)	<0.0001	-233	(-292; -175)	<0.0001
Moroccan-Arabic		2	(-20; 25)	0.84	-14	(-32; 4)	0.13	-31	(-64; 2)	0.07	-82	(-159; -4)	0.04
Moroccan-Berber		3	(-14; 19)	0.76	14	(-1; 28)	0.03	26	(2; 50)	0.03	9	(-47; 65)	0.75
Surinamese-Creole		-1	(-19; 16)	0.91	-60	(-75; -46)	<0.0001	-120	(-146; -93)	<0.0001	-200	(-262; -138)	<0.0001
Surinamese-Hindustani		-10	(-27; 7)	0.26	-60	(-74; -46)	<0.0001	-110	(-135; -84)	<0.0001	-298	(-358; -238)	<0.0001
Turkish		2	(-9; 13)	0.74	-6	(-15; 4)	0.22	-14	(-30; 3)	0.12	-57	(-97; -18)	0.005
Other		-2	(-14; 9)	0.66	-30	(-39; -21)	<0.0001	-58	(-75; -42)	<0.0001	-105	(-144; -66)	<0.0001
Maternal age (reference: <= 27 yr)	8162												
28 to 32 yr		-1	(-7; 6)	0.87	20	(15; 25)	<0.0001	18	(3; 33)	0.02	92	(69; 115)	<0.0001
>= 33 yr		1	(-7; 8)	0.89	35	(29; 41)	<0.0001	70	(59; 80)	<0.0001	131	(106; 155)	<0.0001
Maternal height (10 cm)	8135	4	(0; 8)	0.04	23	(18; 28)	<0.0001	78	(59; 96)	<0.0001	140	(127; 153)	<0.0001
Paternal height (10 cm)	5960	2	(-2; 6)	0.36	25	(22; 29)	<0.0001	48	(42; 54)	<0.0001	106	(92; 120)	<0.0001
Maternal weight (10 kg)	6644	3	(0; 5)	0.03	18	(15; 21)	<0.0001	58	(46; 70)	<0.0001	81	(73; 89)	<0.0001
Maternal smoking (Yes – No)	7197	-2	(-10; 6)	0.58	-28	(-39; -17)	<0.0001	-135	(-179; -92)	<0.0001	-165	(-193; -137)	<0.0001

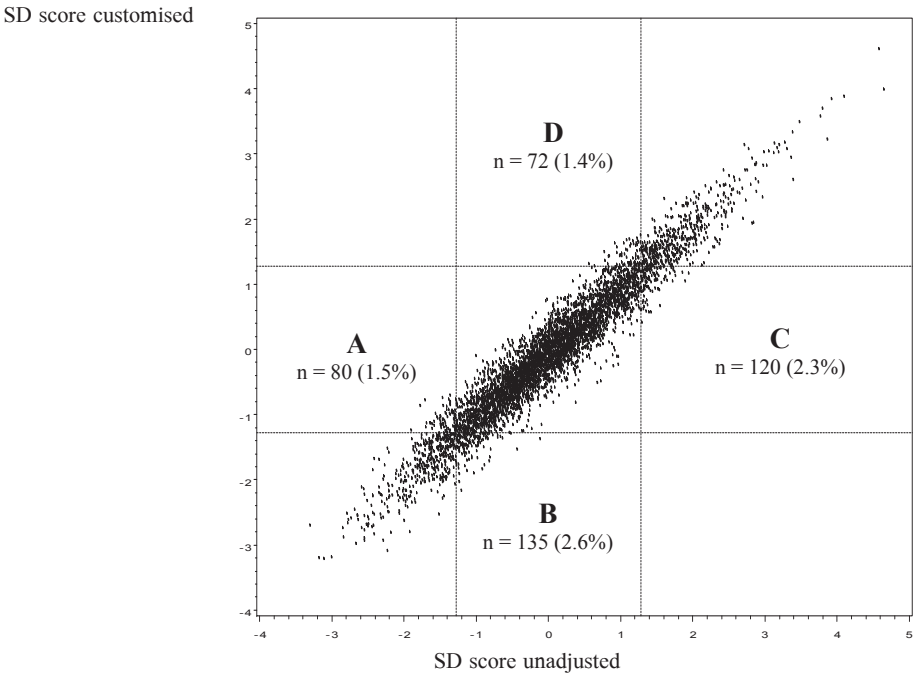
[a]All models are adjusted for gestational age (GA) including terms GA^{-2}, $GA^{-2}*\ln(GA)$, $GA^{-2}*\ln(GA)^2$

CI = Confidence interval

Table 4. Estimated differences for mean estimated fetal weight and birth weight between categories of the determinants, using a multivariate model

	Differences (gr) in estimated fetal weight									**Differences (gr) in birth weight**		
	Gestational age (weeks)									Gestational age (weeks)		
	20			28			36			40		
Determinant	Difference	95% CI	P-value	Difference	95% CI	P-value	Difference	95% CI	P-value	Difference	95% CI	P-value
Gender fetus (Male – Female)	0	(-7; 7)	0.98	12	(7; 18)	<0.0001	25	(15; 35)	<0.0001	112	(90; 134)	<0.0001
Parity (Para 1 or more – Para 0)	3	(-5; 10)	0.46	4	(-5; 14)	0.36	75	(41; 109)	<0.0001	176	(153; 200)	<0.0001
Ethnicity (reference: Dutch and other European)												
Dutch Antilles	5	(-18; 28)	0.69	-6	(-25; 13)	0.53	-17	(-51; 18)	0.34	-92	(-170; -14)	0.02
Cape Verdian	-2	(-20; 17)	0.87	-29	(-45; 14)	0.0002	-57	(-85; -30)	<0.0001	-117	(-180; -55)	0.0002
Moroccan-Arabic	5	(-23; 32)	0.75	-6	(-28; 16)	0.62	-16	(-56; 24)	0.44	-33	(-124; 58)	0.48
Moroccan-Berber	3	(-17; 23)	0.79	19	(3; 34)	0.02	35	(7; 63)	0.02	15	(-49; 80)	0.65
Surinamese-Creole	-2	(-22; 18)	0.84	-55	(-71; -39)	<0.0001	-108	(-137; -78)	<0.0001	-161	(-228; -94)	<0.0001
Surinamese-Hindustani	-6	(-26; 14)	0.54	-27	(-43; 11)	0.001	-48	(-78; -19)	0.001	-163	(-230; -97)	<0.0001
Turkish	2	(-12; 16)	0.75	15	(4; 26)	0.01	27	(7; 48)	0.009	30	(-17; 76)	0.21
Other	0	(-14; 13)	0.95	-3	(-14; 7)	0.54	-6	(-26; 13)	0.53	38	(-6; 83)	0.09
Maternal age (reference: <= 27 yr)												
28 to 32 yr	-2	(-10; 7)	0.67	7	(0; 14)	0.04	16	(4; 29)	0.01	1	(-27; 30)	0.93
>= 33 yr	-1	(-10; 9)	0.89	22	(14; 30)	<0.0001	44	(31; 58)	<0.0001	12	(-19; 43)	0.45
Maternal height (10 cm)	2	(-3; 8)	0.45	13	(9; 18)	<0.0001	24	(16; 33)	<0.0001	101	(82; 119)	<0.0001
Maternal weight (10 kg)	2	(-1; 5)	0.34	14	(10; 17)	<0.0001	57	(42; 71)	<0.0001	56	(46; 65)	<0.0001
Maternal smoking (Yes – No)	-1	(-10; 8)	0.80	-37	(-44; -29)	<0.0001	-72	(-86; -59)	<0.0001	-164	(-194;-133)	<0.0001

Figure 1. Relation between SD score obtained by the unadjusted reference (x-axis) and by individually customised references (y-axis). Reference lines are drawn at P10 and P90.



mean value and standard deviation of EFW at a certain gestational age can be computed and these can be used to convert an observed EFW into a standard deviation (SD) score and a percentile. These provide a measure of fetal size, relative to fetuses with the specified characteristics. The calculations can easily be done in an Excel-worksheet available on our website.

As example, we examined a hypothetical observation for EFW of 1500 gr, obtained at a gestational age of 32 weeks for a female fetus, first child of a Surinamese-Creole mother, with maternal height 155 cm, pre-pregnancy weight 45 kg and age 20 yr. Using the unadjusted formula, we derived in our data (Table 2) the expected EFW at 32 weeks is 1865 gr and the SD 197 gr. So an EFW of 1500 gr at 32 weeks is converted to an SD score of (1500 - 1865) / 197 = - 1.85. The corresponding percentile is P3. When a customised growth chart is constructed, using the characteristics of this fetus, the expected EFW at 32 weeks is 1669 gr, with SD 191 gr. So taking the physiological determinants into account, the SD score for this observed EFW is (1500 - 1669) / 191 = - 0.89, corresponding percentile P19.

The relation between the two SD scores for the same EFW observations is depicted in Figure 1. From the subgroup with complete data, for each fetus the SD scores of the ultrasound measurement in the third trimester (gestational age ≥ 27 weeks) was plotted. On the x-axis the SD score obtained by the unadjusted reference was drawn and on the

y-axis the SD score obtained by individually customised references. On both axes, lines were drawn at - 1.28 SD scores (= P10) and at 1.28 SD score (= P90). Using the customised instead of the unadjusted reference resulted in reclassification from too low EFW to normal for 1.5% of the cases (compartment A), and from normal to too low EFW for 2.6% (compartment B). Cases plotted in compartment C (2.3%) were in the upper 10% of EFW when the unadjusted reference was used and classified as normal using the customised references. For compartment D (1.4%) the opposite was true.

Discussion

In this study, we developed a model to construct individually customised fetal growth curves. It is the first time that such a model is fitted using observations of EFW obtained by ultrasound measurements in a large population-based prospective study. With the customised growth charts, fetal growth can be evaluated, taking into account the following physiological characteristics: fetal gender, parity, ethnicity, maternal height, pre-pregnancy weight and age. All effects increased with advancing gestational age. The effects were largest for parity and ethnicity.

The design of the Generation R Study is optimal for identification of determinants of fetal weight. Our study cohort comprises contemporary urban children including about 40% from ethnic minorities in the Netherlands. The largest ethnic minority groups in this population are from the Turkish, Moroccan and Surinamese groups. Of all eligible children at birth, 61% participated in the study. National and regional registries do not have subject characteristics in all eligible children and their parents that enable detailed non-response analyses in our study. The percentages of mothers from ethnic minorities and lower socio-economic status among the participants were slightly lower than expected from the population figures in Rotterdam (21).This resulted in a more healthy study population, possibly affecting the generalizibility of the results.

Pregnancy dating in this study was done by ultrasound measurements of CRL or BPD at first visit, which is found to be superior over dating by last menstrual period (22-24). However, this procedure neglects possible differences in CRL or BPD, which might be correlated with fetal size, at the time of dating. It is possible that this caused underestimation of the effects of the determinants, especially in early pregnancy. The earlier in pregnancy the dating, the smaller this bias will be. We excluded pregnancies dated later than at 24 weeks and in our study population 73% of the pregnancies was dated before 18 weeks of gestation. Therefore, we think underestimation of the effects in mid pregnancy is possible, but will be very small in relation to the effects in late pregnancy.

The customised antenatal growth charts developed by Gardosi et al. (15) are based on a regression model for birth weight, fitted in a very large group of over 40,000 neonates. The

determinants in this model are maternal height and weight, ethnic origin, parity and fetal gender. After calculation of the "term optimal weight" for a child, a fetus-specific intrauterine growth curve for EFW can be constructed, using a proportionality equation linking EFW during gestation to birth weight. So an important assumption for this approach is that this proportionality equation is correct for each fetus. It also assumes that the effect of each determinant is proportional during pregnancy. Applying the model of Gardosi, we derived the effects of the determinants at gestational ages of 28 and 36 weeks and compared these with our estimated effects. For example, using the model of Gardosi, the estimated difference between male and female fetuses at 28 weeks is 32 gr, using our model it is 12 gr. At 36 weeks these differences are estimated as 76 gr and 25 gr respectively. For the other determinants, our estimated effects were also smaller. The model of Gardosi does not include maternal age and parity is coded in a different way (separate effects for parity 1, 2, 3 and ≥ 4). This may influence the estimations of some effects (e.g. maternal weight, which might be related to maternal age), but not all (e.g. fetal gender). Because our model is based on EFW derived from ultrasound measurements, like in clinical practice, we think our curves are better applicable than the curves of Gardosi, which are based on several assumptions.

Other studies on factors influencing fetal growth, using ultrasound measurements, have been published before (4-8). Jacquemyn (4) only studied the differences between some ethnic groups, while Schwärzler (7) only studied sex-differences. Mongelli (5) compared different subgroups based on maternal weight, maternal height, fetal gender, parity or ethnicity, but did not develop a model with all determinants included. Pang (6) developed models for BPD, HC, AC and FL but not for EFW to avoid potential problems in erroneous estimation of fetal weight and to be able to assess growth restriction in biometric parameters separately. Most comparable to our study is the study of Johnson (8). This study used data of 635 women visiting a low-risk antenatal clinic.

Using customised growth charts enables to identify pathological smallness instead of constitutional small size, with normal intrauterine growth. This can prevent unnecessary classification as growth restricted. On the other hand, studies have shown that failure to detect fetal growth restriction is an important reason for suboptimal perinatal care (35). Which should be the precise factors to take into account when evaluating fetal growth is not obvious. Some factors may represent both physiological and pathological effects. For example, ethnicity may reflect constitutional growth potential, but ethnic differences might also reflect differences in feeding habits and other lifestyle factors for which adjustment is undesirable. Parity is another determinant of which inclusion in the model is debatable. We adjusted for parity, because it is a determinant of the growth potential of a fetus. However, nulliparous women are at higher risk of obstetric complications (36) and stillbirths (37). It is not clear, whether nulliparity must be seen as a physiological determinant, or it should be considered as a possible cause of intrauterine growth restriction. The effects on

EFW for these disputable determinants parity and ethnicity are the largest in our model. If one prefers a customised growth chart adjusted for a selection of the determinants, the unselected determinants can be disregarded.

Awareness of the limitations and possible measurement errors in ultrasound measurements are important for the appropriate use of reference charts of estimated fetal weight. It seems unwise to depend only on estimated fetal weight to select fetuses at risk. Clinical factors like medical history, maternal blood pressure and fetal Doppler measurements should be included in a proper decision in the management of pregnancies (1, 2, 38).

Conclusion

The use of customised growth charts may improve fetal growth monitoring and prenatal care. Follow-up studies are needed to examine whether and to what extend the use of customised growth charts can improve the prediction of which children are at risk for perinatal or later morbidity, and which factors should be used for customisation.

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Appendix

The formula for the mean EFW of an individually customised growth chart is:

$$\begin{aligned}EFW = {} & 17877 - 62328362*GA^{-2} + 49529740*GA^{-2}*\ln(GA) - 10323705*GA^{-2}*(\ln(GA))^2 \\ & - 15*Sexe + 150*Parity + 24*Age_2 - 33*Age_3 - 2.58*Height + 11.27*Weight \\ & + 32*Ethn_2 + 68*Ethn_3 + 30*Ethn_4 - 38*Ethn_5 + 130*Ethn_6 + 46*Ethn_7 - 29*Ethn_8 \\ & + 7*Ethn_9 \\ & + GA*(0.78*Sexe - 12.83*Parity - 52.45*Age_1 - 53.58*Age_2 - 50.76*Age_3 \\ & + 0.1395*Height - 1.0473*Weight \\ & - 1.35*Ethn_2 - 3.48*Ethn_3 - 1.28*Ethn_4 + 2.02*Ethn_5 - 6.60*Ethn_6 \\ & - 2.63*Ethn_7 + 1.57*Ethn_8 - 0.37*Ethn_9) \\ & + GA^2*(0.2694*Parity + 0.02476*Weight)\end{aligned}$$

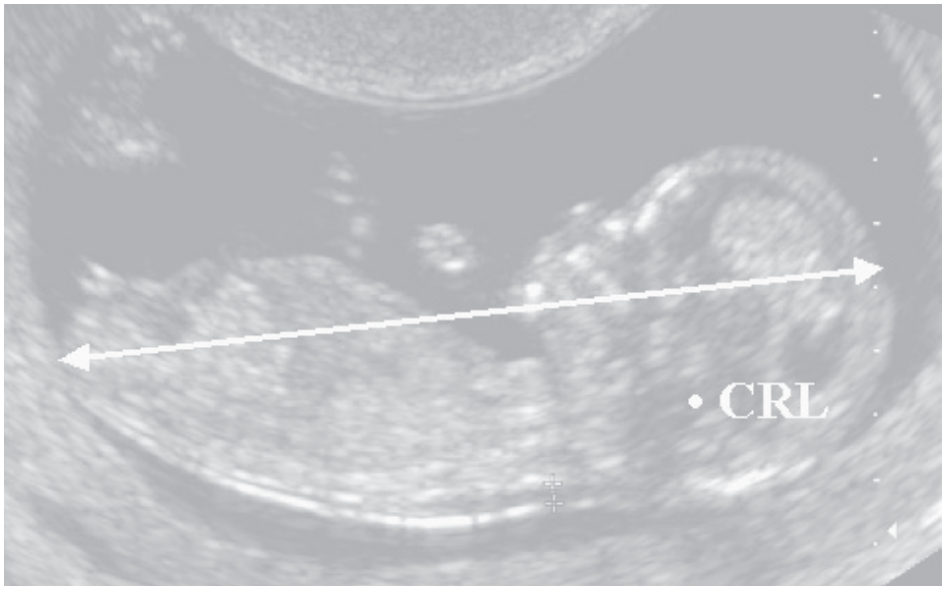
EFW	Estimated Fetal Weight (gr)
GA	Gestational age (weeks)
Sexe	Female = -1, Male = 1
Parity	Nulliparity = -1, Other = 1
Age group	Age_1: <= 27 yr = 1, other = 0
	Age_2: 28 to 32 yr = 1, other = 0
	Age_3: >= 33 yr = 1, other = 0
Height	Maternal height (cm)
Weight	Pre-pregnancy weight (kg)
Ethnicity	$Ethn_2$: Dutch Antilles = 1, other = 0
	$Ethn_3$: Cape Verdian = 1, other = 0
	$Ethn_4$: Moroccan-Arabic = 1, other = 0
	$Ethn_5$: Moroccan-Berber = 1, other = 0
	$Ethn_6$: Surinamese-Creole = 1, other = 0
	$Ethn_7$: Surinamese-Hindustani = 1, other = 0
	$Ethn_8$: Turkish = 1, other = 0
	$Ethn_9$: Other non-European = 1, other = 0

The formula for the standard deviation (SD) for the individually customised growth charts is:

SD EFW = - 23.0315 + 0.006523*GA

Chapter 3.4

Maternal smoking and fetal growth characteristics in different periods of pregnancy. The Generation R Study



Abstract

The authors examined the associations of maternal smoking in pregnancy with various fetal growth characteristics in 7,098 pregnant women participating in the Generation R Study, a population-based prospective cohort study in pregnant women and their children in Rotterdam, the Netherlands. Maternal smoking was assessed by questionnaires in early, mid- and late pregnancy. Fetal growth characteristics included head circumference, abdominal circumference and femur length measured repeatedly in mid- and late pregnancy. Maternal smoking during pregnancy was associated with reduced growth of the head circumference (-0.56 (95% confidence interval (CI): -0.73, -0.40) mm per week), abdominal circumference (-0.58 (95% CI: -0.81, -0.34) mm per week) and femur length (-0.19 (95% CI: -0.23, -0.14) mm per week). This reduced growth resulted in a smaller femur length from mid-pregnancy (gestational age 18 - 25 weeks) onwards and smaller head and abdominal circumference from late pregnancy (gestational age ≥ 25 weeks). Analyses with standard deviation scores for the growth characteristics demonstrated the largest effect estimates for femur length. The authors concluded that maternal smoking during pregnancy is associated with reduced growth of fetal head circumference, abdominal circumference and femur length. The larger effect on femur length suggests that smoking in pregnancy affects primarily peripheral tissues.

Introduction

Maternal smoking in pregnancy is the most important modifiable risk factor for low birth weight in Western countries (1, 2). Smoking in pregnancy leads to low birth weight by decreased fetal supplies of nutrients and oxygen. Birth weight is 150 to 250 grams lower in the offspring of mothers who smoke in pregnancy (1, 2). Low birth weight is an inappropriate measure for assessing the adverse effects of smoking in pregnancy on fetal growth and development. Reduced fetal growth may lead to normal birth weight if the fetus is actually supposed to grow on the upper percentiles based on the genetic growth potential.

A limited number of previous studies examined the effect of maternal smoking in pregnancy on fetal growth (3-8). These studies suggested that smoking in pregnancy is associated with impaired fetal growth from a gestational age of 20 weeks onwards. However, these studies were conducted in small groups or in hospital-based populations, were not able to adjust for potential confounders and did not examine the effects of smoking on various fetal growth characteristics in different periods of pregnancy (3-8). This may be relevant for identifying specific critical periods for the effect of maternal smoking on fetal growth.

We examined in a population-based prospective cohort study in pregnant women the associations of maternal smoking in pregnancy with longitudinally measured fetal growth characteristics.

Materials and Methods

Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood. This study is designed to identify early environmental and genetic determinants of growth, development and health in fetal life, childhood and adulthood and has been described previously in detail (9, 10). Briefly, the cohort includes 9,778 mothers and their children of different ethnicities living in Rotterdam, the Netherlands. Enrolment was aimed in early pregnancy (gestational age < 18 weeks) but was possible until birth of the child. Mothers were informed about the study by routine health care workers in pregnancy (midwives, obstetricians) and were enrolled in the study at their routine fetal ultrasound examination. Mothers who were missed in pregnancy, were approached and enrolled in the first month after birth of their child at the routine child health centres. Assessments in pregnancy, including physical examinations, fetal ultrasound examinations and questionnaires, were planned in early pregnancy (gestational age < 18 weeks), mid-pregnancy (gestational age 18 – 25 weeks) and late pregnancy (gestational age ≥ 25 weeks). Mothers enrolled in early pregnancy (69%) had

three assessments planned (in early, mid- and late pregnancy) whereas those enrolled in mid-pregnancy (19%) had two assessments (in mid- and late pregnancy) and those enrolled in late pregnancy (3%) had one assessment (in late pregnancy) planned. The individual time scheme of these assessments depended on the specific gestational age at enrolment (10). All children were born between April 2002 and January 2006. Of all eligible children in the study area, 61% participated at birth in the study (10). The study has been approved by the Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam. Written informed consent was obtained from all participants.

Maternal smoking

Information about maternal smoking was obtained by postal questionnaires in early, mid- and late pregnancy. Response rates for these questionnaires were 91%, 80% and 77%, respectively (10). Maternal smoking at enrolment was assessed in the first questionnaire by asking whether mother smoked in pregnancy (no, until pregnancy was known, continued after pregnancy was known). This questionnaire was sent to all mothers, independent of their gestational age at enrolment. In the second and third questionnaire, the mothers were asked whether they smoked in the past 2 months (no, yes) in mid- and late pregnancy, respectively. Mothers who reported in the first questionnaire to have smoked until pregnancy was known (n = 861) but still reported to smoke in the second or third questionnaire (n = 270), were reclassified into the 'continued smoking after pregnancy was known' category. The same strategy was used for mothers who reported not to smoke in the first questionnaire (n = 5,372) but smoked in the second or third questionnaire (n = 83). Among the smoking mothers, the number of cigarettes was assessed in the following six categories: less than one per day; one to two per day; three to four per day; five to nine per day; ten to nineteen per day; and twenty or more per day. To increase the number of subjects, these categories were combined and reclassified into the following categories: 1) non-smoking; 2) less than five cigarettes per day; 3) five to nine cigarettes per day; and 4) ten or more cigarettes per day.

Fetal ultrasound examinations

Fetal ultrasound examinations were carried out at the visits at one of the research centres in early, mid- and late pregnancy. These fetal ultrasound examinations were used for both establishing gestational age and assessing fetal growth characteristics. Gestational age was established by fetal ultrasound examination because using last menstrual period has several limitations including the large number of women who do not know their exact last menstrual period date or have irregular menstrual cycles (11). Pregnancy dating curves were constructed on subjects in the study with complete data on gestational age measured by ultrasound and last menstrual period. Crown-rump length was used for pregnancy dating until a gestational age of 12 weeks and 5 days (crown-rump length smaller than 65

mm) and biparietal diameter was used for pregnancy dating thereafter (gestational age from 12 weeks and 5 days onwards, biparietal diameter larger than 23 mm) (12, 13). Fetal growth measurements used in the present study included head circumference, abdominal circumference and femur length measured in mid- and late pregnancy. Growth characteristics were measured to the nearest millimetre using standardized ultrasound procedures (14). Longitudinal growth curves and gestational age adjusted standard deviation scores curves were constructed for all fetal growth measurements. The median (95% range) gestational age for the fetal ultrasound examinations in early, mid- and late pregnancy was 13.1 (9.3 - 17.5) weeks, 20.5 (18.4 - 23.3) weeks and 30.4 (27.9 - 33.0) weeks, respectively.

Covariates

Information about educational level, ethnicity and parity was obtained by the first questionnaire at enrolment in the study. At the first ultrasound examination, maternal anthropometrics, including height (m) and weight (kg), were measured without shoes and heavy clothing. Body mass index was calculated (weight/height^2 (kg/m^2)). Information about pre-pregnancy weight was collected by questionnaire. Because of the large number of missing values and the suboptimal data quality, we used the measured weight and body mass index in the analyses.

Population for analysis

Of the total of 9,778 mothers, 91% (n = 8,880) was enrolled in pregnancy (10). Mothers without information about smoking in pregnancy in the first questionnaire were excluded from the present study (14%, n = 1,249). Of the remaining 7,631 mothers, those with twin pregnancies (n = 81), fetal deaths (n = 100) or missing birth outcomes (n = 352) were excluded since main interest was in low risk singleton pregnancies. Categories of active smoking habits were similarly distributed at baseline among those with singleton live birth as pregnancy outcome and those lost to follow-up. The associations of maternal smoking habits during pregnancy with longitudinally measured fetal growth characteristics were analysed in the remaining 7,098 mothers. Of these mothers, 4.1% were second (n = 284) or third pregnancies (n = 6) in the study. Since there were no differences in results after exclusion of these subjects, they were included in the analyses presented. Analyses focused on the effects of smoking categories on fetal growth in mid- and late pregnancy were restricted to mothers who were enrolled in the study in early pregnancy (n = 5,502) to minimize misclassification of smoking in pregnancy period. Of these mothers, information about smoking and fetal growth was available in 85% (n = 4,655) in mid-pregnancy and in 83% (n = 4,542) in late pregnancy.

Data analysis

The associations between maternal smoking habits during pregnancy and repeatedly measured growth characteristics (head circumference, abdominal circumference, femur length) were analysed with unbalanced repeated measurement regression analyses using the Statistical Analysis System (SAS) version 8.2 for Windows, including the Proc Mixed module (15). The best fitting models were constructed using fractional polynomials of gestational age (16). Maternal smoking during pregnancy (no, until pregnancy was known, continued after pregnancy was known) was included in these models as interaction term with gestational age (p-value < 0.1). These models can be written as:

Head circumference = β_0 + β_1*smoking + β_2*gestational age^2 + β_3*gestational age^2*ln(gestational age) + β_4*smoking*gestational age.

Femur length = β_0 + β_1*smoking + β_2*gestational age + β_3*gestational age^3 + β_4*smoking*gestational age.

The model structure for abdominal circumference was similar as the model for head circumference. In these models, 'β_0 + β_1*smoking' reflects the intercept and 'β_2*gestational age^2 + β_3*gestational age^2*ln (gestational age)' for head circumference and abdominal circumference and 'β_2*gestational age + β_3*gestational age^3' for femur length reflect the slope of growth per week. The terms including 'β_4' reflect the differences in growth of each fetal characteristic between the maternal smoking categories. All models were additionally adjusted for lifestyle and socio-economic status related confounders (maternal body mass index at enrolment, educational level) and other known determinants of fetal growth (maternal age, height, ethnicity and parity and fetal gender) (1). The patterns of increase with gestation and distribution of these curves were similar as curves presented in other studies (17-19). Using the same strategy, additional models were constructed for standard deviation scores of these growth characteristics. The best fitting model for these growth characteristics included the terms:

Standard deviation score = β_0 + β_1*smoking + β_2*gestational age + β_3*gestational age^{-2} + β_4*smoking*gestational age

This model was used for head circumference, abdominal circumference and femur length. The associations between categories of the number of cigarettes smoked per day with these standard deviation scores in mid- and late pregnancy were assessed using multiple regression models. These models were adjusted for maternal body mass index, educational level, age, height, ethnicity and parity and fetal gender. All measures of association are

presented with their 95% confidence intervals (CI). All statistical analyses were performed using SAS version 8.2 for Windows (15).

Table 1. Maternal characteristics (means (SD) or percentages) among smoking categories in the Generation R Study cohort, Rotterdam, the Netherlands, 2002-2006

	Smoking in pregnancy (n = 7,098)*		
	Non-smoking	**Until pregnancy was known†**	**Continued after pregnancy was known†**
	n = 5,289	**n = 591**	**n = 1,218**
Age (years)	30.1 (5.1)	29.4 (5.2) §	28.3 (5.8) §
Height (cm)	167.4 (7.5)	168.3 (7.0) §	167.1 (7.0)
Weight (kg)	69.3 (13.2)	69.4 (12.8)	70.2 (14.0) §
Body mass index (kg/m^2)	24.7 (4.5)	24.5 (4.5)	25.2 (4.8) §
Parity ≥ 1 (%)	45.2	31.7§	45.7
Education (%)			
Primary school	10.6	12.2	21.0 §
Secondary school	41.6	44.9	60.5 §
Higher education	47.8	42.9 #	18.6 §
Ethnicity (%)			
Dutch, other-European	57.6	62.9 #	57.1
Surinamese	8.4	12.4 §	10.4 #
Turkish	7.7	7.5	16.2 §
Moroccan	8.1	1.2 §	1.9 §
Cape Verdian	3.7	4.6	4.8
Antillean	3.4	2.9	3.8
Others	11.0	8.5	5.8 §
1st Child of the same mother in study (%)	95.8	95.2	96.2
Enrolment in study in early pregnancy (%)	76.4	85.8 §	78.2
Ultrasound for fetal growth (%)			
Mid-pregnancy	93.8	96.6	94.0
Late pregnancy	93.9	97.0	93.7
Birth outcomes			
Birth weight (grams)	3454 (552)	3441 (559)	3251 (545) §
Gestational age (weeks) †	40.0 (37.0 – 42.0)	40.0 (36.9 – 42.0)	39.9 (36.1 – 42.0) §

*Data were missing on height (n = 11), weight (n = 27), body mass index (n = 38), parity (n = 409), educational level (n = 218) and ethnicity (n =60).
† Median (90% range).
‡ Differences of maternal characteristics were compared with the non-smoking category using independent sample t-tests for continuous variables and chi-square test for proportions. Gestational age was log transformed for the t-test.
§ p-value < 0.01.
# p-value < 0.05.

Results

Subject characteristics

Characteristics of the mothers per smoking category are presented in table 1. Of all mothers, 25.5% (n = 1,809) reported to actively smoke in early pregnancy and 17.2% (n = 1,218) continued smoking after pregnancy was known. The age in the whole cohort ranged from 15.3 to 46.3 years with a mean of 29.8 years and was lowest in mothers who continued smoking after pregnancy was known. Also the percentage of mothers with higher educational level was lowest in this group. In the whole cohort, the largest ethnic groups were the Dutch and other-European (58.0%), Surinamese (9.1%), Turkish (9.1%) and Moroccan women (6.5%). Table 1 demonstrates that the percentages of Turkish mothers

Table 2. Associations between maternal smoking habits during pregnancy and fetal growth in the Generation R Study cohort, Rotterdam, the Netherlands, 2002-2006

	Head circumference growth (mm/week)			
Smoking category	Unadjusted difference	95% CI *	Adjusted difference †	95% CI
Non-smoking	Reference		Reference	
Until pregnancy was known	0.05	-0.16, 0.26	0.10	-0.11, 0.31
Continued after pregnancy was known	-0.62	-0.77, -0.46‡	-0.56	-0.73, -0.40‡
	Abdominal circumference growth (mm/week)			
Smoking category	Unadjusted difference	95% CI	Adjusted difference	95% CI
Non-smoking	Reference		Reference	
Until pregnancy was known	0.23	-0.07, 0.52	0.35	0.05, 0.65§
Continued after pregnancy was known	-0.68	-0.90, -0.46 ‡	-0.58	-0.81, -0.34‡
	Femur length growth (mm/week)			
Smoking category	Unadjusted difference	95% CI	Adjusted difference	95% CI
Non-smoking	Reference		Reference	
Until pregnancy was known	0	-0.06, 0.06	0	-0.05, 0.06
Continued after pregnancy was known	-0.18	-0.22, -0.14‡	-0.19	-0.23, -0.14 ‡

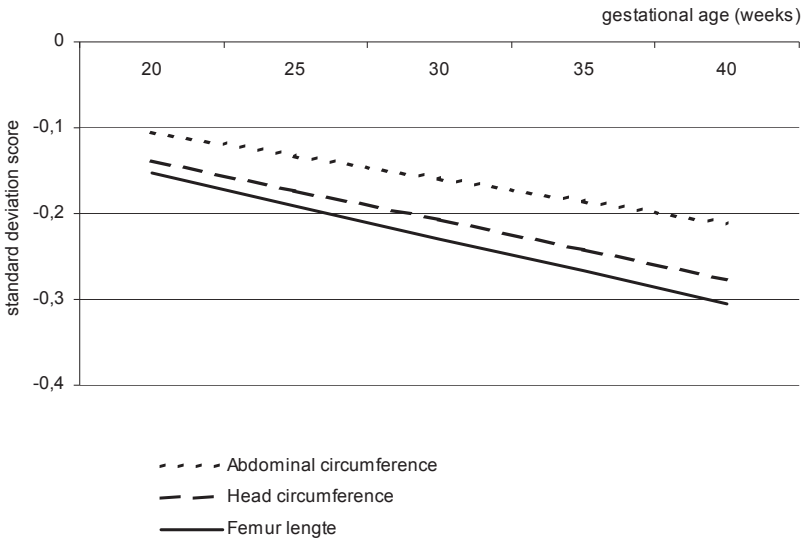
* CI, confidence interval.
† Adjusted for maternal age, body mass index at enrolment, height, educational level, ethnicity and parity and fetal gender.
‡ p-value < 0.01.
§ p-value < 0.05.

and Moroccan mothers were highest (16.2%) and lowest (1.9%), respectively, among those who continued smoking after pregnancy was known. Mean offspring birth weight was 3454 (SD 552) grams for mothers who did not smoke in pregnancy and 3251 (SD 545) grams for mothers who continued smoking after pregnancy was known.

Maternal smoking habits during pregnancy

The associations between maternal smoking habits during pregnancy and longitudinally measured fetal growth characteristics are presented in table 2. Compared to non-smoking, smoking until pregnancy was known was not associated with growth differences in head circumference and femur length. Growth of the fetal abdominal circumference was higher among mothers who quitted smoking after their pregnancy was known compared to non-smokers (0.35 (95% CI: 0.05, 0.65) mm per week). In the fully adjusted models, continued smoking after pregnancy was known was inversely associated with fetal growth of the head circumference (-0.56 (95% CI: -0.73, -0.40) mm per week), abdominal circumference (-0.58 (95% CI: -0.81, -0.34) mm per week) and femur length (-0.19 (95% CI: -0.23, -0.14) mm per week). Figure 1 presents the estimated differences in standard deviation scores for fetal head circumference, abdominal circumference and femur length between mothers who did not smoke in pregnancy and mothers who continued to smoke after pregnancy was known. Differences the between non-smoking and continued smoking after pregnancy

Figure 1. Standard deviation scores of fetal head circumference, abdominal circumference and femur length in mothers who continued to smoke after pregnancy was known in the Generation R Study cohort, Rotterdam, the Netherlands, 2002-2006



Values are estimates based on repeated measurements regression models and reflect the standard deviation score for each growth characteristic in offspring of mothers who continued smoking after pregnancy was known compared to offspring from mothers who did not smoke in pregnancy.

was known categories, increased with increasing gestational age for all three fetal growth characteristics. The largest effect was seen for femur length. No differences in fetal growth characteristics, expressed as standard deviation scores, were found between non-smoking mothers and mothers who smoked until pregnancy was known (not presented in figure 1).

