SCREENING FOR ADVERSE PREGNANCY & CHILDHOOD OUTCOMES

JAN STEVEN ERKAMP

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Screening for Adverse Pregnancy and Childhood Outcomes

Screening voor ongewenste uitkomsten gedurende de zwangerschap en kindertijd

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Promotoren:	Prof.dr. V.W.V. Jaddoe
	Prof.dr. I.K.M. Reiss
Overige leden:	Prof.dr. A. Franx
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	Prof.dr. M.E.A. Spaanderman
Co-promotor:	Dr. R. Gaillard

Paranimfen:	Drs. I.C. van der Marel
	Dr. W.P.A. Kelders

CONTENTS

MANUSCRIPTS BASED ON THIS THESIS

Chapter 2.1: Jan S. Erkamp, Vincent W.V. Jaddoe, Annemarie G.M.G.J. Mulders, Liesbeth Duijts, Irwin K.M. Reiss, Eric A.P. Steegers, Romy Gaillard. Associations of maternal age at the start of pregnancy with placental function throughout pregnancy: The Generation R Study. *Eur J Obstet Gynecol Reprod Biol. 2020 Aug; 251:53-59. Doi: 10.1016/j.ejogrb.2020.04.055*

Chapter 2.2: Jan S. Erkamp, Madelon L. Geurtsen, Vincent W.V. Jaddoe, Liesbeth Duijts, Irwin K.M. Reiss, Eric A.P. Steegers, Romy Gaillard. Associations of maternal early-pregnancy glucose concentrations with placental hemodynamics, blood pressure and gestational hypertensive disorders. *Am J Hypertens. 2020 Jul; 33(7):660-669. Doi: 10.1093/ajh/hpaa070*

Chapter 2.3: Clarissa Wiertsema, **Jan S. Erkamp,** Annemarie G.M.G.J. Mulders, Liesbeth Duijts, Romy Gaillard, Eric A.P. Steegers, Anton de Koning, Vincent W.V. Jaddoe. Reproducibility of first trimester embryonic volume and fetal body proportion measurements in a population-based sample. *Submitted*

Chapter 3.1: Jan S. Erkamp, Vincent W.V. Jaddoe, Liesbeth Duijts, Irwin K.M. Reiss, Annemarie G.M.G.J. Mulders, Eric A.P. Steegers, Romy Gaillard. Population screening for gestational hypertensive disorders using maternal, fetal and placental characteristics: A population-based prospective cohort study. *Prenat Diagn. 2020 May; 40(6):746-757. Doi: 10.1002/pd.5683*

Chapter 3.2: Jan S Erkamp, Ellis Voerman, Eric A.P. Steegers, Annemarie GMGJ Mulders, Irwin K.M. Reiss, Liesbeth Duijts, Vincent W.V. Jaddoe, Romy Gaillard. Second and third trimester fetal ultrasound population screening for risks of preterm birth and small-size and large-size for gestational age at birth: A population-based prospective cohort study. *BMC Medicine 2020 Apr; 18(1):63. Doi: 10.1186/s12916-020-01540-x*

Chapter 3.3: Jan S. Erkamp, Vincent W.V. Jaddoe, Annemarie G.M.G.J. Mulders, Eric A.P. Steegers, Irwin K.M. Reiss, Liesbeth Duijts, Romy Gaillard. Customized versus population birth weight charts for identification of newborns at risk of long-term adverse cardio-metabolic and respiratory outcomes: A population-based prospective cohort study. *BMC Medicine 2019 Oct; 17(1):186. Doi: 10.1186/s12916-019-1424-4.*

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Rationale

The imminent risk of morbidity and mortality due to pregnancy and birth complications makes intra-uterine development and being born a dangerous time in any person's life. Due to risks of maternal complications, pregnancy and childbirth is also a dangerous endeavour for the mother. Globally, every year between 280 and 300 thousand women die because of complications during pregnancy or childbirth[1]. In 2017, almost 900 thousand children died of complications due to preterm birth[1]. In the Netherlands, maternal mortality is rare, but the perinatal mortality rate calculated as the sum of fetal deaths after 28 weeks of pregnancy, and neonatal deaths before 7 days after birth is 10.45 per 1000 births[2]. Pregnancy complications such as gestational hypertensive disorders and fetal growth abnormalities are major contributors to morbidity and mortality. In big Dutch cities like Rotterdam and The Hague, the percentages of complications are even higher than in other areas of the Netherlands[3].

Maternal health can be strongly impacted by pregnancy complications. Worldwide, gestational hypertensive disorders, defined as new-onset hypertension with or without proteinuria, affect 5 to 10% of all pregnancies[4-7]. These women are at risk of serious complications, such as eclampsia, liver rupture, stroke, pulmonary oedema and kidney failure[8-10]. 26% of maternal deaths in low-resource countries can be attributed to gestational hypertensive disorders, but also in high income countries, 16% of maternal deaths can be assigned to hypertensive disorders[7]. Beyond these immediate complications, research in the last decade has shown that women with pregnancies affected by gestational hypertensive disorders also have higher risk of important long-term adverse health outcomes, affecting maternal health far beyond pregnancy, such as hypertension, obesity, dyslipidaemia, and insulin resistance[11].

Besides maternal consequences, pregnancy complications have major consequences for child health. Preterm birth, small-size for gestational age (SGA) and large-size for gestational age (LGA) at birth explain up to 30% of neonatal death, and are strong risk factors for short-term and long-term morbidity[12, 13]. Short-term adverse outcomes include birth trauma, higher risk of assisted vaginal or operative delivery, low Apgar score, respiratory problems, neonatal intensive care unit admission and death. Next to these short-term adverse outcomes, gestational age and weight at birth are important determinants for health in later life[14, 15]. Children born SGA or LGA have increased risks

of suboptimal growth, cardio-metabolic and respiratory development throughout childhood, leading to increased risks of obesity, coronary heart disease, type 2 diabetes and obstructive respiratory disease in later life[14, 15].

Currently, management of gestational hypertensive disorders is focused on symptoms caused by an underlying mechanism that still remains largely unknown[16]. Management of a pregnancy with suspected abnormal fetal growth is largely based on intensified monitoring by more frequent consultations, ultrasound assessments and cardiotocography. Previous studies have shown that early identification of these pregnancy complications is important: SGA or LGA newborns who have not been identified antenatally have strongly increased risks of morbidity and mortality, compared to those who have been identified antenatally[17-20]. Although abnormal fetal growth and gestational hypertensive disorders mostly manifest in third trimester, they likely find their cause and start developing in earlier pregnancy[7]. Also, certain maternal, placental and fetal characteristics have been shown to be associated with pregnancy outcomes[8, 16, 21-25]. This brings about an opportunity for screening for women at risk of pregnancy complications, before severe disease develops. The presence or absence of these characteristics during pregnancy, or possibly even before pregnancy, could help selecting women at higher risk of developing these pregnancy complications. Similarly, after pregnancy, newborns born SGA at birth have a higher risk of suboptimal growth, cardio-metabolic and respiratory development leading to increased risks of diseases in later life. Finding the optimal way to select those newborns at risk for long-term adverse outcomes could give healthcare providers a window of opportunity to monitor and intervene if necessary. Thus, screening for, and early identification of women and their offspring at risk of pregnancy and childhood complications with subsequent monitoring and management may prevent adverse pregnancy outcomes, and improve later life health.

Studies for the identification of maternal, fetal and placental characteristics which could be used for screening for pregnancy complications, adverse birth outcomes and long-term adverse health consequences in the offspring in the general population, are necessary. These studies should focus on low-risk, multi-ethnic populations of mothers and newborns to increase their applicability for clinical practice. **Figure 1.1** shows an overview of the hypotheses for the pathophysiological mechanisms underlying pregnancy complications and long-term adverse health outcomes in the offspring. Additionally, it shows the corresponding clinical measurements which could be used for screening for pregnancy complications and long-term adverse offspring health outcomes.

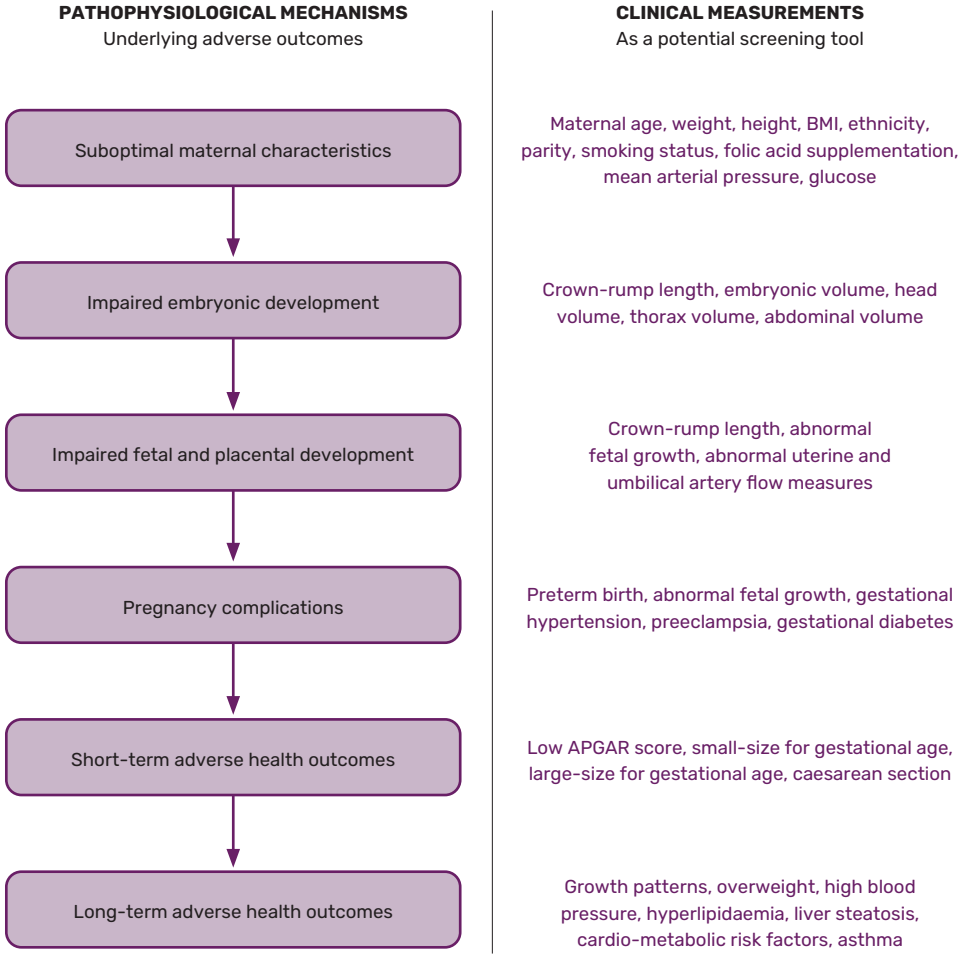

Figure 1.1

Characteristics for screening

Characteristics for screening for adverse outcomes in this thesis can be divided into maternal characteristics, placental characteristics and fetal characteristics.

Maternal characteristics

Maternal characteristics are associated with risk of pregnancy complications. Maternal ethnicity, parity, body mass index (BMI) and educational level is associated with risk of gestational hypertensive disorders[8, 16, 21-23]. For example, women who have an African ethnicity have a higher risk of preeclampsia. Maternal age, height, BMI, ethnicity,

smoking and parity are important determinants of fetal growth[24, 25]. For example, women who smoke are more likely to give birth to a smaller baby, and children born SGA have a higher risk of adverse health outcomes in later life. Thus, maternal characteristics already known in early pregnancy may contain valuable information about risk of those pregnancy complications and adverse health outcomes beyond pregnancy. Often, these maternal characteristics are known or routinely measured at the start of pregnancy, and could therefore be used in screening models. Thus, maternal characteristics are associated with pregnancy complications and health outcomes far beyond pregnancy, and could be used for screening. It is unlikely that only one screening model is optimal for several different pregnancy complications and health outcomes. The optimal screening model for these outcomes remains to be determined.

Placental characteristics

Placental function can be assessed during pregnancy and at birth. Thus far, mostly placental weight at birth has been used as an indicator of placental function, with a lower placental weight representing impaired placental function. During pregnancy, placental flow measures represent the blood flow through and the resistance in the uterine and umbilical arteries, and can be derived using Doppler ultrasound[26]. Increased uterine artery resistance index (UtA-RI) and or umbilical artery pulsatility index (UA-PI) are related to placental disease and may lead to abnormal intra-uterine growth and adverse perinatal outcomes[27-32]. Maternal blood concentrations of placental growth factor (PlGF), which is a proangiogenic factor playing a key role in placental development and functioning, are associated with risk of preeclampsia[33]. The value of these parameters in screening for adverse pregnancy outcomes among low-risk, multi-ethnic populations remains debated, especially in the presence of maternal characteristics. With recent advancements in 2D-ultrasound, 3D-ultrasound and Power Doppler technology, more advanced parameters of early placentation, such as placental bed vascular volume and placenta volume in early pregnancy can be measured. The use of these measurements in screening needs to be further established.

Fetal characteristics

Ultrasound technology is commonly used for assessment of fetal size during pregnancy. Abnormal fetal size is strongly associated with iatrogenic preterm birth, but studies have also shown that impaired or accelerated fetal growth often precedes spontaneous preterm birth[20, 30]. Abnormal fetal size is an important risk factor for SGA and LGA at birth, with health implications far beyond pregnancy as mentioned earlier[30, 34-36]. Furthermore, abnormal fetal growth may be an early sign of other underlying placental pathology, such as preeclampsia[37]. The strongest associations of abnormal ultrasound estimates

of fetal size and adverse birth outcomes can be observed in the third trimester[30]. Thus, ultrasound assessment of fetal size has been established as a proxy for actual fetal size at any gestational age, and can be used for selection of pregnancies at risk for adverse outcomes. Current pregnancy care protocols in the Netherlands include dating ultrasounds and detailed structural ultrasounds at 20 weeks gestational age to assess congenital anomalies and fetal size[38, 39]. In the general population, between 20 weeks of gestation and birth, fetal size is not routinely assessed using ultrasound. Third trimester ultrasound screening is only recommended in selected populations, and the value of routine third trimester ultrasound for preterm birth, SGA or LGA in the general population remains debated[40, 41]. It is unclear which periods of pregnancy are optimal for ultrasound screening to identify fetuses at risk for these adverse birth outcomes. Technological developments in obstetric ultrasound may lead to future changes in ultrasound screening protocols, such as early-pregnancy size and congenital anomalies assessment, and third trimester growth assessment using conventional 2-dimensional and novel 3-dimensional ultrasound. The value of these novel parameters needs to be tested and validated in future studies, aiming for improved screening for fetuses at risk of pregnancy complications.

General aim of this thesis

To identify which maternal, fetal and placental parameters can be used for screening for common pregnancy complications with implications for short-term and long-term neonatal and childhood health outcomes in a healthy, low-risk, multi-ethnic population.

General design

This thesis consists of studies embedded in the Generation R Study and the Generation R *Next* Study. The Generation R Study is a population-based prospective cohort study from fetal life until adulthood in Rotterdam, The Netherlands. The Generation R Study aims to identify early environmental and genetic determinants of growth, development and health. Written consent was obtained from all participating women. All pregnant women were enrolled between 2001 and 2005. Response rate at birth was 61%. Enrollment was possible in pregnancy and at birth but aimed at early pregnancy (n=9,778, 91% of all participants were included in pregnancy). In early, mid and late pregnancy, physical examinations, body sample collections and questionnaires were planned. Ultrasound examinations were carried out in two dedicated research centers in first (median 13.2 weeks gestational age, interquartile range (IQR) 12.2 to 14.7), second (median 20.5 weeks gestational age, IQR 19.9 to 21.3) and third trimester (median 30.4 weeks gestational age, IQR 29.8 to 30.9)[30]. We established gestational age by using data from the first ultrasound.[30] From birth onwards, data collection was performed using

information from municipality health centers, questionnaires and visits to a dedicated research center in Erasmus MC – Sophia Children's Hospital at the ages of 6 years and 9 years.