Mid-pregnancy and late pregnancy smoking categories

Maternal smoking in mid-pregnancy was not associated with head circumference and abdominal circumference (table 3). Smoking of less than five cigarettes per day was associated with a smaller femur length (standard deviation score -0.12 (95% CI: -0.23, -0.01)). We did not find an association between smoking five to nine cigarettes per day and femur

Table 3. Associations of maternal smoking in pregnancy with fetal growth characteristics in mid-pregnancy (18 to 25 weeks) in the Generation R Study cohort, Rotterdam, the Netherlands 2002-2006

	Head circumference (standard deviation score)			
Smoking in mid-pregnancy *	Unadjusted difference	95% CI †	Adjusted difference ‡	95% CI
Non-smoking	Reference		Reference	
< 5 per day	-0.14	-0.25, -0.03 §	-0.09	-0.21, 0.02
5 - 9 per day	-0.10	-0.24, 0.04	-0.04	-0.19, 0.10
> 9 per day	-0.11	-0.29, 0.07	-0.03	-0.23, 0.16
	Abdominal circumference (standard deviation score)			
Smoking in mid-pregnancy *	Unadjusted difference	95% CI	Adjusted difference ‡	95% CI
Non-smoking	*Reference*		*Reference*	
< 5 per day	-0.10	-0.21, 0	-0.06	-0.17, 0.05
5 - 9 per day	-0.03	-0.16, 0.11	-0.01	-0.15, 0.13
> 9 per day	0	-0.17, 0.17	0.07	-0.11, 0.25
	Femur length (standard deviation score)			
Smoking in mid-pregnancy *	Unadjusted difference	95% CI	Adjusted difference ‡	95% CI
Non-smoking	*Reference*		*Reference*	
< 5 per day	-0.12	-0.23, -0.02 §	-0.12	-0.23, -0.01 §
5 - 9 per day	0	-0.13, 0.14	-0.02	-0.16, 0.12
> 9 per day	-0.31	-0.49, -0.14 #	-0.37	-0.55, -0.18 #

* Total number of subjects: n = 4,655; non-smoking: n = 3,940; less than five per day: n = 376; five to nine per day: n = 212; more than nine per day: n = 127.
† CI, confidence interval.
‡ Adjusted for maternal age, body mass index at enrolment, height, educational level, ethnicity and parity and fetal gender.
§ p-value < 0.05.
# p-value < 0.01.

length. A strong inverse association with femur length was found for smoking more than nine cigarettes per day (standard deviation score -0.37 (95% CI: -0.55, -0.18)).

The associations of maternal smoking in late pregnancy with fetal growth characteristics are given in table 4. In the adjusted models, all categories of maternal smoking were inversely associated with head circumference, abdominal circumference and femur length. For all three growth characteristics, the largest effect estimates were found for the highest smoking category, which includes mothers smoking more than nine cigarettes per day (standard deviation scores 0.26 (95% CI: -0.45, -0.08) for head circumference, -0.25 (95% CI: -0.43, -0.06) for abdominal circumference and -0.40 (95% CI: -0.57, -0.22) for femur length).

Table 4. Associations of maternal smoking in pregnancy with fetal growth characteristics in late-pregnancy (≥ 25 weeks) in the Generation R Study cohort, Rotterdam, the Netherlands, 2002-2006

	Head circumference (standard deviation score)			
Smoking in late pregnancy *	Unadjusted difference	95% CI †	Adjusted difference ‡	95% CI
Non-smoking	Reference		Reference	
< 5 per day	-0.24	-0.35, -0.12 §	-0.17	-0.29, -0.05 §
5 - 9 per day	-0.15	-0.29, -0.02 #	-0.08	-0.23, 0.06
> 9 per day	-0.29	-0.47, -0.11 §	-0.26	-0.45, -0.08 §
	Abdominal circumference (standard deviation score)			
Smoking in late pregnancy *	Unadjusted difference	95% CI	Adjusted difference ‡	95% CI
Non-smoking	*Reference*		*Reference*	
< 5 per day	-0.21	-0.32, -0.10 §	-0.15	-0.27, -0.03 #
5 - 9 per day	-0.22	-0.35, -0.08 §	-0.19	-0.32, -0.05 §
> 9 per day	-0.22	-0.40, -0.05 #	-0.25	-0.43, -0.06 §
	Femur length (standard deviation score)			
Smoking in late pregnancy *	Unadjusted difference	95% CI	Adjusted difference ‡	95% CI
Non-smoking	*Reference*		*Reference*	
< 5 per day	-0.16	-0.26, -0.05 §	-0.17	-0.29, -0.06 §
5 - 9 per day	-0.28	-0.41, -0.15 §	-0.29	-0.42, -0.16 §
> 9 per day	-0.41	-0.58, -0.24 §	-0.40	-0.57, -0.22 §

* Total number of subjects: n = 4,542; non-smoking: n = 3,864; less than five per day: n = 322; five to nine per day: n = 225; more than nine per day: n = 131.
† CI, confidence interval.
‡ Adjusted for maternal age, body mass index at enrolment, height, educational level, ethnicity and parity and fetal gender.
§ p-value < 0.01.
# p-value < 0.05.

Discussion

This population-based prospective cohort study showed associations between maternal smoking in pregnancy and impaired growth of fetal head circumference, abdominal circumference and femur length. Differences between non-smoking mothers and mothers who continued to smoke after pregnancy was known, increased with increasing gestational age, resulting in smaller femur length from mid-pregnancy and smaller head circumference and abdominal circumference from late pregnancy.

Methodological considerations

The strength of this study is the population-based cohort with a large number of subjects studied from early pregnancy with information about a large number of potential confounders available. To our knowledge, this is the largest cohort study examining the associations of maternal smoking in pregnancy with fetal growth characteristics. Of all eligible children, 61% participate in the study at birth. Information about smoking in pregnancy at enrolment was missing in 14% of all participating mothers. Birth weight was 41 (95% CI: 6, 76) grams lower in the offspring of these mothers. Non-response would lead to biased effect estimates if the association of maternal smoking in pregnancy with fetal growth differs between those with and without complete data. This seems unlikely. Biased estimates in large cohort studies primarily arise from loss to follow-up rather than from non-response at baseline (20). Since follow-up information at birth was available in 93%, we don not expect biased results due to loss to follow-up. The percentage of smoking mothers may be higher among those not included than included in the present analysis. This would probably lead to loss of power and underestimation of the estimated effects of smoking on fetal growth characteristics.

Information about maternal smoking in pregnancy was collected by questionnaires without reference to fetal growth characteristics. Although assessing smoking habits in pregnancy by questionnaires seems to be a valid method, misclassification may occur (21). Underreporting of maternal smoking across the various smoking categories may be present and would lead to misclassification. The estimated difference in fetal growth between the offspring of non-smoking and low to moderate smoking mothers would be overestimated if this underreporting would be selectively present among heavy smoking mothers who report low to moderate smoking. To overcome these limitations, other studies used biomarkers of tobacco exposure, including cotinine, in maternal urine samples (22, 23). However, low correlations between cotinine and self reported smoking habits have been demonstrated (24). Possible explanations for these low correlations include inaccurate maternal reporting of smoking habits in pregnancy, use of categories of number of cigarettes smoked in questionnaires and individual differences in inhalation, absorption and metabolism. Previous studies demonstrated that using cotinine levels is

not superior to self-report in studying the effect of maternal smoking in pregnancy on birth weight (25). We assessed smoking as categorical and not as continuous variable since data collection and data cleaning are more easily conducted in such a large cohort. Inherently, this diminishes power to identify exposure effects because information within categories is lost.

Gestational age was established by fetal ultrasound examination. This methods seems to be superior to last menstrual period because of the large number of women who do not know their exact last menstrual period date or have irregular menstrual cycles (11). The major disadvantage of establishing gestational age by ultrasound is that growth variation of the fetal characteristics used for pregnancy dating is assumed to be zero. Therefore, in our study, crown-rump length and biparietal diameter were used for pregnancy dating but not for assessing fetal growth (12, 13). Since pregnancy dating characteristics and growth characteristics are correlated throughout pregnancy, growth variation in head circumference, abdominal circumference and femur length may be reduced by pregnancy dating on the other fetal characteristics. This may potentially lead to underestimation of our effect estimates. However, we expect this effect to be small in our results. First, the longitudinal analyses (Table 2 and Figure 1) were focused on fetal growth or change in size during pregnancy within individuals. This change in size is unlikely to be affected materially by our pregnancy dating method. Second, the analyses to assess the associations of maternal smoking and fetal growth characteristics in mid and late pregnancy (Table 3 and 4), were restricted to mothers who were enrolled and dated in early pregnancy (78% of the population for analysis). Since gestational age and fetal growth were not established concurrently, we expect to minimize the effect of pregnancy dating on growth variation.

Smoking in pregnancy and fetal growth patterns

The associations of continued maternal smoking after pregnancy was known with lower growth of fetal head circumference, abdominal circumference and femur length were independent of potential confounders. No adverse effects on fetal growth were found in mothers who quitted smoking after their pregnancy was known. Unexpectedly, a higher fetal abdominal circumference growth rate was found in mothers who quitted smoking after pregnancy was known, compared to non-smokers. These findings are in line with previous studies demonstrating that quitting to smoke in early pregnancy is associated with a normal or even slightly increased birth weight (2, 26). This association seems not to be explained by an increase in body mass index (26). The number of studies examining the effects of maternal smoking in pregnancy on fetal growth is limited (3-8). Results from these studies are not conclusive and cannot easily be compared to our results because of the differences in study populations and growth measurements. More importantly, results from these studies were not able to adjust appropriately for possible confounders. After full adjustment for lifestyle and socio-economic status related variables, including maternal

body mass index and educational level, and for known determinants of birth weight, including maternal age, height, ethnicity and parity and fetal gender, our study demonstrated strong associations of maternal smoking with femur length from mid-pregnancy onwards and of maternal smoking with head circumference and abdominal circumference from late pregnancy. In mid-pregnancy both smoking less than five cigarettes and smoking more than nine cigarettes was associated with smaller femur length. We did not find an effect of smoking five to nine cigarettes on femur length in mid-pregnancy. This seems inconsistent and may be a chance finding, since we cannot explain this inconsistency and the effects were not caused by outliers in the data. Further studies are needed to assess these dose-response trends. Our findings are in line with studies demonstrating that maternal smoking in the third trimester has the largest effect on birth weight in the offspring (27). Both the timing of the effect and the size of the effect estimates suggest that maternal smoking in pregnancy affects first peripheral tissues and second central tissues. In late pregnancy, maternal smoking affected all fetal growth characteristics.

Perspectives for future studies

Our findings are in line with previous studies that showed postnatal effects of fetal exposure to nicotine. Maternal smoking in pregnancy seems to be associated with an increased risk of sudden infant death syndrome and impaired cognitive and behavioural development in their offspring (28, 29). More recently, it has been suggested that maternal smoking in pregnancy is also associated with postnatal blood pressure development (30). The underlying causal pathways for these associations are not known. The effects of maternal smoking on fetal growth characteristics shown in our study may reflect developmental adaptations in fetal organ growth and development that may have consequences in childhood and adulthood. Further follow-up studies are needed to examine the gestational age specific effects of maternal smoking in pregnancy on fetal and postnatal organ growth and function and to examine whether these effects explain the previously demonstrated associations between maternal smoking in pregnancy and various health outcomes in postnatal life.

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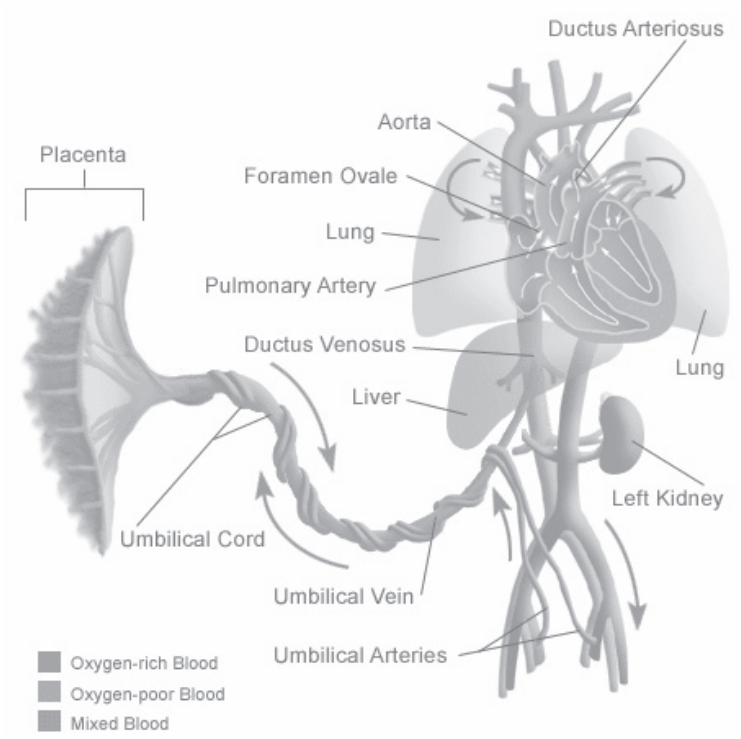
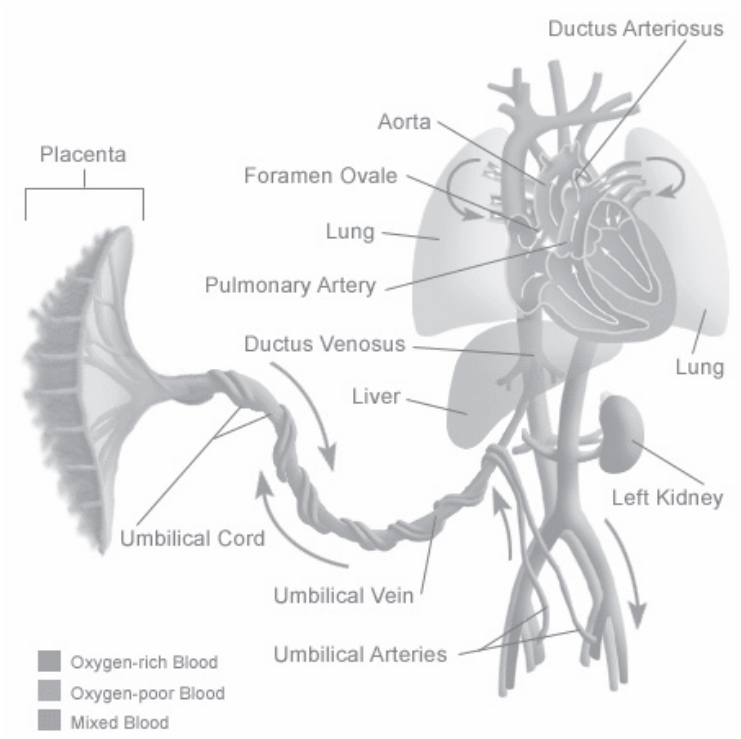
Chapter 4

Fetal organ development and growth restriction



Chapter 4.1

Fetal haemodynamic adaptive changes related to intrauterine growth. The Generation R Study.



Abstract

Background: It has been suggested that an adverse fetal environment increases susceptibility to hypertension and cardiovascular disease in adult life. This increased risk may result from suboptimal development of the heart and main arteries in utero and to adaptive cardiovascular changes in reduced fetal growth. The aim of the present study was to evaluate whether reduced fetal growth is associated with fetal circulatory changes and cardiac dysfunction.

Methods and Results: This study was embedded in a population-based, prospective cohort study starting in early fetal life. Fetal growth characteristics and fetal circulation variables were assessed with ultrasound and Doppler examinations in 1,215 healthy women. The fetal circulation was examined in relation to estimated fetal weight.

Higher placental resistance indices were strongly associated with decreased fetal growth. Cerebral resistance showed a gradual decline with reduced fetal growth. Cardiac output, peak systolic velocity of the outflow tracts and cardiac compliance showed a gradual reduction with diminished fetal growth, while intraventricular pressure gradually increased.

Conclusions: Decreased fetal growth is associated with adaptive fetal cardiovascular changes. Cardiac remodelling and cardiac output changes are consistent with a gradual increase in afterload and compromised arterial compliance in case of decreased fetal growth. These changes already start to occur before the stage of clinically apparent fetal growth restriction and may contribute to the increased risk of cardiovascular disease in later life.

Introduction

Epidemiological studies have demonstrated fetal growth restriction and low birth weight to be risk factors contributing to cardiovascular disease and hypertension in adult life (1-3). This increased risk may result from suboptimal development of the fetal heart and main arteries in utero, and to adaptive cardiovascular changes in fetal growth restriction (4). This hypothesis is supported by studies in fetal and postnatal life that found lower cardiac compliance and increased arterial stiffness in subjects with fetal growth restriction. These adaptations may predispose individuals to an increased risk of hypertension and ventricular hypertrophy in later life (5-9). Cardiovascular changes due to fetal growth restriction might already be present in fetal life. To our knowledge there are no population-based prospective studies that relate fetal growth characteristics with cardiovascular function in fetal life.

Frequently, fetal growth restriction is a consequence of impaired placental perfusion or placental insufficiency (10). In response to general fetal malnutrition, due to placental insufficiency or other factors, like smoking or maternal nutrient restriction, there is preferential blood flow to the brain and heart, depriving other organs from oxygen and nutrients. The increased flow to the brain is caused by vasodilatation in the brain resulting in a lower peripheral resistance ('brain sparing effect') (11). This is part of the phenomena known as fetal redistribution. Evidence of redistribution or centralization of the arterial circulation with fetal cardiac output in favour of the left ventricle has been described in compromised, small for gestational age fetuses (12,13). These haemodynamic changes are quantifiable by Doppler measurements of the fetal and placental circulation and are associated with increased perinatal mortality, low birth weight and hypoxia (14-16). Therefore Doppler surveillance of the fetal circulation represents an important tool for the management of the growth restricted fetus and provides information about fetal circulation characteristics (17,18).

Traditionally, a fetus is considered growth restricted when fetal abdominal circumference or estimated fetal weight is below the 10th percentile. However this state of overt fetal growth restriction is preceded by a period of diminished fetal growth within the normal estimated fetal weight range. Not much is known about adaptive haemodynamic mechanisms during this stage of reduced fetal growth. Changes occurring in the fetal circulation preceding overt fetal growth restriction may improve our understanding of compensatory adaptive fetal mechanisms in response to an adverse fetal environment. Furthermore, adaptations of the fetal circulation preceding fetal growth restriction could elucidate pathophysiological pathways responsible for the increased susceptibility of subjects with fetal growth restriction to hypertension and cardiovascular disease in adult life.

The aim of the present study was to evaluate whether reduced fetal growth is associated with adaptive fetal cardiovascular changes.

Methods

Design

This study was embedded in the Generation R Study, a population-based, prospective cohort study from fetal life until young adulthood. This study is designed to identify early environmental and genetic determinants of growth, development and health from fetal life until young adulthood and has been described previously in detail (19,20). Briefly, the cohort includes 9,778 mothers and their children of different ethnicities living in Rotterdam, the Netherlands. Enrolment was aimed in early pregnancy (gestational age < 18 weeks) but was possible until birth of the child. Mothers were informed about the study by routine health care workers in pregnancy (midwives, obstetricians) and were enrolled in the study at their routine fetal ultrasound examination. A vast majority of mothers (69%) was enrolled in early pregnancy 20. Assessments in pregnancy included physical examinations, fetal ultrasounds, biological samples and questionnaires and were planned in early (gestational age < 18 weeks), mid- (gestational age 18-25 weeks) and late pregnancy (gestational age >25 weeks) to collect information about fetal growth and its main determinants. Maternal smoking habits were assessed by questionnaire in early, mid- and late pregnancy. We categorized smoking habits during pregnancy as 'Non-smoking' and 'Continued smoking'. The children were born between April 2002 and January 2006 and form a prenatally recruited birth-cohort that is currently followed until young adulthood. In total, 61% of all eligible children in the study area participated at birth. Additionally, more detailed assessments of fetal growth and development were conducted in a subgroup of 1,232 Dutch mothers and children, referred to as the Generation R Focus Study. Of all approached women, 80% were enrolled in this subgroup in late pregnancy. This subgroup is ethnical homogeneous to exclude possible confounding or effect modification by ethnicity. For the present study, fetal circulation variables were assessed in this subset between 28 to 34 weeks of gestation. Written informed consent was obtained from all participants. The study has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam.

Population for analysis

In total, 1,232 women were enrolled in the Generation R Focus Study in late pregnancy. All these women were already participating in the Generation R Study from early pregnancy. Twin pregnancies (n = 15) and pregnancies leading to perinatal death (n = 2) were excluded from the analysis. No major cardiac anomalies were present other than small ventricular septum defects (n=3). The present analysis was performed in a total of 1,215 subjects.

Ultrasound measurements

Fetal biometry

Routine ultrasound examinations were carried out in the whole Generation R cohort in a research setting at a regional health facility in the centre of Rotterdam in early, mid and late pregnancy. These fetal ultrasound procedures were used for both establishing gestational age and assessing fetal growth characteristics (21). Estimated fetal weight was calculated with the formula by Hadlock using head circumference, abdominal circumference and femur length (22). For the present study, only late pregnancy measurements were used in the analysis.

Fetal circulation

Fetal circulation variables were assessed by pulsed wave Doppler between 28 and 34 weeks gestation in the Generation R Focus Group, obtained once in each participant. For all Doppler measurements, colour imaging was used to optimize placement of the pulsed wave Doppler gate. The insonation angle was kept as close to zero degrees as possible and always below 20 degrees. The sample volume was adjusted to cover the entire vessel. For each measurement three consecutive uniform waveforms were recorded by pulsed Doppler ultrasound, during fetal apnoea and without fetal movement. The mean of three measurements was used for further analysis. Three experienced sonographers performed all measurements.

To entirely appreciate all aspects of the fetal circulation one should integrate Doppler measurement in different vascular beds (18). Placental vascular resistance was evaluated using recorded flow velocity waveforms from the umbilical and uterine arteries. Raised umbilical artery pulsatility index (PI) and uterine artery resistance index (RI) indicate increased placental resistance (10). Umbilical artery PI was measured in a free-floating loop of the umbilical cord. Uterine artery RI was measured in the uterine arteries near the crossover with the external iliac artery. Umbilical vein diameter was derived from a cross-section of a free-floating loop of the umbilical cord, using the mean of three tracings of the inner edge of the vessel. Volume flow was determined online, using the inner diameter and placing the sample volume over the entire venous vessel, parallel to the ultrasound beam with maximal time averaged velocity (23). Umbilical vein volume flow in ml/min was used for the analysis and the volume flow per kg fetal weight was calculated as well.

The redistribution of blood flow in favour of the fetal brain was quantified by the middle and anterior cerebral artery PI. A reduction in middle and anterior cerebral artery PI are valid indicators of 'brain sparing effect' and fetal redistribution (11,24). The middle and anterior cerebral artery Doppler measurements were performed with colour Doppler visualization of the circle of Willis in the fetal brain and the flow velocity waveforms were obtained in the proximal part of the cerebral arteries.

Cardiac flow velocity waveforms at the level of the mitral and tricuspid valves were recorded from the apical four-chamber-view of the fetal heart, placing the sample volume just below the atrioventricular valves. Colour Doppler visualization of the blood flow allowed us to align the Doppler beam in the direction of the blood flow. The E wave, representing early passive ventricular filling and the A wave, representing active atrial contraction filling, peak velocities were recorded. The E/A ratio was calculated, which is an index for the ventricular diastolic function and expresses both cardiac compliance and preload conditions. Cardiac outflow flow velocity waveforms from the aorta and pulmonary artery were recorded from the five-chamber view and the short-axis view of the fetal heart just above the semilunar valves, respectively. Peak systolic velocity (PSV), time velocity integral (TVI), fetal heart rate and the inner diameter during systole of both arteries were recorded. Left and right cardiac output was calculated in ml/min by multiplying the vessel area x TVI x fetal heart rate. Combined cardiac output was calculated by adding left and right ventricular output. Weight adjusted cardiac output was determined (cardiac output / estimated fetal weight).

Flow assessment at the level of the ductus venosus was carried out in both a transversal or parasagittal oblique scanning plane of the fetal abdomen immediately after the origin of the ductus from the umbilical vein. The venous pulsatility index (PIV) was used to assess atrial contraction and preload conditions (25).

To assess reproducibility of ultrasound measurements, the intraclass correlation coefficient (ICC) and coefficient of variation (CV) between and among observers were calculated in 12 subjects for various Doppler measurements. One observer performed the measurements; subsequently the other observer did the same, after which the first examiner repeated the process. The sonographers left the ultrasound room during each other's assessment and were blinded to the measurements on the screen and printouts. The ICC is defined as the ratio of the variance between subjects to the total variance. The ICC measures the strength of the agreement of the variables (26). Additionally the CV was calculated, which, expressed as a percentage, is the ratio of the SD of the measurement error and the overall mean (27). Table 1 shows results of the intra- and interobserver

Table 1. Intra- and interobserver intraclass correlation coefficients (ICC) and coefficients of variation (CV)

Doppler measurement	Intraobserver ICC	Intraobserver CV	Interobserver ICC	Interobserver CV
Uterine artery	0.82	9.9 %	0.84	8.1 %
Umbilical artery	0.93	6.2 %	0.91	7.2 %
Middle cerebral artery	0.98	6.4 %	0.82	8.9 %
Aorta ascendens	0.84	4.4 %	0.86	4.3 %
Mitral valve	0.82	9.6 %	0.90	8.1 %
Pulmonary artery	0.85	5.2 %	0.84	6.6 %
Ductus venosus	0.86	7.8 %	0.72	12.3 %

variability analysis. The results show high ICC (> 0.80) with corresponding low CV (< 10%) values, indicating adequate reproducibility for all Doppler measurements.

All ultrasound examinations were performed using an ATL-Philips® Model HDI 5000 (Seattle, Washington, USA) equipped with a 5.0 MHz, high frequency curved array transducer.

Statistical analysis

Gestational age adjusted standard deviation scores (sds), which is equivalent to a z-score, for estimated fetal weight were developed. Fetal weight was categorized in ten groups according to deciles of sds estimated fetal weight, indicating small (group 1) to large (group 10) for gestational age fetuses. The means for fetal circulation measurements of these groups were plotted to detect differences over the range from small to large for

Table 2. Subject characteristics (n=1215)

	Boys (n = 629)	**Girls (n = 586)**
Maternal characteristics		
Age (years)	31.8 (21.1 - 39.2)	32.0 (22.7 - 39.0)
Late pregnancy characteristics		
Gestational age (weeks)	30.4 (28.6 - 32.9)	30.3 (28.3 - 32.5)
Estimated fetal weight (grams)	1624 (1168 - 2251)	1574 (1163 - 2203)*
Fetal heart rate	138 (119 - 154)	140 (122 - 155)*
Uterine artery (RI)	0.49 (0.37 - 0.68)	0.48 (0.35 - 0.67)
Umbilical artery (PI)	0.95 (0.66 - 1.30)	0.98 (0.68 - 1.36)*
Umbilical vein volume flow (ml/min)	237 (137 - 386)	239 (136 - 378)
Umbilical vein diameter (cm)	0.75 (0.60 - 0.90)	0.74 (0.60 - 0.88)*
Ductus venosus PIV	0.55 (0.27 - 1.00)	0.53 (0.29 - 0.88)*
Middle cerebral artery PI	1.97 (1.33 - 2.60)	1.97 (1.35 - 2.67)
Middle cerebral artery PSV (cm/s)	42.2 (27.7 - 60.2)	43.1 (27.7 - 60.3)
Anterior cerebral artery PI	1.75 (1.18 - 2.37)	1.74 (1.23 - 2.43)
Combined cardiac output (ml/min)	1388 (838 - 2437)	1417 (850 - 2240)
Aorta ascendens PSV (cm/s)	90.2 (66.4 - 114.0)	91.7 (67.8 - 120.0)
Pulmonary artery PSV (cm/s)	72.2 (56.5 - 92.8)	73.7 (56.5 - 92.0)*
Aorta ascendens diameter (cm)	0.65 (0.52 - 0.80)	0.64 (0.51 - 0.78)*
Pulmonary artery diameter (cm)	0.79 (0.65 - 0.98)	0.78 (0.65 - 0.95)
Postnatal characteristics		
Gestational age at birth (weeks)	40.3 (35.9-42.4)	40.1 (35.6-42.4)
Birth weight (grams)	3570 (2388 - 4563)	3478 (2177 - 4505)*

Values are medians (95% range).
RI, resistance index; PI, pulsatility index; TVI, time velocity integral; PSV, peak systolic velocity.
Differences between boys and girls were compared using independent samples t-tests.
*p-value<0.05

gestational age fetuses. The relations between estimated fetal weight, using the actual z-scores (sds), and the fetal circulation measurements were assessed using multiple linear regression models, additionally adjusted for fetal gender. Regression coefficients were calculated with their 95% confidence intervals (95% CI) and standardized coefficients are provided to compare effect estimates.

Furthermore we looked at the effect of maternal smoking on estimated fetal weight and fetal cardiovascular measurements.

To visualise normal development during gestation, scatterplots of individual measurements of the fetal circulation variable against gestational age with the 5th and 95th percentiles were constructed. Formulas for normal ranges of fetal circulation variables between 28-34 weeks of gestational age were derived. The association of fetal circulation measurements with gestational age was assessed using multiple linear regression models adjusted for fetal gender.

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

Results

Characteristics of the study population are presented in Table 2. The percentage of boys was 51%. The mean gestational age at the time of the measurements was 30.4 weeks. Estimated fetal weight and birth weight were higher in boys than in girls. Umbilical artery PI, pulmonary artery PSV and fetal heart rate were lower in boys than in girls, whereas umbilical vein diameter, aortic diameter and ductus venosus PIV were larger in boys.

Table 3. Descriptive statistics of cardiac parameters, left versus right

Parameter	Aorta ascendens / Mitral valve	Pulmonary artery / Tricuspid valve
Diameter outflow tract (cm)	0.64 (0.52 - 0.79)	0.78 (0.65 - 0.97)*
PSV outflow (cm/s)	91.4 (66.9 - 114.4)	73.4 (56.5 - 92.4)*
TVI outflow	13 (10 - 18)	12 (9 - 16)*
Cardiac output (ml/min)	584 (327 - 978)	800 (445 - 1411)*
% of total cardiac output	42 (31 - 53)	58 (47 - 69)*
E-wave (cm/s)	39.8 (28.7 - 53.7)	42.9 (32.1 - 54.9)*
A-wave (cm/s)	51.0 (37.3 - 68.4)	55.2 (41.5 - 72.0)*
E/A ratio	0.77 (0.61 - 0.99)	0.77 (0.62 - 0.95)

Values are medians (95% range)
TVI, time velocity integral; PSV, peak systolic velocity; E, early passive ventricular filling; A, active atrial contraction filling.
Differences between left and right were compared using paired samples t-tests.
*p-value<0.01

Table 3 presents the cardiac measurements for the left and right ventricle and outflow tracts. Pulmonary diameter, right cardiac output, E wave and A wave velocities of the right ventricle were larger compared to the left side of the heart. Peak systolic velocity and time velocity integral were larger in the aorta compared to the pulmonary artery. E/A ratio was the same in left and right ventricle. There was right heart dominance with a right /left cardiac output ratio of 1.38.

Table 4 gives the regression coefficients of the relation between gestational age adjusted estimated fetal weight and the fetal circulation measurements. Uterine artery RI and umbilical artery PI were negatively associated with estimated fetal weight. Left, right and combined cardiac output, E and A wave of the mitral valve and E wave of the tricuspid valve, including PSV of the outflow tracts were positively associated with estimated fetal weight. Likewise were umbilical vein volume flow, anterior cerebral artery PI and middle cerebral artery PSV positively associated with estimated fetal weight.

Table 4. Regression coefficients of estimated fetal weight with circulation parameters

Doppler parameter	Number (% of total)	Regression coefficient (95% CI)	Standardized Beta- coefficient
Uterine artery RI	1155 (95%)	-0.009 (-0.013, -0.004)**	-0.115
Umbilical artery PI	1191 (98%)	-0.02 (-0.032, -0.013)**	-0.132
Umbilical vein volume flow	1052 (95%)	14.1 (10.4, 17.8)**	0.226
Middle cerebral artery PI	1165 (96%)	0.015 (-0.004, 0.032)	0.045
Middle cerebral artery PSV	1166 (96%)	0.84 (0.36, 1.32)**	0.101
Anterior cerebral artery PI	1061 (87%)	0.02 (0.002, 0.038)*	0.068
Ductus venosus PIV	1087 (89%)	-0.001 (-0.012, 0.009)	-0.007
Cardiac parameter			
Aorta ascendens PSV	1062 (87%)	1.47 (0.71, 2.23)**	0.117
Left cardiac output (ml/min)	1038 (85%)	37.3 (26.7, 48.1)**	0.210
Pulmonary artery PSV	1046 (86%)	1.03 (0.45, 1.62)**	0.106
Right cardiac output (ml/min)	1017 (83%)	49.2 (33.9, 64.6)**	0.195
Combined cardiac output (ml/min)	985 (81%)	88.2 (64.3, 112.1)**	0.226
Ratio left / right cardiac output	975 (80%)	0.004 (-0.008, 0.015)	0.020
Mitral valve E wave	1152 (95%)	0.72 (0.36, 1.08)**	0.114
Mitral valve A wave	1152 (95%)	1.07 (0.61, 1.53)**	0.134
Mitral valve E/A ratio	1165 (96%)	-0.003 (-0.008, 0.003)	-0.028
Tricuspid valve E wave	1126 (93%)	0.29 (-0.05, 0.63)	0.050
Tricuspid valve A wave	1126 (93%)	0.60 (0.13, 1.06)*	0.075
Tricuspid valve E/A ratio	1135 (93%)	-0.003 (-0.008, 0.002)	-0.040

Values are regression coefficients (95% confidence interval) or standardized beta- coefficients and reflect the difference in Doppler measurement per unit increase in gestational age adjusted sds estimated fetal weight. Models adjusted for fetal gender.
*p-value<0.05, **p-value<0.01

Figures 1-4 show the means of the fetal circulation Doppler measurements depicted per sds decile of estimated fetal weight, ranging from small (group 1) to large (group 10) for gestational age. The distribution and mean values of the characteristics gestational age, fetal gender and smoking within the different groups were similar. P values in the figures represent the p values for the regression coefficients given in Table 4.

Figure 1. Placental resistance parameters and venous return with gestational age adjusted estimated fetal weight

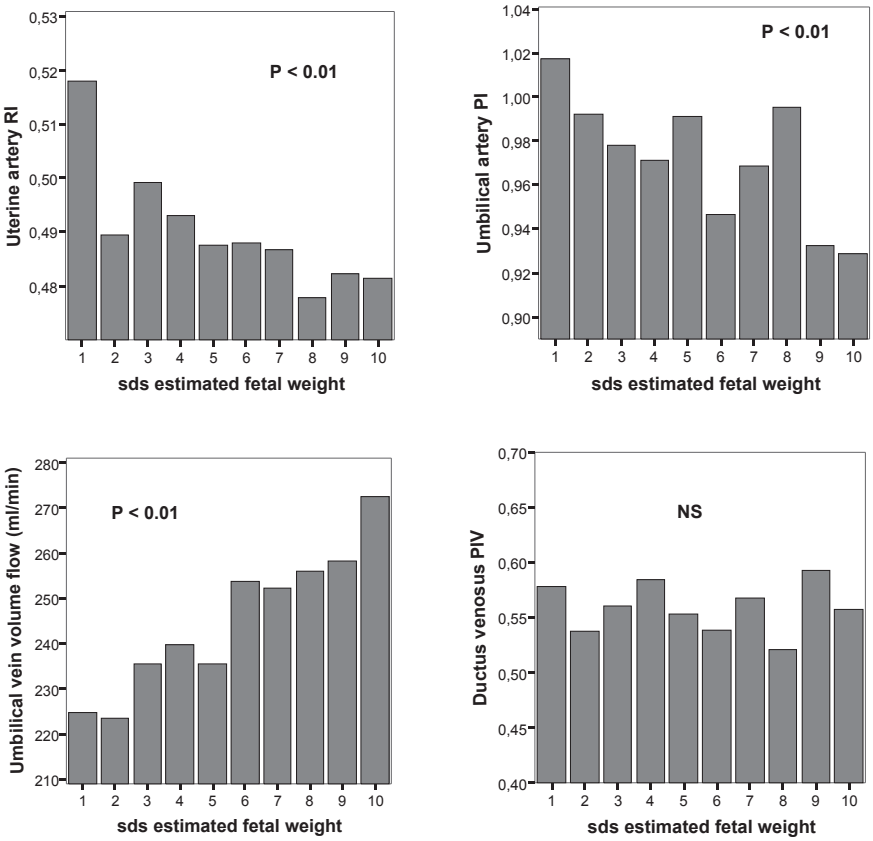


Figure 1 depicts the placental resistance and venous return measurements in relation with estimated fetal weight. Placental resistance increased with decreasing fetal weight, with lower umbilical vein volume flow in smaller fetuses. Ductus venosus PIV did not change with increasing estimated fetal weight.