The Generation R *Next* Study is a population based prospective cohort study, from preconception and the embryonic phase until adulthood in Rotterdam, The Netherlands. The Generation R *Next* Study aims to identify environmental and genetic determinants of growth, development and health, from preconception and the embryonic phase onwards. Written consent was obtained from all participating women. Enrollment, which started in 2017 and is ongoing, is possible for women that wish to conceive, and throughout pregnancy. Information of mother is gathered using physical examinations, body sample collections and questionnaires. 2-dimensional and 3-dimensional abdominal and transvaginal ultrasound examinations were carried out in three dedicated research centers in preconception period, and at 7, 9, 11 and 30 weeks of pregnancy. Using 3-dimensional ultrasound technology and virtual reality technology, detailed measurements of embryonic volume and body proportions can be measured. The reproducibility of these novel parameters in a healthy population needs to be established, before its value for epidemiological and clinical research can be determined. From birth onwards, data collection is planned using information from municipality health centers, questionnaires and visits to a dedicated research center in Erasmus MC – Sophia Children's Hospital.

Outline of this thesis

The objectives of the studies in the current thesis are presented in the various chapters. **Chapter 2** describes association studies of maternal characteristics with placental function and pregnancy outcomes. In **Chapter 2.1**, we examined the associations of maternal age in early pregnancy across the full range with second and third trimester uterine and umbilical artery flow indices, and placental weight. We examined the associations of early-pregnancy glucose concentrations with placental hemodynamics, blood pressure and risks of gestational hypertensive disorders in **Chapter 2.2**. In **Chapter 2.3** we examined the reproducibility of first trimester embryonic volumes and fetal body proportion measurements in a population-based sample. In **Chapter 3**, we assessed the role of maternal, fetal and placental characteristics in screening for common adverse birth and childhood outcomes. In **Chapter 3.1** we assessed the use of maternal, placental and fetal characteristics in screening for preeclampsia and gestational hypertension. We examined the role of second and third trimester fetal ultrasound population screening for risks of preterm birth, SGA and LGA in **Chapter 3.2**. In **Chapter 3.3** we examined the superiority of customized versus population-based birth weight charts, for identification

of newborns at risk of long-term adverse outcomes. Finally, **Chapter 4** provides a general discussion in which the studies in this thesis are further discussed, and implications and suggestions for future research are given.

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ETIOLOGICAL STUDIES

2.1: ASSOCIATIONS OF MATERNAL AGE AT THE START OF PREGNANCY WITH PLACENTAL FUNCTION THROUGHOUT PREGNANCY: THE GENERATION R STUDY

Eur J Obstet Gynecol Reprod Biol. 2020

Jan S. Erkamp
Vincent W.V. Jaddoe
Annemarie G.M.G.J. Mulders
Liesbeth Duijts
Irwin K.M. Reiss
Eric A.P. Steegers
Romy Gaillard

ABSTRACT

Objective

To examine the associations of maternal age at the start of pregnancy across the full range with second and third trimester uterine and umbilical artery flow indices, and placental weight.

Study design

In a population-based prospective cohort study among 8,271 pregnant women, we measured second and third trimester uterine artery resistance and umbilical artery pulsatility indices and the presence of third trimester uterine artery notching using Doppler ultrasound.

Results

Compared to women aged 25-29.9 years, higher maternal age was associated with a higher third trimester uterine artery resistance index (difference for women 30-34.9 years was 0.10 SD (95% Confidence Interval (CI) 0.02 to 0.17), and for women aged ≥40 years 0.33 SD (95% CI 0.08 to 0.57), overall linear trend 0.02 SD (95% CI 0.01 to 0.03) per year). Compared to women aged 25-29.9 years, women younger than 20 years had an increased risk of third trimester uterine artery notching (Odds Ratio (OR) 1.97 (95% CI 1.30 to 3.00)). A linear trend was present with a decrease in risk of third trimester uterine artery notching per year increase in maternal age (OR 0.96 (95% CI 0.94 to 0.98)). Maternal age was not consistently associated with umbilical artery pulsatility indices or placental weight.

Conclusions

Young maternal age is associated with higher risk of third trimester uterine artery notching, whereas advanced maternal age is associated with a higher third trimester uterine artery resistance index, which may predispose to an increased risk of pregnancy complications.

INTRODUCTION

Young maternal age, defined as childbearing in women aged <20 years, and advanced maternal age, defined as childbearing in women aged ≥35 years, are associated with adverse pregnancy outcomes, including fetal growth restriction, preterm birth, and fetal and neonatal death[1-5]. Mechanisms underlying these observed associations are not fully understood but are likely multi-factorial, including pre-existing medical conditions, obstetrical history and social characteristics[5, 6]. Next to these factors, both young or advanced maternal age might affect placental vascular development and function throughout pregnancy, predisposing to an increased risk of pregnancy complications[2]. A better understanding of the role of maternal age in suboptimal placental development may aid screening for and early detection of symptoms associated with suboptimal placental development and the subsequent risk of pregnancy complications.

Placental function and growth can be assessed during pregnancy and at birth. Doppler ultrasound can be used to assess resistance and blood flow in uterine and umbilical arteries throughout pregnancy[7]. Utero-placental vascular resistance, measured in uterine arteries, is a parameter of downstream placental vascular resistance, and may increase as a result of impaired placentation. Feto-placental vascular resistance, measured in umbilical arteries, is a parameter of downstream placental vascular resistance at the fetal side, and may increase as result of suboptimal placentation or suboptimal fetal vascular development[8, 9].

We hypothesized that both young and advanced maternal age leads to suboptimal placental development and function, which may subsequently lead to alterations in utero-placental and feto-placental blood flow and placental weight, predisposing to an increased risk of pregnancy complications. Therefore, in a population based, prospective cohort study among 8,271 pregnant women, we assessed associations of maternal age across the full range with measures of placental vascular function throughout pregnancy and placental weight at birth.

METHODS

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards in Rotterdam, the Netherlands[10] (MEC 198.782/2001/31). Written consent was obtained from all participating women. Pregnant women were enrolled between 2001 and 2005. Response rate at birth was 61%. 8,879

women were enrolled during pregnancy. We excluded non-singleton live births (n=246), and participants with no information available on placental measurements (n=362). The population for analysis comprised 8,271 pregnant women (**Figure 1**).

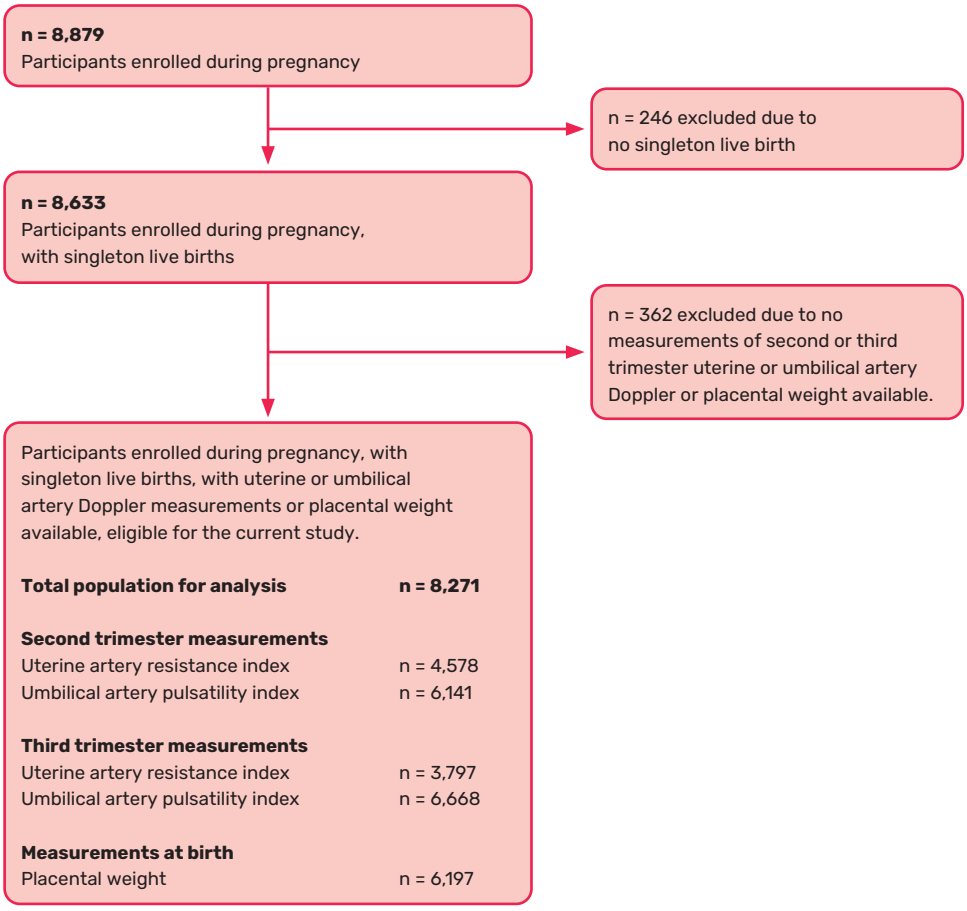

Figure 1. Flowchart population for anaysis

Maternal age

Maternal age was assessed at enrolment by questionnaire. We used maternal age as continuous variable and categorized in six groups: <20 years (n=338); 20–24.9 years (n=1,391); 25–29.9 years (n=2,256); 30–34.9 years (n=3,045); 35–39.9 years (n=1,102); >40 years (n=139)[11]. We used the 25-29.9 years age group as reference.

Placental vascular function and placental weight at birth

Ultrasound examinations were carried out in two dedicated research centers in first trimester (median 13.2 weeks gestational age, interquartile range (IQR) 12.2-14.8), second trimester (median 20.5 weeks gestational age, IQR 19.9-21.3) and third trimester (median 30.3 weeks gestational age, IQR 29.8-30.9)[12]. We established gestational age by using data from the first ultrasound examination[13]. In second and third trimester, uterine artery resistance indices were measured in the uterine arteries near the crossover with the external iliac artery and umbilical artery pulsatility indices were measured in a free-floating loop of the umbilical cord as described previously[8]. The mean of three measurements was used for further analysis. Third trimester uterine artery notching was diagnosed if a notch was present uni- or bilaterally, as a result from increased blood flow resistance, which is a sign of placental insufficiency[14]. Placental weight was obtained from medical records and measured according to standard protocols[15]. Birth weight:placental weight ratio was calculated, as indicator of the ability of the placenta to maintain adequate nutrient supply to the fetus, and is associated with neonatal morbidity and mortality[16]. Small-size for gestational age (SGA) was defined as gestational age adjusted birth weight <10th percentile. Large-size for gestational age (LGA) is defined as gestational age adjusted birth weight >90th percentile.

Covariates

Maternal height (cm) and weight (kg) were measured without shoes and heavy clothing at enrolment. Body mass index (BMI, kg/m^2) was calculated and categorized: normal weight (BMI<25 kg/m^2), overweight (BMI 25.0-30.0 kg/m^2) and obese (BMI≥30.0 kg/m^2)[17]. Information about ethnicity (European/non-European), education (higher education yes/no), folic acid supplementation (yes/no) and parity (nulliparous/multiparous), was obtained at enrolment by questionnaire[18]. Smoking status was assessed by questionnaires and categorized into non-smoking, early-pregnancy only and continued smoking during pregnancy[13].

Statistical analyses

First, we used linear and logistic regression models to assess the associations of maternal age categories with second and third trimester uterine artery resistance indices and umbilical artery pulsatility indices, uterine artery notching, placental weight, birth weight and birth weight:placental weight ratio. P-values for trend were obtained by entering maternal age to the models as a continuous instead of a categorical variable. These models were adjusted for gestational age at each measurement, maternal education, ethnicity, parity, smoking, BMI, folic acid supplementation and fetal sex. These covariates were selected based on previous studies and their associations with the outcomes[11, 19].

[20]. As a secondary analysis, we took forward significant associations of maternal age with placental vascular resistance and explored whether changes in placental vascular resistance partly explained the already established association of maternal age with birth weight[20]. We therefore additionally added placental vascular resistance parameters to linear regression models focused on the associations of maternal age with birth weight, and to logistic regression models focused on the associations of maternal age with risk of delivering an SGA newborn. We used multiple imputation for missing values according to Markov Chain Monte Carlo method[21]. Five imputed datasets were created and pooled for the analyses. Analyses were performed using the Statistical Package of Social Sciences version 24.0 for Windows (IBM Corp., Armonk, NY, USA).

RESULTS

Population characteristics

Table 1 shows population characteristics according to maternal age categories. Younger women were more likely to be of non-Dutch or European ethnicity, to smoke, to have a lower BMI, and to deliver an SGA newborn. Older women were more likely to be of Dutch or European ethnicity and parous, and to deliver an LGA newborn.

Maternal age and placental vascular function

Maternal age was not associated with second trimester uterine artery resistance index (**Table 2**). Compared to women aged 25-29.9 years, higher maternal age was associated with a higher third trimester uterine artery resistance index (difference for women 30-34.9 years was 0.10 SD (95% Confidence Interval (CI) 0.02;0.17), and for women aged ≥40 years 0.33 SD (95%CI 0.08;0.57), overall linear trend 0.02 SDS (95%CI 0.01;0.03) per year). As compared to women aged 25-29.9 years, women younger than 20 years had an increased risk of third trimester uterine artery notching (Odds Ratio 1.97 (95%CI 1.30;3.00)). A linear trend was present with a decrease in risk of third trimester uterine artery notching per year increase in maternal age (OR 0.96 (95%CI 0.94;0.98)). We did not observe associations of maternal age with second or third trimester umbilical artery pulsatility index (**Table 3**).

Table 1. Characteristics of women (N =8,271)

Characteristics	<20 years n=338	20-24.9 years n=1,391	25-29.9 years n=2,256	30-34.9 years n=3,045	35-39.9 years n=1,102	≥ 40 years n=139
Maternal characteristics						
Age, years (median, IQR)	19.0 (18.2 to 19.5)	22.8 (21.6 to 24.0)	27.8 (26.4 to 28.9)	32.4 (31.2 to 33.6)	36.6 (35.7 to 37.9)	41.2 (40.5 to 42.3)
Height, mean (SD) (cm)	165.1 (6.4)	165.3 (7.1)	166.4 (7.4)	168.3 (7.3)	168.2 (7.5)	167.9 (8.2)
Weight, mean (SD) (kg)	65.3 (13.4)	67.8 (13.8)	69.7 (14.2)	69.6 (12.5)	71.0 (12.1)	72.3 (13.6)
Body Mass Index, mean (SD) (kg/m^2)	23.9 (4.6)	24.8 (4.7)	25.1 (4.9)	24.6 (4.3)	25.1 (4.1)	25.6 (4.6)
Parity, No. nulliparous (%)	295 (88.9)	985 (71.9)	1,341 (60.1)	1,538 (51.0)	370 (33.9)	49 (36.0)
Education, No. higher education (%)	2 (0.7)	101 (8.2)	718 (35.1)	1,686 (59.2)	611 (58.9)	73 (57.1)
Race / Ethnicity, No. (%)						
Dutch or European, No. (%)	75 (25.0)	389 (30.3)	1,107 (51.8)	2,105 (71.6)	725 (67.9)	81 (60.0)
Surinamese, No. (%)	61 (20.3)	192 (15.0)	210 (9.8)	165 (5.6)	74 (6.9)	9 (6.7)
Turkish, No. (%)	32 (10.6)	236 (18.4)	253 (11.8)	155 (5.3)	49 (4.6)	6 (4.4)
Moroccan, No. (%)	19 (6.3)	157 (12.2)	192 (9.0)	118 (4.0)	50 (4.7)	12 (8.9)
Cape Verdian or Dutch Antilles, No. (%)	72 (23.9)	190 (14.8)	154 (7.2)	132 (4.6)	49 (4.6)	6 (4.4)
Smoking, No. (%)						
None, No. (%)	161 (55.9)	747 (61.8)	1,454 (73.8)	2,110 (78.0)	717 (73.8)	89 (77.4)
Early-pregnancy only, No. (%)	27 (9.4)	102 (8.4)	172 (8.7)	238 (8.8)	74 (7.4)	7 (6.1)
Continued, No. (%)	100 (34.7)	360 (29.8)	343 (17.4)	358 (13.2)	181 (18.6)	19 (16.5)
Folic acid use No. used (%)	71 (30.5)	809 (83.4)	1,156 (70.0)	1,942 (82.1)	637 (76.7)	73 (71.6)
2nd trimester uterine artery RI, mean (SD)	0.56 (0.09)	0.54 (0.09)	0.54 (0.09)	0.54 (0.09)	0.55 (0.09)	0.56 (0.09)
2nd trimester umbilical artery PI, mean (SD)	1.24 (0.19)	1.23 (0.19)	1.21 (0.18)	1.19 (0.18)	1.18 (0.19)	1.17 (0.19)
3rd trimester uterine artery RI, mean (SD)	0.48 (0.08)	0.48 (0.07)	0.48 (0.08)	0.48 (0.08)	0.50 (0.08)	0.51 (0.07)