Figure 2 shows the cerebral circulation variables. Middle cerebral artery PSV and anterior cerebral artery PI gradually decreased with diminished fetal growth. Middle cerebral

Figure 2. Cerebral circulation parameters with gestational age adjusted estimated fetal weight

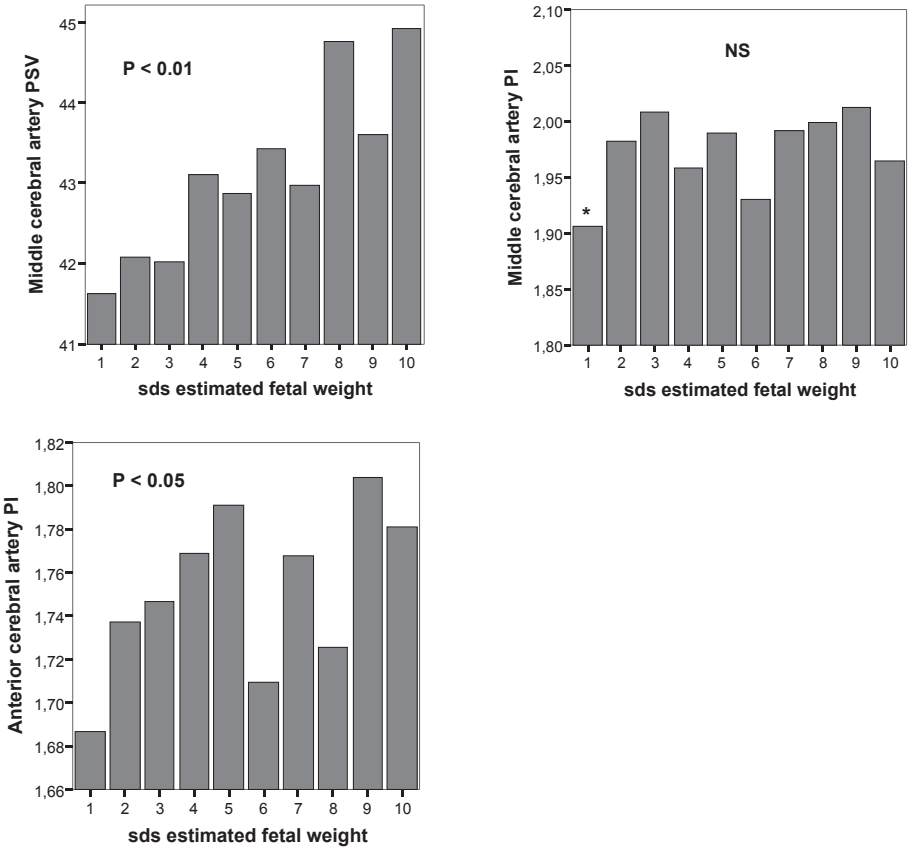
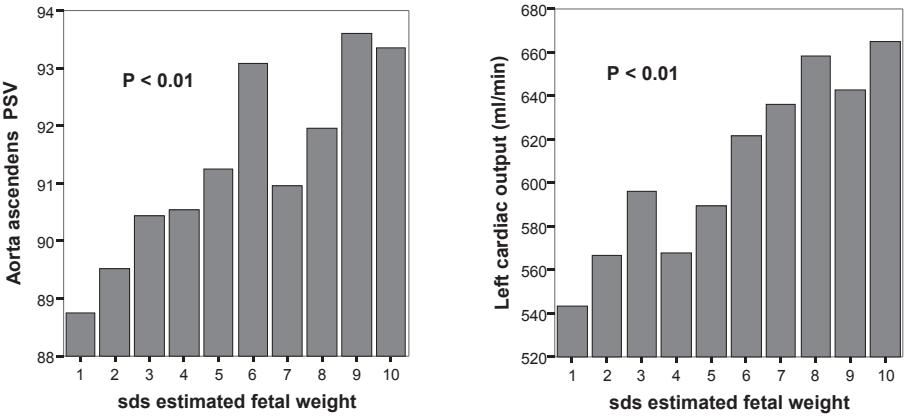
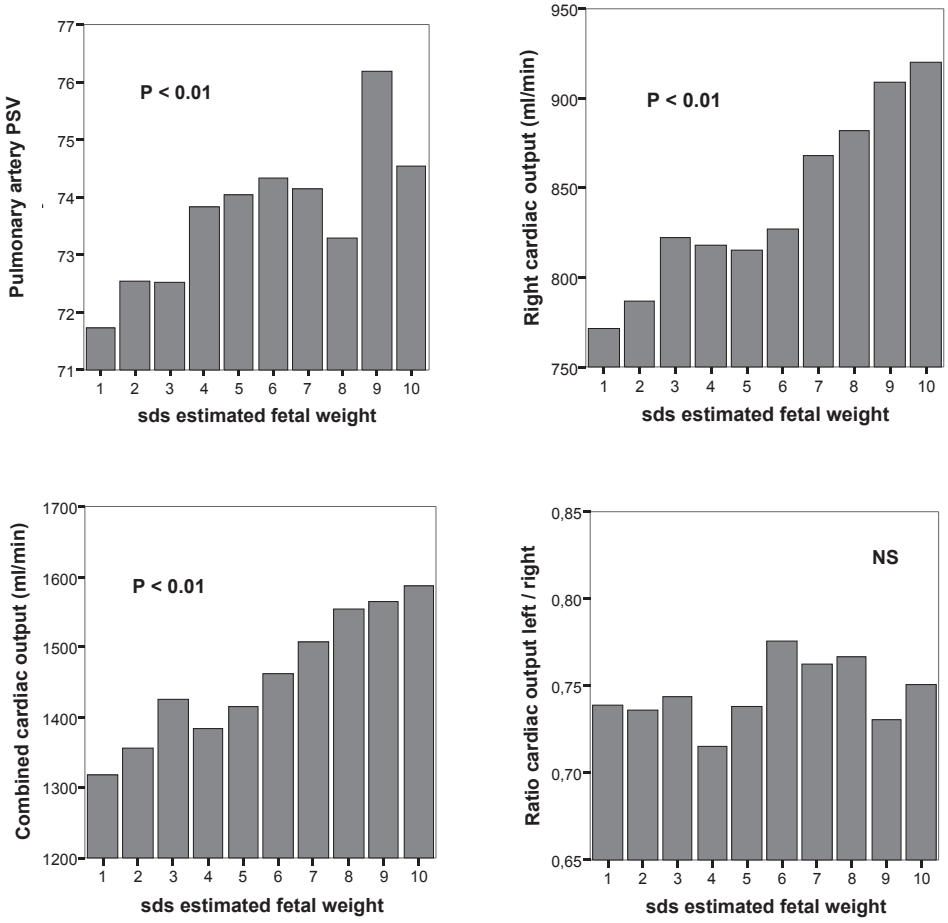


Figure 3 A. Cardiac performance parameters at the level of the outflow tracts with gestational age adjusted estimated fetal weight





artery PI showed no association with estimated fetal weight, although the smallest for gestational age fetuses displayed a significant lower PI than the other fetuses.

In Figure 3A & 3B the cardiac performance measurements are illustrated. Both left and right cardiac outflow PSV and TVI and cardiac output decreased with reduced fetal growth, as did combined cardiac output. The right cardiac output/left cardiac output ratio remained constant. Transmitral E wave velocity decreased with diminished fetal growth. A wave velocity was reduced with decreased fetal growth for both atrioventricular valves. E/A ratio was constant for both atrioventricular valves.

Figure 4 represents the cardiac output and umbilical vein volume flow per unit estimated fetal weight. Reduced fetal growth was associated with a higher volume flow per unit fetal weight.

In our population 162 (13.3%) mothers smoked during pregnancy. Smoking during pregnancy significantly reduced estimated fetal weight at the time of the

Figure 3 B. Cardiac parameters at the level of the atrioventricular valves with gestational age adjusted estimated fetal weight

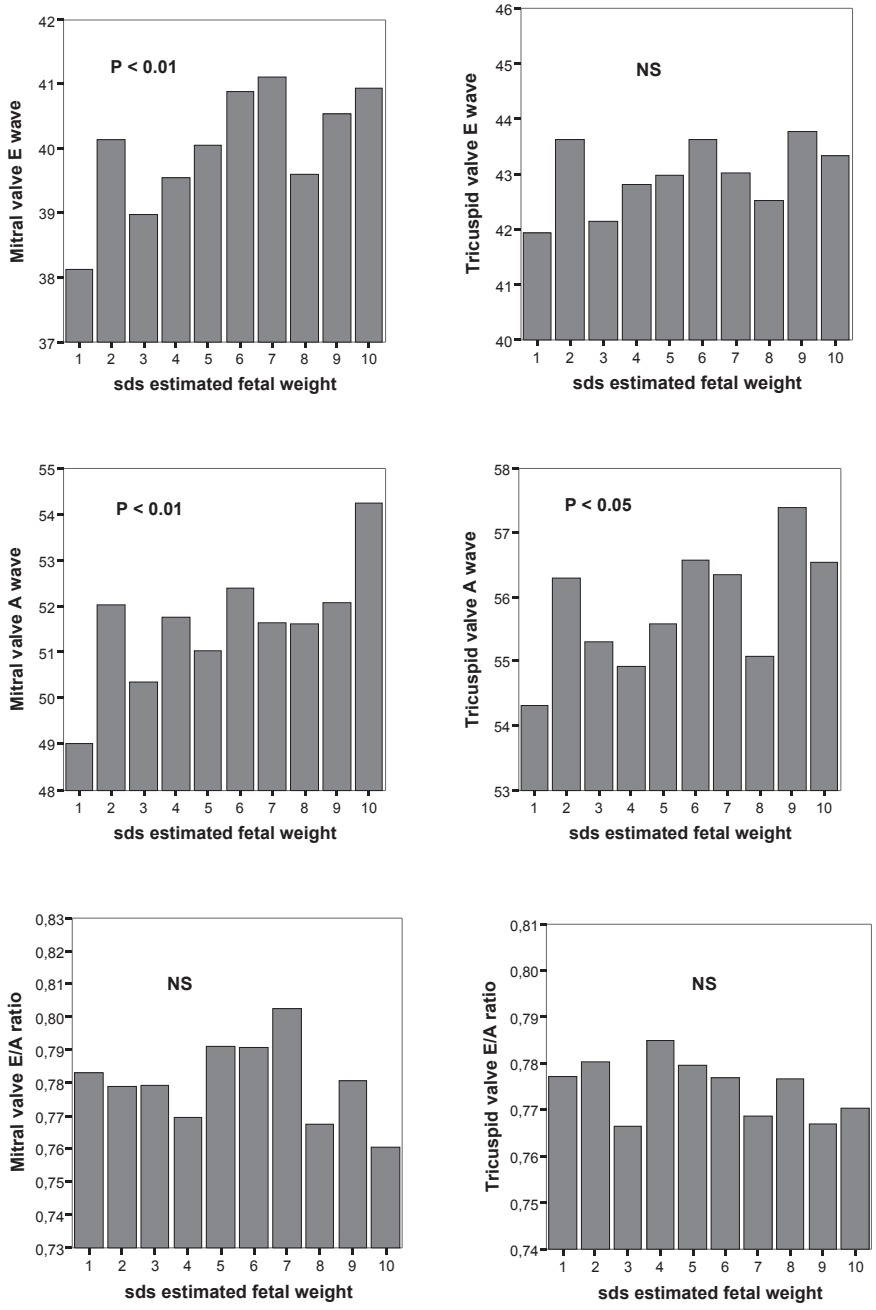
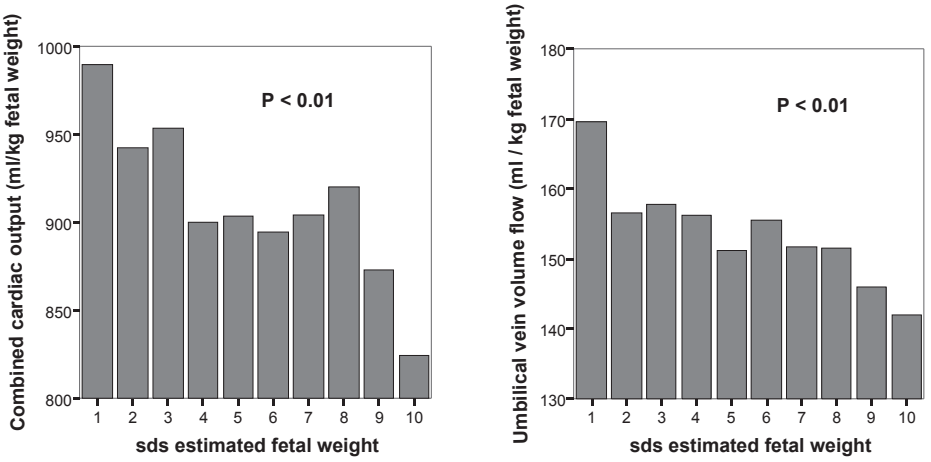


Figure 4. Cardiac output and umbilical vein volume flow per unit estimated fetal weight



measurements with 74 grams (95% CI: 31, 118) and birth weight with 188 grams (95% CI: 97, 278). Smoking resulted in increases of the umbilical artery PI and middle cerebral artery PI of 0.03 (95% CI: 0.01, 0.06) and 0.06 (95% CI: 0.01, 0.12) respectively.

Formulas for the relation between gestational age (28-34 weeks) and fetal circulation measurements are presented in Table 5 (Appendix IV). Scatterplots of individual measurements for the fetal circulation variables against gestational age with the 5th and 95th percentiles are shown in Figures 5 A-D (Appendix IV).

Discussion

The main finding of this study is that fetal haemodynamic patterns already change in the presence of reduced fetal growth whilst still within the normal estimated fetal weight range. Placental resistance indices were increased whereas cerebral resistance was decreased in smaller fetuses. Cardiac output and cardiac performance measurements were consistent with higher afterload and decreased vascular compliance in diminished fetal growth. Reduced cardiac compliance was found mainly in the left heart with decreased fetal growth. Higher end-diastolic ventricular filling pressure was present in reduced fetal growth.

The main strength of our study is the prospective design from early fetal life within a large population-based cohort. Of all mothers of the Generation R Study who were approached for the detailed subgroup, 80% participated in the focus study. Non-participation was mainly due to lack of time. No differences in offspring birth weight and maternal characteristics were found between mothers participating and not participating in the present study. To our knowledge, this is the largest population-based cohort study

in which fetal circulation variables in late pregnancy were established. The population-based setting enabled us to assess fetal circulation physiology unbiased over the whole range of estimated fetal weight, rather than in fetuses with growth restriction or other complications only.

Placental circulation

Inadequate placental perfusion is an important cause of fetal growth restriction with higher risk of adverse fetal outcome (10). Our results support the notion that increased placental impedance is highly associated with decreased fetal growth and reduced umbilical vein volume flow. Umbilical venous volume flow has been reported to be reduced prior to and during fetal growth restriction and adverse Doppler measurements (23).

Cerebral circulation

The presence of blood flow redistribution can be detected by demonstrating the 'brain-sparing' phenomenon. This has been described to precede fetal deterioration and hypoxemia (16,28). However, our study provides evidence that this is an adaptive process, which may already exist in suboptimal fetal growth. Anterior cerebral artery PI gradually decreased suggesting that a continuous modelling takes place for the anterior cerebral artery resulting in optimized cerebral perfusion in diminished fetal growth. We found a decrease in middle cerebral artery PI in the smallest fetuses only. This might indicate that it is a late sign of cerebral redistribution that could predict fetal compromise. Middle cerebral artery PI has been described to be a poor prognostic factor alone, and is performing better in combination with the umbilical artery PI, the cerebro-umbilical ratio (29). Our study suggests that the anterior cerebral artery PI might perform better to describe the 'brain-sparing' effect. The finding of a lower PSV in the middle cerebral artery with declining fetal growth may directly reflect the lower PSV in the cardiac outflow tracts in smaller fetuses. Alternatively, it may be caused by decreased vascular compliance at the level of the cerebral arteries.

Cardiac circulation

Increased right ventricular afterload resulting from high impedance to flow in the feto-placental vascular bed, and decreased left ventricular afterload resulting from cerebral vasodilatation, is suggested to cause redistribution of cardiac output in favour of the left ventricle (12,13). In our study, however, we did not see any change in cardiac output ratio with decreasing fetal growth. A consequence of the existence of the parallel disposition of the two ventricles with shunts is that they share the same systemic ejection pressure. The function of the aortic isthmus as a watershed and a regulator between the cerebral and placental circulation has already been highlighted (30). With the shunts in operation the fetal heart appears to be a very adaptive organ that plays a crucial role in stabilizing

cardiac output in the normally developing fetus. So changes in cardiac distribution do not seem to take place in suboptimal fetal growth in this study, despite increased placental impedance and decreased cerebral resistance.

Peak systolic velocities in the cardiac outflow tracts showed a gradual decrease with declining fetal growth. Lower peak systolic velocities of the cardiac outflow tracts may indicate reduced cardiac function or raised afterload. Progressive decrease of peak systolic velocity in the cardiac outflow tracts has been observed in growth restricted fetuses (31,32). Lower peak systolic velocities do not necessarily reflect poor ventricular function but may reflect a physiological adaptation to a higher afterload or decreased vascular compliance. This is supported by findings of lower peak systolic velocities in the outflow tracts in the presence of raised afterload (33).

Decreased total cardiac output is described in other studies to suggest deterioration of cardiac function in compromised fetuses (31,34). Cardiac output declined with decreasing fetal growth in the present study. This is consistent with increasing afterload or decreased contractility. The gradual decline in left and right cardiac output in reduced fetal growth within the normal range supports the hypothesis that this is due to increased afterload. A deterioration of cardiac function is unlikely and not supported by our finding of normal preload conditions.

Pulse wave velocity has been studied in small for gestational age fetuses. They appear to have a lower pulse wave velocity, possibly caused by stiffer arteries with decreased vessel wall pulsations (7,35). The pulse wave velocity correlates with mean blood pressure and is associated with the peak systolic velocities of the cardiac outflow tracts (7). Our finding that in fetuses with suboptimal fetal growth, cardiac output and peak systolic velocity are reduced is consistent with decreased pulse wave velocity in small fetuses and suggests increased afterload with raised mean arterial pressure.

Diastolic left ventricular performance in reduced fetal growth was characterized by lower transmitral E wave velocities, which suggests decreased ventricular compliance. This was not observed, however, at the level of the tricuspid valve. This could indicate that maturational changes of the diastolic filling properties of the fetal heart are less efficient in reduced fetal growth resulting in decreased left ventricular compliance (36).

A wave velocities for both atrioventricular valves, decreased with declining fetal growth. This implies a gradual increase in ventricular end-diastolic pressure with decreasing fetal growth. So cardiac diastolic function is different in smaller fetuses, suggesting the existence of intracardiac haemodynamic changes. This is a gradual process, seen throughout the whole range of fetal growth. It is already proposed that the leading cause of ventricular remodelling in the small for gestational age fetuses is increased afterload, mainly due to increased placental vascular resistance (14).

Apart from modifications in afterload and ventricular compliance, cardiac haemodynamics are also altered by changes in preload. Diastolic dysfunction and filling properties

at the level of the atrioventricular valves are illustrated by a lower E/A ratio in fetal growth restriction (13). Another study, however, described this to be constant in fetal compromise (37). In the present study E/A ratios were constant over the estimated fetal weight ranges, indicating no cardiac dysfunction in smaller fetuses and suggest that preload conditions had not changed. Even though venous return may be reduced in smaller fetuses as shown by decreased umbilical vein volume flow, no effects were seen in ductus venosus PIV. We therefore presume the preload to be constant in these fetuses with unaffected shunting through the ductus venosus, supporting the assumption that blood flow in the ductus venosus is maintained within normal ranges as long as possible (38).

Relative cardiac output and umbilical vein volume flow, which is the flow per unit fetal weight, appeared to be increased in smaller fetuses. This tendency has been described before and implies that cardiac output and umbilical vein volume flow in fetuses with decreased growth is reduced in absolute values, but increased relative to that fetal size (39). This suggests some kind of adaptation to a suboptimal environment in reduced fetal growth with a relative increase in cardiac output and umbilical vein volume flow to ensure adequate oxygen supply. Possible errors in fetal weight estimates can be larger in small or large fetuses due to a change in the volumetric proportion between head and abdomen. Volume flow indices per unit estimated fetal weight should therefore be interpreted with care (40).

It has been hypothesized that an adverse fetal environment and subsequent reduced fetal growth results in fetal blood flow redistribution and permanent fetal cardiovascular changes like increased arterial wall stiffness and left ventricular dysfunction (1,4,6,8). Studies in infants and adults have shown an association between small size at birth and increased arterial wall stiffness (8,41). Arterial compliance in fetal life may be influenced by a decrease in elastin deposition, a major determinant of stiffness, caused by changing haemodynamic conditions or abnormal pressure (4). Human elastin synthesis is at its maximum in the last weeks of pregnancy and decreases thereafter. Blood flow is an important haemodynamic stimulus for arterial wall development and the finding of increased fetal afterload and increased ventricular filling pressure may indicate that these phenomena may be involved in the association between reduced fetal growth and increased arterial wall stiffness. Arterial wall stiffness in adults is related with high blood pressure and has been recognized as an early marker of cardiovascular disease.

Furthermore, increased afterload and left ventricular dysfunction in fetuses with impaired growth could lead to permanent physiological and morphological changes of the left ventricle and predispose individuals to left ventricular hypertrophy (5,6). Fetuses with increased afterload have been described to show signs of myocardial damage (15). Ventricular hypertrophy in children and adults is a known independent predictor of cardiovascular mortality (42).

Multiple stimuli have been identified as being capable of inducing fetal cardiac programming, including nutrient restriction, chronic hypoxia and smoking. Smoking creates a state of chronic hypoxia, due to carbon dioxide in the maternal bloodstream, crossing the placenta as well as to the vasoactive effects of nicotine (43). Increased umbilical and middle cerebral artery pulsatility indices indicate that smoking causes increased resistance in the vascular beds of the brain and the placenta. The latter may explain the reduction of estimated fetal weight. The effect of smoking might interfere with the brain-sparing effect in fetal growth restriction, because it increases peripheral resistance in the brain, whereas in the physiological situation fetal growth restriction causes increased blood flow to the brain. This finding could potentially harm brain development and merits further investigation. Smoking related reduction in fetal cardiac output was mainly explained by differences in estimated fetal weight. Thus smoking during pregnancy did not seem to cause significant effects on cardiac function itself and arterial compliance in this study.

Conclusion

Cardiovascular performance in reduced fetal growth is consistent with an increase in afterload and raised end-diastolic ventricular filling pressure. Furthermore, cardiac and arterial compliance are compromised in case of diminished fetal growth. These adaptive fetal haemodynamic changes already occur before the stage of clinically apparent fetal growth restriction. These fetal circulation alterations may be part of the underlying associations between low birth weight and hypertension in adult life. Follow-up studies in our children are currently performed to examine whether and to what extend changes in fetal circulation haemodynamics persist during childhood and whether they are related to cardiac function and blood pressure development in postnatal life.

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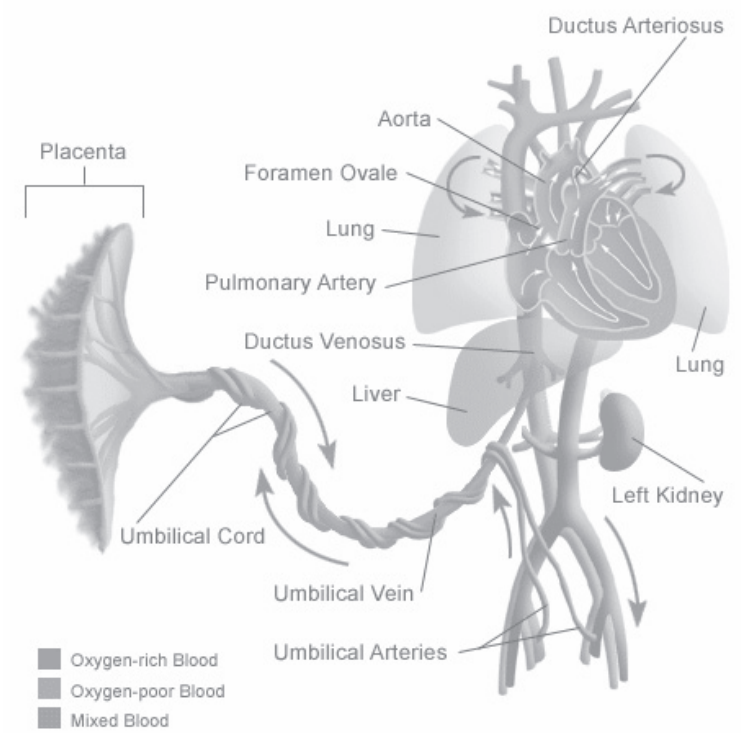
Chapter 4.2

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



Abstract

It has been suggested that an adverse fetal environment leads to permanent smaller kidneys and subsequently to hypertension and renal disease in adult life. The aim of this study was to examine whether maternal characteristics, fetal growth, fetal blood flow redistribution and inadequate placental perfusion in different periods of fetal life affect kidney volume in late fetal life. We also examined whether fetal kidney volume was associated with amniotic fluid quantity. In a population-based prospective cohort study from early fetal life, fetal growth characteristics and fetal blood flow parameters were assessed with ultrasound and Doppler examinations in 1,215 women in mid- and late pregnancy. Kidney volume was assessed in late pregnancy. Maternal height and pre-pregnancy weight were associated with kidney volume. After adjustment for the same characteristics in late pregnancy, fetal growth and blood flow parameters in mid-pregnancy were not associated with kidney volume in late pregnancy. In late pregnancy, all fetal growth characteristics were positively associated with kidney volume. The largest effect on kidney volume was found for abdominal circumference (increase of 1.77 cm^3; 95%CI: 1.46 - 2.08, per increase in standard deviation score). Signs of fetal blood flow redistribution and raised placental resistance were associated with reduced kidney volume in late pregnancy. Kidney volume was positively associated with amniotic fluid quantity. We conclude that maternal anthropometrics, fetal growth characteristics, raised placental resistance and fetal blood flow redistribution affect kidney volume.

Introduction

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

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

Amniotic fluid is known to represent fetal wellbeing (18). An adverse fetal environment as shown by raised placental resistance often results in decreased amniotic fluid indices as well (19). The main component of amniotic fluid is fetal urinary production, which may therefore be related to kidney volume and reflect kidney function.

The first aim of this population-based prospective cohort study was to evaluate the associations of maternal characteristics and fetal growth with kidney volume during pregnancy. The second aim was to examine the associations of placental resistance indices and fetal blood flow redistribution, as a measure of adverse fetal environment, with kidney volume. Finally, we assessed the relation of fetal kidney volume with amniotic fluid as a measure of fetal urine production. If associations of maternal and fetal growth character-

istics with kidney volume exist, further studies designed to identify the causal genetic and environmental mechanisms underlying these associations would be needed.

Methods

Design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood. This study is designed to identify early environmental and genetic determinants of growth, development and health from fetal life until young adulthood and has been described previously in detail (25, 26). In total, the cohort includes 9 778 mothers and their children living in Rotterdam, the Netherlands. A vast majority (69%) of all mothers were enrolled in the first trimester of pregnancy (26). Gestational age was determined by ultrasound during the first visit in early pregnancy. Assessments in pregnancy included physical examinations, fetal ultrasounds, biological samples and questionnaires and were planned in early (gestational age < 18 weeks), mid- (gestational age 18-25 weeks) and late pregnancy (gestational age >25 weeks) to collect information about fetal growth and its main determinants. The children were born between April 2002 and January 2006 and form a prenatally recruited birth-cohort that is currently followed until young adulthood. Of all eligible children born in the study area, 61% participated at birth in the study (26). Additional more detailed assessments of fetal growth and development were conducted in a subgroup of 1 232 Dutch children and their parents, referred to as the Generation R Focus cohort. For the present study, kidney size was assessed at the fetal ultrasound examination in late pregnancy in this subgroup. This subgroup is ethnic homogeneous to exclude possible confounding or effect modification by ethnicity. Of all approached women, 80% were enrolled in this subgroup study in the third trimester of pregnancy (gestational age of 30 weeks). Written informed consent was obtained from all participants. The Medical Ethics Committee of the Erasmus Medical Center, Rotterdam, has approved the study.

Population for analysis

In total, 1 232 women were enrolled in the Generation R Focus Study at a gestational age of 30 weeks. Twin pregnancies (n = 15) and pregnancies leading to perinatal death (n = 2) were excluded from the analysis. No renal or uterovesical anomalies other then mild pyelectasis over 10 mm (n=3) were present in our study population. Renal ultrasounds were only partially performed in 6 subjects due to unfavourable fetal position or maternal adipositas. The present analysis was performed in a total of 1 215 subjects.

Ultrasound Measurements

Fetal biometry

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

Placental and fetal blood flow profiles

Placental resistance as a proxy of placental function was assessed using recorded flow velocity waveforms from the umbilical and uterine arteries, in mid and late pregnancy. Raised umbilical artery pulsatility index (PI) and uterine artery resistance index (RI) indicate increased placental resistance (16). Umbilical artery PI was measured in a free-floating loop of the umbilical cord. Uterine artery RI was measured in the uterine arteries near the crossover with the external iliac artery. The redistribution of blood flow in favour of the fetal brain was quantified by the middle cerebral artery PI and the cerebro RI / umbilical RI ratio, in late pregnancy. A reduction in middle cerebral artery PI and a decreasing cerebro-umbilical ratio are valid indicators of 'brain sparing effect' due to fetal redistribution (17, 31). The middle cerebral artery Doppler was performed with colour Doppler visualization

of the circle of Willis in the fetal brain and the flow velocity wave forms were obtained in the proximal part of the middle cerebral artery.

Kidney measurement

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

Amniotic fluid

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

Intra- interobserver reproducibility

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

Data analysis

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

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

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

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

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

Results

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

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

Table 1. Subject characteristics (n=1 215)

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

Values are means (standard deviation) or medians (95% range).
Differences between boys and girls were compared using independent samples t-tests.
*p-value<0.05, **p-value<0.01

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

Table 2. Fetal kidney characteristics in late pregnancy

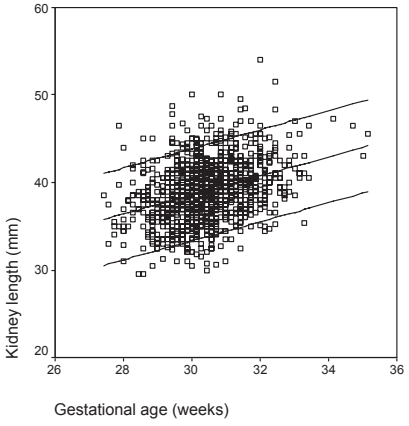
	Boys (n = 629)	**Girls (n = 586)**
Left kidney structures		
Length (mm)	39.5 (3.8)	38.4 (3.5)*
Width (mm)	22.7 (2.9)	22.1 (2.5)*
Depth (mm)	21.6 (2.8)	21.1 (2.5)*
Volume (cm^3)	10.3 (3.0)	9.5 (2.5)*
Right kidney structures		
Length (mm)	39.6 (3.8)	38.5 (3.5)*
Width (mm)	23.2 (3.0)	22.9 (2.7)**
Depth (mm)	22.4 (2.9)	22.0 (2.7)**
Volume (cm^3)	11.0 (3.3)	10.3 (2.8)*
Combined kidney volume (cm3)	21.3 (5.9)	19.9 (5.0)*

Values are means (standard deviation)
Differences between boys and girls were compared using independent samples t-tests.
*p-value<0.05, **p-value<0.01

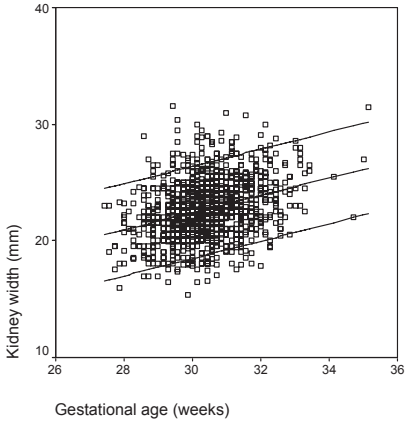
Table 3 gives the associations of maternal characteristics with combined (left plus right) kidney volume. Maternal pre-pregnancy weight and height were positively associated with kidney volume. Other maternal characteristics like obesity, blood pressure, preeclampsia, diabetes or smoking were not associated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

Table 4 presents the associations of fetal growth characteristics and placental resistance indices in mid-pregnancy with combined kidney volume measured in late pregnancy. In

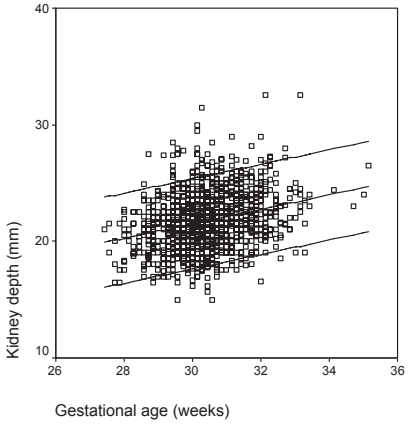
Figure 1. Individual measurements of mean kidney length, width, depth and volume with fitted median, 5th and 95th centiles with gestational age and formulas for normal values

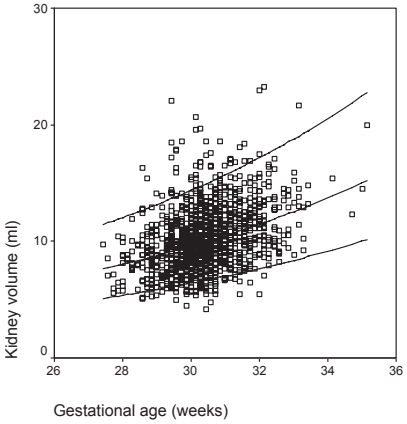


Mean length= 6 + (1.086*GA)
SD = 3.2



Mean width = 0.19 + (0.74*GA)
SD = 2.39





Mean depth (AP) = 2.91 + (0.62*GA)
SD = 2.35

Mean volume = exp(-0.406 + 0.089*GA)
SD = exp 0.245

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

Maternal characteristics	**Difference in total kidney volume (cm3) (95% confidence interval)**
Age (years)	-0.01 (-0.07, 0.06)
Height (cm)	0.07 (0.02, 0.11)**
Pre-pregnancy weight (kg)	0.03 (0.01, 0.05)*
Pre-pregnancy BMI (kg/m^2)	0.04 (-0.03, 0.11)
Weight gain during pregnancy (kg)	0.05 (-0.04, 0.13)
Systolic blood pressure in late pregnancy (mmHg)	-0.01 (-0.03, 0.02)
Diastolic blood pressure in late pregnancy (mmHg)	-0.01 (-0.04, 0.02)
Hypertension (yes vs. no)	-0.03 (-1.28, 1.33)
Pre-existent or pregnancy induced diabetes (yes vs. no)	-0.60 (-3.49, 2.30)
Preeclampsia (yes vs. no)	2.14 (-0.25, 4.53)
Smoking during pregnancy (yes vs. no)	-0.06 (-0.88, 0.77)

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

model A, adjusted for gestational age and fetal gender only, all fetal growth characteristics were positively associated with combined kidney volume and umbilical artery PI negatively associated (Model A). After additional adjustment for the same fetal growth characteristic or blood flow parameter in late pregnancy, measured at the same time as kidney volume, associations were no longer significant (model B). These results suggest

Table 4. Associations of fetal growth characteristics and placental resistance indices in mid-pregnancy with combined fetal kidney volume in late pregnancy

Measurements in mid-pregnancy	Difference in combined kidney volume (cm3) (95% confidence interval)	
	Model A	**Model B**
Growth characteristic		
Head circumference (sds)	0.49 (0.16, 0.82)**	0.30 (-0.03, 0.64)
Abdominal circumference (sds)	0.80 (0.46, 1.14)**	0.20 (-0.13, 0.53)
Femur length (sds)	0.48 (0.14, 0.82)**	0.34 (-0.02, 0.69)
Ratio abdominal circumference / head circumference (sds)	0.59 (0.18, 1.00)**	0.18 (-0.22, 0.58)
Estimated fetal weight (sds)	0.84 (0.50, 1.19)**	0.04 (-0.14, 0.56)
Placental resistance indices		
Umbilical artery, pulsatility index (PI)	-2.23 (-4.09, -0.37)*	-1.38 (-3.22, 0.53)
Uterine artery, resistance index (RI)	-2.24 (-6.52, 2.03)	-1.30 (-5.91, 3.31)

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

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

Measurements in late-pregnancy	Difference in combined kidney volume (cm3) (95% confidence interval)
Growth characteristic	
Head circumference (sds)	0.91 (0.57, 1.23)*
Abdominal circumference (sds)	1.76 (1.47, 2.05)*
Femur length (sds)	1.03 (0.71, 1.35)*
Ratio head circumference /abdominal circumference (sds)	1.71 (1.34, 2.09)*
Estimated fetal weight (sds)	1.77 (1.46, 2.08)*
Placental resistance indices	
Umbilical artery, pulsatility index (PI)	-2.74 (-4.55, -0.92)*
Uterine artery, resistance index (RI)	-6.40 (-10.4, -2.43)*
Redistribution parameters	
Middle cerebral artery (PI)	0.46 (-0.41, 1.33)
Cerebro-umbilical (C/U) ratio	0.87 (0.22, 1.51)*

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

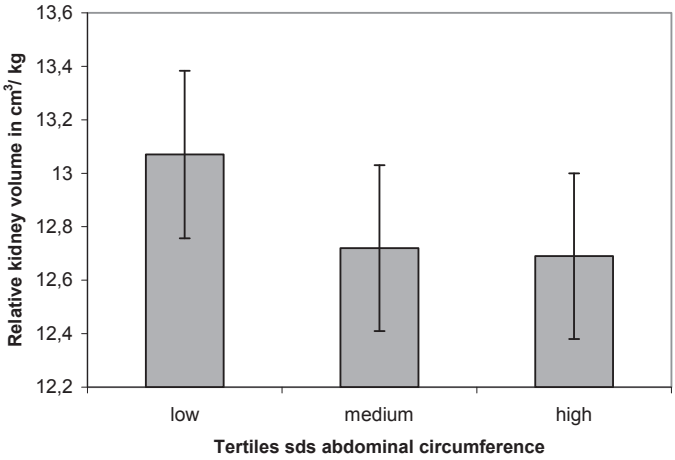
that the associations of mid-pregnancy growth characteristics and placental resistance indices with late pregnancy kidney volume are largely explained by the same characteristics in late-pregnancy.

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

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

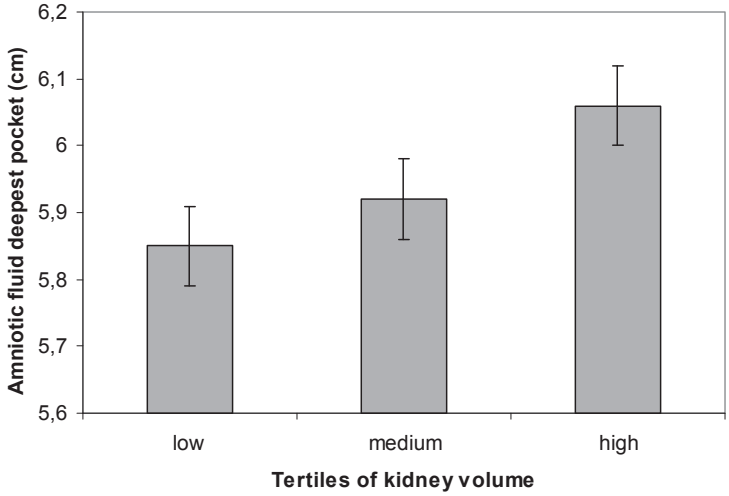
Figure 3 shows that in late pregnancy, kidney volume is positively associated with amniotic fluid deepest pocket.

Figure 2. The association between fetal abdominal circumference and fetal weight adjusted combined kidney volume in late pregnancy



SDS gestational age adjusted standard deviation score
P=0.07 P-value for trend using linear regression models

Figure 3. Relation of kidney volume with amniotic fluid deepest pool



Model adjusted for gestational age, abdominal circumference and fetal gender
$P < 0.05$ P-value for trend using linear regression models

Discussion

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

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

Both environmental and genetic factors are important determinants of fetal growth (20-22). We examined the association of maternal characteristics with fetal kidney volume in late pregnancy. Maternal weight and height were positively associated with kidney volume. This association may be explained by both environmental (maternal nutritional status) and common genetic factors that are important in the determination of kidney

volume during pregnancy. Maternal smoking, obesity, blood pressure and diabetes did not considerably influence fetal kidney size in this study. Even though these factors have a known influence on overall fetal size (20, 21).

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

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

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

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

A hypothesis for a decrease in renal size in growth restricted fetuses is alteration in renal perfusion caused by a preferential blood flow to the brain (23). In our study we did not find any relation with middle cerebral artery PI and kidney volume. Another parameter is the cerebro-umbilical ratio, which did show a relation with kidney size. It is not unlikely that

redistribution with decrease in middle cerebral artery PI is a later sign in fetal growth restriction what is not yet eminent in this population-based study. A direct measure of renal blood flow would be renal artery PI, which we did not measure. A previous study showed that renal artery blood flow was not altered in growth restricted fetuses (5). However, abnormal renal artery Doppler flow velocity waveforms were demonstrated in hypoxic growth restricted fetuses in another study (23). Altered renal artery flow velocity seems to be a late effect that is not present in growth restricted fetuses that do not yet show signs of redistribution. We think that the reduced kidney volume in our study is not solely explained by redistribution because it is already present in smaller fetuses when signs of redistribution are absent.

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

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

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

Conclusion

Our findings suggest that reduced fetal growth, signs of raised placental resistance and fetal blood flow redistribution result in a decreased kidney volume in late fetal life. Impaired fetal development results in smaller kidneys and may result in increased risk of hypertension and renal disease in later life. Follow-up studies in our children are currently performed to examine whether and to what extend changes in fetal kidney size persist during childhood and whether they are related to renal function and blood pressure development in postnatal life.

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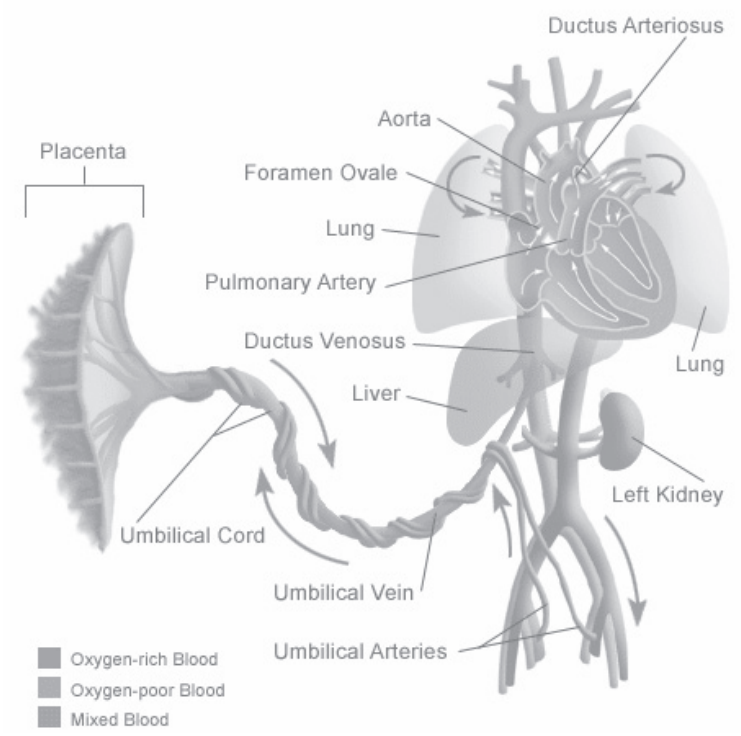
Chapter 4.3

Maternal anthropometrics and fetal growth are associated with kidney size in infancy. The Generation R Study.



Abstract

Background: It has been suggested that an adverse fetal environment leads to permanent smaller kidneys with a reduced nephron number and subsequently to renal disease and hypertension. The aim of this study was to examine whether maternal anthropometrics during pregnancy and fetal growth characteristics are associated with kidney volume in infancy.

Methods: This study was embedded in a population-based prospective cohort study from fetal life until adulthood. Maternal anthropometrics were measured in early pregnancy. Fetal growth characteristics were assessed in mid- and late pregnancy. Kidney size was measured by ultrasound in a subgroup of 767 infants aged 6 months.

Results: Maternal height was positively associated with combined kidney volume (increase of 0.38 (95% confidence interval 0.12, 0.63) cm^3 in kidney volume per cm increase in maternal height. No association was found of maternal weight with kidney volume. Combined kidney volume increased by 3.15 (95% confidence interval 1.31, 5.00) and 1.93 (95% confidence interval 1.18, 3.69) cm^3 per standard deviation score fetal abdominal circumference measured in mid- and late pregnancy, respectively. We found tendencies towards an inverse association between fetal femur length and kidney characteristics. All analyses were adjusted for infant gender, age, length and weight.

Conclusion: This study shows for the first time that early postnatal kidney size is at least partly established in fetal life. Follow-up studies are needed to examine whether these changes in kidney size persist during childhood and whether they are related to measures of renal function and blood pressure development in later life.

Introduction

Low birth weight is associated with hypertension and cardiovascular mortality in adult life (1, 2). More recently, is has been demonstrated that low birth weight is also related to kidney diseases or its risk factors in childhood and adulthood (3-8). The underlying mechanisms for these associations are unknown. It has been hypothesized that low birth weight infants have smaller kidneys with a reduced number of nephrons, which leads to compensatory higher glomerular pressure, progressive glomerular sclerosis and subsequently predispose the individual to impaired kidney function and hypertension (9).

This hypothesis is supported by several studies. Nephrogenesis continues until 36 weeks of gestation and the induction of nephron number ceases thereafter (10, 11). Animal studies have shown that low protein intake, relative vitamin A deficiency, reduced placenta perfusion or administration of steroids in late pregnancy lead to fetal growth restriction and a permanent nephron deficit (12-15). Human studies demonstrated that low birth weight infants have lower kidney weight with a reduced number of nephrons (16-18). Recently, it was demonstrated that hypertensive subjects have lower nephron numbers (19). Currently, there is no satisfactory method of assessing nephron number in vivo. The best surrogate measure for assessing nephron number in epidemiological studies appears to be kidney weight or size measured by ultrasound (11). Studies that examined prospectively the effect of fetal growth characteristics and their main environmental determinants on postnatal kidney size and function are lacking.

We examined in a population-based prospective cohort study whether maternal anthropometrics, as a measure of fetal nutrition, and fetal growth characteristics measured in mid- and late pregnancy are associated with kidney size in infancy.

Methods

Design

This study was embedded in the Generation R Study, a population-based, prospective cohort study from fetal life until young adulthood. This study is designed to identify early environmental and genetic determinants of growth, development and health from fetal life until young adulthood and has been described previously in detail (20, 21). In total, the cohort includes 9,778 mothers and their children living in Rotterdam, the Netherlands. A vast majority of mothers were enrolled in the first trimester of pregnancy (21). Assessments in pregnancy included physical examinations, fetal ultrasounds, biological samples and questionnaires and were planned in early (gestational age < 18 weeks), mid- (gestational age 18-25 weeks) and late pregnancy (gestational age >25 weeks) to collect information about fetal growth and its main determinants. Partners were assessed once during this

period. The children were born between April 2002 and January 2006 and form a prenatally recruited birth-cohort that is currently followed until young adulthood. Additionally, more detailed assessments of fetal and postnatal growth and development are conducted in a subgroup of 1,232 Dutch children and their parents, referred to as the Generation R Focus cohort. In this subgroup, postnatal renal ultrasounds were performed in infants at the age of 6 months. This subgroup is ethnic homogeneous to exclude possible confounding or effect modification by ethnicity, and includes children of parents from Dutch origin. Of all approached women, 80% were enrolled in this subgroup study in the third trimester of pregnancy (gestational age of 30 weeks). The study has been approved by the Medical Ethics Committee of the Erasmus MC, Rotterdam. Written informed consent was obtained from all participants.

Maternal anthropometrics

Maternal weight (kg) and height (cm) were measured light clothed in early (gestational age < 18 weeks), mid- (gestational age 18-25 weeks) and late (gestational age > 25 weeks) pregnancy. We used maternal weight and height at intake as measure of maternal nutritional status in the present study.

Fetal growth characteristics

Fetal ultrasound examinations were carried out during visits to the research centres in early, mid- and late pregnancy. These fetal ultrasounds were used for both establishing gestational age and assessing fetal growth characteristics. Pregnancy dating curves were constructed on subjects in the study with complete data on gestational age measured by ultrasound and last menstrual period. Crown-rump length was used for pregnancy dating in early pregnancy (gestational age until 12 weeks and 5 days, crown-rump length smaller than 65 mm), and biparietal diameter was used for pregnancy dating thereafter (gestational age from 12 weeks and 5 days onwards, biparietal diameter larger than 23 mm) (22). Fetal growth measurements used for the present study included head circumference, abdominal circumference and femur length in mid- and late pregnancy, measured to the nearest millimetre using standardized ultrasound procedures (23-25). Growth measures in early pregnancy were not included since these fetal ultrasound examinations were primarily performed to establish gestational age. Gestational age adjusted standard deviation scores were constructed for these fetal growth measurements. These were based on reference growth curves based on the whole study population. The median (95% range) gestational age for the visits in early, mid- and late pregnancy was 12.6 (9.6 – 16.9) weeks, 20.4 (18.6 - 22.5) weeks and 30.2 (28.5 – 32.5) weeks, respectively.

Kidney volume

Two-dimensional ultrasounds of the kidneys were performed on Kretz Voluson 530D equipment in children at the age of 6 months. The examination was carried out in a quiet room with the child quietly awake in a supine position. One radiographer performed the vast majority (86%) of these measurements. Two other radiographers performed the measurements in the remaining children. The kidney was identified in the sagittal plane along its longitudinal axis. Measures of maximal bipolar kidney length, width and depth were obtained from both the left and right kidney. Kidney width and depth were measured at the level of the kidney hilum. All dimensions were measured to the nearest millimetre (26, 27). Mean length, width and depth were calculated as the average of three measurements and used for data analysis. Kidney volume was calculated in cubic centimetres using the equation of an ellipsoid: volume (cm^3) = 0.523 x mean length (mm) x mean width (mm) x mean depth (mm) (27, 28). Left and right kidney volume were summed to establish combined kidney volume (cm^3) (29).

Covariates

All anthropometrics in the infants were measured without clothes at the same visits as the kidney ultrasounds at the age of 6 months. Weight was measured to the nearest gram using electronic scales. Length was measured in supine position to the nearest millimetre using a neonatometer. Date of birth, birth weight and gender were obtained from midwife and hospital registries.

Population for analysis

In total, 1,232 women were enrolled in the Generation R Focus Study at a gestational age of 30 weeks. The present analysis was limited to singleton live births (n = 1,215). Twin pregnancies (n = 15) and pregnancies leading to intrauterine death or perinatal death (n = 2) were excluded from the analysis. Of these singleton live births, 74% (n = 903) participated in the postnatal assessments at the age of 6 months. Kidney ultrasounds were successfully performed in 85% (n = 767) of these infants. No renal or uterovesical anomalies were observed in these infants. Missing values were mainly due to crying behaviour or unavailability of equipment or radiographer.

Data analysis

Associations of maternal anthropometrics (height, weight) and fetal growth characteristics (standard deviation scores of head circumference, abdominal circumference and femur length in mid- and late pregnancy) with postnatal kidney size were assessed using multiple linear regression models. First, we assessed the associations of maternal anthropometrics and fetal growth characteristics with combined left and right kidney volume. These models were first adjusted for infant gender and age, and subsequently additionally

adjusted for infant weight and length to get the effect estimates for absolute and relative kidney volume, respectively. Second, we assessed the associations of maternal anthropometrics and fetal growth characteristics with mean kidney length, width and depth. These models were adjusted for infant gender, age and current weight and length. All measures of association are presented with their 95% confidence intervals (CI). All statistical analyses were performed using the Statistical Package of Social Sciences version 11.0 for Windows (SPSS Inc, Chicago, IL, USA).

Table 1. Subject characteristics

	Boys (n = 389)	**Girls (n = 378)**
Maternal characteristics		
Age (years)	31.9 (4.0)	32.2 (3.8)
Weight (kg)	71.0 (13.2)	71.6 (12.7)
Height (cm)	170.7 (6.1)	171.1 (6.7)
Mid-pregnancy fetal growth characteristics		
Head circumference (sds)	0.20 (0.98)	-0.12 (0.96)
Abdominal circumference (sds)	0.19 (1.1)	0.13 (1.1)
Femur length (sds)	-0.14 (0.92)	0.04 (0.99)
Late pregnancy fetal growth characteristics		
Head circumference (sds)	0.29 (0.98)	-0.05 (0.88)
Abdominal circumference (sds)	0.18 (1.0)	0.11 (1.0)
Femur length (sds)	-0.21 (0.92)	0.05 (0.99)
Postnatal characteristics		
Birth weight (grams)	3544 (527)	3456 (538)
Gestational age (weeks)	40.3 (36.0 - 42.4)	40.3 (35.7 - 42.4)
Age at visit (weeks)	27.4 (23.8 – 34.9)	27.3 (23.5 – 36.1)
Weight at visit (grams)	8200 (848)	7638 (805)
Length at visit (cm)	70 (3)	68 (3)
Kidney structures		
Length (mm)	118.7 (9.6)	116.0 (8.1)
Width (mm)	56.3 (5.9)	55.1 (5.8)
Depth (mm)	54.3 (5.5)	52.5 (5.2)
Volume (cm^3)	47.8 (9.4)	44.1 (8.5)

Values are means (standard deviation) or medians (95% range).
Of the total group, data were missing on mid-pregnancy abdominal circumference (n=15), late pregnancy abdominal circumference (n=18), birth weight (n=4), gestational age (n=8), weight at 6 months (n=3), length at 6 months (n=12), kidney length (n=19), kidney width (n=62) and kidney depth (n=67).

Results

Characteristics of infants who participated in the postnatal renal ultrasound studies and their mothers are presented in Table 1. Infants who had a postnatal renal ultrasound (n = 767) did not differ from the postnatal non-responders (n = 448) in fetal characteristics (difference in birth weight 18 (95% CI: -48, 83) grams). The percentage of boys was 51%. The overall median age of infants at their 6 months visit was 27.3 (95% range: 23.7 – 35.6) weeks. Weight, length and absolute renal size were larger in boys than in girls.

Table 2 presents the associations of maternal anthropometrics and fetal growth characteristics measured in mid- and late pregnancy with postnatal absolute and relative combined kidney volume. Maternal height was associated with both absolute and relative combined kidney volume (increase in combined kidney volume of 0.26 (95% CI: 0.15, 0.36) cm^3 and of 0.38 (95% CI: 0.12, 0.63) cm^3 per cm increase in maternal height, respectively). Maternal weight was only associated with absolute combined kidney volume. In mid-pregnancy, abdominal circumference was associated with both absolute and relative combined kidney volume (increase of 1.42 (95% CI: 0.67, 2.17) cm^3 and 3.15 (95% CI: 1.31, 5.00) cm^3 per increase in standard deviation score, respectively). No associations were found for fetal head circumference and femur length in mid-pregnancy with combined kidney volume. Late pregnancy abdominal circumference was also associated with

Table 2. Associations of maternal anthropometrics and fetal growth characteristics with postnatal combined kidney volume at the age of 6 months

	Difference in absolute kidney volume		**Difference in relative kidney volume**	
	(cm3)	**(%)**	**(cm3)**	**(%)**
Maternal anthropometrics				
Height (cm)	0.26 (0.15, 0.36)*	0.6	0.38 (0.12, 0.63)*	0.3
Weight (kg)	0.07 (0.02, 0.13)*	0.2	0.10 (-0.03, 0.23)	0.1
Mid-pregnancy fetal growth characteristics				
Head circumference (sds)	0.40 (-0.35, 1.15)	0.9	0.83 (-1.01, 2.67)	0.7
Abdominal circumference (sds)	1.42 (0.67, 2.17)*	3.1	3.15 (1.31, 5.00)*	2.5
Femur length (sds)	-0.12 (-0.88, 0.64)	-0.3	-0.55 (-2.41, 1.32)	-0.5
Late pregnancy fetal growth characteristics				
Head circumference (sds)	1.13 (0.33, 1.93)*	2.5	1.69 (-0.91, 3.16)	1.4
Abdominal circumference (sds)	1.34 (0.63, 2.05)*	2.9	1.93 (0.18, 3.69)$	1.6
Femur length (sds)	-0.04 (-0.82, 0.74)	-0.1	-1.70 (-3.60, 0.22)	1.4

Values are regression coefficients (95% confidence interval) and reflect the difference in absolute and relative combined kidney volume per unit increase in maternal or fetal characteristic. Models with absolute kidney volume are adjusted for age and gender. Models with relative kidney volume are additionally adjusted for current weight and length. *p-value <0.01; $p-value <0.05

absolute and relative combined kidney volume (increase of 1.34 (95% CI: 0.63, 2.05) cm^3 and 1.93 (95% CI: 0.18, 3.69) cm^3 per increase in standard deviation score, respectively). Fetal head circumference and femur length measured in late-pregnancy were not associated with relative combined kidney volume.

Table 3 presents the associations of maternal anthropometrics and fetal growth characteristics measured in mid- and late pregnancy with mean kidney length, width and depth. Maternal height was only associated with kidney depth (increase of 0.06 (95% CI: 0, 0.12) cm per cm increase in maternal height). No associations were found for maternal weight. In mid-pregnancy, fetal abdominal circumference was associated with kidney depth (increase of 0.81 (95% CI: 0.38, 1.24) cm per increase in standard deviation score). No associations were found of fetal head circumference and femur length in with the kidney structures. In late pregnancy, inconsistent results were found. Fetal head circumference was only associated with kidney length (increase of 0.91 (95% CI: 0.19, 1.64) cm per increase in standard deviation score). Fetal abdominal circumference was associated with kidney depth (increase of 0.45 (95% CI: 0.03, 0.88) cm per increase in standard deviation score), whereas fetal femur length was inversely associated with kidney width (decrease of -0.52 (95% CI: -1.01, -0.03) per increase in standard deviation score. Remarkably, all associations of fetal femur length measured in mid- and late pregnancy with kidney volume, length, width and depth showed inverse tendencies.

Table 3. Associations of maternal anthropometrics and fetal growth characteristics with mean postnatal kidney length, width and depth at the age of 6 months

	Kidney length (mm)	**Kidney width (mm)**	**Kidney depth (mm)**
Maternal anthropometrics			
Height (cm)	0.07 (-0.03, 0.17)	0.03 (-0.04, 0.10)	0.06 (0, 0.12)$^{\$}$
Weight (kg)	0.04 (-0.01, 0.09)	0 (-0.04, 0.02)	0.02 (-0.01, 0.05)
Mid-pregnancy fetal growth characteristics			
Head circumference (sds)	0.47 (-0.22, 1.15)	-0.21 (-0.67, 0.25)	0.35 (-0.08, 0.78)
Abdominal circumference (sds)	0.33 (-0.36, 1.03)	0.40 (-0.06, 0.87)	0.81 (0.38, 1.24)*
Femur length (sds)	-0.11 (-0.80, 0.58)	-0.26 (-0.72, 0.21)	-0.13 (0.56, 0.31)
Late pregnancy fetal growth characteristics			
Head circumference (sds)	0.91 (0.19, 1.64)$^{\$}$	0.04 (-0.46, 0.54)	0.33 (-0.14, 0.80)
Abdominal circumference (sds)	0.19 (-0.47, 0.85)	0.27 (-0.18, 0.72)	0.45 (0.03, 0.88)*
Femur length (sds)	-0.28 (-1.00, 0.45)	-0.52 (-1.01, -0.03)$^{\$}$	-0.45 (-0.91, 0.02)

Values are regression coefficients (95% confidence interval) and reflect the difference in kidney length, width and depth per unit increase in maternal or fetal characteristic. All models are adjusted for age, gender and current weight and length.

*p-value <0.01; $^{\$}$p-value <0.05

DISCUSSION

Our population-based prospective cohort study showed positive associations of maternal height and fetal abdominal circumference in mid- and late pregnancy with kidney size in infancy. Surprisingly, we found tendencies towards inverse associations of fetal femur length with kidney size. Our findings strongly suggest that maternal anthropometrics in pregnancy and fetal growth in mid- and late pregnancy affect kidney size in postnatal life.

Previous studies have suggested associations of birth weight with postnatal kidney size (30). Since the same birth weight may be the result of various fetal growth patterns, studies relating prenatally measured characteristics of fetal growth and environmental with postnatal kidney size and function are needed for identifying biological pathways leading to small kidneys. To our knowledge, our study is the first that examines the associations of maternal anthropometrics during pregnancy and longitudinally measured fetal growth characteristics with kidney size in infancy. Of all children participating in the measurements at the age of 6 months, kidney measurements were successfully performed in 85%. Missing values were mainly due to crying behaviour or unavailability of equipment or radiographer. The effect estimates would be biased if the associations of maternal anthropometrics and fetal growth characteristics with kidney structures differ between those included and not included in the present analyses. This seems unlikely.

Our findings suggest that impaired growth in fetal life has consequences for kidney size in postnatal life. Fetal abdominal circumference from mid-pregnancy was, independent of postnatal weight and length, associated with kidney size in infancy. Unexpectedly, we found inverse associations of fetal femur length with postnatal kidney size. Although the effect of fetal abdominal circumference on kidney size is expected to be larger than the effect of fetal femur length, we expected the same direction of these effect estimates. One explanation for our findings is that subtle fetal growth retardation that is only characterized by a reduced femur length leads to compensatory abdominal organ sparing effects and larger kidneys. Only when growth of the abdominal circumference is compromised, kidney volume will be smaller. Further studies are necessary to replicate these findings. The associations of fetal growth characteristics with detailed kidney size measures were not consistent. Fetal head circumference was associated with kidney length, whereas fetal abdominal circumference and fetal femur length were associated with kidney depth and width, respectively. We can not explain these findings. They may represent biological mechanisms. However, for each fetal growth characteristics, the directions of all effect estimates on the kidney structures were similar. Chance finding of significant findings can therefore not be excluded.

Animal studies have shown that various determinants of fetal nutrition including low protein intake, relative vitamin A deficiency, reduced placenta perfusion and administration

of steroids in late pregnancy cause fetal growth restriction, smaller kidneys and a permanent reduced nephron number (12-15). Maternal anthropometrics is a major determinant of fetal nutrition, fetal growth and birth weight in humans (31). We showed that maternal height but not weight is associated with kidney volume in their offspring. This association may be explained by both genetic and environmental determinants. The effect of maternal height on postnatal kidney growth persisted after adjustment for current height in the infant, suggesting the causal pathway does involve at least other mechanism than larger heights in both mother and child. Further studies focused on potential genetic variants and detailed maternal dietary habits during pregnancy are needed to identify the underlying mechanisms.

Our study suggests that prenatal exposures and fetal growth patterns lead postnatally to smaller kidneys. Since the number of nephrons is largely determined in prenatal life, suboptimal kidney growth and development in fetal life leads to a smaller number of nephrons (10, 11). The kidneys respond to this reduced number of nephrons by hyperperfusion and remodelling (9). This process may be in favour of short-term renal function but may eventually lead to glomerular hypertrophy and damage (9). Finally, this might result in renal failure and hypertension. It has been shown that low birth weight is associated with early onset chronic renal failure. In subjects aged less than 50 years, those who weighted less than 2.5 kg at birth had a higher risk for end-stage renal disease than people who weighted 3-3.5 kg at birth (5). This association was shown in all groups of primary causes of end stage renal failure in adults (hypertension, diabetes and other causes). Studies in younger subjects have focused on urine albumin excretion, a predictor of cardiovascular and renal disease in diabetic and non-diabetic subjects (32). Low birth weight is associated with microalbuminuria in children and adults independent of blood pressure and measures of insulin resistance (3, 4, 6). The pathway leading from small kidneys to hypertension may include the renin-angiotensin system, which has been demonstrated to be altered in the early phase of primary hypertension (33). An increased activity of the renin-angiotensin system could be a compensatory mechanism in a decreased number of nephrons in order to maintain normal renal filtration. It has been demonstrated that renin activity in umbilical cord blood is inversely related with the size of the kidney at birth (34).

Our study demonstrated for the first time in large population-based cohort that kidney size is already partly established in fetal life. Follow-up studies in our children are currently planned to examine whether and to what extend these changes in kidney size in infancy persist during childhood and whether they are related to renal function and blood pressure development in later life.

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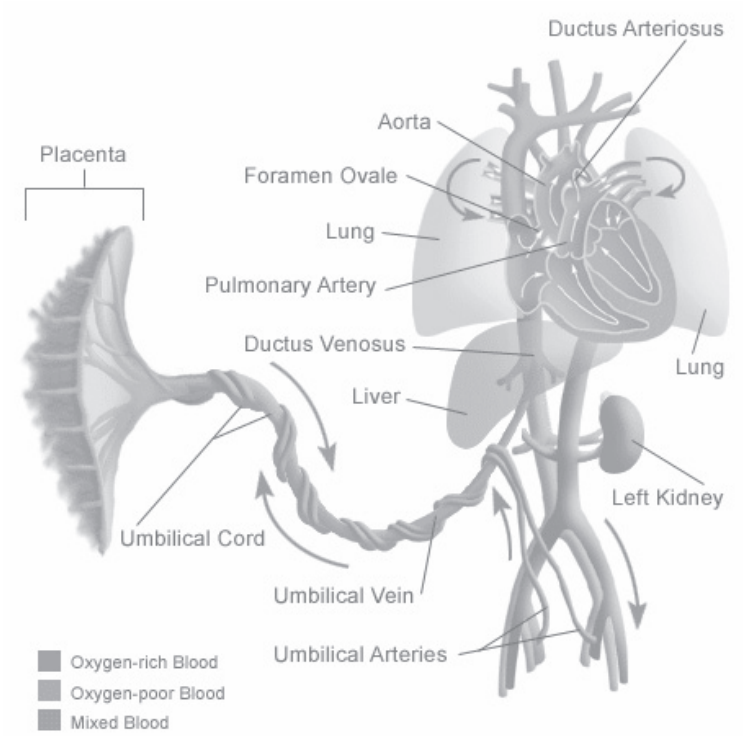
Chapter 4.4

Effects of maternal smoking in pregnancy on prenatal brain development. The Generation R Study.



Abstract

Nicotine, as has been shown in animal studies, is a neuroteratogen, even in concentrations that do not cause growth-retardation. In humans, there is only indirect evidence for negative influences of nicotine on brain development from studies on the association between maternal smoking in pregnancy and behavioural and cognitive development in the offspring. We investigated the associations of maternal smoking in pregnancy with fetal head growth characteristics in 7,042 pregnant women. This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until adulthood. Maternal smoking was assessed by questionnaires in early, mid- and late pregnancy. Head circumference, biparietal diameter, transcerebellar diameter and atrial width of lateral ventricle were repeatedly measured by ultrasound. When mothers continued to smoke during pregnancy, fetal head circumference showed a growth reduction of 0.13 mm (95% confidence interval -0.18; -0.09) per week compared to fetuses of mothers who never smoked during pregnancy. Biparietal diameter of fetuses with smoking mothers grew 0.04 mm (95% confidence interval -0.05; -0.02) less per week than that of fetuses of non-smoking mothers. Atrial width of lateral ventricle was 0.12 mm (95% confidence interval -0.22; -0.02) smaller and transcerebellar diameter was 0.08 mm (95% confidence interval -0.15; -0.00) smaller if mothers smoked, but growth per week was not affected by maternal smoking in pregnancy. In conclusion, continuing to smoke during pregnancy leads to reduced growth of the fetal head. Further research should focus on the causal pathway from prenatal cigarette exposure via brain development to behavioural and cognitive functions.

Introduction

Research has demonstrated the several negative effects of maternal smoking in pregnancy on the developing fetus. Maternal cigarette smoking is an established risk factor for intrauterine growth restriction, perinatal morbidity and mortality and postnatal growth (1, 2). This is assumed to be the result of fetal hypoxia due to nicotine-induced vasoconstriction, which leads to reduced blood flow to the fetus, and decreased oxygen availability. In addition, carbon monoxide exposure induces higher levels of carboxyhemoglobin, which also leads to intrauterine hypoxia (1, 3). Several reports described reduced fetal head circumference and biparietal diameter as parameters of total growth restriction in fetuses of smoking mothers (4-8). So far, only animal studies, but not human studies, indicate that nicotine directly influences fetal brain development, even in concentrations that do not cause growth retardation (9, 10). Altered cell proliferation and differentiation due to prenatal exposure of nicotine affects neural cell survival and the development of several neurotransmitter systems (10), including the cholinergic, the dopaminergic and the serotonergic system (11). In humans, there is only indirect evidence for negative influences of nicotine on brain development from studies on the association between maternal cigarette smoking in pregnancy and behavioural and cognitive development in the offspring. The consistent finding of higher rates of behaviour problems in children whose mothers smoked during pregnancy is striking, but still difficult to interpret because of numerous confounding environmental and genetic factors (12). Whether the relationship between maternal smoking in pregnancy and behavioural and cognitive development is mediated by brain deficits, is yet unknown.

To explore the first step in this causal pathway, the present study investigated the associations of maternal smoking in pregnancy with longitudinally measured fetal head growth characteristics. We hypothesized that 1) maternal smoking in pregnancy adversely affects growth of fetal head circumference and biparietal diameter, 2) this effect is independent of total fetal growth restriction and 3) specific brain parameters, i.e. transcerebellar diameter and atrial width of lateral ventricle, are negatively influenced by prenatal nicotine exposure.

Materials and methods

Setting

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood. The Generation R Study, designed to identify early environmental and genetic determinants of growth, development and health, has been described previously in detail (13, 14).

Briefly, the cohort includes 9,778 mothers and their children of different ethnicities living in Rotterdam, one of the major cities of the Netherlands. Enrolment was aimed in early pregnancy (gestational age < 18 weeks) but was possible until birth of the child. Assessments in pregnancy, including physical examinations, ultrasound assessments and questionnaires, were planned in early pregnancy (gestational age < 18 weeks), mid-pregnancy (gestational age 18-25 weeks) and late pregnancy (gestational age ≥ 25 weeks). The study has been approved by the Medical Ethics Committee of the Erasmus Medical Centre, Rotterdam. Written informed consent was obtained from all participants.

Study population

All pregnant women who were resident in the study area at their delivery date from April 2002 until January 2006 were invited to participate. Of the total of 9,778 mothers (response rate 61%), 91% (n=8,880) was enrolled in pregnancy (14) and was eligible for present analyses. Mothers without information on smoking habits in pregnancy on the basis of the three questionnaires were excluded from the present study (4.9%, n=433). Mothers with twin pregnancies (n=90) were excluded since growth potentials for individual fetuses in multiple pregnancies are not comparable to singleton pregnancies. Of the remaining 8,357 mothers, 4.4% (n=364) had only one ultrasound assessment during pregnancy. 1,852 (22.2%) mothers had two ultrasound assessments, while most participating mothers (n=6,141; 73.5%) had three ultrasound assessments. In the present study, we restricted analyses to mothers with measurements in early, mid- and late pregnancy for head circumference (n = 5,180) and biparietal diameter (n = 5,501). Since transcerebellar diameter and atrial width of lateral ventricle can only be measured reliably from mid-pregnancy onwards, we restricted the analyses of the association between maternal smoking in pregnancy and these parameters to two measurements in mid- and in late pregnancy. Analyses were based on 5,675 subjects for transcerebellar diameter and 3,071 subjects for atrial width of lateral ventricle. Overall, 7,042 mothers were included in one or more analyses.