Table 1. Continued

Characteristics	<20 years n=338	20-24.9 years n=1,391	25-29.9 years n=2,256	30-34.9 years n=3,045	35-39.9 years n=1,102	≥ 40 years n=139
3rd trimester umbilical artery PI, mean (SD)	0.99 (0.16)	0.99 (0.17)	0.99 (0.17)	0.98 (0.17)	0.98 (0.17)	0.97 (0.19)
3rd trimester uterine artery notching, No. (%)	40 (22.5)	113 (14.8)	137 (10.8)	146 (7.9)	50 (7.6)	6 (8.1)
Birth characteristics						
Males, No.(%)	164 (48.5)	713 (51.3)	1131 (50.1)	1536 (50.4)	572 (51.9)	72 (51.8)
Gestational age at delivery, weeks (IQR)	39.9 (38.7 to 40.8)	40.0 (39.1 to 40.9)	40.1 (39.0 to 41.0)	40.1 (39.4 to 41.0)	40.3 (39.3 to 41.0)	40.3 (38.7 to 41.1)
Birth weight, mean (SD) grams	3,184 (516)	3,315 (522)	3,399 (551)	3,461 (576)	3,487 (549)	3,424 (656)
Small-size for gestational age, No. (%)	53 (15.7)	191 (13.7)	214 (9.5)	254 (8.3)	97 (8.8)	13 (9.4)
Large-size for gestational age, No. (%)	16 (4.7)	75 (5.4)	209 (9.3)	379 (12.4)	122 (11.1)	20 (14.4)
Preterm birth	27 (8.0)	65 (4.7)	128 (5.7)	153 (5.0)	50 (4.5)	9 (6.5)
Assisted vaginal delivery	29 (9.8)	156 (12.4)	301 (14.6)	409 (14.7)	128 (12.7)	10 (7.7)
Caesarean delivery	28 (9.5)	111 (8.8)	249 (12.1)	363 (13.0)	142 (14.0)	32 (24.6)
Gestational hypertension	13 (4.1)	42 (3.2)	80 (3.8)	117 (4.1)	41 (3.9)	4 (3.1)
Preeclampsia	10 (3.2)	23 (1.8)	58 (2.8)	57 (2.0)	15 (1.5)	3 (2.3)
APGAR <7 after 5 minutes, No. (%)	5 (1.6)	18 (1.3)	27 (1.2)	38 (1.3)	11 (1.0)	1 (0.7)
Placental weight (grams) median (IQR)	600 (500 to 695)	600 (520 to 700)	620 (540 to 713)	630 (545 to 725)	619 (530 to 724)	650 (530 to 732)

Abbreviation: IQR: inter quartile range. Values are observed data and represent means (SD), medians (IQR) or number of subjects (valid %).

Table 2. Associations of maternal age with uterine artery resistance indices and notching

	Difference in uterine artery resistance index[a]		Uterine artery notching[b]
	Second trimester n=4,578 SDS difference (95% CI)	Third trimester n=4,479 SDS difference (95% CI)	Third trimester n=4,762 Odds ratio (95% CI)
Maternal age			
<20 years	0.12 (-0.05 to 0.29)	-0.02 (-0.19 to 0.15)	1.97 (1.30 to 3.00)*
	n=159	n=169	n=178
20-24.9 years	-0.02 (-0.05 to 0.08)	-0.00 (-0.10 to 0.09)	1.25 (0.994 to 1.66)
	n=715	n=717	n=755
25-29.9 years	*reference*	*Reference*	*Reference*
	n=1,268	n=1,179	n=1,272
30-34.9 years	-0.00 (-0.08 to 0.07)	0.10 (0.02 to 0.17)*	0.79 (0.61 to 1.03)
	n=1,760	n=1,745	n=1,826
35-39.9 years	0.04 (-0.07 to 0.14)	0.18 (0.08 to 0.29)*	0.77 (0.54 to 1.11)
	n=611	n=601	n=657
≥40 years	0.18 (-0.07 to 0.44)	0.33 (0.08 to 0.57)*	0.85 (0.36 to 2.01)
	n=65	n=68	n=74
Trend[c]	**0.00 (-0.00 to 0.01)**	**0.02 (0.01 to 0.03)***	**0.96 (0.94 to 0.98)***

CI: Confidence Interval; SDS: Standard deviation score;
Models are adjusted for maternal age at intake, smoking, parity, education, BMI, ethnicity, folic acid intake, fetal sex and gestational age at ultrasound measurement.
[a] Values are regression coefficients (95% confidence interval) that reflect the difference in SDS score or odds ratio per measurement per maternal age-group compared to the reference group of women aged between 25 and 29.9 years. Tests for trend were based on multiple linear regression models with maternal age as a continuous variable. The trends are differences in measurements per additional maternal year.
[b] Values are odds ratios (95% confidence interval) compared to the reference group of women aged between 30 and 34.9 years. Tests for trend were based on logistic regression models with maternal age as a continuous variable.
[c] Tests for trend were based on multiple linear and logistic regression models with maternal age as a continuous variable. The trends are differences in regression coefficients and odds ratio per additional maternal year.
** Significant value (p<0.05).*

Table 3. Associations of maternal age with umbilical artery pulsatility indices

	Difference in umbilical artery pulsatility index	
	Second trimester n=6,141 SDS difference (95% CI)	Third trimester n=6,668 SDS difference (95% CI)
Maternal age		
<20 years	0.04 (-0.10 to 0.18)	-0.08 (-0.21 to 0.06)
	n=228	n=259
20-24.9 years	0.06 (-0.02 to 0.14)	-0.01 (-0.09 to 0.07)
	n=978	n=1,104
25-29.9 years	*reference*	*reference*
	n=1,693	n=1,804
30-34.9 years	-0.00 (-0.07 to 0.06)	-0.03 (-0.09 to 0.07)
	n=2,333	n=2,502
35-39.9 years	-0.01 (-0.09 to 0.08)	0.02 (-0.07 to 0.10)
	n=821	n=888
≥40 years	-0.03 (-0.24 to 0.18)	0.02 (-0.17 to 0.21)
	n=88	n=111
Trend	**-0.00 (-0.01 to 0.00)**	**0.00 (-0.00 to 0.01)**

CI: Confidence Interval; SDS: Standard deviation score;
Models are adjusted for maternal age at intake, smoking, parity, education, BMI, ethnicity, folic acid intake, fetal sex and gestational age at ultrasound measurement.
Values are regression coefficients (95% confidence interval) that reflect the difference in SDS per measurement per maternal age-group compared to the reference group of women aged between 25 and 29.9 years. Tests for trend were based on multiple linear regression models with maternal age as a continuous variable. The trends are differences in SDS per additional maternal year.

Maternal age and placental weight, birth weight and birth weight:placental weight ratio

Compared to women aged 25-29.9 years, women aged 35-39.9 years had a lower placental weight (-12 grams (95% CI -24.0;-0.17) and gave birth to newborns with a lower birth weight (-34 grams (95% CI -66;-1.3)), and a higher birth weight:placental weight ratio (ratio difference 0.12 (95% CI 0.03;0.22)) (**Table 4**). Women aged ≥40 gave birth to newborns with a lower birth weight (p-value<0.05), but no difference in placental weight was present. A decreasing trend for birth weight was present across the full range of maternal age (-2.5 grams per additional year (95% CI -4.7;0.3)), but not for placental weight. As higher maternal age was significantly associated with higher third trimester uterine artery vascular resistance and lower birth weight, we explored whether third trimester uterine artery vascular resistance partly explained this observed association

of higher maternal age with a lower birth weight and the risk of delivering an SGA newborn. **Table S1 and S2** show that additional adjustment for third trimester uterine artery vascular resistance partly attenuated the association of maternal age with birth weight, and the risk of delivering an SGA newborn.

Table 4. Associations of maternal age with placental weight, birth weight and birth weight:placental weight ratio

	Placental weight at birth	**Birth weight**	**Birth weight: Placental weight ratio**
	n=6,197 Difference in grams (95% CI)	n=8,224 Difference in grams (95% CI)	n=6,197 Difference in ratio (95% CI)
Maternal age			
<20 years	-6 (-25 to 13)	-23(-74 to 29)	0.04 (-0.10 to 0.19)
	n=249	n=332	n=249
20-24.9 years	-1 (-12 to 10)	0 (-30 to 30)	0.00 (-0.08 to 0.09)
	n=1,064	n=1,381	n=1,064
25-29.9 years	*reference*	*Reference*	*reference*
	n=1,707	n=2,247	n=1,707
30-34.9 years	-0.0 (-9 to 9)	-1 (-25 to 23)	0.00 (-0.07 to 0.07)
	n=2,230	n=3,029	n=2,230
35-39.9 years	-12 (-24 to -0)*	-34 (-66 to -1.3)*	0.12 (0.03 to 0.22)*
	n=833	n=1,096	n=833
≥40 years	-9 (-35 to 18)	-80 (-155 to -6)*	0.01 (-0.20 to 0.22)
	n=114	n=139	n=114
Trend	**-0 (-1 to 0)**	**-2.5 (-4.7 to -0.3)***	**0.00 (-0.00 to 0.01)**

CI: Confidence Interval.

Models are adjusted for maternal age at intake, smoking, parity, education, BMI, ethnicity, folic acid intake, fetal sex and gestational age at birth.

Values are regression coefficients (95% confidence interval) that reflect the difference in grams or ratio per maternal age-group compared to the reference group of women aged between 25 and 29.9 years. Tests for trend were based on multiple linear regression models with maternal age as a continuous variable. The trends are differences in grams or ratio per additional maternal year.

** Significant value (p<0.05)*

DISCUSSION

Principal findings

We observed that after adjustment for socio-demographic and lifestyle factors, young maternal age was associated with an increased risk of third trimester uterine artery notching, whereas advanced maternal age was associated with an increased third trimester uterine artery resistance index. Maternal age was not associated with second trimester uterine artery resistance index or second and third trimester umbilical artery pulsatility indices. Advanced maternal age tended to be associated with lower placental and birth weight and higher birth weight:placental weight ratio, but this association was not consistent across the full range of maternal age.

Results

Both young and advanced maternal age are associated with an increased risk of pregnancy complications[2]. Suboptimal placental function may play a key role in the pathophysiology of these placenta-related complications, but studies focusing on pathophysiological mechanisms are scarce[22, 23]. Obtaining a better insight into potential placenta-related pathophysiological mechanisms underlying the observed associations of young and advanced maternal age with pregnancy complications is important to develop future prevention, screening and treatment strategies for a population that is increasingly of advanced maternal age during pregnancy.

For young maternal age, previous studies have only focused on the associations of young maternal age with placental weight and showed conflicting findings[24, 25]. In a study among 31 adolescent pregnancies, young maternal age had no effect on placental weight, morphometry or cell turnover[25]. A study among 552 mothers and their healthy singleton newborns, found no association of young maternal age with placental weight[26]. However, a study among 431 uncomplicated singleton near-term deliveries, showed that young maternal age was associated with a low birth weight:placental weight ratio[27].We observed that the risk of third trimester uterine artery notching was increased among women aged <20 years, but we did not observe associations with uterine artery resistance or umbilical artery pulsatility indices across the full range. There were no associations of young maternal age with placental weight or placental weight:birth weight ratio. Thus, our findings seem to suggest that young maternal age may specifically be associated with a suboptimal third trimester utero-placental vascular function. Normally, in early pregnancy, trophoblast invasion and spiral artery remodelling takes place to ensure adequate blood flow to the placenta, leading to larger vessels with lower resistance and increased end diastolic flow[28]. If these processes are inadequate,

abnormal uterine artery flow patterns with higher resistance indices and notching may be observed, which is strongly associated with the risk of pregnancy complications. This may be the case in biologic immaturity among adolescent pregnancies[29]. It might be that young maternal age mostly affects placentation leading to an increased risk of notching, but due to overall adequate vascular quality and dynamics of young women, small changes in utero-placental flow and resistance can be more easily compensated. The lack of associations with other placental vascular function markers and placental weight might be due to the relatively high (19 years) young maternal age in our study.

The effects of advanced maternal age on placental function have been studied in larger populations. A study among 536,954 singleton births showed that older women had larger placentas[30]. It was suggested that this enlargement represents a biological compensatory mechanism for suboptimal placental function, to secure a threatened pregnancy[30]. Possibly, other maternal characteristics which influence placental weight and are strongly related to maternal age, such as parity, may be responsible for the larger placentas among older women[30]. A observational prospective study among 24,152 singleton livebirths, found that after correction for maternal characteristics, such as parity, BMI, cigarette use, socio-economic status and race, higher maternal age was associated with lower placental weight[31]. A cross-sectional study among 884 pregnant women showed that after adjustment for gestational age and parity, advanced maternal age was associated with an increased uterine artery pulsatility index in the second half of pregnancy[32]. We observed that after correction for socio-demographic and lifestyle factors, higher maternal age was associated with an increased third trimester uterine artery resistance index, and that the effect of maternal age on uterine artery vascular resistance is already visible from 30 years onwards. These effects of advanced maternal age on third trimester uterine artery vascular resistance were small and within the normal range. However, several studies have shown that small increases in utero-placental vascular resistance, even within the normal range, are associated with pregnancy complications[33-36]. Importantly, the direction of the normal changes in hemodynamics during pregnancy seems to be opposite to the changes that occur in ageing[37]. Previous studies have shown that with ageing, uterine blood flow diminishes, uterine blood vessels are less compliant, and endothelium-dependent function is altered[32, 37, 38]. The increased uterine artery vascular resistance may indeed be explained by general reduced vascular compliance among older women, whereas newly constructed fetal vasculature is not affected by the effects of advanced maternal age on vascular quality, which could explain lack of effect on feto-placental vascular function in our study. As differences in third trimester uterine artery vascular resistance were within the normal range and we observed no associations with the risk of third trimester notching, our findings may suggest that not suboptimal placentation explains these observed associations,

but rather overall reduced vascular quality due to advanced maternal age. We further observed that higher maternal age was associated with a lower birth weight, and an increased risk of delivering an SGA newborn, and that this association attenuated after considering third trimester uterine artery vascular resistance. This suggests that even this small difference in third trimester utero-placental vascular function among older women may play a pathophysiological role in the established associations of advanced maternal age with an increased risk of pregnancy complications, such as an abnormal birth weight.

Our findings provide insight into potential pathophysiological mechanisms explaining observed associations of young and advanced maternal age with pregnancy complications. From a clinical perspective, measurement of utero-placental vascular function among pregnant women with a young or advanced maternal age could possibly aid in screening for those pregnancies at risk of adverse pregnancy outcomes. However, the additional value of using utero-placental vascular function for screening for adverse pregnancy outcomes may depend upon specific populations and pregnancy outcomes of interest. We have previously shown within the same study population that among low-risk, multi ethnic women combined second and third trimester utero-placental vascular function ultrasound results in addition to maternal characteristics improved screening for pre-eclampsia but not for gestational hypertension[39]. A systematic review has shown that model performance for screening for gestational hypertensive disorders varies with the use of different maternal, fetal and placental characteristics among low-risk and high-risk populations[40]. A meta-analysis comprising seventeen observational studies showed that among SGA fetuses and newborns, which is considered a high-risk population, concluded that an increased UtA-PI increased the risk of adverse perinatal outcomes, but because of limited predictive capacity as a standalone test, UtA-PI should be combined in combination with other tests[41]. Although causality cannot be established in observational research, these findings suggest that maternal age may, through suboptimal utero-placental vascular function, influence pregnancy outcomes. Among young maternal age pregnancies, impaired placental development may be due to biologic immaturity, whereas among advanced maternal age pregnancies, reduced vascular quality due to ageing may play a key role. Further mechanistic studies are needed to obtain a better understanding of these potential pathways, by using more advanced placental imaging techniques from early pregnancy onwards, placental biomarkers or detailed assessments of placental vasculature at birth through placental biopsy. Large meta-analyses on patient level data are necessary to enable assessment of associations at the extremes of the maternal age spectrum where numbers are smaller and to enable identification of the optimal maternal age at pregnancy for various pregnancy outcomes.