Maternal smoking in pregnancy

Information about maternal smoking was obtained by postal questionnaires in early, mid- and late pregnancy. Maternal smoking at enrolment was assessed in the first questionnaire by asking whether mother smoked in pregnancy (no, until pregnancy was known, continued during pregnancy). In the second and third questionnaire, mothers were asked whether they smoked in the past 2 months (yes, no) in mid- and late pregnancy, respectively. Maternal smoking during pregnancy was categorized on the basis of all three questionnaires into 'no', 'until pregnancy was known' and 'continued during pregnancy'. Mothers who reported in the first questionnaire to have smoked until pregnancy was known but reported to smoke in the second or third questionnaire, were classified as 'continued smoking during pregnancy'. Similarly, mothers who reported not to smoke

in the first questionnaire but acknowledged to smoke in the second or third questionnaire were classified as 'continued during pregnancy'. When information was missing on maternal smoking at enrolment, information from the second and / or third questionnaire was used to classify mothers into 'no smoking in pregnancy' or 'continued smoking during pregnancy'.

Fetal ultrasound examinations

Sonographers carried out fetal ultrasound examinations at the visits to the research centres in early, mid- and late pregnancy. Most assessments (88%) were performed at the Generation R research centre in Rotterdam, the remaining were carried out at the five collaborating hospitals. The fetal ultrasound examinations were used for both establishing gestational age and assessing fetal growth characteristics. Crown-rump length was used for pregnancy dating until a gestational age of 12 weeks and biparietal diameter was used for pregnancy dating thereafter. In additional analyses, we used history of last menstrual period to date pregnancy. To avoid bias from irregularity of menstrual cycles or recall problems, we only included ultrasound measurements when gestational duration on the basis of last menstrual period dating and gestational duration on the basis of ultrasound dating differed less than three weeks.

The median (95% range) gestational age for the fetal ultrasound examinations in early, mid- and late pregnancy was 13.1 (9.1 – 17.5) weeks, 20.5 (18.4 – 23.5) weeks and 30.3 (27.2 – 33.0) weeks. Online measurements used for the present study included head circumference, biparietal diameter, transcerebellar diameter and atrial width of lateral ventricles and were done using standardized techniques. The biparietal diameter represents the widest diameter of the fetal head in a transverse plane, measured outer to outer of the fetal skull, perpendicular to the midline. The head circumference was measured at the level of the biparietal diameter and represents the outer perimeter of the fetal skull. The atrial width of the lateral ventricle is the widest diameter of the atrium of one of the lateral ventricles that can be measured in an axial plane. A slightly caudal rotated axial plane was used for measuring the transcerebellar diameter as the widest diameter across both hemispheres of the cerebellum. Sonographers were blinded to smoking status of the pregnant women. High intraobserver and interobserver reproducibility for biparietal diameter, head circumference and atrial width of lateral ventricle have been reported (15, 16).

The intra- and interobserver reliability of fetal biometry measurements in early pregnancy within the Generation R Study is good (intraclass correlation coefficients for head circumference 0.995 (intraobserver) and 0.988 (interobserver) and for biparietal diameter 0.995 (intraobserver) and 0.994 (interobserver) with coefficients of variation between 1.8 and 3.8%).

Covariates

The following variables were considered as possible confounders: maternal age, fetal gender, maternal height, maternal body mass index, maternal educational level, maternal ethnicity, parity, maternal alcohol consumption, maternal prenatal anxiety and maternal prenatal depression. Maternal age and maternal anthropometrics were assessed at enrolment in one of the research centres. Height and weight were measured without shoes and heavy clothing and body mass index was calculated from height and weight (weight / height^2). Information on maternal educational level, maternal ethnicity and parity was obtained by the first questionnaire at enrolment in the study. Maternal alcohol consumption was assessed in early, mid- and late pregnancy by questionnaire and categorized into 'no alcohol use', 'alcohol use until pregnancy was known' and 'continued using alcohol during pregnancy'. Maternal anxiety and depression were assessed in mid-pregnancy using two scales of the Brief Symptom Inventory (BSI), a validated self-report questionnaire with 53 items scored on a 5-point scale (17, 18). Fetal gender was obtained from midwife and hospital registries at birth.

Data analysis

The associations between maternal smoking habits during pregnancy and repeatedly measured brain parameters (head circumference, biparietal diameter, transcerebellar diameter and atrial width of lateral ventricle) were analysed using longitudinal multilevel analysis (19) to account for the dependency between measurements in the same subject. First, the best fitting model with the outcome as a function of gestational age was constructed using fractional polynomials (20). Second, maternal smoking as main determinant was brought into the model. The final curve was fitted with random effects for both intercept and gestational age. The interaction term of maternal smoking with gestational age was included in the model to compare the slope of the curves between the different smoking categories. When this interaction term did not result in a significant improvement of the model (evaluated by comparing the –2 log likelihood of the model with the interaction term to the –2 log likelihood of the model without the interaction term), the term was left out in further analyses.

The best fitting models were the following:

Head circumference = β_0 + β_1*smoking + β_2*gestational age^2 + β_3*gestational age^2*ln(gestational age) + β_4*smoking*gestational age.

Biparietal diameter = β_0 + β_1*smoking + β_2*gestational age + β_3*gestational age^3 + β_4*smoking*gestational age.

Transcerebellar diameter = β_0 + β_1*smoking + β_2*gestational age^2.

Atrial width of lateral ventricle = $\beta_0 + \beta_1$*smoking + β_2*gestational age.

Models were based on 15,540 observations for head circumference, 16,503 observations for biparietal diameter, 11,350 observations for transcerebellar diameter and 6,142 observations for atrial width of lateral ventricle. All models were adjusted for lifestyle and socio-economic status related confounders (maternal body mass index and educational level) and other known determinants of fetal growth (maternal age, height, ethnicity, parity and fetal gender). Maternal alcohol use, maternal prenatal anxiety and maternal prenatal depression did not significantly improve the models and did not change the regression coefficients for maternal smoking. These covariates were therefore excluded in final analyses. History of last menstrual period of reasonable quality, i.e. less than 3 weeks difference from ultrasound-defined age, was available for 4,592 subjects. A p-value of 0.05 was taken to indicate statistical significance. Statistical analyses were carried out using SAS v.8.2 (Stata Corporation, College Station, TX, USA), including the Proc Mixed module for longitudinal multilevel analysis.

Results

Characteristics of pregnant women per smoking category are presented in table 1. Of all mothers, 7.7% (n=545) reported to smoke until pregnancy was known and 17.0% (n=1,199) continued smoking during pregnancy. Mothers who continued smoking in pregnancy were younger, lower educated and more often used alcohol during pregnancy compared to mothers who never smoked during pregnancy. Anxiety and depression scores were higher in mothers who continued to smoke during pregnancy than in non-smoking mothers. More Turkish women (30.3%) continued smoking during pregnancy compared to Dutch women (16.2%), while only few Moroccan mothers (5.4%) smoked during pregnancy.

Table 2 presents mean values for head circumference, biparietal diameter, transcerebellar diameter and atrial width of lateral ventricle at the median gestational ages in early, mid- and late pregnancy. There was a strong significant correlation between head circumference and biparietal diameter (r=0.7) measured at the median gestational age in late pregnancy. Head circumference (r=0.3) and biparietal diameter(r=0.3) were moderately correlated to transcerebellar diameter, while the correlation of the atrial width of lateral ventricle with head circumference (r=0.2) and biparietal diameter (r=0.2) was low, but significant. Transcerebellar diameter was not significantly related to atrial width of lateral ventricle.

Table 1. Maternal characteristics

	Smoking in pregnancy (n = 7,042)		
	Non-smoking	**Quit smoking when pregnancy known**	**Continued smoking during pregnancy**
	n =5,298	*n =545*	*n = 1,199*
Age (years)	30.1 (5.0)	29.4 (5.0)*	28.3 (5.7)**
Height (cm)	167.3 (7.4)	168.5 (7.0)*	166.9 (7.1)
Body mass index (kg / m^2)	24.6 (4.3)	24.5 (4.4)	24.9 (4.6)*
Educational level (%)			
Primary level	9.7	11.7	20.9
Secondary level	40.7	43.3 χ^2=4.6	60.0 χ^2=343**
Higher education	49.4	45.0 df=2	19.0 df=2
Ethnicity (%)			
Dutch	51.6	55.5	49.4
Turkish	7.3	7.0	15.5
Moroccan	7.5	1.1	1.9
Cape Verdian	3.8	4.6 χ^2=39**	5.4 χ^2=141**
Antillean	3.1	2.2 df=7	4.2 df=7
Surinamese	8.1	11.3	10.1
Other Western	8.8	9.6	7.9
Other non-Western	9.8	8.7	5.6
Parity (% nulli)	55.4	68.2**	54.7
Alcohol use in pregnancy (%)			
No	52.3	25.2	46.5
Until pregnancy was known	11.3	27.2 χ^2=188**	11.8 χ^2=13.6**
Continued during pregnancy	36.4	47.6 df=2	41.6 df=2
Depression score	0.20 (0.4)	0.21 (0.5)	0.42 (0.7)**
Anxiety score	0.26 (0.4)	0.30 (0.5)	0.44 (0.6)**

Values are means (SD) for continuous variables and percentages for categorical variables.
* p<0.01, **p<0.001; ANOVA or Kruskal-Wallis with post-hoc comparisons for continuous variables, chi-squared tests for categorical covariates, compared to non-smoking mothers.

Table 2. Mean (standard deviation) of head circumference, biparietal diameter, transcerebellar diameter and atrial width of lateral ventricle at median gestational age in early, mid- and late pregnancy

	Early pregnancy (median 13.1 weeks)	**Mid-pregnancy** (median 20.4 weeks)	**Late pregnancy** (median 30.4 weeks)
Head circumference (mm)	82.3 (5.3)	176.4 (6.6)	284.6 (9.3)
Biparietal diameter (mm)	23.6 (1.4)	49.8 (2.1)	80.3 (3.1)
Transcerebellar diameter (mm)	-	20.7 (1.0)	37.7 (1.8)
Atrial width of lateral ventricle (mm)	-	5.7 (1.1)	4.9 (1.7)

The associations between maternal smoking habits in pregnancy and longitudinally measured growth and developmental brain parameters are presented in table 3. Adjustment for the covariates maternal age, body mass index, height, educational level, ethnicity, parity and fetal gender changed the effect of maternal smoking by 1.2–14.1%. In the models with head circumference and biparietal diameter, both the separate smoking variables and the interaction terms of smoking with gestational age significantly contributed to the model. The main (positive) effect of continued maternal smoking on head circumference

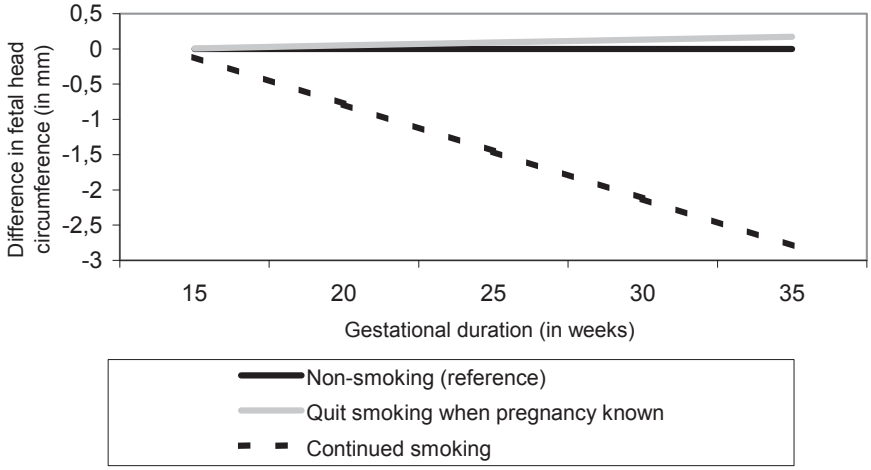
Table 3. Associations between maternal smoking habits in pregnancy and repeatedly measured head- and brain parameters

	Head circumference	**Biparietal diameter**	**Transcerebellar diameter**	**Atrial width lateral ventricle**
Non-smoking	*Reference*	*Reference*	*Reference*	*Reference*
Smoking until pregnancy	-0.12 (-1.15; 0.92)	-0.29 (-0.60; 0.03)	0.01 (-0.09; 0.11)	0.01 (-0.12; 0.14)
Continued smoking	1.89 (1.12; 2.67)**	0.53 (0.30; 0.76)**	-0.08 (-0.15; -0.00)	-0.12 (-0.22; -0.02)*
Gestational age(GA)	5.83 (4.36; 7.30)**	3.98 (3.97; 4.00)**	-0.12 (-0.22; -0.01)*	-0.09 (-0.09; -0.08)**
GA^2	1.09 (0.93; 1.25)**	-	0.04 (0.03; 0.04)**	-
GA^3	-	-0.0005 (-0.0; -0.0)**		-
GA^2*ln (GA)	-0.27 (-0.30; -0.23)**	-	-	-
GA*non-smoking	*Reference*	*Reference*	-	-
GA*quit smoking	0.008 (-0.05; 0.07)	0.02 (-0.00; 0.04)	-	-
GA*continued smoking	-0.13 (-0.18; -0.09)**	-0.04 (-0.05; -0.02)**	-	-

Models were constructed using fractional polynomials for gestational age. Values are regression coefficients (95% confidence interval). All values are adjusted for maternal age, body mass index, height, educational level, ethnicity, parity and fetal gender.
*p-value < 0.05 **p-value < 0.01

(regression coefficient 1.89 (1.12; 2.67)) should not be interpreted because the interaction effect was included in the model. The interaction effect of continued maternal smoking with gestational age shows that fetal head circumference in mothers who continued smoking during pregnancy grew 0.13 mm less per week compared to fetuses of mothers who never smoked in pregnancy. Figure 1 shows the difference in fetal head circumference of mothers who continued smoking during pregnancy and mothers who quit smoking when pregnancy was known compared to fetal head circumference of mothers who never smoked during pregnancy. This figure shows differences instead of the crude effects of maternal smoking on head circumference since the effect sizes were small (max. 2.8 mm

Figure 1. Size difference of fetal head circumference due to maternal smoking in pregnancy compared to fetuses of mothers who never smoked during pregnancy.



Values are estimated differences in foetal head circumferences based on the linear mixed models, adjusted for maternal age, body mass index, height, educational level, ethnicity, parity and fetal gender.

difference) and the growth rate was high (from 80 to 300 mm during pregnancy). Total effect size of continued smoking during pregnancy on fetal head circumference varied from 0.11% (at 15 weeks of gestational age) to 0.87% (at 35 weeks of gestational age). When we additionally adjusted the model for abdominal circumference measured at the same gestational age, the effect of continued maternal smoking on fetal head circumference became smaller (0.09 mm / week growth reduction; 95% confidence interval (–0.13; -0.04)), but remained statistically significant. In fetuses of smoking mothers biparietal diameter grew 0.04 mm less per week than that of fetuses of non-smoking mothers. The effect size of continued smoking varied from 0.10% - 0.80% for biparietal diameter over time. When fetuses, dated on biparietal diameter were left out of the analyses, the effect of maternal smoking on growth of biparietal diameter did not materially change (data not shown). Continued smoking in pregnancy significantly decreased the transcerebellar diameter (0.08 mm smaller) and atrial width of the lateral ventricle in fetuses of smoking mothers was 0.11 mm smaller compared to fetuses of mothers who never smoked during pregnancy. The interaction term of smoking with gestational age did not result in a better fit in the models of transcerebellar diameter or atrial width of lateral ventricle, i.e. the slope of the growth curve during pregnancy did not differ between the three smoking groups.

Finally, we analysed our data using last menstrual period for pregnancy dating. Head circumference grew 0.10 mm less per week (95% confidence interval –0.16;-0.05) in fetuses of continued smoking mothers compared to fetuses of non-smoking mothers. Fetal biparietal diameter, when exposed to nicotine throughout pregnancy, showed 0.03 mm per week decreased growth (95% confidence interval: –0.04;-0.01). Transcerebellar diameter

was significantly smaller in fetuses of mothers who continued smoking in pregnancy (-0.14 mm, 95% confidence interval: -0.26;-0.01) compared to fetuses of mothers who never smoked in pregnancy. Atrial width of lateral ventricle was not significantly affected by maternal smoking in pregnancy in the analyses based on last menstrual period dating (0.02 mm, 95% confidence interval –0.11;0.14).

Discussion

This study showed that maternal smoking in pregnancy is associated with reduced growth of the fetal head. Small but highly significant associations were found for head circumference and biparietal diameter. Maternal smoking did also result in smaller atrial width of lateral ventricle and smaller transcerebellar diameter but the differences between fetuses of smoking and non-smoking mothers remained constant throughout pregnancy.

Three different mechanisms to explain the untoward effects of maternal smoking in pregnancy on neurobehavioral development have been proposed. First, cigarette smoking leads to intrauterine growth restriction due to fetal hypoxia (1, 4, 5). Research, which initially focused on decreased birth weight, now mostly utilizes ultrasound to measure fetal growth characteristics. Cigarette exposure seems to cause a more general, symmetrical growth restriction with also decreased head circumference (6, 7, 21, 22), while brain maturation is usually maintained in mild fetal growth restriction (the 'brain sparing effect'). Our study also showed reduced growth of the fetal head circumference and biparietal diameter, even after controlling for the effects of maternal smoking on abdominal circumference. Growth of the transverse cerebellum diameter was not affected by cigarette exposure in our study, which is consistent with the fact that the cerebellum is the least affected in growth restriction (22).

The second mechanism proposed is the direct effect of nicotine on the developing brain, which has mainly been shown in animal studies. Cell replication and differentiation, the most prominent features in fetal brain development, are controlled partly by neurotransmitter-induced stimulation. Doses of nicotine below the threshold that causes growth retardation, induct premature stimulation of this receptor-mediated process, which results in cell damage, cell loss and synaptic dysfunction (23). Increased cell death has been demonstrated in both cortex and cerebellum (11), whereas changes in expression of nicotinic and muscarinic acetylcholine receptors were seen in human brainstem and cerebellum in the first trimester (16). The long-term effects of functional alteration of nicotinic acetylcholine receptors in the human brain remain unclear. No studies examined these subtle changes or their relation to developmental outcomes in living human brains. In the present study, we found structural alterations in the cerebellum by prenatal cigarette

exposure, which indicates that nicotine induces cell loss in this region. To our knowledge, effects of nicotine on the ventricular system have not been described in animal or human studies until now. Atrial width of the lateral ventricle, a measurement for verifying the state of the ventricular system, provides information on the growth of the cerebral hemispheres during mid- and late pregnancy. In contrast to an earlier report on 100 healthy fetuses (12, 24, 25), our study on more than 5,000 fetuses showed a decrease of the mean lateral ventricle atrium diameter throughout gestation. Most likely, the narrowing of the ventricles is due to growth of the cerebral hemispheres. Cigarette exposure did not influence the narrowing of these cavities.

A third explanation for the effect of prenatal maternal smoking on neurodevelopment in the offspring are the epiphenomena of smoking, e.g. parental psychopathology, co-abuse of other substances, poor prenatal care, dietary restriction and low socio-economic status. The large amount of studies on associations between maternal smoking during pregnancy and subsequent mental health problems in offspring has provided strikingly consistent evidence for an etiologic role for prenatal cigarette exposure in the onset of conduct disorder, antisocial behaviour and attention deficit hyperactivity disorder as well as subtle intellectual decrements and neurocognitive impairments (12, 24, 25). However, the interpretation of these findings still leads to discussion. Some reviewers have pointed out that the found effects, especially those on cognitive functioning and academic achievement, attenuate completely after control for a variety of co-varying influences (26). In line with other studies (27), we found increased scores on depression and anxiety in mothers who continued to smoke during pregnancy. These mothers were also lower educated than non-smoking pregnant women. It is clear that maternal smoking in pregnancy frequently occurs in the context of other factors that place the child at increased developmental risk, and it remains a challenge to fully control for these confounding variables.

It is tempting to speculate that reduced growth of the head circumference and biparietal diameter is one of the mediators in the relationship between maternal smoking in pregnancy and behaviour in the offspring. The neurobehavioral consequences of subnormal head circumference are well-known: in (very) low birth weight children, this indicator of brain volume was negatively associated with cognitive function and neuropsychological abilities at early school age (28). Reductions in brain volume, as measured by structural magnetic resonance imaging studies, were associated with poorer cognitive outcome (29). Furthermore, several MRI investigations in clinical samples revealed information on alterations in total cerebral volume in ADHD, schizophrenia and autism. Both children with ADHD and children with schizophrenia seem to have decreased total brain volumes (29). In addition, reduced cerebellar volumes and decreased growth of the lateral ventricular system have been found in children and adolescents with ADHD (30). Our data suggest that the structural changes induced by prenatal exposure to tobacco smoke are similar to the structural changes found in ADHD. However, whether the morphological alterations in

this and other child psychiatric disorders are due to prenatal nicotine exposure or caused by genetic and other environmental influences cannot be inferred from our data.

Strengths of this study are the large number of participating pregnant women, its prospective population-based design, the repeated measurements of fetal head growth parameters during pregnancy and the information on numerous potential confounding variables. However, some methodological issues need to be considered. Firstly, the specific brain parameters transcerebellar diameter and atrial width of lateral ventricle were not measured in all participating pregnant women. This was mainly due to the fact that these parameters were added to the study protocol during data collection. These missing outcomes are assumed to be random across participants. However, we cannot rule out non-random effects as ultrasound data probably were more complete in healthier, Dutch-speaking and higher-educated participants who also might have different smoking patterns. Secondly, information about maternal smoking habits during pregnancy was collected by postal questionnaires. Using self-reports may have introduced misclassification mainly due to underreporting of cigarette consumption, which could lead to underestimation of the effects. Thirdly, the main disadvantage of using fetal ultrasound examinations for pregnancy dating is that growth variation before the first measurement is set to zero. This makes it impossible to notice effects of maternal smoking on fetal growth in early pregnancy. Furthermore, Henriksen *et al*. (1995) showed that this method can distort effects of maternal smoking in pregnancy on preterm and postterm delivery (31). However, the use of last menstrual period for pregnancy dating can be biased by recall problems, irregularity of menstrual cycles or contraceptive use. Thus, we performed analyses with both dating methods and found very small differences in results. The similar results indicate that the found effects of maternal smoking in pregnancy on fetal head growth characteristics are not biased by the method of pregnancy dating. Finally, despite our efforts to control for several determinants of fetal growth and lifestyle related confounders, it cannot be ruled out that the associations are confounded by diet-related determinants or other, unknown, factors.

Conclusion

Prenatal cigarette exposure leads to reduced growth of the fetal brain. Growth of the cerebellum or ventricular system was not affected by maternal smoking, but molecular alterations and changes in neurotransmitter systems can still be present. The effects of maternal smoking in pregnancy were significant, even after controlling for numerous important confounders. One cannot conclude from our results which components in tobacco smoke induce the structural changes within the developing fetus. We recommend

clinical and public health strategies aimed at the primary and secondary prevention of prenatal tobacco exposure of children. In this respect, it is important to know that quitting smoking as soon as pregnancy is known positively affects fetal growth and development. Primary caregivers should inform women about the (long-term) consequences of smoking during pregnancy. Future research should comprise large prospective studies, which relate the structural and functional changes in the human fetal brain after prenatal nicotine exposure to children's behavioural and cognitive development.

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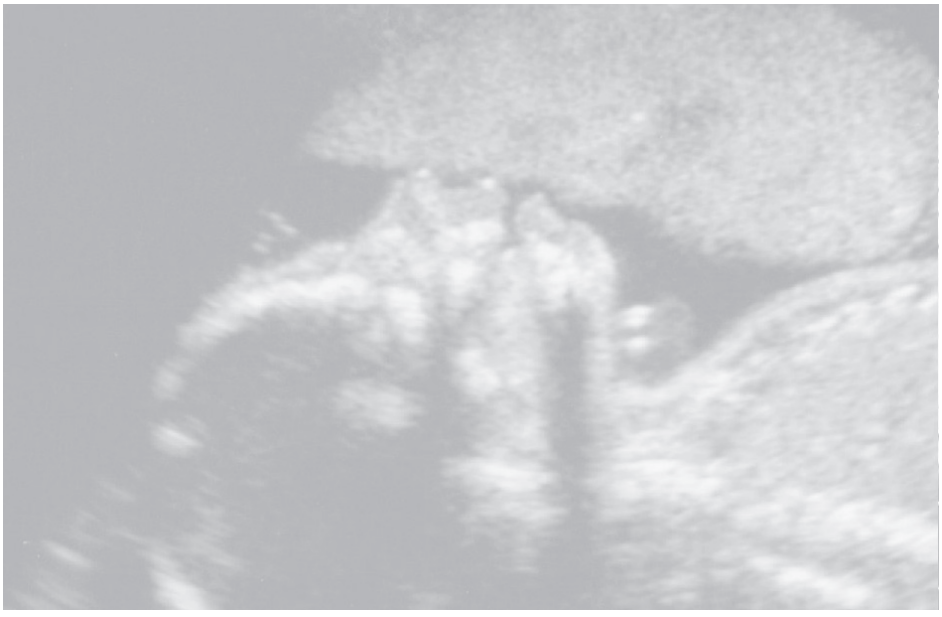
Chapter 5

General discussion



Background

Gestational age is historically derived on the basis of the first day of the last menstrual period (LMP). Naegele described the human birth to take place 280 days after the first day of the last menstrual period (1). However, in about 40% of pregnancies the LMP cannot be used since the date is not known, women have only recently stopped the use of oral contraceptives, or report to have irregular or prolonged menstrual cycles (2, 3). Even when the LMP is known and the cycle was regular, there may be subtle variations in true gestational age due to early or delayed ovulation, fertilisation or nidation (2-6). Even in pregnancies conceived with artificial reproductive techniques, pregnancy dating by ultrasound proved to be similar or superior to menstrual history (7, 8). Furthermore, it has been assumed that embryos follow the same growth pattern in early pregnancy (9, 10). Therefore, more reliable information on gestational age can be provided by early ultrasound assessment and this is widely recognised to be the method of choice (11-13).

The fetal biparietal diameter (BPD) was the first sonographic parameter used to determine gestational age and to assess fetal growth described by Cambell in 1969 (14). This was followed in 1975 by the crown-rump length (CRL), described by Robinson to determine gestational age in early pregnancy (15). Later, improved ultrasound signal processing allowed anatomical definition of the standard BPD plane (16). Since the identification of variability in fetal head shape, measurement of the fetal BPD only for estimation of gestational age and fetal growth has become much less common. The move toward measurements of several parts of the fetal anatomy has been called fetal biometry.

Nowadays, measuring multiple fetal ultrasound parameters is the most effective way for dating pregnancies, as well as the evaluation of fetal growth. An individual approach to each pregnancy is recommended for fetal growth assessment. The various epidemiological factors involved in fetal growth should be considered and specific charts for different communities should be used when possible (17). In general, the earlier the ultrasound, the more accurate is the assessment of dates. This is logical because all fetuses begin at the same size yet may vary dramatically in size by term. Because of the high degree of accuracy, dating by ultrasound in the first half of pregnancy has become a routine part of antenatal care in many institutions around the world. Accurate pregnancy dating is the most important initial step in modern obstetric management. Precise knowledge of gestational age is essential for the management of pregnancies and in particular to monitor (ab)normal fetal growth.

In clinical practice, when abnormal growth is suspected, serial ultrasound examinations are more helpful than a single point on the growth curve. We now realise that fetal environment and conditions shape the fetus. Regulation of fetal growth is multifactorial and complex. Several factors including intrinsic fetal conditions as well as maternal and environmental factors influence fetal growth.

Fetal size and growth can be evaluated using reference charts of different fetal measurements with gestation. Most reference charts start at 12 to 14 weeks of gestation. Additionally, substantial differences exist depending on the population and the method of pregnancy dating (18, 19). Numerous studies have been conducted to publish charts (standards) for fetal size. Many however have a suboptimal design, used a hospital-based population or do not have an appropriate sample size (19, 20). Furthermore, charts might be outdated, due to improved ultrasound equipment with higher resolution and changing health status (21). In clinical practice charts are being extrapolated which is not what they were designed for, resulting in false gestational age estimates. There is a clear need for lucent dating methods and new charts derived from a large population based cohort study, using serial measurements in a multi-ethnic population.

Unfavourable fetal environment may disrupt normal developmental processes and have lifelong consequences. In the past two decades, epidemiological studies have demonstrated associations of fetal growth restriction and low birth weight with cardiovascular disease and diabetes (22, 23). The fetal origins hypothesis postulates that an adverse fetal environment leads to developmental adaptations that permanently program the fetus' structure, physiology and metabolism. This programming is in favour of short-term survival and leads to fetal growth restriction and low birth weight and eventually to increased susceptibility for adult diseases. Long-term effects of this programming would be detrimental and lead to several health problems in adulthood including cardiovascular disease and diabetes.

Although birth weight is easily measured and available from obstetric records, it is not the best marker for an adverse fetal environment or exposure. The same birth weight may be the result of various fetal exposures and growth patterns. Therefore, birth weight is not likely to be the causal factor per se. Maternal lifestyle habits, including smoking in pregnancy, along with placental insufficiency are important determinants of low birth weight in Western countries. Identification of variables that have an adverse effect on fetal growth in utero are important markers for adverse fetal environment that may lead to developmental changes and subsequently increased susceptibility for adult diseases. Unravelling the mechanisms underlying the associations of fetal growth restriction and low birth weight with adult disease may eventually lead to new strategies for identification of groups at risk and prevention. To this purpose, the adverse effect of different variables, including smoking, on growth and development of fetal organ systems was investigated. Special emphasis has been put on fetal growth restriction and suboptimal fetal organ and the fetal haemodynamic development in utero.

Main findings

Fetal growth and development

Chapter 3.1

Studies examining fetal growth and development were conducted in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood among 9,778 mothers and their children (24, 25). Before fetal growth and development can be assessed, gestational age should be properly established. Sonographic measurement of fetal biometry is the basis for accurate determination of gestational age. Selection of the most useful single biometric parameter depends on the timing and is influenced by specific limitations. Crown-rump length was the best parameter for early dating of pregnancy. Biparietal diameter maintained the closest correlation with gestational age in the second trimester. Charts for ultrasound dating of pregnancy based on crown-rump length and biparietal diameter, were derived in this study. Crown-rump length measurements observed before 11 weeks of gestation were smaller than those reported by Robinson (15). This might be due to improved high-resolution ultrasound equipment and standardisation of the measurement technique. Our result suggests that gestational age may be underestimated if pregnancies are dated according to the CRL curve from Robinson, before 11 weeks of gestational age. BPD measurements were consistent with pregnancy dating curves derived by Altman and Chitty (26). An additional advantage of our study is that we derived our charts for ultrasound dating of pregnancy from 10 weeks of gestational onwards, compared to 12-14 weeks in other studies (26, 27). Pregnancy dating could be optimised using CRL from 20 until 65 mm and BPD from 23 mm onwards. An inverse relation was present between the time of ultrasound assessment and a correct prediction of the date of delivery. The earlier the ultrasound assessment in pregnancy, preferably between 10 and 12 weeks, the better is the prediction of gestational age. Internal validation with the actual date of delivery showed that ultrasound provided reliable gestational age estimates. Up to 92% of deliveries took place within 37 to 42 weeks of gestation if gestational age was derived from ultrasound data, compared to 87% based on a reliable last menstrual period. From 24 weeks onwards a reliable LMP provided a better prediction of estimated date of delivery. Increasing uncertainty of predictions by ultrasound due to variability in fetal size as pregnancy proceeds makes ultrasound less reliable in later pregnancy. In conclusion, ultrasound seems to be a more reliable and more consistent method for dating of pregnancies, compared to the use of the last menstrual period up to 20 weeks of pregnancy.

To obtain growth reference curves, fetuses should be measured on a series of occasions in a longitudinal study (18, 19). Reference data should relate to the population women originate from, and therefore it is important to have a group as unselected as possible.

The starting point of creating reference curves for fetal growth with gestational age is a clear definition of gestational age. In our study, gestational age was derived from ultrasound data, which proved to be superior to LMP. Reference curves for normal fetal growth were developed from 10 weeks of gestational age onwards for BPD, HC, TCD, AC, and FL. Reference curves for fetal growth of BPD, HC, AC and FL demonstrated a similar pattern of increase with gestation and no large inconsistencies with other frequently used curves (27-30). The distributions and SD of the growth characteristics are similar compared to those of the curves developed by Chitty (28-30). However, our curves did differ from existing curves in various aspects. Snijders found a higher increase in SD and different distributions of SD for BPD, HC and AC as pregnancy proceeded compared to our study (27). Before 16 weeks of gestational age, the median of BPD, HC, AC and FL was significantly smaller in our study than found by Snijders (27). These differences are likely to arise from different statistical methods and the way pregnancy was dated. Improving ultrasound resolution and standardization of technique in our study might have some influence as well. TCD measurements in our study were markedly larger in late pregnancy compared to those previously described by Goldstein and Snijders (27, 31). TCD measurement is not yet commonly established and difficult to conduct, especially in later pregnancy, this makes it less reliable when evaluated retrospectively. Furthermore, different ultrasound planes and techniques may explain differences in TCD curves.

In conclusion, validation with the actual date of delivery and their derivation in a large non-hospital based, urban population could make our curves appropriate for common use. Early ultrasound dating and the use of reliable growth curves can improve obstetrical management in pregnancy. Furthermore, accurate growth curves can enhance early detection of fetal growth anomalies.

Chapter 3.2

Reproducibility of fetal biometry in later pregnancy is well established (32). Even though early pregnancy ultrasound is widely used for clinical purposes, reproducibility analyses are scarce and have never been conducted properly. Before reference charts of fetal growth are used for clinical practice in early pregnancy, one should analyse the intra- and interobserver reproducibility to test reliability of the measurements. This study demonstrated good reproducibility of measurements of fetal biometry in early pregnancy by abdominal ultrasound. CRL and BPD showed high reproducibility and agreement from 9 weeks onwards and to a lesser extend HC and AC. FL had a poorer reproducibility before 12 weeks of gestational age, so prudence is necessary with this measurement in an early stage of pregnancy for clinical purposes. Narrow limits of agreement ascertained that we can reliably construct charts for fetal size in early pregnancy for clinical use. These limits of agreement give enough confidence that the influence of measurement errors would not considerably change gestational age estimates.

Chapter 3.3

Environmental as well as genetic factors are important determinants of fetal growth. Using standard reference growth curves neglects normal variation in fetal growth due to these characteristics, which hampers the identification of fetuses with growth abnormalities. Customisation of fetal growth curves attempts to adjust for physiological characteristics to estimate optimal fetal growth or growth potential for an individual. It is shown that the use of individually customised growth charts improves the distinction between normal and abnormal weight and reduces the false-positive rate for the diagnosis of growth restriction. To establish the optimal growth for a fetus, one should consider aspects like ethnicity, fetal gender, and parental anthropometrics (33-35). Previous studies found no effects of these factors in the first trimester, but they have a known influence in later pregnancy (36-39). These studies found small influences from these variables on fetal growth parameters. These identified differences seemed hardly clinically significant, suggesting that specific normograms are of limited value (17). However, all variables together may have a clinically significant impact. The best overall measure of fetal size is obtained by estimating fetal weight (40). Estimated fetal weight is the most common parameter to assess fetal growth restriction. This paper identified the most important determinants of fetal growth and the effects on estimated fetal weight.

A model was obtained for individually customised growth charts for estimated fetal weight. We evaluated how fetal growth is assessed using these customised growth charts, compared to assessment using unadjusted charts. As determinants of fetal growth, we considered physiological factors that are fixed at the start of pregnancy: fetal gender, gravidity, parity, ethnicity, maternal age, height, and pre-pregnancy maternal weight. All considered determinants had significant influence on estimated fetal weight and birthweight, and all effects were significantly larger with advancing gestational age. The estimated differences in EFW between categories of the determinants are given for three timepoints during gestation. At 20 weeks gestation, the differences were small and only significant for maternal weight. At 28 and 36 weeks, almost all differences were significant. With the customised growth charts, fetal growth can be evaluated, taking into account the following physiological characteristics: fetal gender, parity, ethnicity, maternal height, pre-pregnancy weight and age. The effects were largest for parity and ethnicity. In conclusion, the use of customised growth charts may improve fetal growth monitoring and prenatal care. Follow-up studies are needed to examine whether and to what extend the use of customised growth charts can improve the prediction of which children are at risk for perinatal or later morbidity, and which factors should be used for customisation.

Chapter 3.4

Active maternal smoking in pregnancy seems to be the most important modifiable risk factor for low birth weight in Western countries (41, 42). Smoking in pregnancy leads to

decreased fetal supplies of both nutrients and oxygen and subsequently to fetal growth restriction and low birth weight (41, 42). Since low birth weight is only a proxy for fetal growth restriction, it is not the best marker for assessing the adverse effect of smoking in pregnancy on fetal growth and development. Fetal growth restriction may lead to normal birth weight if the fetus was actually supposed to grow on the upper percentiles based on the genetic growth potential. Previous studies suggested that maternal smoking in pregnancy is associated with impaired fetal growth from a gestational age of 20 weeks onwards (43-46). However, these studies were conducted in small groups or in hospital-based populations and were not able to adjust for all potential confounders. We found that continued active maternal smoking in pregnancy was associated with reduced growth of fetal head circumference, abdominal circumference and femur length. These impaired fetal growth rates led to smaller femur length from mid-pregnancy onwards and smaller head circumference and abdominal circumference from late pregnancy. The earlier and larger effects on femur length suggest that maternal smoking in pregnancy may affect primarily peripheral tissues. This may indicate some kind of organ sparing with limb wasting in fetal growth restriction or maternal tobacco use (47, 48). Another explanation can be that femur length is variable compared to the parameters used for monitoring of fetal growth, which might explain controversial findings.

Fetal organ development and cardiovascular performance in relation to fetal growth

Chapter 4.1

Epidemiological studies have demonstrated fetal growth restriction and low birth weight to be risk factors contributing to cardiovascular disease and hypertension in adult life (23, 49). The increased risk may result from suboptimal development of the heart and main arteries in utero, and to haemodynamic changes in fetal growth restriction. This hypothesis is supported by studies that found lower cardiac compliance and increased arterial stiffness in fetal growth restriction, predisposing these subjects to an increased risk of cardiovascular disease in later life (60-64).

Traditionally a fetus is considered growth restricted when fetal abdominal circumference or estimated fetal weight is below the 10^{th} centile. However this state of overt fetal growth restriction is preceded by a period of diminished fetal growth within the normal estimated fetal weight range. Little is known about adaptive haemodynamic mechanisms during this stage of reduced fetal growth. Adaptations of the fetal circulation preceding fetal growth restriction could elucidate pathophysiological pathways responsible for increased susceptibility to hypertension and cardiovascular disease in adult life. Furthermore, changes occurring in the fetal circulation preceding fetal growth restriction can improve our understanding of compensatory adaptive fetal mechanisms to adverse fetal environment.

The aim of the study was to assess fetal circulation parameters and the haemodynamic adaptive changes (with Doppler sonography) in relation with decreased fetal growth in a healthy population-based study. Decreased fetal growth is associated with adaptive fetal haemodynamic changes, consistent with an increase in cardiac afterload. These changes already start to occur before the stage of clinically apparent fetal growth restriction. Cardiac remodelling, placental resistance indices and cardiac output changes are consistent with a gradual increase in afterload and mean arterial pressure with decreasing fetal growth. Furthermore in case of reduced fetal growth left cardiac compliance and arterial compliance is compromised. These (patho)physiological changes to an adverse fetal environment may very well predispose these fetuses to an increased risk of hypertension and cardiovascular disease in later life. Long-term studies are needed to examine whether and to what extend changes in circulation haemodynamics observed in utero persist during childhood and whether they are related to cardiac function and blood pressure development in postnatal life.

Chapter 4.2

Epidemiological studies have demonstrated intrauterine growth restriction and low birth weight to be risk factors contributing to renal disease, hypertension and smaller kidney size in adult life (23, 49, 50). It has been hypothesized that an adverse fetal environment leads to fetal growth retardation and smaller kidneys with a reduced number of nephrons (51, 52). A permanently reduced number of nephrons would lead to compensatory higher glomerular pressure, progressive glomerular sclerosis and would subsequently predispose the individual to impaired kidney function and hypertension (51). This hypothesis is supported by studies in animals and humans. Animal studies have shown that low protein intake and reduced placental perfusion lead to fetal growth restriction and a permanent nephron deficit (53, 54). Human studies demonstrated that low birth weight infants and hypertensive subjects have lower kidney weight with a reduced number of nephrons in adult life (55-58). This suggests that an adverse environment in utero leads to fetal growth restriction and impairs kidney development with a nephron deficit, eventually leading to hypertension (51, 59). The cause of fetal growth restriction and low birth weight is multifactorial. Nutritional deficiencies, smoking and inadequate placental perfusion are some causes that might provoke intrauterine growth restriction and low birth weight infants. Our study showed no associations of fetal growth characteristics in mid-pregnancy with kidney volume measured in late pregnancy. In late pregnancy, all fetal growth characteristics, indices of raised placental resistance, and fetal redistribution parameters were associated with kidney volume. Also, we found that placental perfusion seems to play an important role in the determination of kidney size in pregnancy. Furthermore, the results show a tendency that smaller fetal body size is associated with smaller kidneys. However, these kidneys were relatively large for that body size, suggesting an organ or kidney sparing

effect in fetal growth restriction. Since we know that the number of nephrons is largely determined in prenatal life, suboptimal kidney growth and development in fetal life may have lifelong consequences (55-59).

Chapter 4.3

We next examined whether maternal anthropometrics, as a measure of fetal nutrition, and fetal growth characteristics measured in mid- and late pregnancy were associated with kidney size in infancy. The observed association between abdominal circumference in late pregnancy and kidney volume in infancy suggests that infant kidney size and structure are established in fetal life. Follow-up studies are needed to examine the consequences for renal function and blood pressure in later life.

Chapter 4.4

In the last chapter we investigated the effect of maternal smoking in pregnancy on prenatal brain development. Research has demonstrated the several adverse effects of maternal smoking in pregnancy on the developing fetus. Maternal cigarette smoking is an established risk factor for intrauterine growth restriction, perinatal morbidity and mortality and postnatal growth (41, 65). This is assumed to be the result of fetal hypoxia due to nicotine-induced vasoconstriction, which leads to reduced blood flow to the fetus, and decreased oxygen and nutrition availability. Several reports described reduced fetal head circumference and biparietal diameter as parameters of total growth restriction in fetuses of smoking mothers (44-46, 66, 67). Behavioural and cognitive delays in the child due to prenatal exposure to tobacco smoke could be, at least partly, explained by changes in fetal brain development. This study showed that maternal smoking in pregnancy is associated with reduced growth of the fetal head. Small but highly significant associations were found for head circumference and biparietal diameter. Maternal smoking did also result in smaller atrial width of lateral ventricle and smaller transverse cerebellar diameter but the differences between fetuses of smoking and non-smoking mothers remained constant throughout pregnancy. Further research should focus on the causal pathway from prenatal cigarette exposure via brain development to behavioural characteristics and cognitive function.

Methodological considerations

Intra- and interoperator reproducibility

Reproducibility studies of fetal biometry are scarce and could be conducted more properly. We describe a new technique to analyse the reproducibility of fetal ultrasound procedures

in depth. This approach is better than what is usually done to perform reproducibility studies and gives a better insight in the structure of the measurements (32, 68, 69).

Various statistical methods were used in this study to assess the reproducibility and agreement of fetal growth measurements. Different methods have been described in the literature regarding the study of reproducibility of ultrasound measurements (32, 68-70).

The first method is to plot the duplicate data in a 2-dimensional plot and draw the line of equality on which all points would lie if the duplicate measurements would be exactly the same. This visualises the degree of agreement and is more useful than drawing a regression line that is essentially concerned with a unilateral effect of an x-variable on a y-variable. Moreover, the corresponding Pearson correlation coefficient is invariant under a systematic difference between the two duplicate measurements (71). Instead of the Pearson correlation coefficient, the Intraclass Correlation Coefficient (ICC) is frequently used as a measure for reliability of measurement (71, 72). The ICC is defined as the ratio of the variance between subjects to the total variance, which is the total of the variance between subjects, the variance between observers (if the duplicate measurements derive from two different observers) and a residual variance. The residual variance can theoretically be considered as the result of within-observer variability, within-subject variability, and variability caused by error of the measurement device. If we calculate the ICC using replicate measurements within a single observer, intra-observer ICC, then we leave the between-observer component out of the total variance, resulting in larger value of the ICC. The interpretation of ICC values is often a source of debate. One problem concerning the ICC is that it is a dimensionless number between 0 and 1, so being more a statistical measure for the strength of agreement than a clinically interpretable measure. Another problem is that the ICC depends on the range of the true value of the variable in the sample. When the range is large, the ICC will be greater than when it is narrow. Measurements over a broad range of values (in a heterogeneous group of smaller and larger fetuses), yield a higher ICC than the same measurements over a smaller range of values in a more homogeneous group of fetuses (71). So this property has to be taken into account when comparing ICCs calculated from different populations.

Another method is described by Bland and Altman (71, 73). Our study carefully followed their statistical approach and we believe this method to be the correct choice for similar studies. In order to have a better insight in the data, differences between two observers are plotted against their means. This allows us to investigate how the measurement error behaves in relation to the true value. The true value is not known, but the mean of the two measurements is taken as the best estimate for it. We can summarize the lack of agreement by calculating the mean difference and the standard deviation of the differences. We would expect most differences to lie between two limits: the mean difference -2SD and the mean difference +2SD. If the differences are Gaussian distributed, 95% of differences will lie between these two limits, which are called 'limits of agreement'. The nearer-to-zero the

mean difference and the smaller the SD of the differences are, the better the agreement. As these limits are measured in the same units as the variable considered, they can be clinically judged. Provided that these limits do not represent clinically important changes within a subject, the measures between and among observers can then be assumed to be interchangeable. In contrast to the ICC, between-subject variability does not play a part anymore in the calculation of limits of agreement. An important remark has to be made about the limits of agreement; each variable has its own clinical boundary below which within-subject differences can be considered clinically irrelevant. Therefore, one should decide in advance for each variable what the clinical value should be for the limits of agreement to be acceptable to draw conclusions about reproducibility of measurements, an important step that seems to be forgotten in some studies (32, 68, 69). Results of observers might be interchangeable from a statistical point of view within the limits of agreement, but the limits of agreement might still indicate clinically important within-subject changes of the variable considered!

Selection of the study population

The most important step in producing fetal growth charts is the choice of an appropriate sample. Studies that include the general population like Generation R, are less likely to result in biased estimates compared with studies that make use of routine clinical data. In the latter, high-risk fetuses and/or pregnancies with clinical indications are more likely to be intensively scanned than those at low risk and will be more prevalent in the study. The best approach for developing reference data is to collect data expressly for the purpose (i.e. prospectively), this is the design of Generation R. For constructing reference curves, it is desirable to obtain an even spread of data across the gestational age range of interest. Unfortunately, the design of Generation R provided less ultrasound assessments after 36 weeks of gestational age. Data collected after 36 weeks of gestational age were deliberately not used for these are likely to be derived from scans performed in the hospital because of clinical concern, for example fetal growth restriction, and thus would bias the distribution. Despite the design of the Generation R study, in which scans were clustered around predefined gestational ages, distribution and mean of growth reference curves did not differ considerably compared to other studies.

Inclusion and exclusion criteria

Reference data should relate to normal fetuses, therefore it is sensible to exclude fetuses shown to have a serious congenital abnormality. The exclusion of neonatal deaths is questionable except for this reason. A condition for inclusion in our study population was a healthy singleton birth. Neonatal deaths were excluded because we could not rule out that their death might have been caused by congenital abnormalities. However, numbers of neonatal death were small and analysis showed no difference in results if we would

have left them in. Twin pregnancies were excluded as well, for there is a high complication rate that affects normal growth (e.g. twin to twin transfusion syndrome). It may also have been appropriate to exclude those pregnancies with a condition that affects growth, such as maternal diabetes. We felt the sample had to be as unselected as possible, therefore we did not exclude smokers or women with obesity. Furthermore, information not known at the time of the ultrasound scan, like preeclampsia developed in later pregnancy, should not be an indication for exclusion. The population-based sample caused us to have relatively low samples of pregnancy-induced diabetes (1%) and preeclampsia (<2%), both conditions often clinically appeared after the scan at 30 weeks. Analyses showed no effects on the fetal growth curves if we would have excluded these conditions.

Size of sample

The larger the sample size the greater precision of the resulting centiles. The tails of the distribution are most important for clinical decisions, i.e. the extreme values, and therefore a large sample is necessary to obtain a good fit and correct estimation of the 5^{th} and 95^{th} centiles (19). It is clear that several hundreds of observations are required and even larger samples would be desirable. Many published studies are too small, which is one reason why their findings vary (19). While a large sample is desirable so that the centiles can be estimated precisely, a large sample cannot compensate for an inappropriate sample or other design deficiency (19). Finally, the high correlation between longitudinal measurements on the same fetus means that the effective sample size will be nearer to the number of fetuses than to the total number of measurements.

Statistical analysis

Confusion between fetal size and growth is regrettably common. Thus, cross-sectional charts of fetal size are frequently, but incorrectly, referred to as growth curves. Cross-sectional data can be used to compare the size of a fetus on a single occasion with reference data and to estimate gestational age from fetal size. Growth curves derived from studies using longitudinal data are in theory necessary to assess the growth of a fetus and determine growth anomalies between multiple occasions (19). Data from longitudinal studies should preferably be used to produce reference centiles for fetal size and fetal growth, although it is arguably better to use cross-sectional data for size centiles (18-20). Royston has shown how to apply a particular type of statistical model to longitudinal data to produce growth centiles and the same model may also be used to calculate valid size centiles. This approach is multilevel modelling which was used in the Generation R Study (18, 74-76). Fetal longitudinal data are hierarchical in structure, with variation between gestational ages within fetuses and variation between fetuses. Multilevel modelling adequately represents the underlying structure of the data and allows efficient models to be constructed. It is of course important to correctly represent the shape of the

relationship between fetal size and gestational age; fractional polynomials are needed to obtain a good fit for each characteristic analysed (18, 75). Briefly steps to construct growth reference charts are as follows. First, to model the mean by fitting the polynomial model to the raw data. Like recommended, we did not absolutely adhere to the P values and incidentally chose a less complex model with a slightly less better fit to the data. Second, to calculate the residuals between the observed values and the fitted line. The residuals were plotted against gestational age to show if and how the variability changes with gestation. Third, to model the variability, by modelling the standard deviation as a function of gestation. Fourth, to calculate the standard deviation scores, as the basis for checks of the assumptions underlying the modelling and for calculating the centile corresponding to any observation. Fifth, to check the goodness of fit of the models by examining a plot of the SDS against gestational age and check if they have a close to normal distribution. Sixth, to derive the centiles and finally superimpose the centiles on a scatter diagram of the observations as a final check of the fit.

Another statistical aspect that has to be discussed is that twenty percent of our pregnancies are dated between 18 and 24 weeks of gestation. As a consequence, biological variation might be underestimated and the SD of the distribution of the measurements may be too small. Other studies included dating based on last menstrual period that differed up to 10 days with the ultrasound measure, resulting in a slightly wider distribution of the measurements than found in our study (20, 27). Earlier studies demonstrated that male fetuses have a significantly larger BPD compared to females from as soon as circa 16 weeks of gestational age onwards (77, 78). A small difference might be present for other variables like ethnicity as well. The impact of these findings on the sonographic estimation of gestational age in an individual pregnancy is not great, but in large samples, like Generation R, a small shift in the gestational age distribution might significantly affect certain outcome measures, like gestational age at birth. These issues are addressed in the relevant chapters. Last but not least, awareness of the limitations of ultrasound and possible measurement errors are important for the appropriate use of reference charts of fetal growth and interpretation of the results.

Clinical implications

Accurate pregnancy dating is important to establish gestational age for evaluation of fetal growth and prediction of the date of delivery. As shown by our results, pregnancy dating in mid pregnancy is less accurate than pregnancy dating in early pregnancy. This has important implications for the timing of the first antenatal visit. Up to 30% of women in some ethnic minorities came after 24 weeks of gestational age for their first antenatal visit. Uncertainty about their last menstrual period is high in these groups as well. When

women would come earlier for their first antenatal visit, gestational age could be properly established and fetal growth subsequently better evaluated, possibly resulting in less perinatal morbidity and mortality. An additional advantage is that some major structural defects can be detected by ultrasound in early pregnancy. These high-risk subjects should be identified and preventive strategies be initiated if necessary.

Appropriate estimation of gestational age is also important for risk estimation of Down's syndrome in first trimester screening. In particular, the biochemical parameters used for screening are dependent on gestational age. Indeed, data from first trimester biochemical studies have already demonstrated that the use of ultrasound-based information on gestational age markedly reduced the false positive rate of the test (80).

Previous studies have shown a relative preservation of normal cerebellar growth in growth-restricted fetuses and found TCD to be a useful measurement to establish gestational age in late pregnancy (31). However, before dating based on TCD is widely implemented, further confirmation of the reliability of existing curves is needed.

Accurate growth charts for fetal biometry and fetusspecific estimated fetal weight charts might have additional value in early detection of growth anomalies. Clinicians should be conscious of the variables that influence fetal growth and realise that under certain conditions fetuses might appear to have growth anomalies, but are constitutionally small or large. The additional value for the improved detection of growth abnormality of factors like fetal gender, parity, ethnicity and parental size, should be investigated.

It is clear that optimal kidney growth and development in pregnancy is important to prevent hypertension in later life. Future preventive strategies could focus on optimal kidney development during pregnancy. Our finding that raised placental resistance plays a role in the development of smaller kidneys, gives a possible indication as to where these strategies could focus on. Furthermore, women with fetuses who show signs of growth restriction should be strongly encouraged to refrain from smoking.

The knowledge that adverse fetal environment can disrupt normal development and cause increased susceptibility to diseases in adult life, should lead to the realisation that optimal fetal growth should be assured were possible. Optimal obstetric management and early detection of fetal growth restriction might improve outcome and provides a target for intervention.

Smoking during pregnancy reduced fetal growth and caused a lower birth weight. Furthermore, our results suggest that brain size was reduced in fetuses of mothers who smoked during pregnancy. These effects might have lifelong consequences. We recommend clinical and public health strategies aimed at the primary and secondary prevention of prenatal tobacco exposure of children. In this respect, it is important to know that quitting smoking as soon as pregnancy is known positively affects fetal growth and development. Primary caregivers should inform women about the (long-term) consequences of smoking during pregnancy.

Pathophysiological considerations and future research

This thesis showed the importance of various environmental as well as genetic factors on fetal growth. Studies suggested that growth variability is small in early pregnancy with increasing importance in later pregnancy (2-6, 9, 10). However, disparities in growth occur at an early stage of pregnancy due to chromosomal or structural abnormalities, early placental maladaptation or environmental factors including nutrition (81). Consistent with this hypothesis is the relative smaller CRL in fetuses with triploidy and trisomy 18 (2, 82). Furthermore, low levels of pregnancy associated protein A (PAPPA) at 9-12 weeks of gestation are associated with an increased risk of stillbirth, growth restriction, pre-term birth and preeclampsia (81, 83, 84). The PAPPA levels are indicators of placental maladaptation, possibly putting fetuses at risk from early pregnancy onwards. Abnormal placental development and vascularization in early pregnancy causes a substantial part of perinatal and maternal morbidity and mortality due to miscarriage and hypertensive disorders. Genetic as well as environmental factors, including nutrition, of both parents can cause abnormal development of the embryo and the placenta in early pregnancy. This may result in congenital abnormalities, abnormal intrauterine development, and chronic disease in later life of both the child and the mother. It is hypothesized that the following periconceptional and early pregnancy nutrient-gene interactions link vascular-related reproductive complications and cardiovascular disease in adulthood (85, 86):

1. Maternal and paternal genetically controlled nutrient status affects the quality of gametes and fertilization capacity;

2 The embryonic genetic constitution, derived from both parents, and the maternal genetically controlled nutrient environment determine embryogenesis and fetal growth;

3.Throphoblast invasion of deciduas and spiral arteries is driven by genes derived from both parents as well as by maternal nutritional factors;

4. Angiogenesis, vasculogenesis, and vascular function are dependent on the genetic constitution of the embryo, derived from both parents, and the maternal genetically controlled nutritional environment.

Early intrauterine programming of vessels may concern the same (in)dependent determinants of vascular-related complications during pregnancy and cardiovascular diseases in later life. Growth requires nutrition, which is provided by genetically controlled metabolic, endocrine adaptations and placental transfer. More scientific research into the aetiology of abnormal embryogenesis and early placentation are therefore necessary to achieve definitive improvements in pregnancy outcome, as well as health in adult life in the future. Primary prevention by removal of certain risk factors related to developmental disorders in early pregnancy is already possible in many cases. Preconception care should therefore be added to the traditional organisation of care surrounding pregnancy and could be of additional value to optimize the course of pregnancy.

After the embryonic period, fetal growth is dependent on various factors. Genetic constitution of the fetus, maternal environment and maternal nutrition are causes that influence fetal growth. Furthermore, determinants as fetal gender, ethnicity and placental perfusion are factors as well. To describe the influence on fetal growth and to investigate the effects of various factors on fetal health, normal fetal growth should first be described. Therefore reference charts of fetal growth should be developed in a multi-ethnic, population-based study. Customised fetal growth charts may have an additional role in distinguishing small for gestational age fetuses and appropriate for gestational age fetuses that are constitutionally small. It is important to determine the individual growth potential of a fetus.

Unfavourable fetal environment may disrupt normal developmental processes and have lifelong consequences. In the past two decades, epidemiological studies have demonstrated associations of fetal growth restriction and low birth weight with cardiovascular diseases, diabetes and renal disease (22, 23, 49, 50). Unravelling the mechanisms underlying the associations of fetal growth restriction and low birth weight with adult disease may eventually lead to new strategies for identification of groups at risk and prevention. Periconceptional and prenatal medicine can be regarded as promising fields of preventive medicine in the future.

There is much uncertainty about the pathophysiological pathways that lead to adult disease. Continuous adaptations of fetal haemodynamics and organ growth in relation to intrauterine environment may result in differential growth and development of organs and the cardiovascular system. Along with the acquisition of further insight in etiologic processes, the associations of suboptimal intrauterine fetal development and health in childhood should be investigated. Increasing scientific, clinical and public health efforts should be made to optimise intrauterine environment and fetal growth. This should be accomplished by starting before pregnancy with preconception care.

The Generation R Study provides a unique opportunity to investigate the aspects mentioned above. The study is designed to identify early environmental and genetic causes of normal and abnormal growth, development and health from early fetal life until young adulthood. Eventually, results forthcoming from the Generation R Study have to contribute to the development of strategies for optimizing health and healthcare for pregnant women and children, now and in the future.

Future research

In our study, new charts for ultrasound dating of pregnancy and reference charts for fetal growth are presented. The beneficial effect of early pregnancy dating and the additional value of HC, AC, and FL in the estimation of gestational age should be validated in subsequent studies

In late pregnancy, the use of accurate fetal growth curves and the additional influence of different variables on fetal growth, like ethnicity, fetal gender and parental size, could improve earlier detection of fetal growth anomalies. Especially to distinguish between small for gestational age fetuses and appropriate for gestational age fetuses that are constitutionally small. The use of customised growth charts might improve the detection of growth-restricted fetuses that merit intensive aftercare. Long-term studies should be performed to examine whether and to what extend the use of customised growth charts would have predicted or could have improved the selection of growth restricted fetuses at risk for perinatal morbidity, and eventually could have prevented postnatal sequelae.

It is possible to identify groups at risk for suboptimal obstetric care because they come late in pregnancy for their first antenatal visit. Future research should focus on preventive strategies for high-risk subjects and the effects of these strategies on morbidity during pregnancy.

The effects of maternal smoking on fetal growth characteristics shown in our study may reflect developmental adaptations in fetal organ growth and development that may have consequences in childhood and adulthood. Further follow-up studies are needed to examine the gestational age specific effects of maternal smoking in pregnancy on fetal and postnatal organ growth and function and to examine whether these effects explain the previously demonstrated associations between maternal smoking in pregnancy and various health outcomes in postnatal life. Furthermore, future research should relate the structural and functional changes in the human fetal brain after prenatal nicotine exposure to children's behavioural and cognitive development.

Our findings suggest that reduced fetal growth, signs of raised placental resistance and fetal redistribution result in a decreased kidney volume in late fetal life. Follow-up studies in our children are currently performed to examine whether and to what extend changes in fetal kidney size persist during childhood and whether they are related to renal function and blood pressure development in postnatal life.

Adaptive haemodynamic mechanisms to changing fetal condition are found. Changing afterload of the fetal heart caused adaptive changes in reduced fetal growth reflecting cardiac remodelling. Along with increased afterload we found decreased arterial and cardiac compliance. Long-term studies are needed to examine whether and to what extend changes in circulation haemodynamics observed in utero persist during childhood and whether they are related to cardiac function and blood pressure development in postnatal life.

It has long been recognized that a growth-restricted fetus has much greater short-term morbidity and mortality compared with its normal counterpart. More recently, the long-term sequelae of this condition are being recognized, illuminating the importance of the prevention and prediction of pregnancies with fetal growth problems. The knowledge that adverse fetal environment can disrupt normal development and cause increased

susceptibility to diseases in adult life, should lead to the realisation that optimal fetal growth must be assured where possible. By focusing on better education and nutrition to prevent pregnancies at risk of becoming growth restricted and by using ultrasound to predict and manage these cases, we can improve the outcome for this group of patients. Early detection of fetal growth restriction and optimal obstetric management may not only reduce perinatal mortality but the risk of diseases in later life as well. Future research should focus on preventive strategies that optimise fetal growth and management in case of fetal growth restriction, to ensure a good start of life.

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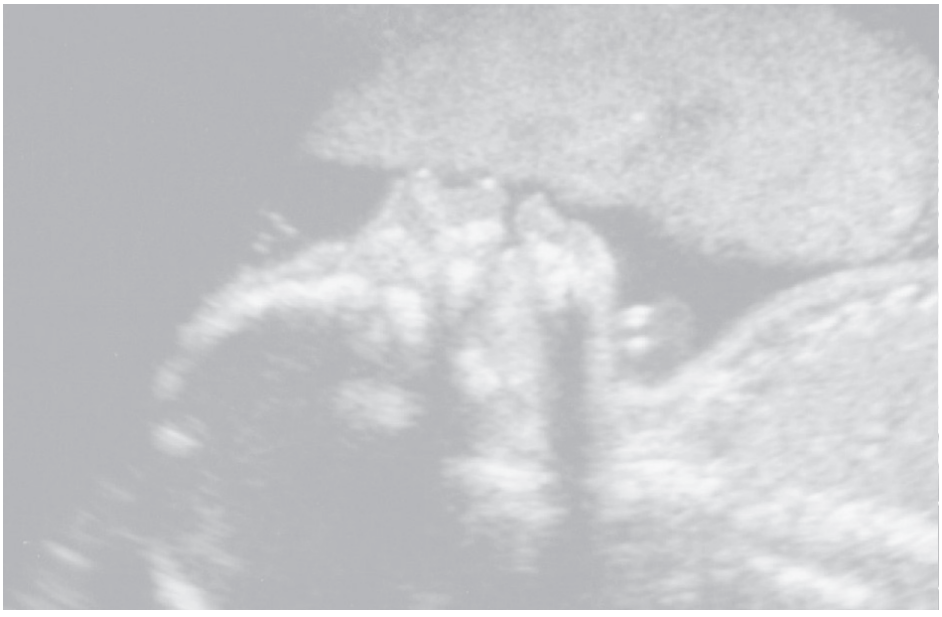
Chapter 6

Appendix



Appendix I

Images of standard fetal biometry measurements

Figure 8. CRL Crown-rump length is measured in a true midsagittal plane with the genital tubercle and the fetal spine longitudinally in view. The maximum length from cranium to the caudal rump is measured as a straight line.

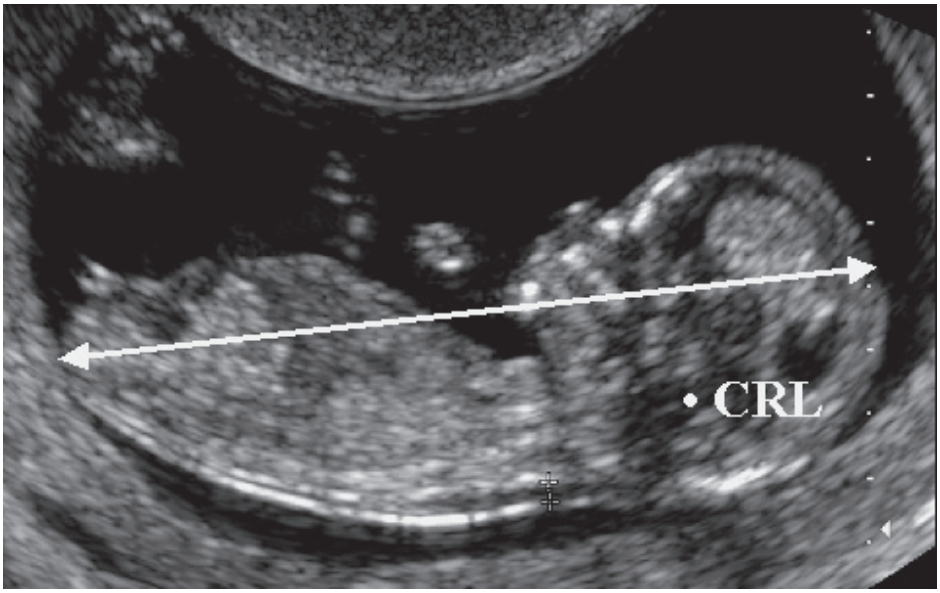


Figure 9a. BPD Biparietal diameter in early pregnancy is measured in a transverse section of the head with the midline horizontally visible with both the lateral ventricles symmetrically in view. To measure the biparietal diameter, callipers are placed perpendicular at the outer borders of the cranium at its maximum width.

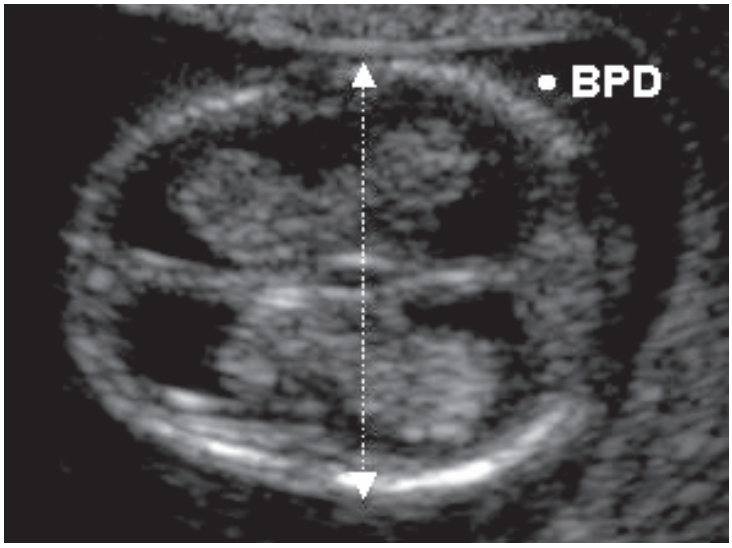


Figure 9b. BPD Biparietal diameter in late pregnancy is measured in a transverse section of the head with a central mid-line echo, interrupted in the anterior third by the cavity of the septum pellucidum (CSP) with the anterior and posterior horns of the lateral ventricles in view. To measure the biparietal diameter, callipers are placed perpendicular at the outer borders of the cranium at its maximum width.

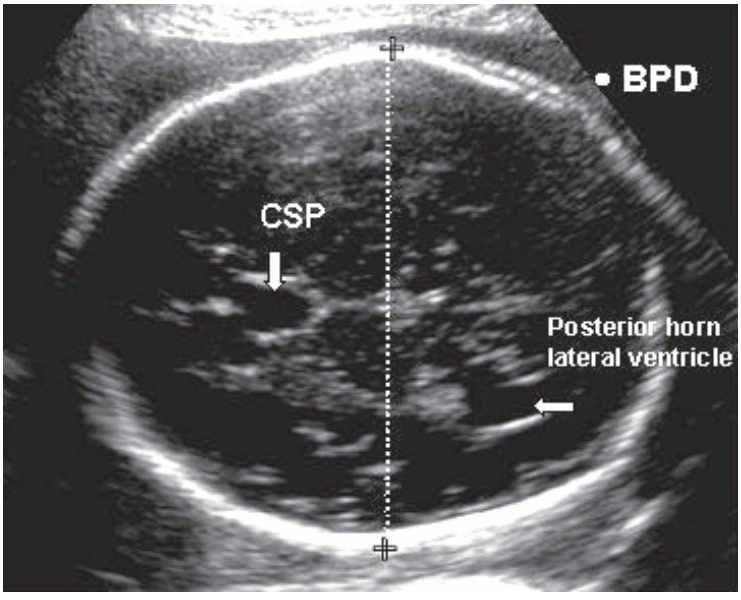


Figure 10. HC Head circumference is measured in a transverse section of the head with a central mid-line echo, interrupted in the anterior third by the cavity of the septum pellucidum (CSP) with the anterior and posterior horns of the lateral ventricles in view. An ellipse is drawn starting from the BPD position around the outline of the skull.

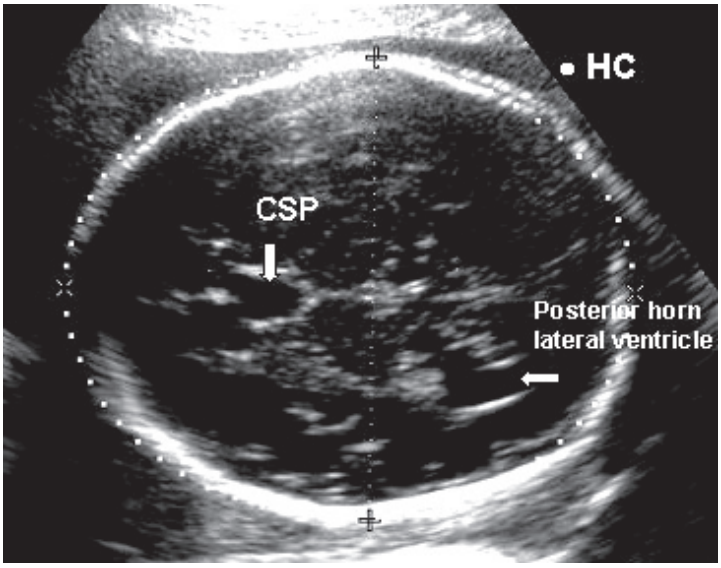


Figure 11. TCD Transcerebellar diameter. From the transverse plane for measurement of the BPD the transducer is rotated towards the cerebellum in the back of the head whilst keeping the cavum septum pellucidi in view. The optimal plane is reached when the peduncles are sighted with a symmetrical shaped cerebellum. The callipers are placed on the outer, lateral edges of the cerebellum.