Strengths and limitations

Bias due to nonresponse at baseline is unlikely because biased estimates in large cohort studies mainly arise from loss to follow-up rather than from nonresponse at baseline[42]. Selection of a healthy population might affect the generalizability of results to higher-risk populations. As clinical practice guidelines during the inclusion period of the current study (2001-2006) did not recommend Aspirin prophylaxis, we do not have information on Aspirin use available. Although we do not think that the use Aspirin prophylaxis, or rather the lack thereof, has biased the results of the current study, it may limit the generalizability of our results to contemporary populations. Finally, we had a relatively small number of women in the age group 40 years and older and these results should be interpreted with caution. Although we adjusted for a number of potential confounders, residual confounding by other lifestyle factors might still be present.

Conclusions

Young maternal age is associated with higher risk of third trimester uterine artery notching, whereas advanced maternal age is associated with higher third trimester uterine artery resistance index, which may predispose to an increased risk of pregnancy complications. These associations are not explained by maternal socio-demographic or lifestyle characteristics.

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SUPPLEMENTARY MATERIALS

Table S1. Associations of maternal age with birth weight and the role of additional adjustment for third trimester uterine artery resistance index

	Absolute birth weight		**Gestational age adjusted SDS for birth weight**	
	Adjusted	Additionally adjusted for 3rd trimester uterine artery resistance index.	Adjusted	Additionally adjusted for 3rd trimester uterine artery resistance index.
Maternal age				
<20 years	-27.9 (-93.1 to 37.3)	-17.3 (-101.4 to 66.8)	-0.07 (-0.19 to 0.05)	-0.03 (-0.19 to 0.14)
20-24.9 years	22.83 (-14.9 to 60.5)	23.0 (-25.5 to 71.5)	-0.00 (-0.07 to 0.07)	-0.03 (-0.12 to 0.07)
25-29.9 years	*Ref*	*Ref*	*Ref*	*Ref*
30-34.9 years	-9.0 (-39.6 to 21.6)	-1.2 (-39.9 to 37.5)	-0.01 (-0.06 to 0.05)	-0.00 (-0.08 to 0.07)
35-39.9 years	-23.8 (-64.6 to 17.1)	-33.5 (-85.3 to 18.2)	-0.07 (-0.15 to 0.00)	-0.07 (-0.17 to 0.03)
≥40 years	-107.8 (-201.7 to -13.8)*	-18.3 (-142.0 to 105.4)	-0.17 (-0.34 to 0.00)	0.00 (-0.23 to 0.24)
Trend	**-3.3 (-6.08 to -0.59)***	**-1.97 (-5.5 to 1.6)**	**-0.01 (-0.01 to -0.00)***	**-0.00 (-0.01 to 0.00)**

All models are adjusted for maternal age at intake, smoking, parity, education, BMI, ethnicity, folic acid intake, fetal sex and gestational age at 3rd trimester uterine artery measurement. Additionally adjusted models are additionally adjusted for 3rd trimester uterine artery resistance index.

Values are regression coefficients (95% confidence interval) that reflect the difference in birth weight in grams or SD score per maternal age-group compared to the reference group of women aged between 25 and 29.9 years. Tests for trend were based on multiple linear regression models with maternal age as a continuous variable. The trends are differences in birth weight in grams or SD score per additional maternal year.

** Significant value (p<0.05)*

Table S2. Associations of maternal age with small-size for gestational age at birth and the role of additional adjustment for third trimester uterine artery resistance index

	Small-size for gestational age	
	Adjusted	Additionally adjusted for 3rd trimester uterine artery resistance index.
Maternal age		
<20 years	0.94 (0.66 to 1.33)	0.92 (0.54 to 1.54)
20-24.9 years	1.07 (0.86 to 1.34)	1.26 (0.92 to 1.73)
25-29.9 years	*Ref*	*Ref*
30-34.9 years	1.10 (0.90 to 1.35)	1.08 (0.81 to 1.45)
35-39.9 years	1.34 (1.03 to 1.75)*	1.31 (0.90 to 1.92)
≥40 years	1.53 (0.84 to 2.81)	1.59 (0.69 to 3.70)
Trend	**1.02 (1.00 to 1.04)***	**1.01 (0.99 to 1.04)**

All models are adjusted for maternal age at intake, smoking, parity, education, BMI, ethnicity, folic acid intake, fetal sex and gestational age at 3rd trimester uterine artery measurement.
Additionally adjusted models are additionally adjusted for 3rd trimester uterine artery resistance index.
Values are regression coefficients (95% confidence interval) that reflect odds ratios for small-size for gestational age compared to the reference group of women aged between 25 and 29.9 years. Tests for trend were based on multiple logistic regression models with maternal age as a continuous variable. The trends are differences in odds ratio for small-size for gestational age per additional maternal year.
** Significant value (p<0.05)*

ETIOLOGICAL STUDIES

2.2: ASSOCIATIONS OF MATERNAL EARLY-PREGNANCY GLUCOSE CONCENTRATIONS WITH PLACENTAL HEMODYNAMICS, BLOOD PRESSURE AND GESTATIONAL HYPERTENSIVE DISORDERS

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Jan S. Erkamp
Madelon L. Geurtsen
Liesbeth Duijts
Irwin K.M. Reiss
Annemarie G.M.G.J. Mulders
Eric A.P. Steegers
Romy Gaillard
Vincent W.V. Jaddoe

ABSTRACT

Background

Gestational diabetes mellitus is associated with increased risks of gestational hypertension and preeclampsia. We hypothesized that high maternal glucose concentrations in early pregnancy are associated with adverse placental adaptations and subsequently altered utero-placental hemodynamics during pregnancy, predisposing to an increased risk of gestational hypertensive disorders.

Methods

In a population-based prospective cohort study from early pregnancy onwards, among 6,078 pregnant women, maternal early-pregnancy non-fasting glucose concentrations were measured. Mid and late pregnancy uterine and umbilical artery resistance indices were assessed by Doppler ultrasound. Maternal blood pressure was measured in early, mid and late pregnancy and the occurrence of gestational hypertensive disorders was assessed using hospital registries.

Results

Maternal early-pregnancy glucose concentrations were not associated with mid or late pregnancy placental hemodynamic markers. A 1 mmol/l increase in maternal early-pregnancy glucose concentrations was associated with 0.71 mmHg (95% Confidence Interval (CI) 0.22 to 1.22) and 0.48 mmHg (95% CI 0.10 to 0.86) higher systolic and diastolic blood pressure in early pregnancy, respectively, but not with blood pressure in later pregnancy. Also, maternal glucose concentrations were not associated with the risks of gestational hypertension or preeclampsia.

Conclusions

Maternal early-pregnancy non-fasting glucose concentrations within the normal range are associated with blood pressure in early pregnancy, but do not seem to affect placental hemodynamics and the risks of gestational hypertensive disorders.

BACKGROUND

Gestational diabetes complicates up to 17% of all pregnancies and is a strong risk factor for gestational hypertensive disorders[1, 2]. In pregnant women with pre-gestational diabetes, hyperglycaemia causes a pro-inflammatory environment and cytokine derangements that acts on the endothelium, and leads to placental vascular changes, whereas insulin may have a direct toxic effect on the placenta[3, 4]. Also, pregnancies complicated by obesity or gestational diabetes show dysregulation of metabolic, vascular and inflammatory pathways[5, 6]. This dysregulation is characterized by increased circulating concentrations of inflammatory molecules and placental overexpression of genes encoding for inflammatory mediators[5, 6]. Studies have shown that hyperglycaemia during pregnancy is associated with reduced invasiveness of the trophoblast, increased oxidative stress in the maternal and fetal milieu, disrupted vasculogenesis, and macroscopically and histologically altered placentae[4, 7-11]. Treatment of gestational diabetes has been shown to reduce the prevalence of preeclampsia[12]. It is not known yet to what extent early-pregnancy non-fasting glucose concentrations may influence early placental adaptations, blood pressure and predispose women to gestational hypertensive disorders.

We hypothesized that high maternal glucose concentrations in early pregnancy are associated with adverse placental adaptations and subsequently altered utero-placental hemodynamics during pregnancy, predisposing to an increased risk of gestational hypertensive disorders. We examined in a low-risk, multi-ethnic, population-based prospective cohort study among 6,078 pregnant women, the associations of maternal early-pregnancy non-fasting glucose concentrations with placental flow measures, blood pressure throughout pregnancy, and gestational hypertensive disorders.

METHODS

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards in Rotterdam, the Netherlands. All pregnant woman and their children who were living within the city of Rotterdam at the time of birth were eligible to participate[13]. The study has been approved by the local Medical Ethical Committee (MEC 198.782/2001/31). Written consent was obtained from all participating women. All pregnant women were enrolled between 2001 and 2005. Response rate at birth was 61%[14]. In total, 8,879 women were enrolled during pregnancy. For the current study, 6,869 women were eligible as they enrolled before 18 weeks of gestational age

and had singleton livebirths. Women with no data on maternal early-pregnancy glucose metabolism or with all outcome measures missing were excluded (n=763). Women with pregestational diabetes (n=21) and women with unreliable glucose concentrations (<1 mmol/l) were excluded (n=7). The population for analysis comprised 6,078 pregnant women (**Figure 1**). All measurements in pregnancy were performed by trained research assistants who were part of the study team.

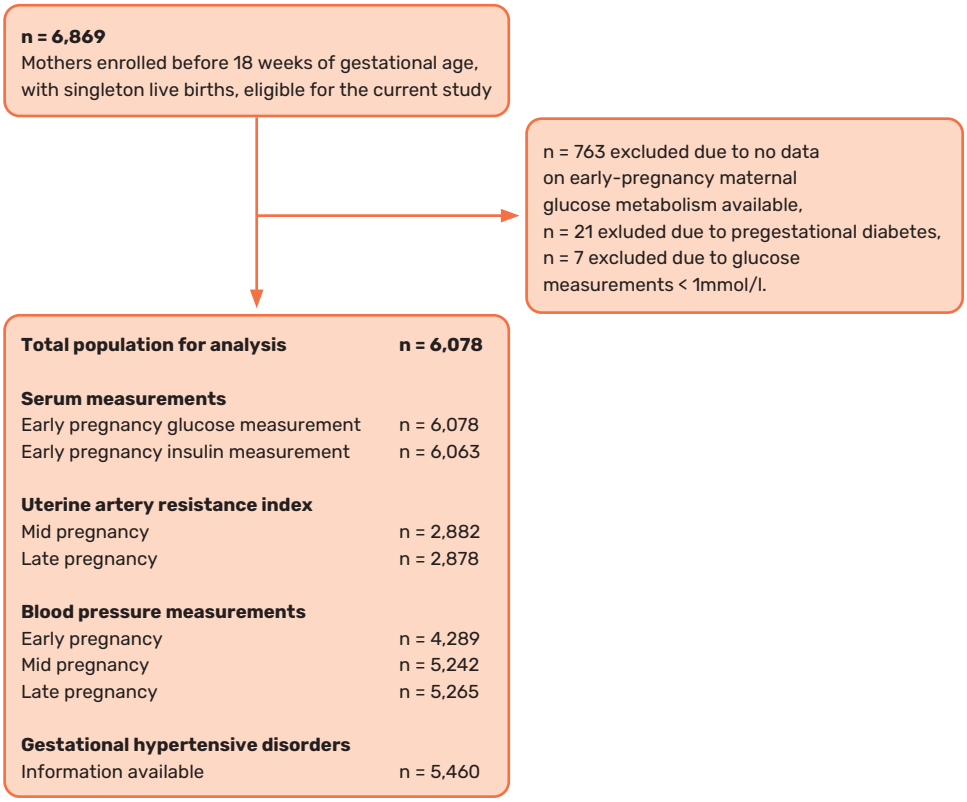

Figure 1. Flowchart population for analysis.

Maternal glucose concentrations

Blood samples were collected once in early pregnancy at 13.2 median weeks' gestation (95% range 9.6;17.6), as described previously[15]. After 30 min of fasting, venous blood samples were collected from pregnant women, by specifically trained research nurses who were part of the research team, and temporally stored at room temperature for a maximum of 3 hours. We considered the 30 min fasting samples non-fasting

samples. This time-interval was chosen because of the design of our study, in which it was not possible to obtain fasting samples from all pregnant women. At least every 3 hours, blood samples were transported to a dedicated laboratory facility (Star-MDC, Rotterdam, The Netherlands), for further processing and storage[16]. Glucose (mmol/l) is an enzymatic quantity and was measured with the c702 module on a Cobas 8000 analyser (Roche, Almere, The Netherlands). Insulin (pmol/l) was measured with electrochemiluminescence immunoassay on a Cobas e411 analyser (Roche, Almere, The Netherlands). Quality control samples demonstrated intra- and inter-assay CVs of 1.30% and 2.50%, respectively. Information on pre-gestational diabetes mellitus was obtained from self-reported questionnaires and on gestational diabetes from medical records after delivery. Gestational diabetes was diagnosed by a community midwife or an obstetrician according to Dutch midwifery and obstetric guidelines using the following criteria: either a random glucose concentration >11.0 mmol/l, a fasting glucose ≥7.0 mmol/l or a fasting glucose between 6.1 and 6.9 mmol/l with a subsequent abnormal glucose tolerance test[17].

Placenta hemodynamic characteristics

Ultrasound examinations were carried out in two dedicated research centers in the city of Rotterdam in early (median 13.2 weeks gestational age, interquartile range (IQR) 12.2;14.9), mid (median 20.4 weeks gestational age, IQR 19.9;21.1) and late pregnancy (median 30.2 weeks gestational age, IQR 29.9;30.6). We established gestational age by using data from the first ultrasound examination[18]. Uterine artery resistance index (UtA-RI) and umbilical artery pulsatility index (UA-PI) were derived from flow velocity waveforms in mid and late pregnancy. Standard deviation scores (SDS) for UtA-RI and UA-PI were based on values from the whole study population and represent the equivalent of z-scores. Late pregnancy uterine artery notching was diagnosed if a notch was present uni- or bilaterally, as a result from increased blood flow resistance, which is a sign of placental insufficiency[19].

Blood pressure and gestational hypertensive disorders

Blood pressure was measured at each pregnancy visit (median gestational age 13.2 weeks, (IQR 12.2;14.9); 20.4 weeks, (IQR 19.9;21.1) and 30.2 weeks, (IQR 29.9;30.6) using an Omron 907 automated digital oscillometer sphyghmanometer (OMRON Healthcare Europe, Hoofddorp, the Netherlands)[20]. The mean value of two blood pressure readings over a 60-s interval was documented for each participant[21].

Information about hypertensive disorders in pregnancy was obtained from medical records[14]. The occurrence of hypertension and related complications were cross-

validated using hospital registries, and defined using criteria of the International Society for the Study of Hypertension in Pregnancy[22, 23]. Gestational hypertension was defined as de novo hypertension alone (an absolute blood pressure 140/90 mmHg or greater), appearing after 20 weeks gestational age. Preeclampsia was defined as de novo hypertension (blood pressure>=140/90 mmHg) after the 20th gestational week with concurrent proteinuria (0.3 grams or greater in a 24-hour urine specimen or 2+ or greater [1 g/l] on a voided specimen or 1+ or greater [0.3 g/l] on a catheterized specimen). Any gestational hypertensive disorder was defined as either gestational hypertension or preeclampsia.

Covariates

Maternal height (cm) and weight (kg) were measured without shoes and heavy clothing at enrolment and body mass index (BMI, kg/m^2) was calculated. Information about pre-pregnancy weight, ethnicity (European/non-European) and education (higher education yes/no) was obtained by questionnaire[14]. Folic acid supplementation, categorized as use versus no use, and parity, categorized as nulliparous or multiparous, were obtained at enrolment by questionnaire[24]. Information about smoking was available from questionnaires, and was classified as 'yes' if the woman smoked until pregnancy was known and if she continued to smoke throughout pregnancy[25].