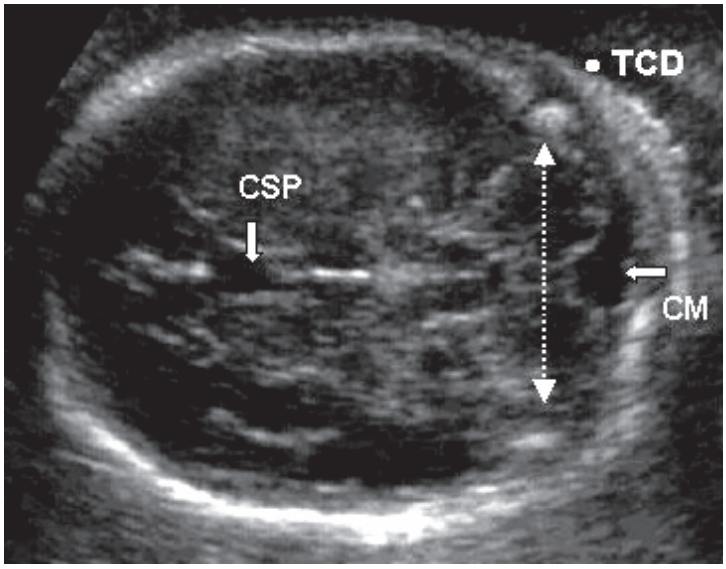
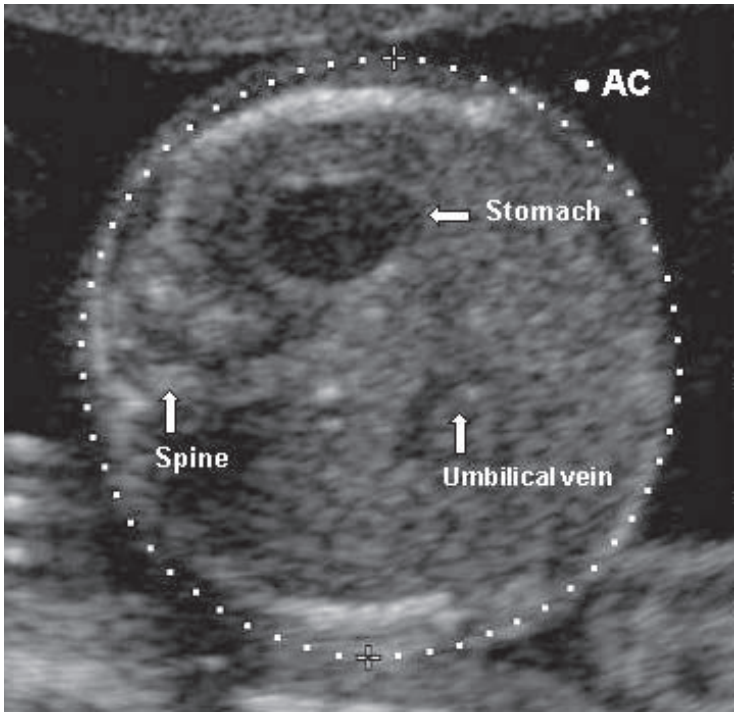


Figure 12. AC Abdominal circumference is measured in a symmetrical, transverse, round section through the abdomen, with visualization of the vertebrae on a lateral position in alignment with the ribs. The measurement is taken in a plane with the stomach and the bifurcation of the umbilical and hepatic veins.



1st trimester AC

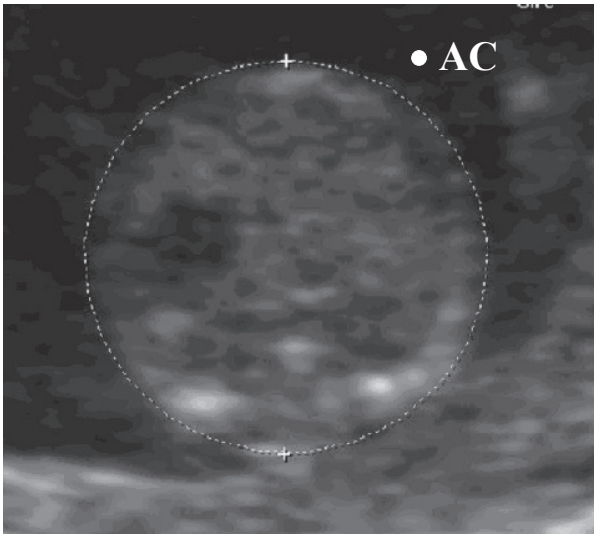
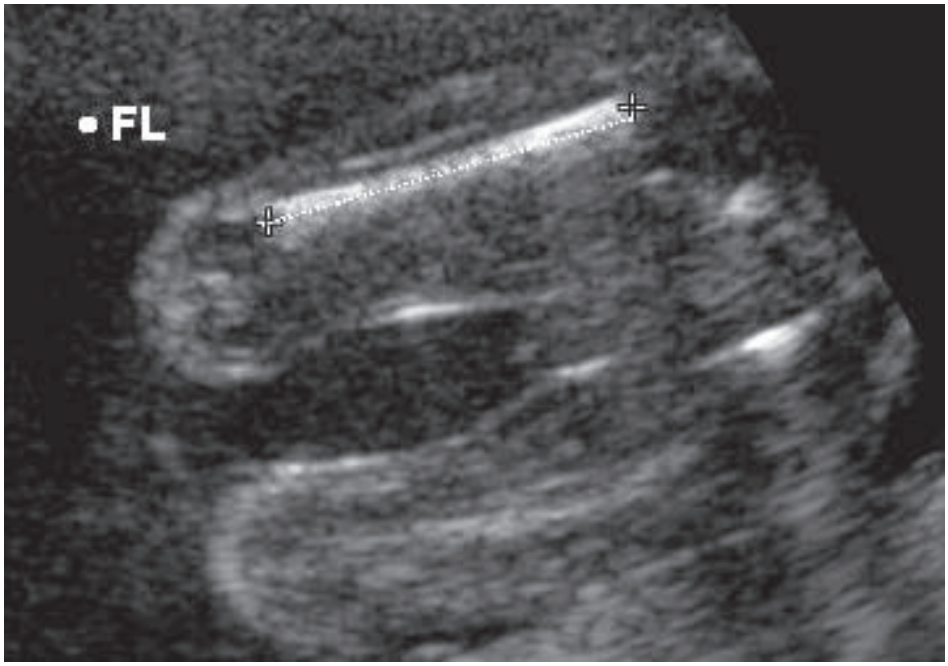
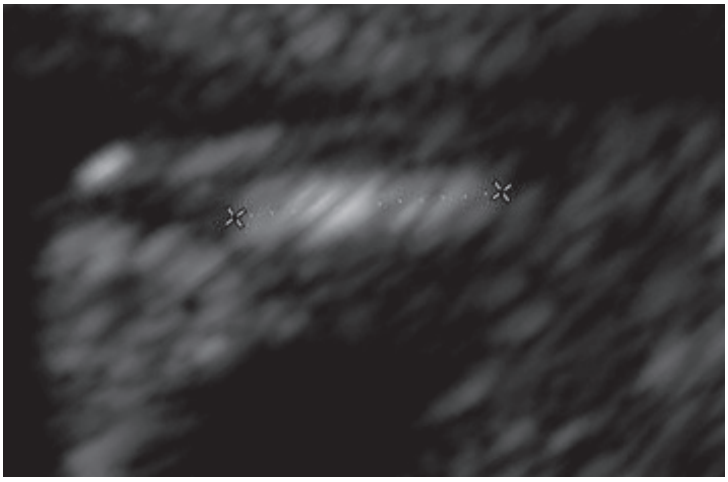


Figure 13. FL Femur length is measured with the full length of the bone in view at a slight angle with the ultrasound beam to avoid lengthening by side lobe artefacts.



1st trimester FL



Appendix II

Fetal biometry measurements in relation to gestational age

Figure 4. Individual values for fetal biparietal diameter (BPD) in mm plotted on the appropriate reference range (median, 3rd, 10th, 90th, 97th) with gestational age

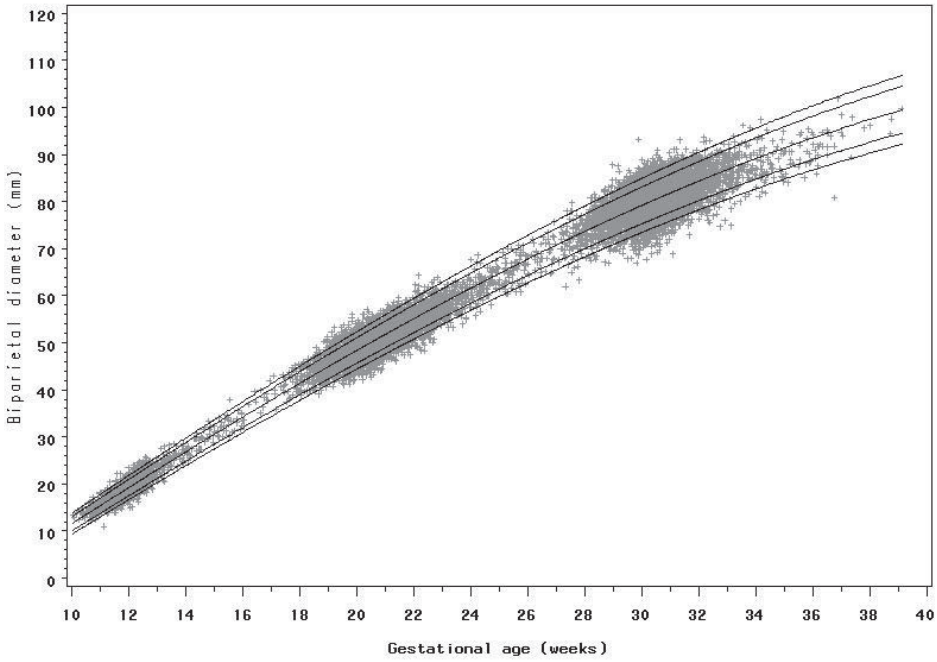


Figure 5. Individual values for fetal head circumference (HC) in mm plotted on the appropriate reference range (median, 3rd, 10th, 90th, 97th) with gestational age

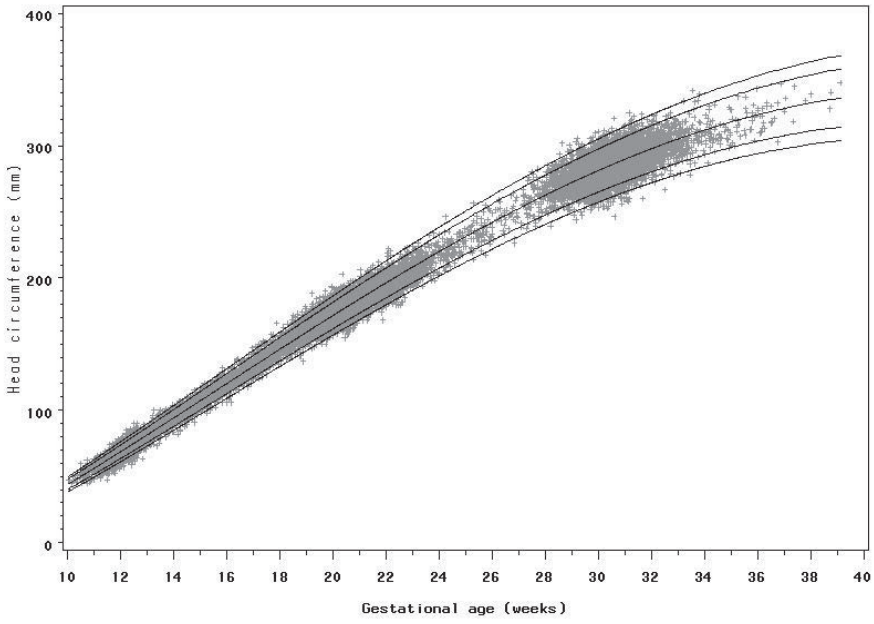


Figure 6. Individual values for fetal transverse cerebellar diameter (TCD) in mm plotted on the appropriate reference range (median, 3rd, 10th, 90th, 97th) with gestational age

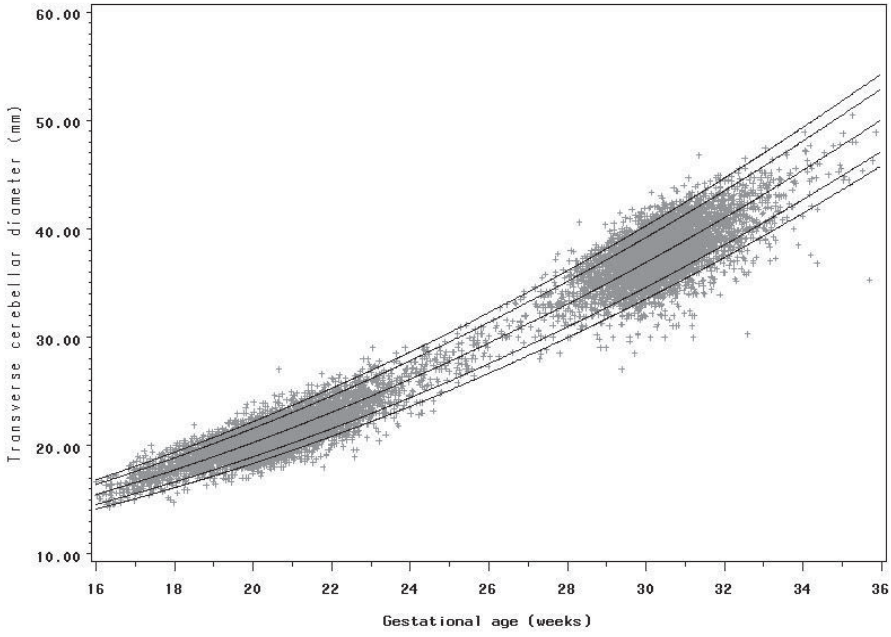


Figure 7. Individual values for fetal abdominal circumference (AC) in mm plotted on the appropriate reference range (median, 3rd, 10th, 90th, 97th) with gestational age

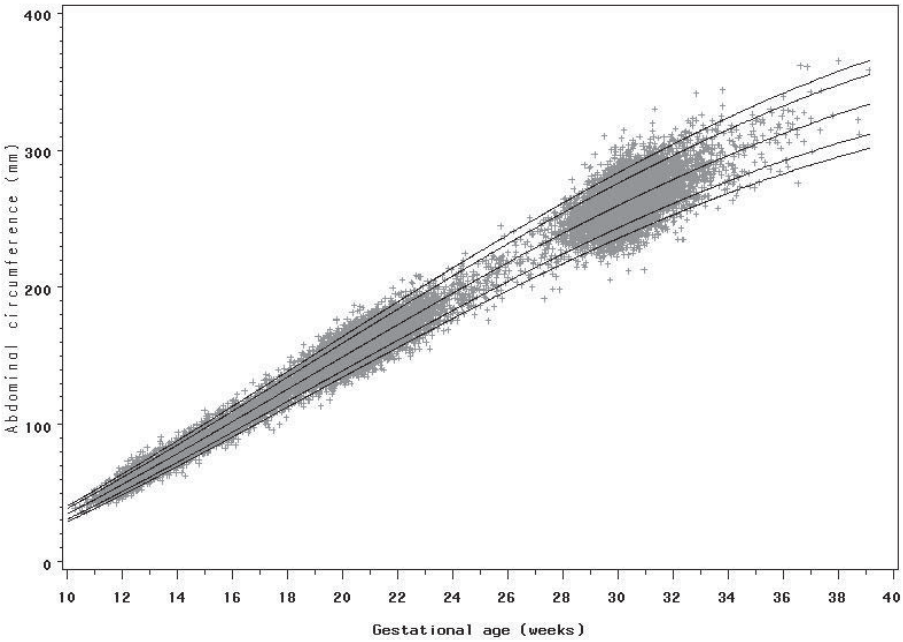
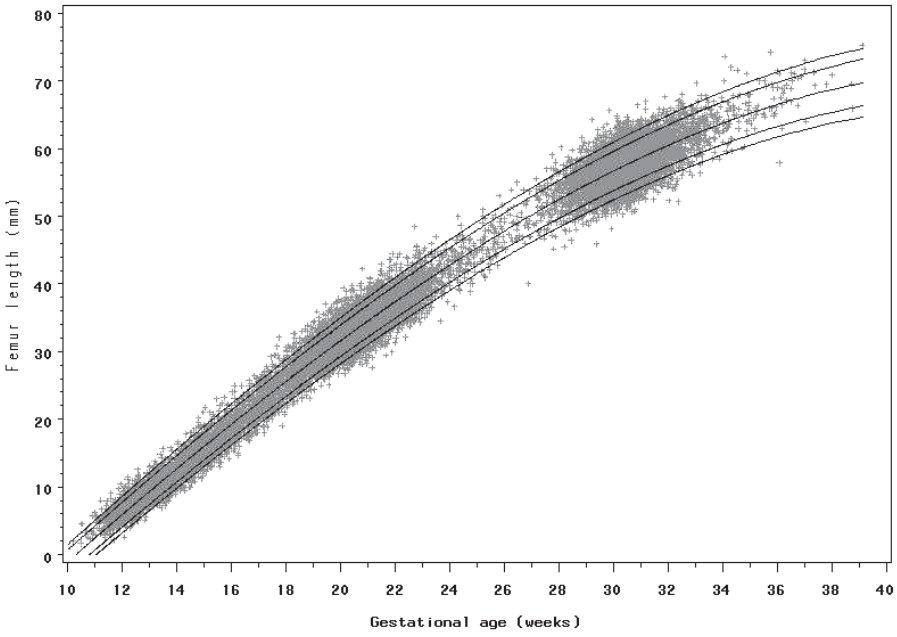


Figure 8. Individual values for fetal femur length (FL) in mm plotted on the appropriate reference range (median, 3rd, 10th, 90th, 97th) with gestational age



Appendix III

Normal ranges for fetal biometry

Table 8. Normal range for fetal biparietal diameter (BPD)

Gestational age (weeks)	Biparietal diameter (mm)				
	3rd centile	10th centile	Median	90th centile	97th centile
10	9,4	10,1	11,6	13,1	13,8
11	13,0	13,8	15,4	17,1	17,8
12	16,7	17,5	19,3	21,0	21,8
13	20,3	21,2	23,1	24,9	25,8
14	23,9	24,8	26,8	28,8	29,8
15	27,5	28,4	30,6	32,7	33,7
16	30,9	32,0	34,2	36,5	37,5
17	34,4	35,5	37,8	40,2	41,3
18	37,8	39,0	41,4	43,9	45,1
19	41,1	42,4	44,9	47,5	48,7
20	44,4	45,7	48,4	51,1	52,4
21	47,6	49,0	51,8	54,6	56,0
22	50,8	52,2	55,1	58,1	59,5
23	53,9	55,3	58,4	61,5	62,9
24	56,9	58,4	61,6	64,8	66,3
25	59,9	61,4	64,7	68,1	69,6
26	62,8	64,4	67,8	71,2	72,8
27	65,6	67,2	70,8	74,3	76,0
28	68,3	70,0	73,7	77,4	79,1
29	70,9	72,7	76,5	80,3	82,1
30	73,5	75,3	79,2	83,1	85,0
31	75,9	77,8	81,8	85,9	87,8
32	78,3	80,2	84,4	88,5	90,5
33	80,5	82,6	86,8	91,1	93,1
34	82,7	84,8	89,2	93,6	95,6
35	84,8	86,9	91,4	95,9	98,0
36	86,7	88,9	93,5	98,2	100,4
37	88,6	90,8	95,6	100,3	102,6
38	90,3	92,6	97,5	102,4	104,7
39	91,9	94,3	99,3	104,3	106,6
40	93,5	95,9	101,0	106,1	108,5

Table 9. Normal range for fetal head circumference (HC)

Gestational age	Head circumference (mm)				
(weeks)	3rd centile	10th centile	Median	90th centile	97th centile
10	38,6	40,3	43,9	47,5	49,2
11	50,2	52,1	56,1	60,2	62,0
12	62,1	64,2	68,6	73,1	75,1
13	74,3	76,5	81,4	86,2	88,5
14	86,6	89,0	94,3	99,5	101,9
15	99,0	101,6	107,2	112,9	115,5
16	111,4	114,2	120,3	126,3	129,1
17	123,8	126,8	133,3	139,7	142,7
18	136,2	139,4	146,2	153,0	156,2
19	148,4	151,8	159,0	166,3	169,7
20	160,5	164,1	171,7	179,3	182,9
21	172,4	176,1	184,2	192,2	196,0
22	184,0	188,0	196,4	204,9	208,8
23	195,4	199,5	208,4	217,2	221,4
24	206,4	210,8	220,0	229,3	233,6
25	217,1	221,7	231,3	240,9	245,5
26	227,5	232,2	242,2	252,3	257,0
27	237,4	242,3	252,7	263,1	268,0
28	246,8	251,9	262,7	273,6	278,7
29	255,8	261,1	272,3	283,6	288,8
30	264,2	269,7	281,4	293,0	298,5
31	272,2	277,8	289,9	301,9	307,6
32	279,5	285,4	297,8	310,3	316,1
33	286,3	292,3	305,2	318,1	324,1
34	292,5	298,7	312,0	325,2	331,4
35	298,0	304,4	318,1	331,7	338,1
36	302,9	309,5	323,5	337,6	344,2
37	307,0	313,8	328,3	342,7	349,5
38	310,5	317,5	332,3	347,2	354,2
39	313,2	320,4	335,7	350,9	358,1
40	315,2	322,6	338,2	353,9	361,3

Table 10. Normal range for fetal transverse cerebellar diameter (TCD)

Gestational age	Transverse cerebellar diameter (mm)				
(weeks)	3rd centile	10th centile	Median	90th centile	97th centile
16	14,1	14,6	15,5	16,4	16,8
17	15,1	15,5	16,6	17,6	18,1
18	16,1	16,6	17,7	18,8	19,4
19	17,2	17,7	19,0	20,2	20,7
20	18,3	18,9	20,3	21,6	22,2
21	19,5	20,2	21,6	23,0	23,7
22	20,8	21,5	23,1	24,6	25,3
23	22,2	22,9	24,6	26,2	26,9
24	23,6	24,4	26,1	27,8	28,6
25	25,1	25,9	27,7	29,6	30,4
26	26,6	27,5	29,4	31,3	32,2
27	28,3	29,2	31,2	33,2	34,2
28	29,9	30,9	33,0	35,1	36,1
29	31,7	32,7	34,9	37,1	38,2
30	33,5	34,6	36,9	39,2	40,3
31	35,4	36,5	38,9	41,3	42,5
32	37,3	38,5	41,0	43,5	44,7
33	39,4	40,6	43,2	45,8	47,0
34	41,5	42,7	45,4	48,1	49,4
35	43,6	44,9	47,7	50,5	51,8
36	45,8	47,2	50,1	53,0	54,3

Table 11. Normal range for fetal abdominal circumference (AC)

Gestational age (weeks)	Abdominal circumference (mm)				
	3rd centile	10th centile	Median	90th centile	97th centile
10	29,1	30,9	34,8	38,7	40,5
11	38,7	40,8	45,3	49,9	52,0
12	48,7	51,1	56,2	61,4	63,8
13	59,0	61,7	67,4	73,2	75,9
14	69,4	72,4	78,8	85,2	88,2
15	80,1	83,4	90,4	97,4	100,6
16	90,9	94,4	102,1	109,7	113,2
17	101,8	105,6	113,8	122,1	125,9
18	112,7	116,8	125,7	134,5	138,7
19	123,6	128,0	137,5	147,0	151,4
20	134,5	139,2	149,3	159,4	164,2
21	145,4	150,4	161,1	171,8	176,8
22	156,1	161,4	172,8	184,1	189,4
23	166,8	172,4	184,3	196,3	201,9
24	177,3	183,2	195,7	208,3	214,2
25	187,6	193,8	207,0	220,2	226,4
26	197,7	204,2	218,0	231,8	238,3
27	207,6	214,4	228,8	243,3	250,0
28	217,3	224,3	239,4	254,4	261,5
29	226,6	234,0	249,7	265,3	272,7
30	235,7	243,4	259,7	276,0	283,6
31	244,5	252,4	269,3	286,2	294,2
32	252,9	261,1	278,6	296,2	304,4
33	260,9	269,5	287,6	305,8	314,3
34	268,6	277,4	296,2	315,0	323,8
35	275,9	285,0	304,4	323,8	332,9
36	282,8	292,1	312,2	332,2	341,6
37	289,2	298,9	319,5	340,1	349,8
38	295,1	305,1	326,4	347,6	357,6
39	300,6	310,9	332,8	354,6	364,9
40	305,6	316,2	338,7	361,2	371,7

Table 12. Normal range for fetal femur length (FL)

Gestational age (weeks)	Femur length (mm)				
	3rd centile	10th centile	Median	90th centile	97th centile
12	3,2	4,1	5,9	7,8	8,6
13	6,5	7,4	9,3	11,2	12,1
14	9,8	10,7	12,7	14,7	15,6
15	13,0	14,0	16,0	18,0	19,0
16	16,2	17,2	19,3	21,3	22,3
17	19,3	20,3	22,4	24,6	25,6
18	22,4	23,4	25,6	27,8	28,8
19	25,3	26,4	28,6	30,9	31,9
20	28,2	29,3	31,6	33,9	35,0
21	31,0	32,2	34,5	36,9	38,0
22	33,8	34,9	37,4	39,8	40,9
23	36,5	37,6	40,1	42,6	43,8
24	39,0	40,2	42,8	45,3	46,5
25	41,5	42,7	45,3	48,0	49,2
26	43,9	45,1	47,8	50,5	51,7
27	46,2	47,5	50,2	52,9	54,2
28	48,4	49,7	52,5	55,3	56,6
29	50,5	51,8	54,6	57,5	58,8
30	52,4	53,8	56,7	59,6	61,0
31	54,3	55,7	58,6	61,6	63,0
32	56,0	57,4	60,5	63,5	64,9
33	57,6	59,1	62,2	65,3	66,7
34	59,1	60,6	63,7	66,9	68,4
35	60,5	62,0	65,2	68,4	69,9
36	61,7	63,2	66,5	69,8	71,3
37	62,8	64,4	67,7	71,0	72,6
38	63,8	65,4	68,7	72,1	73,7
39	64,6	66,2	69,6	73,1	74,7
40	65,2	66,9	70,4	73,9	75,5

Appendix IV

Normal ranges for fetal circulation measurements with gestational age

Table 5. Reference curves formulas for Doppler parameters with gestational age between 28-34 weeks: equations for the mean of the measurement and standard deviation

Doppler parameter	Number (% of total)	Regression equations	
Uterine artery RI	1155 (95%)	Mean	0.56 - 0.002 * GA
		SD	0.07
Umbilical artery PI *	1191 (98%)	Mean	1.92 - 0.031 * GA
		SD	0.14
Umbilical vein volume flow *	1052 (95%)	Mean	-203 + 14.8 * GA
		SD	53.1
Middle cerebral artery PI *	1165 (96%)	Mean	2.6 - 0.021 * GA
		SD	0.28
Middle cerebral artery PSV (cm/s) *	1166 (96%)	Mean	-39.5 + 2.71 * GA
		SD	6.60
Anterior cerebral artery PI	1061 (87%)	Mean	1.86 - 0.003 * GA
		SD	0.26
Ductus venosus PIV	1087 (89%)	Mean	0.80 - 0.008 * GA
		SD	0.15
Cardiac Doppler parameters			
Aorta ascendens PSV (cm/s) *	1062 (87%)	Mean	61.5 + 0.98 * GA
		SD	11.0
Left cardiac output (ml/min) *	1038 (85%)	Mean	-862 + 48.3 * GA
		SD	151
Pulmonary artery PSV (cm/s) *	1046 (86%)	Mean	39.5 + 1.12 * GA
		SD	8.52
Right cardiac output (ml/min) *	1017 (83%)	Mean	-1140 + 65 * GA
		SD	218
Combined cardiac output (ml/min) *	985 (81%)	Mean	-2019 + 114 * GA
		SD	341
Mitral valve E-wave *	1152 (95%)	Mean	30 + 0.33 * GA
		SD	5.34
Mitral valve A-wave	1152 (95%)	Mean	55.8 - 0.14 * GA
		SD	6.8
Mitral valve E/A ratio *	1165 (96%)	Mean	0.51 + 0.009 * GA
		SD	0.08
Tricuspid valve E-wave *	1126 (93%)	Mean	31.4 + 0.38 * GA
		SD	5.00
Tricuspid valve A-wave	1126 (93%)	Mean	57.0 - 0.04 * GA
		SD	6.8
Tricuspid valve E/A ratio *	1135 (93%)	Mean	0.55 + 0.008 * GA
		SD	0.07

* $P<0.05$ against gestational age.

SD, standard deviation; GA, gestational age in exact weeks; PSV, peak systolic velocity; PI, pulsatility index; RI, resistance index.

Figure 5 A. Scatterplots of individual measurements with gestational age, placental resistance indices and venous return parameters (mean with the 5th and 95th percentiles)

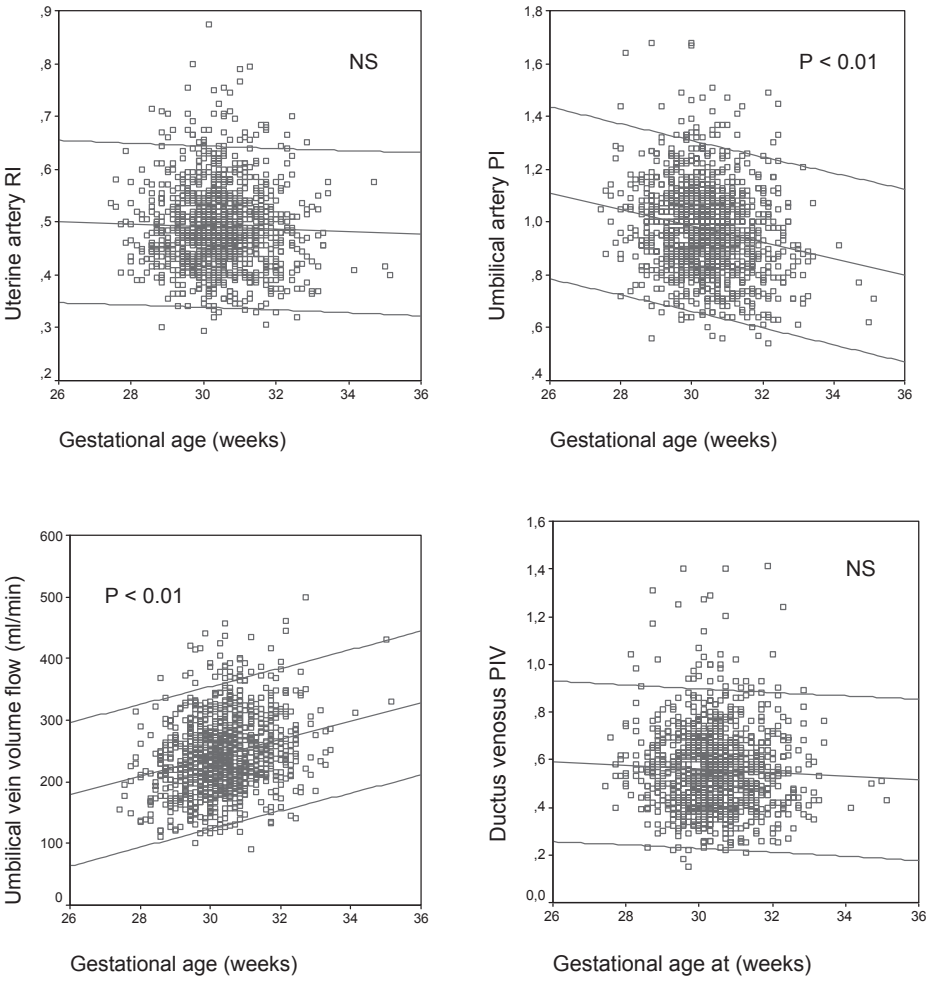
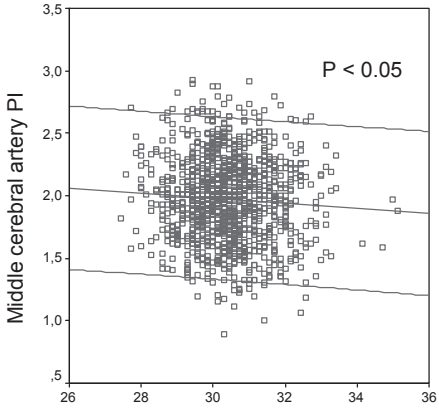
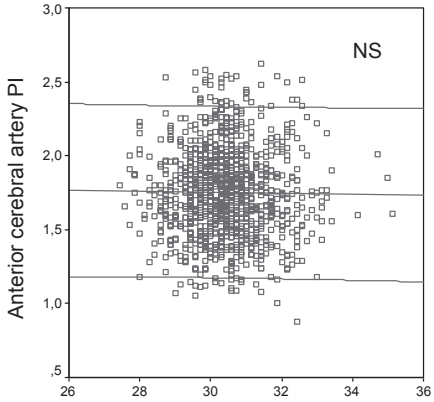


Figure 5 B. Scatterplots of individual measurements with gestational age with, fetal cerebral circulation parameters (mean with the 5th and 95th percentiles)





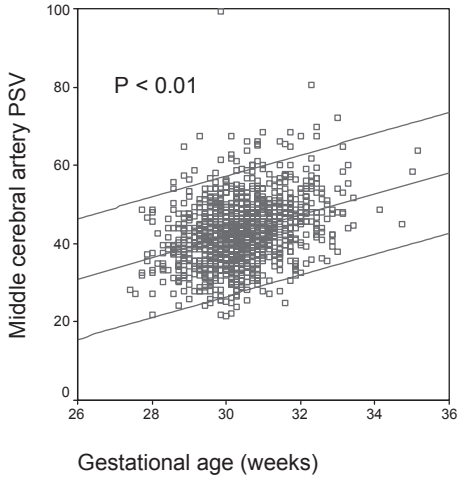
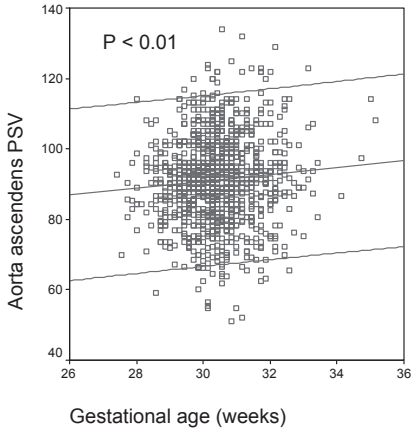
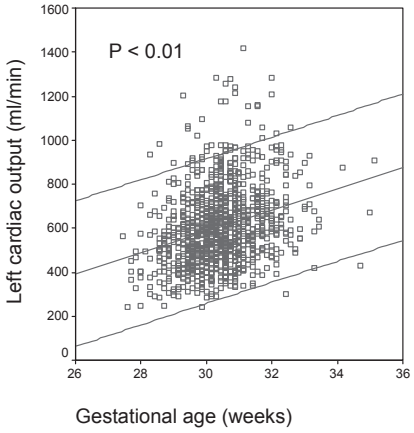
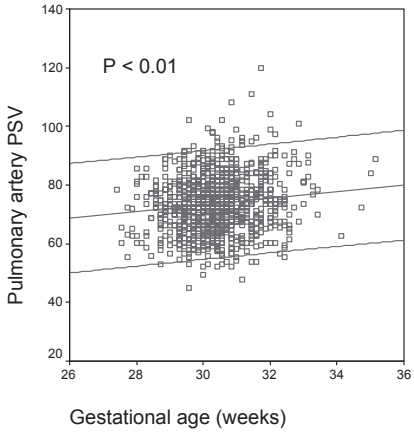
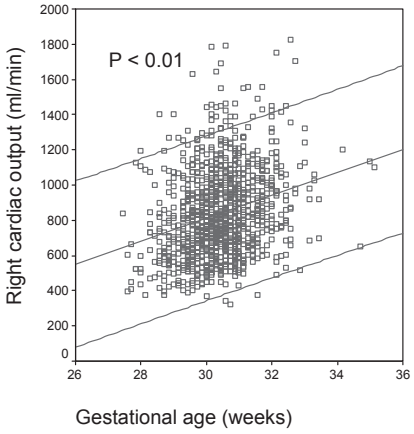


Figure 5 C. Scatterplots of individual measurements with gestational age, fetal cardiac outflow parameters (mean with the 5th and 95th percentiles)