Statistical analyses

First, we conducted a non-response analysis to compare characteristics of women with and without glucose measurements available. Second, we assessed the associations of maternal early-pregnancy non-fasting glucose concentrations continuously with mid and late pregnancy uterine artery and umbilical artery resistance indices and late pregnancy uterine artery notching, and with blood pressure in early, mid and late pregnancy, using linear and logistic regression models. We also analysed the longitudinal systolic and diastolic blood pressure patterns in women using unbalanced repeated measurement regression models[26]. These models take the correlation between repeated measurements of the same subject into account, and allow for incomplete outcome data. Using fractional polynomials of gestational age, the best-fitting models were constructed. For presentation purposes, we constructed tertiles of maternal glucose concentrations for these analyses. Third, we assessed the associations of maternal early-pregnancy non-fasting glucose concentrations continuously with gestational hypertensive disorders (gestational hypertension, preeclampsia), using logistic regression models. For all analyses, we constructed different models to explore whether any association was explained by maternal socio-demographic and lifestyle factors. The basic model was adjusted for gestational age at glucose measurement; the main model

was additionally adjusted for gestational age at assessment, maternal ethnicity, age, parity, educational level, smoking, and folic acid supplement use; and the maternal BMI model was additionally adjusted for maternal pre-pregnancy BMI. Included covariates were based on previous studies, strong correlations with exposure and outcomes, and changes in effect estimates of >10%. We further tested but did not observe statistical interactions between maternal pre-pregnancy BMI and maternal early-pregnancy non-fasting glucose concentrations for the associations with uterine and umbilical artery resistance indices and blood pressure. Statistical interaction terms were tested by including the term maternal pre-pregnancy BMI * maternal early-pregnancy non-fasting glucose concentrations in the regression model. We performed three sensitivity analyses. First, analyses were repeated using maternal early-pregnancy non-fasting insulin concentrations. Second, to test whether the associations of maternal early-pregnancy non-fasting glucose concentrations with high blood pressure we excluded women with gestational diabetes (n=66). Third, to test whether a cut-off effect was present, we tested for differences in associations with blood pressure between women in quintiles of glucose concentrations, with the lowest quintile used as the reference group. We used multiple imputation for missing values of covariates according to Markov Chain Monte Carlo method[27]. The percentage of missing data was <10%, except for smoking (15%) and folic acid supplement use (31.2%). Five imputed datasets were created and pooled for analyses. No significant differences in descriptive statistics were found between the original and imputed datasets. The repeated measurement analysis was performed using the Statistical Analysis System version 9.4 (SAS, Institute Inc., Gary, NC, USA), including the Proc Mixed module for unbalanced repeated measurements. All other analyses were performed using the Statistical Package of Social Sciences version 24.0 for Windows (IBM Corp., Armonk, NY, USA).

RESULTS

Population characteristics

Population characteristics are shown in **Table 1**. Mean maternal early-pregnancy glucose concentrations were 4.4 mmol/l. In total, 64(1.1%) women were diagnosed with gestational diabetes. Late pregnancy uterine artery notching occurred in 312(10.2%) participants. Gestational hypertension developed in 203(3.8%) women and preeclampsia developed in 131(2.4%) women. Non-response analyses showed that women without glucose measurements were more often parous, had a lower level of educational attainment,

used folic acid supplementation more often, were more often of non-European descent, and had a higher mid pregnancy and a lower late pregnancy uterine artery resistance index (**Table S1**). Histogram for maternal glucose concentrations given in **Figure S1**.

Table 1. Characteristics of mothers (n=6,078)

Characteristics	
Maternal characteristics	
Age, mean (SD), years	29.8 (5.1)
Height, mean (SD), cm	167.5 (7.4)
Weight before pregnancy, mean (SD), kg	66.4 (12.7)
Body Mass Index, median (IQR), kg/m^2	22.6 (20.7 to 25.4)
Parity, No. nulliparous (%)	3,458 (57.4)
Education, No. higher education (%)	2,538 (44.9)
Race / Ethnicity	
Dutch or European, No. (%)	3,558 (61.0)
Surinamese, No. (%)	503 (8.6)
Turkish, No. (%)	472 (8.1)
Moroccan, No. (%)	352 (6.0)
Cape Verdian or Dutch Antilles, No. (%)	410 (7.1)
Smoking	
None, No. (%)	3,712 (72.2)
Early-pregnancy only, No. (%)	452 (8.8)
Continued, No. (%)	974 (19.0)
Folic acid use No. used (%)	2,943 (47.4)
Pre-gestational diabetes mellitus, No. (%)	0 (0)
Blood pressure, mean (SD), mmHg	
Early pregnancy	115 (12.3) /68 (9.6)
Mid pregnancy	116 (12.0) /67 (9.4)
Late pregnancy	118 (12.0) / 69 (9.4)
Mid pregnancy uterine artery resistance index, mean (SD)	0.54 (0.09)
Late pregnancy uterine artery resistance index, mean (SD)	0.49 (0.08)
Late pregnancy uterine artery notching, No. (%)	312 (10.2)
Glucose, mean (SD), mmol/l	4.4 (0.84)
Insulin, median (IQR), pmol/l	115.1 (55.4 to 233.4)
Gestational diabetes mellitus, No. (%)	64 (1.1)
Gestational hypertension, No. (%)	203 (3.8)
Preeclampsia, No. (%)	131 (2.4)

Table 1. Continued

Characteristics	
Birth characteristics	
Males, No.(%)	3,076 (50.6)
Gestational age at delivery, median (IQR), weeks	40.1 (39.1 to 41.0)
Preterm birth, No (%)	310 (5.1)
Birth weight, mean (SD), grams	3,417 (564)
Placental weight, median (IQR), grams	610 (530 to 720)

Values are observed data and represent means (SD), medians (IQR) or number of subjects (valid %). Abbreviation: IQR: inter quartile range.

Early pregnancy glucose concentrations and placental hemodynamics

Maternal early-pregnancy glucose concentrations were not associated with mid and late pregnancy uterine artery resistance indices, umbilical artery pulsatility indices, and risk of late pregnancy uterine artery notching (**Table 2**).

Table 2. Associations of maternal early-pregnancy glucose concentrations with mid and late pregnancy placental flow measures (n=4,236)

Maternal early-pregnancy glucose concentrations (mmol/l)	**Uterine artery**		**Umbilical artery**
	Resistance index (95% Confidence Interval)	**Notching** (95% Confidence Interval)	**Pulsatility index** (95% Confidence Interval)
Mid pregnancy			
Basic model	-0.00 (-0.02 to 0.02)	Not available	0.03 (-0.01 to 0.07)
Main model	-0.00 (-0.05 to 0.04)	Not available	0.03 (-0.01 to 0.07)
BMI model	-0.02 (-0.07 to 0.03)	Not available	0.02 (-0.02 to 0.06)
Late pregnancy			
Basic Model	-0.00 (-0.03 to 0.02)	0.96 (0.84 to 1.09)	-0.02 (-0.06 to 0.01)
Main model	-0.00 (-0.05 to 0.04)	0.95 (0.82 to 1.09)	-0.02 (-0.06 to 0.02)
BMI model	-0.03 (-0.08 to 0.02)	0.92 (0.79 to 1.08)	-0.02 (-0.07 to 0.02)

Values are SDSs (95% CI) from linear regression models, reflecting differences in measures of uterine and umbilical artery flow measures, and OR (95% CI) reflecting difference in risk of late pregnancy uterine artery notching, per 1 mmol/l increase in maternal early-pregnancy non-fasting glucose concentrations. Estimates are from multiple imputed data.
Basic model: Adjusted for gestational age at glucose measurement.
Main model: Gestational age at glucose measurement, gestational age at ultrasound, maternal ethnicity, age, parity, educational level, smoking, and folic acid supplement use.
BMI model: Main model additionally adjusted for maternal pre-pregnancy BMI.

Early pregnancy glucose concentrations, blood pressure and gestational hypertensive disorders

Associations of maternal early-pregnancy glucose concentrations with blood pressure in early, mid and late pregnancy are shown in **Table 3**. A 1 mmol/l increase in maternal early-pregnancy glucose concentrations was associated with 0.71 mmHg (95% Confidence Interval (CI) 0.22;1.22) and 0.48 mmHg (95%CI 0.10;0.86) higher systolic and diastolic blood pressure in early pregnancy, respectively, but not with blood pressure in later pregnancy. Using repeated measurements analysis (**Figure 2**), we observed that tertiles of maternal early-pregnancy glucose concentrations were not associated with blood pressure over time (p value for interaction of early-pregnancy glucose concentrations with gestational age >0.05, **Table S5**). Also, maternal early-pregnancy glucose concentrations were not associated with the risks of gestational hypertensive disorders (**Table 4**).

Table 3. Associations of maternal early-pregnancy glucose concentrations with early, mid and late pregnancy blood pressure (n=5,265)

Maternal early-pregnancy glucose concentrations (mmol/l)	**Systolic blood pressure,** mmHg (95% Confidence Interval)	**Diastolic blood pressure,** mmHg (95% Confidence Interval)
Early pregnancy		
Basic model	0.37 (-0.08 to 0.81)	0.40 (0.06 to 0.75)*
Main model	0.47 (0.03 to 0.92)*	0.40 (0.06 to 0.75)*
BMI model	0.71 (0.22 to 1.22)*	0.48 (0.10 to 0.86)*
Mid pregnancy		
Basic model	0.13 (-0.30 to 0.48)	-0.13 (-0.44 to 0.18)
Main model	0.19 (-0.21 to 0.59)	-0.12 (-0.43 to 0.20)
BMI model	0.36 (-0.09 to 0.80)	-0.02 (-0.37 to 0.33)
Late pregnancy		
Basic model	0.21 (-0.18 to 0.61)	0.19 (-0.12 to 0.50)
Main model	0.25 (-0.15 to 0.65)	0.18 (-0.13 to 0.49)
BMI model	0.36 (-0.08 to 0.80)	0.24 (-0.10 to 0.59)

Values are mmHg (95% CI) from linear regression models, reflecting differences in systolic and diastolic blood pressure, per 1 mmol/l increase in maternal early-pregnancy non-fasting glucose concentrations Estimates are from multiple imputed data.

Basic model: Adjusted for gestational age at glucose measurement.

Main model: Gestational age at glucose measurement, gestational age at blood pressure measurement, maternal ethnicity, age, parity, educational concentrations, smoking, and folic acid supplement use.

BMI model: Main model additionally adjusted for maternal pre-pregnancy BMI.

**p-value < 0.05*

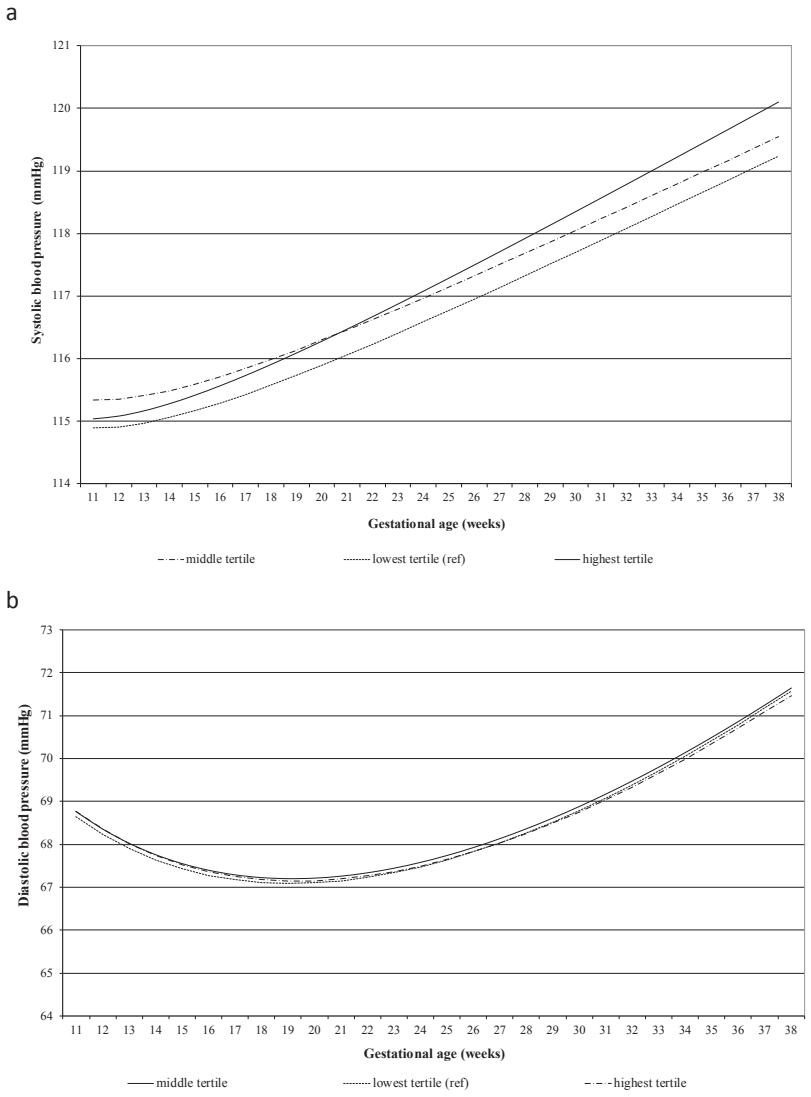

Figure 2. Longitudinal associations between tertiles of early-pregnancy glucose concentrations and blood pressure (n=6,078).

Blood pressure patterns in different maternal early-pregnancy glucose tertiles. (a) Systolic and (b) diastolic blood pressure in different maternal early-pregnancy glucose tertiles (n=6,078). Results reflect the change in mmHg in mothers with early-pregnancy glucose concentrations in the second (4.0-4.6 mmol/l) and third (4.6-10.3 mmol/l) tertile, compared to those with glucose levels in the first tertile (1.0-4.0 mmol/l). (a) Systolic blood pressure = $\beta_0 + \beta_1 \times$ glucose tertile + $\beta_2 \times$ gestational age + $\beta_3 \times$ gestational age^{-2} + $\beta_4 \times$ glucose tertile × gestational age. (b) Diastolic blood pressure = $\beta_0 + \beta_1 \times$ glucose tertile + $\beta_2 \times$ gestational age + $\beta_3 \times$ gestational age$^{0.5}$ + $\beta_4 \times$ glucose tertile × gestational age. The models were adjusted for gestational age at intake. The interaction term of maternal early-pregnancy glucose tertile with gestational age in weeks was not significant. Similarly, when glucose was used continuously in the models, no significant interaction of maternal early-pregnancy glucose concentration with gestational age in weeks was observed. Estimates are given in **Table S5**.

Table 4. Associations of maternal early-pregnancy glucose concentrations with the risks of gestational hypertensive disorders (n=5,459)

Maternal early-pregnancy glucose concentrations (mmol/l)	**Gestational hypertension** (95% Confidence Interval) n=203	**Preeclampsia** (95% Confidence Interval) n=131	**Any gestational hypertensive disorder** (95% Confidence Interval) n=334
Basic model	1.01 (0.86 to 1.20)	0.98 (0.81 to 1.17)	0.95 (0.83 to 1.09)
Main model	1.02 (0.86 to 1.20)	0.87 (0.70 to 1.09)	0.96 (0.84 to 1.10)
BMI model	0.98 (0.82 to 1.18)	0.88 (0.69 to 1.11)	0.94 (0.81 to 1.09)

Values are ORs (95% CI) from logistic regression models, reflecting differences in risk of gestational hypertensive disorders, per 1 mmol/l increase in maternal early-pregnancy non-fasting glucose concentrations. Estimates are from multiple imputed data.

Basic model: Adjusted for gestational age at glucose measurement.

Main model: Gestational age at glucose measurement, maternal ethnicity, age, parity, educational level, smoking, and folic acid supplement use.

BMI model: Main model additionally adjusted for maternal pre-pregnancy BMI.

Sensitivity analyses

In mid pregnancy, higher insulin concentrations were associated with a higher umbilical artery pulsatility index in the basic and main model, but the association attenuated in the BMI model (**Table S2**). In the BMI model, higher early-pregnancy insulin concentrations were associated with a higher early-pregnancy systolic blood pressure (**Table S3**). We found similar results to the main findings when we excluded women with gestational diabetes (data not shown). Finally, no differences in associations with blood pressure between women with non-fasting glucose concentrations in quintiles were observed (data not shown).