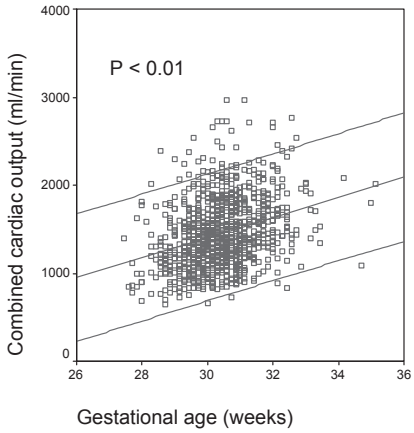
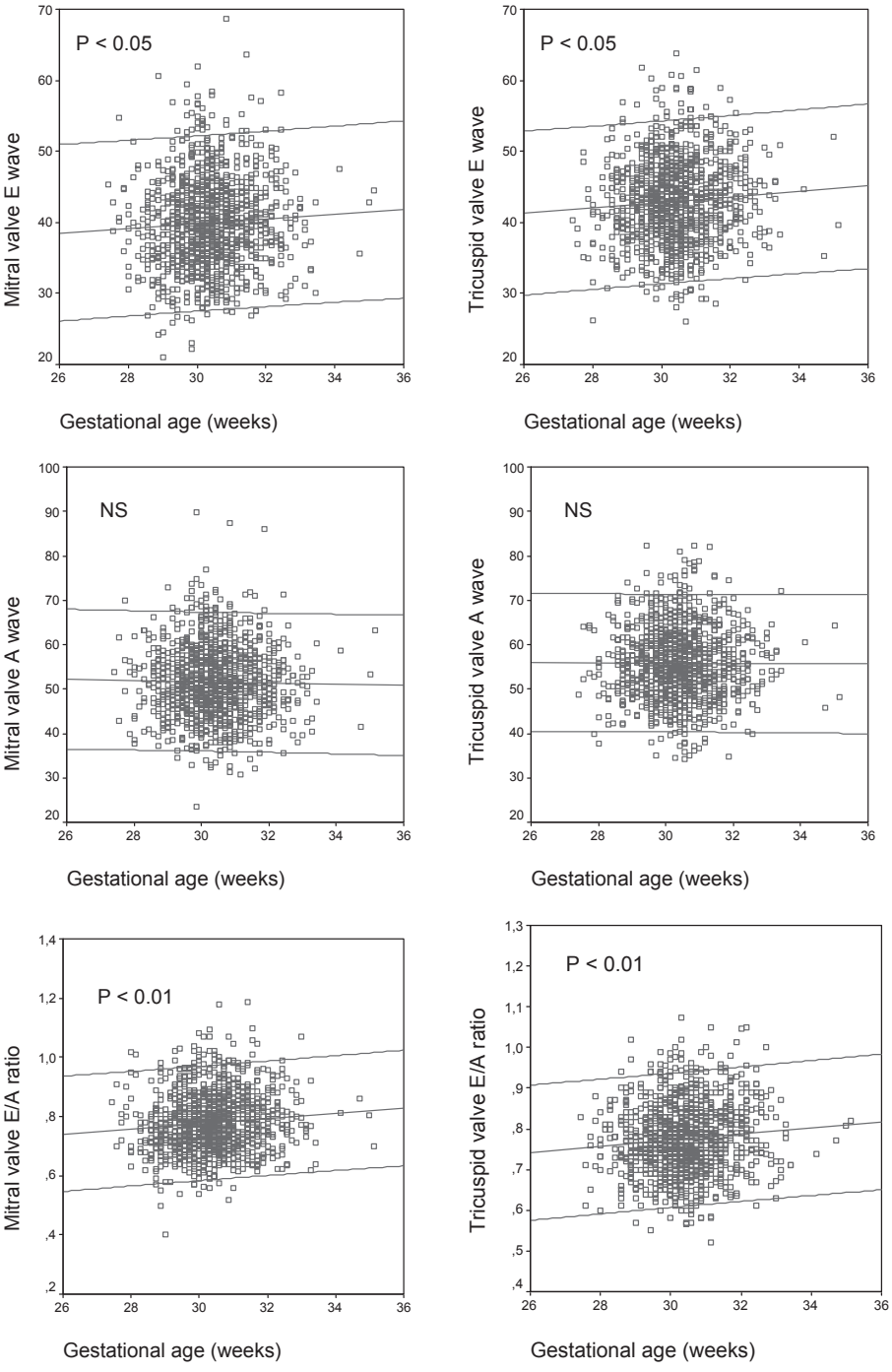


Figure 5 D. Scatterplots of individual measurements with gestational age, fetal cardiac parameters at the level of the atrioventricular valves (mean with 5th and 95th percentiles)



Appendix V

Normal ranges for fetal kidney measurements with gestational age

Table 6. Normal range for kidney length in mm

Gestational age (weeks)	Kidney length (mm)		
	5th centile	Median	95th centile
28	31,1	36,4	41,7
29	32,2	37,5	42,8
30	33,3	38,6	43,8
31	34,4	39,7	44,9
32	35,5	40,8	46,0
33	36,6	41,8	47,1
34	37,7	42,9	48,2

Table 7. Normal range for kidney depth, antero-posterior diameter in mm

Gestational age (weeks)	Kidney depth, antero-posterior diameter (mm)		
	5th centile	Median	95th centile
28	16,4	20,3	24,1
29	17,0	20,9	24,8
30	17,6	21,5	25,4
31	18,3	22,1	26,0
32	18,9	22,7	26,6
33	19,5	23,4	27,2
34	20,1	24,0	27,9

Table 8. Normal range for fetal kidney width, transverse diameter in mm

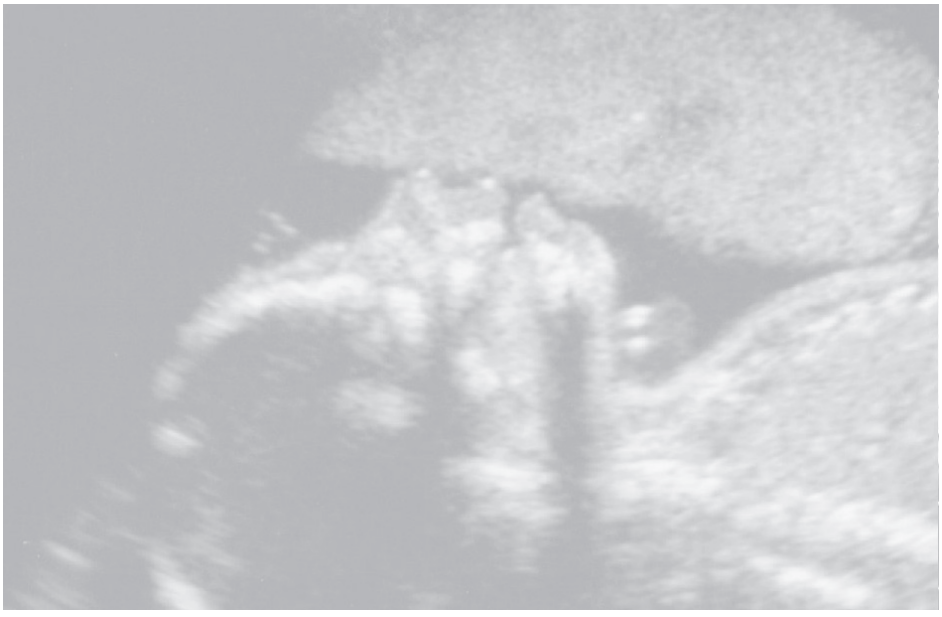
Gestational age (weeks)	Kidney width, transverse diameter (mm)		
	5th centile	Median	95th centile
28	17,0	20,9	24,9
29	17,7	21,7	25,6
30	18,5	22,4	26,4
31	19,2	23,2	27,1
32	20,0	23,9	27,8
33	20,7	24,6	28,6
34	21,5	25,4	29,3

Table 9. Normal range for fetal kidney volume, in cm^3

Gestational age (weeks)	Kidney volume (cm3)		
	5th centile	Median	95th centile
28	5,4	8,0	12,0
29	5,9	8,8	13,2
30	6,4	9,6	14,4
31	7,0	10,5	15,7
32	7,7	11,5	17,2
33	8,4	12,5	18,8
34	9,2	13,7	20,5

Chapter 7

Summary



Summary

The general aim of the studies presented in this thesis was to accurately describe normal and abnormal fetal growth and development. Additionally, the influence of determinants on fetal growth was investigated. The fetal origins hypothesis was the main point of departure for the studies presented in the second part of this thesis. Unravelling the mechanisms underlying the associations of fetal growth restriction and low birth weight with adult disease may eventually lead to new strategies for identification of groups at high risk and preventive measures.

In **Chapter 2**, the Generation R Study is presented. The Generation R Study is a population-based prospective cohort study from fetal life until young adulthood. The study is designed to identify early environmental and genetic causes of normal and abnormal growth, development and health from fetal life until young adulthood. The study focuses on four primary areas of research: 1) growth and physical development; 2) behavioural and cognitive development; 3) diseases in childhood; and 4) health and healthcare for pregnant women and children. In total, 9,778 mothers with a delivery date from April 2002 until January 2006 were enrolled in the study. Of these mothers, 91% (n= 8,880) was enrolled in pregnancy. This population forms the basis for all studies described in this thesis. Data collection in the prenatal phase included physical examinations, questionnaires, fetal ultrasound examinations and biological samples. In addition, more detailed assessments are conducted in a subgroup of 1,232 pregnant women and their children, referred to as the Generation R Focus Study. The children form a prenatally recruited birth-cohort that will be followed until young adulthood. Eventually, results forthcoming from the Generation R Study have to contribute to the development of strategies for optimizing health and healthcare for pregnant women and children, now and in the future.

Chapter 3.1 provides new charts for ultrasound dating of pregnancy and reference charts for normal fetal growth. Precise knowledge of gestational age is essential for the management of pregnancies and in particular to monitor fetal growth. In this study, new charts for ultrasound dating of pregnancies based on crown-rump length and biparietal diameter are presented. The results showed that the earlier the ultrasound assessment in pregnancy, preferably between 10 and 12 weeks, the better is the prediction of gestational age. Pregnancy dating could be optimised using crown-rump length from 20 until 65 mm and biparietal diameter from 23 mm onwards. Internal validation with the actual date of delivery showed that ultrasound provided reliable gestational age estimates. Up to 92% of deliveries took place within 37 to 42 weeks of gestation if gestational age was derived from ultrasound data, compared to 87% based on a reliable report of last menstrual period. A reliable report of last menstrual period is better than ultrasound from 24 weeks of gestational age onwards. Increasing fetal variability caused increasing uncertainty of

prediction. To monitor fetal growth and to identify growth anomalies, reference curves for normal fetal growth were developed from 10 weeks of gestational age onwards for biparietal diameter, head circumference, transverse cerebellar diameter, abdominal circumference, and femur length. Early ultrasound dating and the use of reliable growth curves can improve obstetrical management in pregnancy.

The study in **Chapter 3.2** examined the intra- and interobserver reproducibility and agreement of ultrasound measurements. Early pregnancy ultrasound is widely used for clinical purposes. Before reference charts of fetal growth are used for clinical practice in early pregnancy, one should analyse the reliability of the measurements. This study demonstrated good reproducibility of measurements of fetal biometry in early pregnancy by abdominal ultrasound. Crown-rump length and biparietal diameter showed high reproducibility and agreement from 9 weeks onwards and to a lesser extend head circumference and abdominal circumference. Femur length had a poorer reproducibility before 12 weeks of gestational age, so prudence is necessary with this measurement in an early stage of pregnancy for clinical purposes. Narrow limits of agreement ascertained that we can reliably construct charts for fetal size in early pregnancy for clinical use. These limits of agreement give enough confidence that the influence of measurement errors would not considerably influence gestational age estimates.

Chapter 3.3 described a study on the effect of different variables on estimated fetal weight and birth weight. To establish the optimal growth for a fetus one should consider aspects like ethnicity, fetal gender, parity and parental anthropometrics. A model is described that provides the possibility to determine a fetus specific growth chart. These customized growth charts might improve the detection of growth anomalies and help to distinguish between small for gestational age fetuses and appropriate for gestational age fetuses that are constitutionally small.

In **Chapter 3.4**, we demonstrated that continued active maternal smoking in pregnancy was associated with reduced growth of fetal head circumference, abdominal circumference and femur length. These impaired fetal growth rates led to smaller femur length from mid-pregnancy onwards and smaller head circumference and abdominal circumference from late pregnancy onwards. Maternal smoking until pregnancy was known and quitting thereafter did not adversely affect fetal growth patterns.

In **Chapter 4**, the fetal origins hypothesis was the main point of departure, the studies presented focus specifically on the development of fetal organs during pregnancy. Brain, kidney and heart development are studied in relation to fetal growth. Special emphasis has been put on fetal growth restriction and suboptimal fetal organ development in utero.

Chapter 4.1 studies the development of the fetal circulation and heart, in relation with fetal growth. Epidemiological studies have demonstrated fetal growth restriction and low birth weight to be risk factors contributing to cardiovascular disease and hypertension in adult life (23, 49). The increased risk may result from suboptimal development of the heart

and main arteries in utero, and to haemodynamic changes in fetal growth restriction. The aim of the present study was to study fetal circulation parameters and the haemodynamic adaptive changes in relation with decreased fetal growth in a population-based study. We found that decreased fetal growth was associated with adaptive fetal haemodynamic changes, consistent with an increase in cardiac afterload and a decrease in cardiac and arterial compliance. These changes already started to occur before the stage of clinically apparent fetal growth restriction. Cardiac remodelling, placental resistance indices, and cardiac output changes are consistent with a gradual increase in afterload with decreasing fetal growth. Long-term studies are needed to examine whether and to what extend changes in circulation haemodynamics observed in utero persist during childhood and whether they are related to cardiac function and blood pressure development in postnatal life.

In **Chapter 4.2** we examined renal development in relation to fetal growth. Epidemiological studies have demonstrated intrauterine growth restriction and low birth weight to be risk factors contributing to renal disease, hypertension and smaller kidney size in adult life (23, 49, 50). Our study showed no associations of fetal growth characteristics in mid pregnancy with kidney volume measured in late pregnancy. In late pregnancy, reduced fetal growth, indices of raised placental resistance and fetal redistribution parameters were associated with reduced kidney volume. The influence of fetal growth on kidney volume seems to exert mainly after mid pregnancy. Also, we found that placental perfusion seems to play an important role in the determination of kidney size in pregnancy. Furthermore, the results show a tendency that smaller fetal body size is associated with smaller kidneys, but these kidneys are relatively large for that body size, suggesting an organ or kidney sparing effect in fetal growth restriction. Since we know that the number of nephrons is largely determined in prenatal life, suboptimal kidney growth and development in fetal life may have lifelong consequences (55-59).

In **Chapter 4.3** we investigated whether maternal anthropometrics, as a measure of fetal nutrition, and fetal growth characteristics measured in mid- and late pregnancy were associated with kidney size in infancy. The observed association between abdominal circumference in late pregnancy and kidney volume in infancy suggests that infant kidney size and structure are established in fetal life. Follow-up studies are needed to examine the consequences for renal function and blood pressure in later life.

In **Chapter 4.4**, the effect of maternal smoking in pregnancy on prenatal brain development was investigated. Research has demonstrated several adverse effects of maternal smoking in pregnancy on the developing fetus. Maternal cigarette smoking is an established risk factor for intrauterine growth restriction, perinatal morbidity and mortality and postnatal growth (41, 65). This study showed that maternal smoking in pregnancy is associated with reduced growth of the fetal head. Small but highly significant associations were found for head circumference and biparietal diameter. Maternal smoking did also

result in smaller atrial width of the lateral ventricle and smaller transverse cerebellar diameter but the differences between fetuses of smoking and non-smoking mothers remained constant throughout pregnancy.

Chapter 5 provides a more general discussion of the main findings, considers general methodological and pathophysiological issues, and gives suggestions for further research.

Samenvatting

Het doel van de onderzoeken die gepresenteerd worden in dit proefschrift was om de normale en abnormale foetale groei en ontwikkeling nauwkeurig te beschrijven. Daarnaast is de invloed van een aantal determinanten op foetale groei bestudeerd. De 'foetale origine van volwassen aandoeningen' hypothese was de belangrijkste aanleiding tot het doen van onderzoek zoals beschreven is in het tweede deel van dit proefschrift. Het ontrafelen van de mechanismen die ten grondslag liggen aan de associatie tussen foetale groeivertraging en laag geboortegewicht enerzijds en ziekten in de volwassenheid anderzijds kan leiden tot nieuwe strategieën om groepen met een verhoogd risico te identificeren en preventieve maatregelen te ontwikkelen.

In **Hoofdstuk 2** wordt het Generation R onderzoek gepresenteerd. Het Generation R onderzoek is een prospectief cohort onderzoek vanaf het vroege foetale leven tot de jongvolwassenheid. Het onderzoek is opgezet om de vroege omgevings- en genetische oorzaken van normale en abnormale groei, ontwikkeling en gezondheid van het foetale leven tot aan de jongvolwassenheid te identificeren. Het onderzoek is gericht op vier primaire onderzoeksgebieden: 1) groei en ontwikkeling; 2) gedrag en cognitieve ontwikkeling; 3) ziekten op de kinderleeftijd; 4) gezondheid en zorggebruik van zwangere vrouwen en kinderen. In totaal zijn 9.778 moeders geïncludeerd met een bevallingsdatum tussen april 2002 en januari 2006. Van deze moeders werd 91% (n=8880) tijdens de zwangerschap geïncludeerd. Deze populatie is de basis van alle in dit proefschrift beschreven studies. Dataverzameling in de prenatale fase omvatte lichamelijk onderzoek, vragenlijsten en foetaal echo-onderzoek. Daarnaast werden in een subgroep van 1.232 zwangere vrouwen en kinderen extra gegevens verzameld; deze subgroep wordt het Generation R Focus onderzoek genoemd. De kinderen vormen een prenataal geïncludeerd geboortecohort, dat gevolgd wordt tot aan de jongvolwassenheid. De resultaten die voortkomen uit het Generation R onderzoek dienen bij te dragen aan de ontwikkeling van strategieën om de gezondheid en het zorggebruik van zwangere vrouwen en kinderen te optimaliseren, nu en in de toekomst.

Hoofdstuk 3.1 verstrekt nieuwe curven voor het dateren van de zwangerschap op basis van echometingen en referentiecurven voor normale foetale groei. Nauwkeurige kennis van zwangerschapsduur is noodzakelijk voor de controle van zwangerschappen en in het bijzonder voor het monitoren van de foetale groei. In deze studie worden nieuwe curven voor zwangerschapsdatering op basis van echometingen gepresenteerd, die gebaseerd zijn op de kruin-romp lengte en de biparietale diameter. De resultaten wijzen uit dat hoe eerder tijdens de zwangerschap de echometing wordt uitgevoerd, bij voorkeur tussen 10 en 12 weken, hoe beter de voorspelling van de zwangerschapsduur is. Zwangerschapsdatering kan geoptimaliseerd worden door de kruin-romp lengte te gebruiken van 20 tot 65 mm en de biparietale diameter te gebruiken vanaf 23 mm. Interne validatie met de

daadwerkelijke bevallingsdatum liet zien dat echometingen tot een betrouwbare schatting van de zwangerschapsduur leiden. Tot 92% van de bevallingen vond plaats tussen 37 en 42 weken zwangerschapsduur, als deze was bepaald met behulp van echometingen, in vergelijking met 87% als de zwangerschapsduur gedateerd was op basis van een betrouwbare rapportage van de laatste menstruatie. Bij een zwangerschapsduur vanaf 24 weken is een betrouwbare rapportage van de laatste menstruatie periode beter dan een echometing, aangezien de toenemende foetale groei variatie tot een toenemende onzekerheid in de voorspelling van de zwangerschapsduur leidt. Om de foetale groei te controleren en om afwijkingen in de groei te identificeren zijn referentiecurven voor normale foetale groei van de biparietale diameter, hoofdomtrek, cerebellaire diameter, buikomtrek en femurlengte ontwikkeld, die te gebruiken zijn bij een zwangerschapsduur vanaf 10 weken. Vroege zwangerschapsdatering op basis van echometingen en het gebruik van betrouwbare groeicurven kan leiden tot verbetering van obstetrisch handelen tijdens de zwangerschap.

Het onderzoek gepresenteerd in **Hoofdstuk 3.2** betreft de intra- en interobserver reproduceerbaarheid en overeenstemming van de echometingen. Echometingen in de vroege zwangerschap worden veelvuldig gebruikt voor klinische doeleinden. Alvorens referentiecurven van foetale groei in de vroege zwangerschap in de klinische praktijk te gebruiken, zal de betrouwbaarheid van de metingen in kaart moeten worden gebracht. Dit onderzoek toonde een goede reproduceerbaarheid van de foetale biometrie metingen met behulp van trans-abdominale echoscopie in de vroege zwangerschap. Vanaf 9 weken zwangerschapduur lieten kruin-romp lengte en biparietale diameter een hoge reproduceerbaarheid en overeenstemming zien. Voor hoofd- en buikomtrek waren de reproduceerbaarheid en overeenstemming iets minder hoog, deze metingen zijn pas betrouwbaar vanaf circa 12 weken zwangerschapsduur. De reproduceerbaarheid van de femurlengte was slecht, waardoor bedachtzaamheid geboden is bij het gebruik voor klinische doeleinden van deze meting in de vroege zwangerschap. De grote mate van overeenstemming van de foetale echometingen sterkt ons in het vertrouwen dat eventuele meetfouten tussen verschillende echoscopisten weinig effect zullen hebben op de schatting van de zwangerschapsduur.

Hoofdstuk 3.3 beschrijft een studie over het effect van verschillende variabelen op geschat foetaal gewicht en geboortegewicht. Om de optimale groei van een foetus vast te stellen dient men ook rekening te houden met factoren zoals etniciteit, foetaal geslacht, pariteit en gewicht en lengte van de ouders. Een model wordt beschreven dat de mogelijkheid geeft om de specifieke groeicurve van een foetus te bepalen. Deze foetusspecifieke groeicurven kunnen de detectie van groeiafwijkingen verbeteren en helpen onderscheid te maken tussen foetussen die klein zijn voor de zwangerschapsduur en foetussen die constitutioneel klein zijn.

In **Hoofdstuk 3.4** hebben we aangetoond dat actief blijven roken in de zwangerschap van de moeder geassocieerd is met een verminderde groei van de foetale hoofdomtrek, buikomtrek en femurlengte. Deze verminderde foetale groei leidt tot een kleinere femurlengte vanaf halverwege de zwangerschap en tot een kleinere hoofd- en buikomtrek vanaf laat in de zwangerschap. Roken van moeder totdat de zwangerschap bekend was had geen negatief effect op de foetale groeipatronen.

In **Hoofdstuk 4**, met de 'foetale origine van volwassen aandoeningen' als achtergrond hypothese, worden onderzoeken beschreven die specifiek gericht zijn op de ontwikkeling van de foetale organen tijdens de zwangerschap. De ontwikkeling van hersenen, nieren en hart &vaten worden bestudeerd in relatie tot de foetale groei. De nadruk ligt hierbij op foetale groeivertraging en suboptimale ontwikkeling van de foetale organen in de baarmoeder.

In **Hoofdstuk 4.1** wordt de relatie tussen de foetale groei enerzijds en de foetale ontwikkeling van het hart en de bloedsomloop anderzijds bestudeerd. Epidemiologische studies hebben aangetoond dat foetale groeivertraging en een laag geboortegewicht risicofactoren zijn voor het ontwikkelen van hart- en vaatziekten en hypertensie in het volwassen leven. Dit verhoogde risico kan zowel het resultaat zijn van een suboptimale foetale ontwikkeling van het hart en de arteriën als van hemodynamische veranderingen bij foetale groeivertraging. Het doel van dit onderzoek was het bestuderen van parameters van de foetale circulatie en van adaptieve hemodynamische veranderingen in relatie tot verminderde foetale groei. We vonden dat verminderde foetale groei geassocieerd was met veranderingen van de foetale bloedsomloop, passend bij een toegenomen (bloed) druk in de linker hartkamer en het arteriële systeem en een afgenomen hart- en vaatwandelasticiteit. Deze veranderingen traden al op voordat de foetale groeivertraging klinisch duidelijk werd. Lange termijn studies zijn nodig om te onderzoeken of en in welke mate de geobserveerde foetale hemodynamische veranderingen persisteren in de kindertijd en of deze veranderingen gerelateerd zijn aan cardiaal (dys)functioneren en de ontwikkeling van hoge bloeddruk in het postnatale leven.

In **Hoofdstuk 4.2** hebben we de relatie tussen foetale groei en de ontwikkeling van de nieren onderzocht. Epidemiologische onderzoeken hebben aangetoond dat intrauteriene groeivertraging en laag geboortegewicht risicofactoren zijn, die bijdragen aan (de ontwikkeling van) nierziekten, hypertensie en kleinere niergrootte in het volwassen leven. Laat in de zwangerschap waren een verminderde foetale groei en tekenen van toegenomen placentaweerstand geassocieerd met een kleiner niervolume. De foetale groei lijkt voornamelijk in de tweede helft van de zwangerschap het niervolume te beïnvloeden. We hebben ook gevonden dat placentaire doorbloeding een belangrijke rol speelt in het bepalen van niergrootte in de zwangerschap. Daarnaast lieten de resultaten een tendens zien dat een kleinere foetale lichaamsgrootte geassocieerd is met kleinere nieren, maar dat deze nieren relatief groot waren voor de lichaamsgrootte, wat wijst op een orgaan- of

niersparend effect bij foetale groeivertraging. Aangezien het aantal nephronen grotendeels bepaald wordt in het prenatale leven, kan een suboptimale foetale groei en ontwikkeling van de nieren levenslange consequenties met zich meebrengen.

In **Hoofdstuk 4.3** hebben we onderzocht of gewicht en lengte van de moeder, als maat voor de foetale voeding, en de foetale groeikenmerken, gemeten halverwege en laat in de zwangerschap, geassocieerd waren met niergrootte in de babytijd. De geobserveerde associatie tussen buikomtrek in de late zwangerschap en niervolume in de babytijd suggereert dat een baby's niergrootte en –structuur vastgelegd worden in de foetale periode. Follow-up studies zijn nodig om de consequenties voor nierfuncties en bloeddruk op latere leeftijd te onderzoeken.

In **Hoofdstuk 4.4** is gericht op het effect van roken van moeder tijdens de zwangerschap op de foetale hersenontwikkeling. Onderzoek heeft diverse negatieve effecten van roken van moeder tijdens de zwangerschap op de ontwikkelende foetus aangetoond. Het roken van sigaretten door moeder is een risicofactor voor intrauteriene groeivertraging, perinatale morbiditeit en mortaliteit en (verminderde) postnatale groei. Dit onderzoek toonde aan dat het roken van moeder tijdens de zwangerschap geassocieerd is met verminderde foetale groei van het hoofd. Kleine, maar zeer significante associaties werden gevonden voor hoofdomtrek en biparietale diameter. Het roken van moeder leidde ook tot een kleinere breedte van de laterale hersenholte en een kleinere cerebellaire diameter. Deze verschillen tussen foetussen van rokende en niet-rokende moeders bleven constant tijdens de zwangerschap.

Hoofdstuk 5 geeft een meer algemene discussie van de belangrijkste bevindingen. Daarnaast worden algemene methodologische en pathofysiologische kwesties besproken en suggesties gegeven voor toekomstig onderzoek.

Dankwoord

Toen mijn vader promoveerde was ik nog jong. Ik weet er niet meer zoveel van, alleen dat een van de commissieleden aan mijn vader vroeg: 'Mijnheer Verburg, denkt u nu echt dat iemand dit boek gaat lezen?' Deze anekdote typeert het promoveren wel een beetje: een dissertatie is er om te schrijven, niet om te lezen. Het dankwoord daargelaten, want dat leest wel iedereen.

Het is een heldere herfstochtend, de zon schijnt. Het manuscript ligt bij de drukker. Het zou toch niet zo moeilijk moeten zijn om iedereen te bedanken die heeft bijgedragen aan de totstandkoming van dit proefschrift? Toch zijn deze laatste pagina's een soort van sentimental journey terug in de tijd.

Ik wil iedereen bedanken die direct of indirect heeft bijgedragen aan dit proefschrift. Er zijn simpelweg teveel mensen om persoonlijk te bedanken, maar ik ben jullie niet vergeten!

In de eerste plaats gaan mijn gedachten uit naar alle deelnemers van Generation R die hun onvoorwaardelijke bijdrage hebben geleverd aan de wetenschap. Het enthousiasme, de volharding en de positieve feedback van de deelnemers geven vertrouwen in de toekomst. Het is een prachtig project met mooie resultaten. De motivatie van de deelnemers is essentieel geweest om de benodigde gegevens te verzamelen. De tijd die ik heb doorgebracht met het maken van echo's bij deelnemers heb ik altijd als heel plezierig ervaren; ik heb me geen moment verveeld. Ik hoop dat ik mijn enthousiasme op de deelnemers heb kunnen overbrengen voor dit unieke project en dat ze nog vele jaren mee blijven doen!

Verder zijn er natuurlijk alle verloskundigen en ziekenhuizen in de regio Rotterdam, die enthousiast hebben meegewerkt om Generation R onder de aandacht van de zwangere vrouwen te brengen. Zonder jullie inzet zou de respons niet zo hoog zijn geweest! Jullie hebben er allemaal aan bijgedragen dat de foetale groei en ontwikkeling beter in kaart zijn gebracht en dat ook obstetrisch management verbeterd kan worden.

Dit brengt mij bij alle echoscopisten en medewerkers van de Star-MDC die in de prenatale fase allemaal heel hard gebikkeld hebben om alle metingen bij elkaar te krijgen. Ondanks alle logistieke problemen bleef de sfeer altijd wonderbaarlijk goed, een heel hecht en efficiënt team met goede echokwaliteiten! (En waar je ook heel gezellig een drankje mee kan drinken of een lang weekendje mee op stap kan in Istanbul.) Ik heb altijd met heel veel plezier met jullie samengewerkt. Zonder jullie onvoorwaardelijke inzet en enthousiasme had Generation R het nooit zo ver geschopt. Eric Smith heeft hierin een speciale rol gespeeld. Hij heeft mij de fijne kneepjes van het vak geleerd. Door zijn enthousiasme, gedrevenheid en uitleg ben ik nu ook bedreven in de echo(cardio)grafie. Hij heeft heel

veel werk verzet voor Generation R. Gelukkig zie ik hem nog regelmatig en is hij een goed maatje geworden. Ik denk met veel plezier terug aan de lange avonden dat we samen echo's maakten voor het Focus. Om daarna even bij te komen met een glas koud bier. Ook bedankt voor het leveren van sommige plaatjes!

Uiteraard verdienen ook alle bureaumedewerkers een blijk van waardering! Het zijn er in de loop der jaren te veel geworden om allemaal op te noemen. Maar één ding is duidelijk: zonder jullie ondersteunende taken zou alles in de soep lopen. Bedankt!

Rosalinde Snijders was de projectleider die zich altijd met veel passie heeft ingezet voor Generation R. Zonder haar was Generation R niet geworden wat het nu is. Ook persoonlijk heeft zij veel voor me betekend. Onder het genot van een borreltje (of wat) zijn er veel Generation R perikelen besproken. Rosalinde, ik vind het een eer dat je als 'extern deskundige' in mijn promotiecommissie zit.

Vincent Jaddoe heeft het stokje van Rosalinde overgenomen en heeft een grote bijdrage geleverd aan dit proefschrift. Eerst in de vorm van collega, later als projectleider en medeauteur, tot slot zelfs als laatste auteur en copromotor. Je epidemiologische aanpak, statistische benadering en duidelijk commentaar zijn altijd een houvast geweest voor de structuur van mijn artikelen. Het was prettig om met je samen te werken en ik wens je nog heel veel succes in je (wetenschappelijke) carrière.

Jacqueline Witteman, jammer dat je benoeming niet op tijd rond was, anders had je gewoon in de commissie gezeten. Ik wil je bedanken voor alle vrijheid die je mij hebt gegeven en het vertrouwen dat je me hebt geschonken. Je weet altijd goed wat er speelt en hebt ook oog voor de menselijke aspecten, daar heb ik veel respect voor. Door jouw brede interesse was je zelfs in staat om al die verloskundige onderwerpen te beoordelen.

Maria de Ridder, bedankt voor alle hulp bij het statistisch gepuzzel. Het was niet altijd even duidelijk wat we nu precies wilden; bovendien was het werk aan de curven soms erg frustrerend, omdat we telkens opnieuw konden beginnen als er weer een nieuwe opgeschoonde dataset werd aangeleverd… Ik weet dat je er veel tijd in hebt gestoken en zonder jou was ik er niet uitgekomen.

Professor Wladimiroff, via u ben ik bij Generation R terechtgekomen. Ik heb met u mee mogen draaien op de afdeling en zo de kneepjes van het vak kunnen leren. Door uw gedrevenheid en kennis van de foetale circulatie is het fascinerend om de resultaten te bespreken. Ik heb de inhoudelijke discussies altijd erg op prijs gesteld.

Professor Steegers, u was altijd enthousiast. Bedankt voor het vertrouwen dat u in mij heeft gesteld en de mogelijkheden die u mij geboden heeft. Uw kijk op de zaken werkte heel verhelderend. U vroeg zich vaak af waarom wij bepaalde resultaten vonden. Dit maakt onderzoek zo interessant; het is geen kunst om te beschrijven wat je vindt, maar veel leuker om te beredeneren waarom dat zo kan zijn en wat de klinische relevatie is. Uw

begeleiding was goud waard: knopen doorhakken, plannen maken, zaken doen, duidelijk en snel commentaar, prettig in de omgang, beter had ik mij niet kunnen wensen. Bedankt voor de geweldige samenwerking en de leuke, inhoudelijke discussies!

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De leescommissie wil ik bedanken voor het lezen en beoordelen van mijn proefschrift en voor de bijdrage die ze willen leveren aan het symposium.

Dokter de Leeuw, de andere gynaecologen en collega's in het Ikazia; bedankt voor de prettige werksfeer en de ruimte die ik gekregen heb. Als je met veel plezier naar je werk gaat, heb je ook nog energie over om in je vrije tijd je boekje af te maken, dat is dus mede dankzij jullie gelukt.

Een boekje schrijf je niet alleen: al mijn co-auteurs heel hartelijk bedankt voor de hulp en ondersteuning. Met name natuurlijk Sabine en Miranda: naast co-auteurs ook vriendinnen, kamergenoten en mijn paranimfen. Thanx voor de hulp, maar ook voor het plezier!

Alle promovendi en collega's van Generation R natuurlijk heel erg bedankt voor alle gezelligheid, creativiteit, de vele borrels en de vriendschappen die er ontstaan zijn. Joost, Sabine, Pauline, Jens, Miranda, Elise en alle anderen: het was een top tijd!

Ook in de periode dat het gezondheidstechnisch iets minder met mij ging ;-), hebben vele mensen mij onvoorwaardelijk gesteund. Met name Bart, Sandra, Anouk, Sebas, Jitske en de (toen nog) kleine Maite hebben mij heel veel energie gegeven om door te gaan. De support, kaartjes en berichten van al mijn vrienden, familie, collega's en kennissen zijn van onschatbare waarde geweest!

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En dan is er natuurlijk nog Heske, het allerliefste zusje van de hele wereld!! Jij hebt ook heel wat te verduren gehad, met mij en met jezelf. Ik ben zó blij dat je mijn zusje bent! Jij bent er gewoon altijd en dat is heel fijn. Samen met Lennart en nu ook Benten. Benten krijgt geloof ik nog de meeste eer, die staat op bijna alle illustraties..

Lieve ouders, gewoonweg geweldig zoals jullie voor me klaar staan, grenzeloos vertrouwen in me hebben en me altijd steunen. Go with the flow, ik houd van jullie. Paps, ik beloof dat ik binnenkort jouw proefschrift zal lezen.....

PW, het was wel een beetje te druk en gestresst de laatste tijd hè...Nu hopelijk een beetje relaxen...

Vrije tijd: 'Ga gewoon even zitten, luister naar alles wat je nooit hoort, kijk naar alles wat je nooit ziet, terwijl je nadenkt over wat je zult gaan doen en zing een liedje' (Winnie the Pooh)

Bero

About the author

Bero Verburg was born on April 4, 1973 in Bussum, The Netherlands. He grew up in Weesp and passed his secondary school in 1991 at the ' Goois Lyceum' in Bussum. In 1991, he started to study medicine at the Leiden University Medical Center (LUMC). The fourth year of his study he followed in Lausanne, Switzerland with the Erasmus student exchange program. The scientific research project for his study he performed in Lille, France, studying the 'Epidemiology of Clostridium Difficile in infants'. Furthermore, he worked in Nyahururu, Kenya in a district hospital for 2 months and followed part of his internships in Beaumont Hospital, Dublin, Ireland. He graduated (cum laude) from Medical School in 1998. Subsequently, he worked as a resident at the department of Obstetrics & Gynecology and Surgery in the Albert Schweitzer Hospital in Dordrecht, completing his training in the field of Tropical Medicine. In 2001, he graduated at the National School of Public Health as a Tropical Doctor. In 2002 he started his work as a junior researcher at the department of Epidemiology and Biostatistics, Erasmus Medical Center, Rotterdam, resulting in the work described in this thesis. During this period he combined his research with a part-time function as Medical Coordinator Ultrasound, training and supervising fetal sonographers at Star, Medical Diagnostic Center, Rotterdam. In 2006, he obtained a Master of Science degree in Clinical Epidemiology from the Netherlands Institute for Health Sciences (Nihes) in Rotterdam. He started his residency in Obstetrics & Gynecology in January 2007, in the Ikazia Hospital, Rotterdam.