DISCUSSION

Our findings suggest that higher maternal early-pregnancy non-fasting glucose concentrations are associated with higher blood pressure in early pregnancy, but no associations were present with blood pressure in mid or late pregnancy. Also, maternal early-pregnancy non-fasting glucose concentrations were not associated with placental hemodynamics or gestational hypertensive disorders.

Meaning of the current study and findings

Hyperglycaemia during pregnancy is associated with miscarriage, fetal structural anomalies, fetal macrosomia, fetal demise, preterm birth and gestational hypertensive disorders[28, 29]. Limited evidence for early pregnancy screening for diabetes in the general

population exists, although testing can be performed as early as the first prenatal visit if a high degree of suspicion of undiagnosed type 2 diabetes exists[28]. Current clinical guidelines advise screening for pre-gestational diabetes among women with overweight and additional risk-factors[28, 30]. In clinical practice, the diagnosis of gestational diabetes is usually made in second half of pregnancy. However, high glucose concentrations may already have contributed to risk of gestational hypertensive disorders and other adverse effects on maternal and fetal health before gestational diabetes and associated complications such as fetal macrosomia and polyhydramnios become apparent[29]. Optimization of glucose regulation in the case of gestational diabetes and pre-gestational diabetes leads to a strong reduction of risk of gestational hypertensive disorders[31]. Therefore, early pregnancy may be a critical period for adverse effects of increased glucose concentrations on fetal and maternal pregnancy outcomes. Previously we reported associations of higher maternal early-pregnancy non-fasting glucose concentrations with decreased fetal growth rates in mid-pregnancy and increased fetal growth rates from late pregnancy onwards, and an increased risk of delivering a large-for-gestational-age infant[15]. Early placental development may play an important role in these associations. Next to its adverse effects on fetal growth, inadequate placental development may play an important role in the development of gestational hypertensive disorders.

Early pregnancy is a critical period for optimal placental development. In this period, trophoblast invasion and spiral artery remodelling takes place to ensure adequate blood flow to the placenta, leading to larger vessels with lower resistance and increased end-diastolic flow[32]. Normally, in early pregnancy, cardiac output increases, peripheral vascular resistance is reduced, and blood pressure decreases until mid-pregnancy, returning to baseline at term[32]. If these processes are inadequate, increased blood pressure, abnormal uterine artery Dopplers with higher resistance indices and notching may be observed, and gestational hypertension or preeclampsia may develop. Previous studies have shown that women with prediabetes defined as HbA_{1c} of 5.7-6.4% in early pregnancy represent a high-risk group for development of gestational hypertensive disorders[33, 34]. It is unclear how early-pregnancy glucose concentrations across the full range influence placental flow measures, blood pressure and gestational hypertensive disorders. We hypothesized that higher early-pregnancy non-fasting glucose concentrations negatively influence placental flow measures, blood pressure and risk of gestational hypertensive disorders.

Previous studies report associations of glucose concentrations with placental flow measures[35, 36]. In a study among 231 pregnant women with polycystic ovarian syndrome, early pregnancy and, more strongly, mid pregnancy fasting glucose concentrations,

were positively associated with an increased mid-pregnancy uterine artery pulsatility index[35]. A retrospective study among 155 pre-gestational diabetic women suggested a positive correlation between concentrations of HbA_{1c} and increased vascular resistance in the uterine and umbilical arteries, suggesting that hyperglycaemia may influence uterine and placental vessel endothelial function[36]. In the current study in a low risk healthy population, we did not observe associations of maternal early-pregnancy glucose concentrations with placental flow measures. The difference in results may be explained by our low-risk, non-diabetic population. Also, glucose concentrations in early pregnancy may not influence placental flow measures measured later in pregnancy.

Diabetes and hypertension often occur simultaneously and show a substantial overlap in disease aetiology and risk-factors, such as genetics, obesity, insulin resistance and inflammation[37-39]. Due to prolonged exposure to effects of hyperglycaemia, we expected to find stronger associations of early-pregnancy glucose concentrations with blood pressure throughout pregnancy. In the current study, we observed associations of maternal early-pregnancy non-fasting glucose concentrations with early-pregnancy blood pressure, but not later in pregnancy. Possibly, this may be due to the fact that the time between the exposure and the outcome is large, and as the effect estimates are already small and within the normal range in early pregnancy, the effect of early-pregnancy glucose concentrations on blood pressure in mid or late pregnancy may not be detectable, or no association may present at all. Possibly, a more pronounced effect on cardiovascular outcomes may be observed in the presence of sustained elevated glucose concentrations. It has been shown that gestational diabetes leads to a strongly increased risk of gestational hypertensive disorders[1, 2]. Simultaneously, associations with gestational hypertensive disorders have not been found in women diagnosed with prediabetes in early pregnancy although these women are at increased risk of development of gestational diabetes[34, 40]. A previous prospective study among 4,589 healthy nulliparous women showed that even within the normal range, the plasma glucose level 1 hour after 50-g oral glucose challenge was positively correlated with the likelihood of preeclampsia[41]. As parity is a strong risk-factor for preeclampsia, the baseline risk of gestational hypertensive disorders among this nulliparous population may be higher. In the current study we did not find associations of early-pregnancy non-fasting glucose concentrations with risk of preeclampsia. This difference might be explained by differences in baseline risk and in glucose measurements. Future studies, using early pregnancy fasting glucose concentrations or glucose concentrations obtained after a standardized oral glucose challenge, are needed to confirm if early pregnancy glucose concentrations are indeed associated with preeclampsia in a low-risk population. We did not observe associations of maternal early-pregnancy glucose concentrations across the full range, with gestational hypertensive disorders.

Findings from our study do not support strong effects of non-fasting glucose concentrations in early pregnancy within the normal range on the risks of gestational hypertensive disorders. In clinical practice, testing for pre-gestational diabetes is only recommended among high-risk populations[28, 30, 42]. As pregnancy physiologically influences the glucose metabolism, future studies focused on pre-pregnancy glucose concentrations may shed an important light on the effects of glucose concentrations on blood pressure, placental flow measures and risk of gestational hypertensive disorders.

Strengths and limitations

We had a prospective data collection from early pregnancy onwards and a large low-risk sample of 6,078 women with detailed glucose measurements, blood pressure, placental flow measures and information on gestational hypertensive disorders available. The response rate at baseline was 61%. The non-response at baseline might have led to selection of a healthier population. We had a population with a relatively low BMI, a low mean non-fasting glucose concentration, and the sample contained a small number of cases of gestational diabetes, indicating selection towards a non-diabetic population and might affect the generalizability of our findings to higher risk populations in which stronger associations are expected. Blood sample collection was performed in a non-fasting state at different time-points in the day. The minimum fasting time until blood sample collection was 30 minutes, due to the design of the study. The samples were therefore considered as non-fasting blood samples. Since glucose and insulin concentrations are sensitive towards carbohydrate intake and vary during the day, this may have led to non-differential misclassification and an underestimation of the observed effect estimates. We had no information available on oral glucose tolerance testing in pregnancy. Although we included many covariates, there still might be some residual confounding, as in any observational study. Further studies are needed to replicate our findings using more detailed maternal glucose metabolism measurements, including fasting glucose concentrations and detailed postprandial glucose measurements among higher risk populations.

Conclusion

Maternal early-pregnancy non-fasting glucose concentrations across the full range are associated with blood pressure in early pregnancy, but not later in pregnancy. Also, maternal early-pregnancy non-fasting glucose concentrations within the normal range are not associated with placental flow measures and gestational hypertensive disorders.

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SUPPLEMENTARY MATERIALS

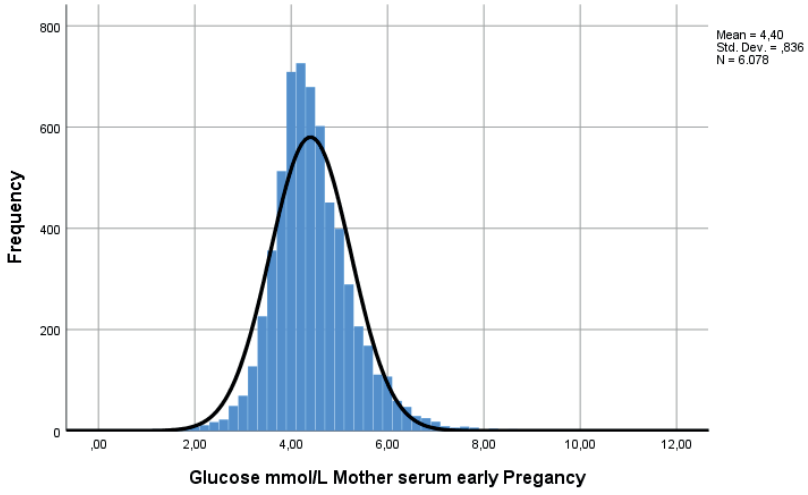

Figure S1. Early pregnancy glucose concentrations.

Table S1. Characteristics of mothers without glucose measurement available (n=6,869)

Characteristics	No glucose measurement n=763	Glucose measurement n=6,078	P-value[a]
Maternal characteristics			
Age, mean (SD), years	29.5 (5.6)	29.8 (5.1)	<0.001
Height, mean (SD), cm	166.7 (7.3)	167.5 (7.4)	0.63
Weight before pregnancy, mean (SD), kg	65.8 (13.6)	66.4 (12.7)	0.48
Body Mass Index, median (IQR), kg/m^2	22.5 (207 to 25.2)	22.6 (20.7 to 25.4)	0.31
Parity, No. nulliparous (%)	414 (55.2)	3,458 (57.4)	0.48
Education, No. higher education (%)	269 (39.4)	2,538 (44.9)	0.02
Race / Ethnicity			<0.001
Dutch or European, No. (%)	373 (52.4)	3,558 (61.0)	
Surinamese, No. (%)	71 (1.0)	503 (8.6)	
Turkish, No. (%)	84 (11.8)	472 (8.1)	
Moroccan, No. (%)	49 (6.9)	352 (6.0)	
Cape Verdian or Dutch Antilles, No. (%)	53 (7.4)	410 (7.1)	
Smoking			0.92
None, No. (%)	471 (72.9)	3,712 (72.2)	
Early-pregnancy only, No. (%)	57 (8.8)	452 (8.8)	
Continued, No. (%)	118 (18.3)	974 (19.0)	
Folic acid use No. used (%)	380 (49.8)	2,943 (47.4)	0.03
Blood pressure, mean (SD) (mmHg)			
Early pregnancy	116 (12.4) / 68 (9.8)	115 (12.3) / 68 (9.6)	0.68 / 0.69
Mid pregnancy	117 (12.1) / 67 (9.3)	116 (12.0) / 67 (9.4)	0.35 / 0.82
Late pregnancy	118 (12.5) / 69 (9.0)	118 (12.0) / 69 (9.4)	0.30 / 0.35
Mid pregnancy uterine artery resistance index, mean (SD)	0.55 (0.08)	0.54 (0.09)	0.04
Late pregnancy uterine artery resistance index, mean (SD)	0.48 (0.07)	0.49 (0.08)	0.04
Third trimester uterine artery notching, No. (%)	40 (10.5)	312 (10.2)	0.81
Glucose, mean (SD), mmol/l	Not available	4.4 (0.84)	
Insulin, median (IQR), pmol/l	Not available	115.1 (55.4 to 233.4)	
Gestational diabetes mellitus, No. (%)	8 (1.1)	64 (1.1)	0.99
Gestational hypertension, No. (%)	28 (4.1)	203 (3.8)	0.63
Preeclampsia, No. (%)	15 (2.2)	131 (2.4)	0.92
Birth characteristics			
Males, No. (%)	384 (50.5)	3,076 (50.6)	0.96
Gestational age at delivery, median (IQR), weeks	40.1 (39.0 to 40.9)	40.1 (39.1 to 41.0)	0.44

Table S1. Continued

Characteristics	No glucose measurement n=763	Glucose measurement n=6,078	P-value[a]
Preterm birth, No. (%)	36 (4.7)	310 (5.1)	0.68
Birth weight, mean (SD), grams	3,381 (570)	3,417 (564)	0.15
Placental weight, median (IQR), grams	630 (540 to 710)	610 (530 to 720)	0.71

Values are observed data and represent means (SD), medians (IQR) or number of subjects (valid %).

[a] Differences in subject characteristics between participants with and without glucose measurements available were evaluated using one-way ANOVA tests for continuous variables and chi-square tests for categorical variables. Abbreviation: IQR: inter quartile range; SD: Standard deviation.

Table S2. Associations of maternal early-pregnancy insulin concentrations with mid and late pregnancy placental flow measures (n=4,236)

	Uterine artery			Umbilical artery
Maternal early-pregnancy insulin concentrations (SDS)	**Pulsatility index** (95% Confidence Interval)	**Resistance index** (95% Confidence Interval)	**3rd Trimester notching** (95% Confidence Interval)	**Pulsatility index** (95% Confidence Interval)
Mid pregnancy				
Basic model	0.00 (-0.02 to 0.02)	0.00 (-0.02 to 0.02)	Not available	0.04 (0.01 to 0.07)*
Main model	0.01 (-0.03 to 0.05)	0.01 (-0.03 to 0.05)	Not available	0.03 (0.00 to 0.06)*
BMI model	0.00 (-0.05 to 0.05)	0.01 (-0.04 to 0.05)	Not available	0.02 (-0.02 to 0.06)
Late pregnancy				
Basic model	0.02 (0.00 to 0.04)	0.01 (0.01 to 0.01)	0.97 (0.91 to 1.03)	0.02 (-0.01 to 0.05)
Main model	0.02 (-0.02 to 0.06)	0.00 (-0.03 to 0.04)	0.96 (0.85 to1.08)	0.01 (-0.02 to 0.04)
BMI model	0.00 (-0.04 to 0.05)	-0.01 (-0.06 to 0.03)	0.94 (0.82 to 1.09)	-0.00 (-0.04 to 0.04)

Values are SDSs (95% CI) from linear regression models, reflecting differences in measures of uterine and umbilical artery flow measures, and OR (95% CI) reflecting difference in risk of 3rd trimester uterine artery notching, per 1 standard deviation increase in maternal early-pregnancy non-fasting insulin concentrations. Estimates are from multiple imputed data. SDS: Standard deviation score.

Basic model: Adjusted for gestational age at insulin measurement.

Main model: Gestational age at insulin measurement, gestational age at ultrasound, maternal ethnicity, age, parity, educational level, smoking, and folic acid supplement use.

BMI model: Main model additionally adjusted for maternal pre-pregnancy BMI.

**P-value <0.05*

Table S3. Associations of maternal early-pregnancy insulin concentrations with early, mid and late pregnancy blood pressure (n=5,265)

Maternal early-pregnancy insulin concentrations (SDS)	**Systolic blood pressure,** mmHg (95% Confidence Interval)	**Diastolic blood pressure,** mmHg (95% Confidence Interval)
Early pregnancy		
Basic model	0.18 (-0.20 to 0.55)	0.11 (-0.18 to 0.40)
Main model	0.28 (-0.10 to 0.66)	0.16 (-0.14 to 0.45)
BMI model	0.49 (0.06 to 0.93)*	0.30 (-0.04 to 0.64)
Mid pregnancy		
Basic model	0.10 (-0.24 to 0.44)	-0.11 (-0.38 to 0.16)
Main model	0.16 (-0.19 to 0.51)	-0.08 (-0.36 to 0.19)
BMI model	0.24 (-0.15 to 0.64)	-0.04 (-0.34 to 0.27)
Late pregnancy		
Basic model	0.06 (-0.29 to 0.40)	-0.06 (-0.33 to 0.21)
Main model	0.15 (-0.21 to 0.50)	-0.03 (-0.31 to 0.24)
BMI model	0.16 (-0.24 to 0.56)	-0.05 (-0.36 to 0.27)

Values are mmHg (95% CI) from linear regression models, reflecting differences in systolic and diastolic blood pressure, per 1 standard deviation increase in maternal early-pregnancy non-fasting insulin concentrations. Estimates are from multiple imputed data. SDS: Standard deviation score.

Basic model: Adjusted for gestational age at insulin measurement.

Main model: Gestational age at insulin measurement, gestational age at ultrasound, maternal ethnicity, age, parity, educational level, smoking, and folic acid supplement use.

BMI model: Main model additionally adjusted for maternal pre-pregnancy BMI.

**P-value <0.05*

Table S4. Associations of maternal early-pregnancy insulin concentrations with the risks of gestational hypertensive disorders (n=5,427)

Maternal early-pregnancy insulin concentrations (SDS)	**Gestational hypertension** (95% Confidence Interval) n=104	**Preeclampsia** (95% Confidence Interval) n=133	**Any gestational hypertensive disorder** (95% Confidence Interval) n=334
Basic model	0.87 (0.73 to 1.02)	0.94 (0.77 to 1.13)	0.89 (0.78 to 1.01)
Main model	0.89 (0.75 to 1.06)	0.93 (0.77 to 1.14)	0.91 (0.79 to 1.03)
BMI model	0.86 (0.71 to 1.04)	0.94 (0.75 to 1.18)	0.89 (0.77 to 1.03)

Values are ORs (95% CI) from logistic regression models, reflecting differences in risk of gestational hypertensive disorders, per 1 standard deviation increase in maternal early-pregnancy non-fasting insulin concentrations. Estimates are from multiple imputed data. SDS: Standard deviation score.

Basic model: Adjusted for gestational age at glucose measurement.

Main model: Gestational age at glucose measurement, maternal ethnicity, age, parity, educational level, smoking, and folic acid supplement use.

BMI model: Main model additionally adjusted for maternal pre-pregnancy BMI.

Table S5. Associations of tertiles of early pregnancy glucose concentrations with longitudinally measured systolic and diastolic blood pressure (n=5,265)[a]

	Difference in systolic blood pressure			
	Intercept (mmHg)	**P-value[b]**	**Slope** (mmHg)	**P-value[b]**
Intercept	111.55	<0.0001		
Glucose lowest tertile	*Ref*			
Glucose middle tertile	0.5033	0.44		
Glucose highest tertile	-0.1433	0.82		
Gestational age in weeks	0.1998	<0.0001		
Glucose lowest tertile * gestational age (weeks)			*Ref*	
Glucose middle tertile * gestational age (weeks)			-0.00512	0.84
Glucose highest tertile * gestational age (weeks)			0.02634	0.31
Ga_2	138.19	0.05		
	Difference in diastolic blood pressure			
	Intercept (mmHg)	**P-value[b]**	**Slope** (mmHg)	**P-value[b]**
Intercept	93.7377	<0.0001		
Glucose lowest tertile	*Ref*			
Glucose middle tertile	0.2180	0.67		
Glucose highest tertile	0.1405	0.78		
Gestational age in weeks	1.3947	<0.0001		
Glucose lowest tertile * gestational age (weeks)			*Ref*	
Glucose middle tertile * gestational age (weeks)			-0.00839	0.67
Glucose highest tertile * gestational age (weeks)			0.001838	0.93
GA05	-12.1936	<0.0001		

[a]Values are based on repeated non-linear regression models and reflect the change in blood pressure per tertile increase in early-pregnancy glucose concentration.

[b]P-value reflects the significance level of the estimate.

ETIOLOGICAL STUDIES

2.3: REPRODUCIBILITY OF FIRST TRIMESTER EMBRYONIC VOLUME AND FETAL BODY PROPORTION MEASUREMENTS IN A POPULATION-BASED SAMPLE

Submitted

Clarissa Wiertsema
Jan S. Erkamp
Eric A.P. Steegers
Annemarie G.M.G.J. Mulders
Irwin K.M. Reiss
Liesbeth Duijts
Vincent W.V. Jaddoe
Romy Gaillard

ABSTRACT

Objective

To establish feasibility and reproducibility of first trimester fetal proportion volumetric measurements using three-dimensional ultrasound and a Virtual Reality (VR) approach.

Methods

We performed reproducibility analyses of three-dimensional ultrasound datasets of 50 late first trimester fetuses. We used V-scope software for VR volumetric measurements of the total fetus, extremities, head-trunk, head, trunk, thorax, and abdomen. Intra- and interobserver agreement was analyzed using Bland and Altman methods.

Results

Volumetric measurements of the total fetus, extremities, head-trunk, head, trunk, thorax and abdomen showed good intra- and inter-observer agreement and reproducibility with ICC above 0.90 and CV < 10% and mean difference <10%. The limits of agreement for interobserver agreement were within the ±10% range for fetal, head-trunk, head and trunk volumes. The limits of agreement for interobserver agreement exceeded the ±10% range for extremities, thorax and abdomen (lower limit of agreement, upper limit of agreement: -26.09%;4.77%; -14.14%,10.00%; -14.47%,8.83%, respectively).

Conclusions

First trimester fetal proportion volumetric measurements using three-dimensional ultrasound combined with a Virtual Reality approach are feasible and reproducible. These measurements enable detailed study of embryonic and first trimester fetal development and growth.

INTRODUCTION

The first trimester of pregnancy is crucial for growth and the initial arrangement of organs[1]. Observational studies suggest that first trimester growth restriction, assessed by ultrasound, is associated with increased risks of adverse birth outcomes and an adverse cardiovascular risk profile in childhood[2, 3]. Recent developments in obstetric two- and three-dimensional (3D) ultrasound techniques enable improved evaluation of early fetal growth and development[4]. In line with these developments, ultrasound is increasingly used for first trimester anomaly screening[5, 6]. Detailed studies on first trimester fetal development may also enable better understanding of early developmental adaptation mechanisms leading to adverse outcomes in later life.

The combination of 3D ultrasound with offline analyses using a Virtual Reality (VR) technique enables measurement of novel first trimester volumetric markers compared to the traditional crown rump length (CRL)[7]. Previously, embryonic volume measurements have shown to be feasible[7]. Additionally, first trimester fetal proportion volumetric measurements using 3D ultrasound combined with VR could have great potential in clinical research settings focused on embryonic and early fetal growth and development[7].

Therefore, we developed novel volumetric measurements of first trimester fetal proportions using three-dimensional ultrasound datasets combined with a VR approach. We assessed the feasibility and the reproducibility considering volumetric measurements of extremities, head-trunk, head, trunk, thorax and abdomen of 50 late first trimester fetuses.

METHODS

Study population

This study was embedded in the Generation R *Next* study, a population-based prospective cohort study from preconception onwards in Rotterdam, the Netherlands. Recruitment started in August 2017 and is still ongoing. Pregnant women are invited to the research center for three appointments in the first trimester of pregnancy, from 7 to 13 weeks of gestational age, with an interval of approximately two weeks. During these 30-minute visits 3D ultrasound datasets were obtained to assess embryonic, early fetal and placental development. Around 30 weeks of gestation participants were invited back to the research centre to evaluate third-trimester growth and placental hemodynamic parameters. For the current analysis, we focused on the 3D ultrasound

recordings collected in the late first trimester. We selected a random sample of 50 participants who visited the research center at Sophia Children's Hospital starting from March 2019 and who had a complete set of 3D ultrasound data available. They were eligible for the current study if the crown rump length was ≥75 mm, to ensure the fetus was evaluated in the fetal developmental phase of the first trimester[6]. All participants gave written informed consent. The medical ethics committee of the Erasmus University Medical Center approved of this study (MEC-2016-589).

Gestational age assessment

Gestational age was calculated from the first day of the last menstrual period (LMP) in spontaneous pregnancies, or from oocyte pick-up plus 14 days in IVF pregnancies. In women with regular menstrual cycles, we adjusted gestational age for cycle duration if the cycle duration was more than 3 days different from 28 days. Gestational age was based on CRL in 5 subjects, because the LMP was unknown, menstrual cycle was irregular or GA determined by crown rump length differed more than 7 days from the LMP[8].

First trimester fetal ultrasound examination

All ultrasound scans were performed by experienced ultrasonographers using a Voluson E10 System (GE Healthcare, Zipf, Austria) with a 5-13 MHz transvaginal transducer (RIC6-12D). Ultrasound settings were predefined to create uniformity. The 3D dataset acquisition was performed with a 90-110° volume angle while the fetus was not moving and preferably facing towards the transducer to provide detailed visualization of the fetal anatomy. The 3D datasets were stored in Cartesian volume files for offline analysis.

Fetal proportion volumetric measurements

We used the Barco I-Space, a CAVE™-like virtual reality (VR) system, for offline analysis of the 3D volume datasets[9]. V-Scope software enables accurate semi-automatic volumetric measurements due to improved depth perception using VR displays[10 11]. Initially, we measured fetal volume as described previously[12]. Subsequently, we performed volumetric measurements of extremities, head-trunk, head, trunk, thorax and abdomen. In preparation of the measurements, the insertion of the umbilical cord and the surrounding uterine wall was erased. To perform the volumetric measurement of the total fetus, automatic segmentation of hyperechoic structures was followed by manual segmentation of hypoechoic structures (e.g. brain ventricles and stomach)[11, 12]. Second, we deselected the segmented voxels of the extremities to perform the volumetric measurements of the extremities and head-trunk. Third, the volumetric measurement of the head and trunk were obtained by deselecting the segmented voxels of the head

with the base of the chin and the fourth ventricle in the midsagittal plane as reference points, as described previously[13]. Fourth, we performed the volumetric measurements of the thorax and abdomen by erasing the segmented volume below the diaphragm. During the fetal proportion volumetric measurements, we used a transparent segmentation color to enable identification of the fourth ventricle and the diaphragm. **Figure 1** shows a step-by-step description for the performance of the fetal proportion volumetric measurements. All fetal proportion volumetric measurements were performed independently by two researchers (C.W. and J.E.) to obtain intra- and interobserver reproducibility. Both researchers performed the offline measurements twice, with an interval of at least one week to prevent recollection bias. The measurements were performed in a blinded setting.

Statistical analysis

We performed statistical analyses described by Bland and Altman[14, 15]. For the intra-observer analysis, the first measurement was compared with the second measurement for each observer. For the inter-observer analysis, the mean of the two measurements of the first observer was compared to the mean of the two measurements of second observer using similar calculations. Similar calculations were performed in the intra- and interobserver analyses. First, we plotted the measurements with the line of equality to visualize the degree of agreement. Second, intraclass correlation coefficients (ICCs) with a 95% confidence interval and the coefficients of variation (CVs) were calculated for each measurement to evaluate consensus within each observer and between observers. Third, intra- and interobserver variability was quantified using Bland and Altman plots, showing the mean difference in percentage measurement error with the 95% limits of agreement (mean difference ±2SD) for the all the measurements. The mean difference in percentage measurement error was calculated as the difference between the measurements divided by the mean of the measurements multiplied by 100. An acceptable mean difference and acceptable limits of agreement are not a statistical but a clinical consideration. To establish that the measurements are useable for future association studies, we decided that an ICC >90%, a CV <10% and mean difference <10% were considered to be proof of good agreement[16]. We decided that the limits of agreement should deviate a maximum of 10% from the mean difference, which indicates that 95% of all differences should be within the ±10% measurement error range[14]. Statistical analyses were performed using IBM SPSS, version 25.

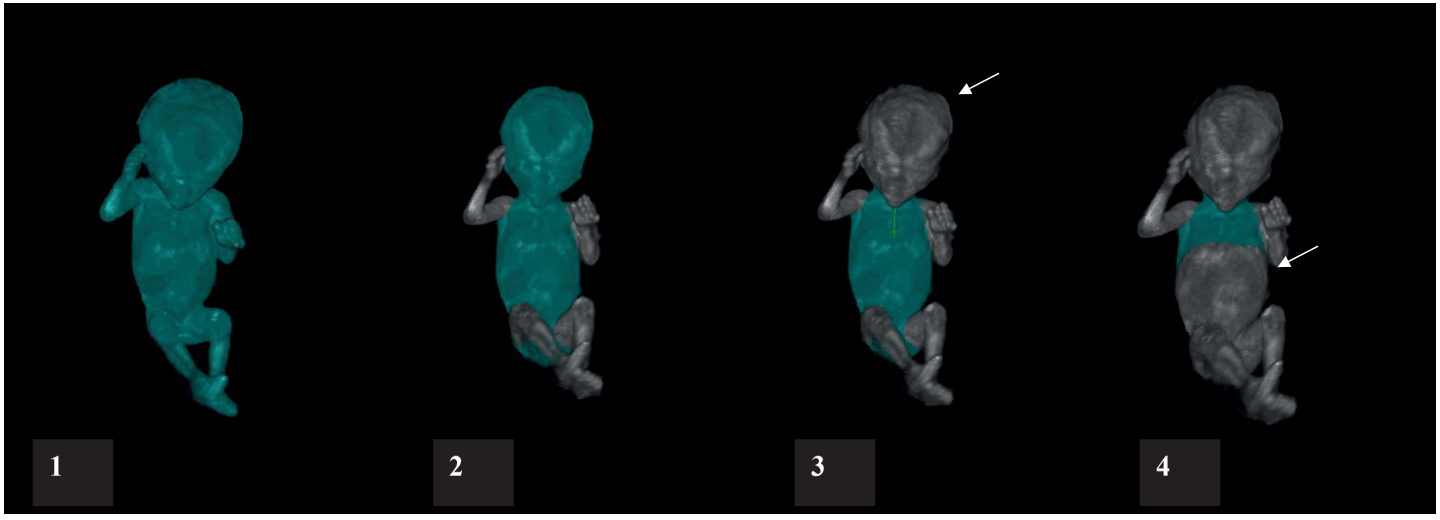

Figure 1. Image of a fetus at 12 weeks and 5 days of gestation in the BARCO I-Space, a step-by-step approach for volumetric measurement of the total fetus and fetal proportions is shown.

Volumetric measurements from left to right: 1) Segmentation of the total fetus in cyan; 2) Segmentation of head-trunk in cyan with indirect measurement of the extremities in grey; 3) Segmentation of the trunk cyan with indirect measurement of the head in grey indicated by the arrow (reference line in green connects chin and fourth ventricle in the midsagittal plane); 4) Segmentation of the thorax in cyan with indirect measurement of the abdomen in grey indicated by the arrow.

RESULTS

Participant characteristics

Participants and pregnancy characteristics are shown in **Table 1**. Of all pregnancies, 90% were spontaneously conceived. The mean gestational age was 12 weeks and 2 days. Median body mass index was within the normal range. A total of 112 3D datasets that included the complete fetus were available for analysis, on average 2.4 per participant.

Table 1. Participant characteristics (n = 50)

	mean (IQR) / N (%)
Maternal age (years)	31.5 (29.8-34.2)
Maternal BMI, median (range (kg/m^2))*	22.5 (20.8-26.7)
Gestational age (weeks. days)	12.2 (11.5, 12.5)
Reproduction	
Spontaneously conceived (%)	45 (90)
IVF (%)	1 (2)
Ovulation induction (%)	4 (8)

BMI, Body Mass Index, IVF, In Vitro Fertilisation.

Intraobserver reproducibility analysis

Table 2 presents the ICCs, CVs, mean differences and corresponding limits of agreement for intraobserver agreement for volumetric measurements of the total fetal, extremities, head-trunk, head, trunk, thorax and abdomen. All measurements for both observers lie in close proximity to the line of equality suggesting small intraobserver differences, except for the volumetric measurements of the extremities (**Figure S1A-B**). Intraobserver ICCs were higher than 0.97 and CVs were lower than 10% for each measurement. The observed mean differences ranged from 2.7% to 9.4% for intraobserver differences of observer 1 and from 1.3% to 8.9% for observer 2. **Figures 2A-B** depicts the Bland and Altman plots for intraobserver agreement for each measurement of both observers, in which the mean difference is plotted against the mean of the assessments accompanied with the limits of agreement. We observed that the limits of agreement for volumetric measurements of the total fetal, head-trunk and trunk were within the limits of ±10%. Intraobserver limits of agreement for volumetric measurements of thorax and abdomen slightly exceeded the 10% limit for both observers. Limits of agreement for volumetric measurement of the extremities ranged between 15% to 20% for both observers.

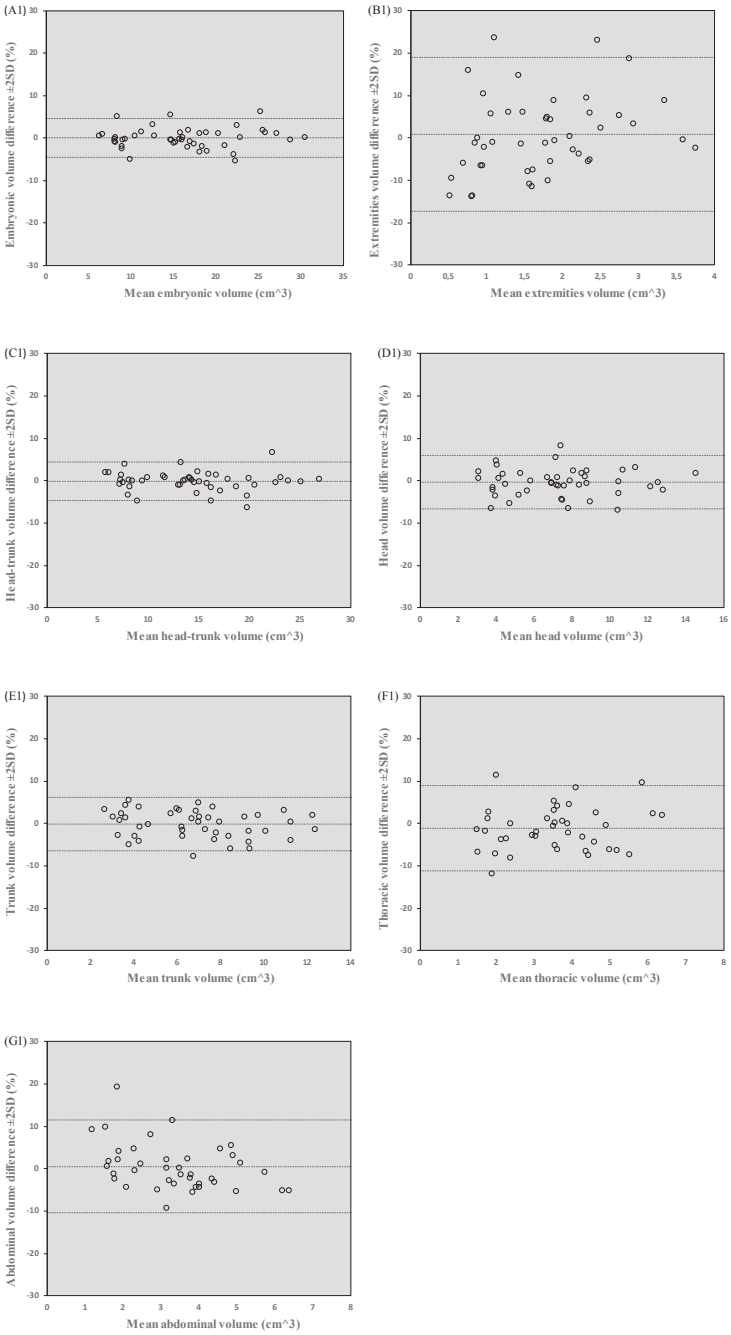

Figure 2A. Bland and Altman plots of intraobserver agreement of observer 1 with corresponding limits of agreement in proportion of the mean ± 2SD for: A) Fetal volume, B) Head-trunk volume, C) Extremities volume, D) Head volume, E) Trunk volume, F) Thorax volume, G) Abdomen volume.

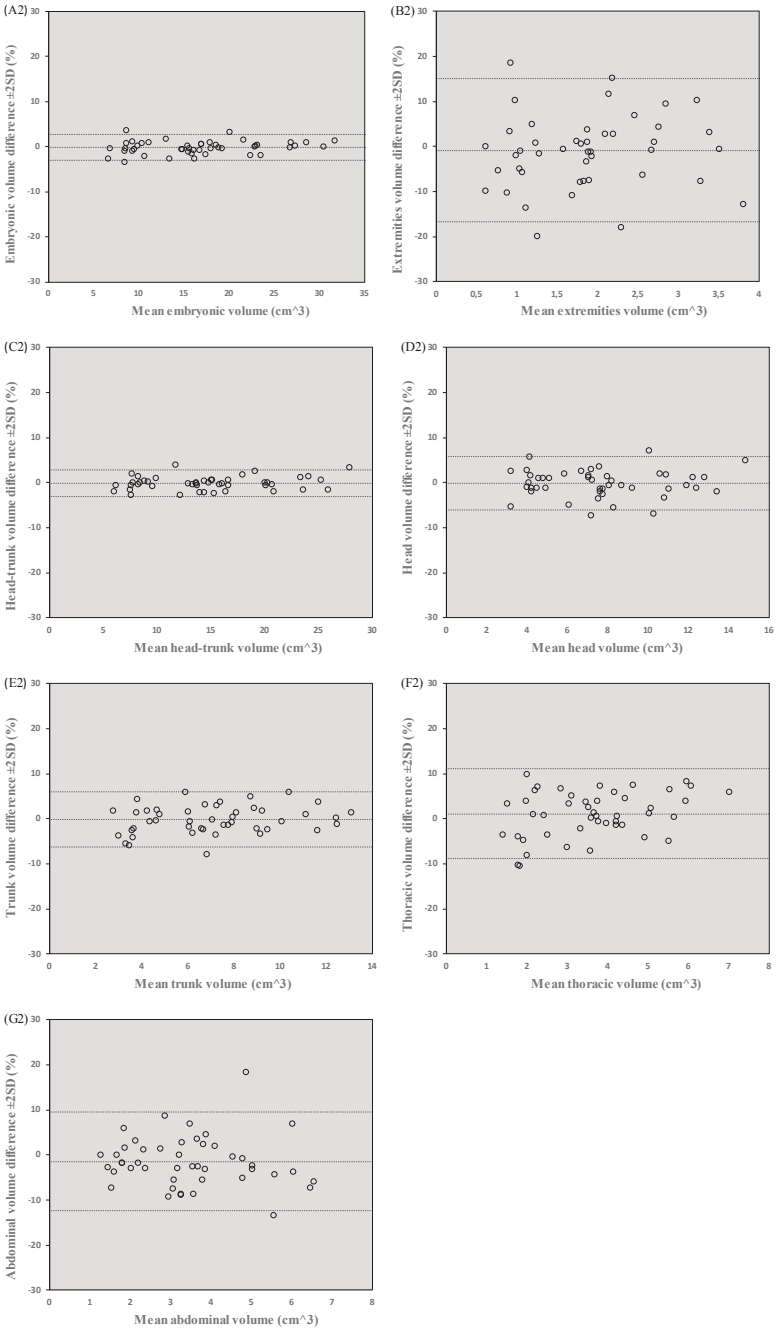

Figure 2B. Bland and Altman plots of intraobserver agreement of observer 2 with corresponding limits of agreement in proportion of the mean ± 2SD for: A) Fetal volume, B) Head-trunk volume, C) Extremities volume, D) Head volume, E) Trunk volume, F) Thorax volume, G) Abdomen volume.

Table 2. Intraobserver agreement of fetal proportion volumetric measurements for both observers (n = 50)

Volumetric measurement	Observer	Mean volume cm^3	ICC (95% CI)	CV %	Mean difference (LLOA, ULOA) %
Total fetus	1	16.6	0.998 (0.996-0.999)	2.7	0.04 (-4.60, 4.67)
	2	16.6	0.999 (0.999-1.000)	1.3	-0.16 (-2.99, 2.67)
Extremities	1	1.7	0.980 (0.982-0.994)	9.4	0.76 (-17.42, 18.94)
	2	1.9	0.983 (0.970. 0.991)	8.9	-0.89 (-16.77, 14,98)
Head-Trunk	1	14.3	0.998 (0.996-0.999)	2.7	-0.00 (-4.61, 4.52)
	2	14.7	0.999 (0.998-0.999)	1.7	-0.11 (-3.04, 2.82)
Head	1	7.3	0.996 (0.994-0.998)	3.3	-0.00 (-6.70, 5,89)
	2	7.6	0.996 (0.994-0.998)	3.4	-0.13 (-6.09, 5,83)
Trunk	1	6.8	0.996 (0.993-0.998)	3.3	-0.00 (-6.42, 6.25)
	2	7.1	0.997 (0.995-0.998)	3.1	-0,17 (-6.23, 5,89)
Thorax	1	3.5	0.989 (0.980-0.994)	5.5	-0.01 (-11.19, 8,85)
	2	3.6	0.991 (0.983-0.995)	5.1	1,07 (-8.90, 11.03)
Abdomen	1	3.4	0.992 (0.992-0.985)	5.0	0.01 (-10.44, 11.47)
	2	3.5	0.985 (0.973-0.992)	7.1	-1,44 (-12.44, 9,56)

CI, confidence interval; ICC, intraclass correlation coefficient; CV, coefficient of variation; LLOA, lower limit of agreement; ULOA, upper limit of agreement.

Interobserver reproducibility analysis

Table 3 presents the results for the interobserver agreement for volumetric measurements of the total fetus, extremities, head-trunk, head, trunk, thorax and abdomen. The plots with the line of equality suggest small intraobserver differences, except for the volumetric measurement of the extremities (**Figure S2**). Interobserver ICCs for all measurements were higher than 0.90 and CVs were lower than 10% for each measurement. The observed mean differences ranged from 2.7 – 10.7% for all measurements. **Figures 2C** depicts the Bland and Altman plots for interobserver agreement. We observed good agreement for volumetric measurement of the total fetus, head-trunk volume, head and trunk with limits of agreement not exceeding ±10%. Interobserver limits of agreement for the volumetric measurements of the extremities, thorax and abdomen exceeded the 10% limit (lower limit of agreement, upper limit of agreement: -26.09%, 4.77%; -14.14%, 10.00%; -14.47%, 8.83%, respectively).

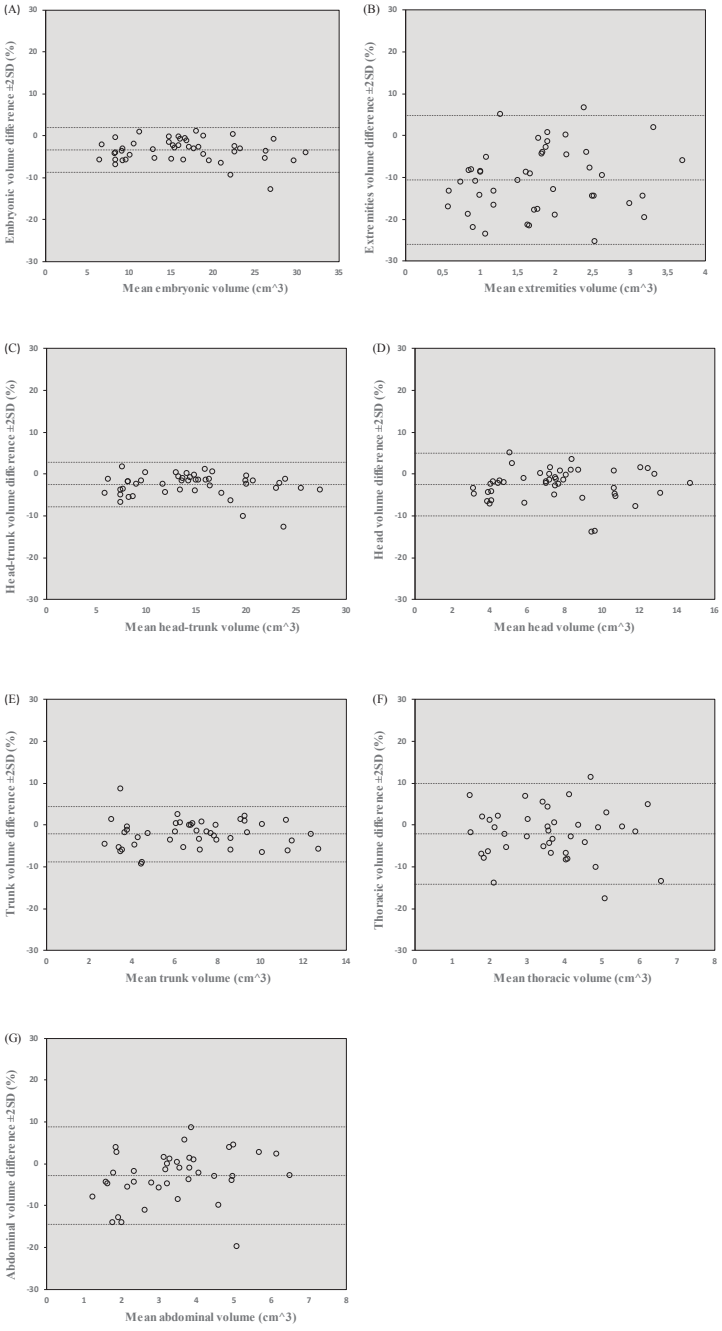

Figure 5C. Bland and Altman plots of interobserver agreement with corresponding limits of agreement in proportion of the mean +/- 1.96 SD for: A) Fetal volume, B) Head-trunk volume, C) Extremities volume, D) Head volume, E) Trunk volume, F) Thorax volume, G) Abdomen volume.

Table 3. Interobserver agreement of fetal proportion volumetric measurements (n = 50)

Volumetric measurement	Mean volume cm^3	N	ICC (95% CI)	CV %	Mean difference (LLOA, ULOA) %
Total fetus	16.3	46	0.991 (0.944-0.997)	3.9	-3.44 (-8.79, 1.91)
Extremities	1.8	46	0.951 (0.697-0.983)	10.9	-10.66 (-26.09, 4.77)
Head-Trunk	14.5	46	0.993 (0.973-0.997)	3.7	-2.57 (-7.90, 2.77)
Head	7.5	45	0.991 (0.977-0.996)	4.5	-2.51 (-10.00, 4.98)
Trunk	6.9	45	0.995 (0.984-0.998)	3.3	-2.14 (-8.76, 4.46)
Thorax	3.6	40	0.979 (0.960-0.989)	7.5	-2.07 (-14.14, 10.00)
Abdomen	3.4	40	0.985 (0.971-0.992)	6.4	2.82 (-14.47, 8.83)

N, number of datasets in which both observers could do the measurements. CI, confidence interval; ICC, intraclass correlation coefficient; CV, coefficient of variation; LLOA, lower limit of agreement; ULOA, upper limit of agreement.

DISCUSSION

We observed good intra- and interobserver reproducibility for volumetric measurements of the total fetus, head-trunk, head, trunk, thoracic and abdominal volume using a VR approach. We found that volumetric measurement of extremities was feasible, but these measurements have lower intra- and interobserver reproducibility.

Interpretation of main findings

With the use of transvaginal ultrasonography and improvements in ultrasound technology over the recent years, evaluation of first trimester fetal anatomy and growth has become feasible[5, 6]. The use of more advanced first trimester measurements in antenatal care may improve early detection of congenital abnormalities and early fetal growth restriction[5, 17, 18]. Two-dimensional ultrasound is currently used in routine first trimester antenatal care for evaluation of crown rump length and biometric measures. It has become apparent that first-trimester growth is not similar in all pregnancies, even though this has been the general assumption in the past[7]. Also, observational studies suggest that impaired first trimester growth is associated with larger risks of adverse birth outcomes and adverse cardiovascular risk profile in childhood[2, 3]. Better insight the embryonic and first trimester fetal development may help to identify clues for later life health and disease.

Advanced ultrasound techniques such as 3D ultrasound in combination with VR volumetric measurements can lead to more accurate first trimester markers when

compared to the routine two-dimensional ultrasound measures[7]. The use of VR enables depth-perception in 3D ultrasound datasets and therefore offers the possibility to reliably conduct complex volumetric measurements. Due to a detailed measurement protocol with predefined ultrasound and VR settings, this technique is highly reproducible[19]. Other techniques like Virtual Organ Computer-aided AnaLysis (VOCAL) can result in structural over-estimation and subsequently larger individual variation because the examiner has to draw a contour around the structure of interest[12, 20]. Thus, further exploration of the use of 3D ultrasound in combination with VR is promising for the development of more advanced fetal measurements.

Previously, 3D ultrasound in combination with a VR system were shown to be feasible and reproducible for several first trimester measurements including the measurement of embryonic volume[12, 13, 21]. As the increase in embryonic volume in the first trimester is much larger than the increase in embryonic length as measured by CRL, it is suggested that these volumetric measurements may have higher sensitivity to assess deviations in first-trimester growth compared to customary biometric measurements[12]. Subsequently, first trimester volumetric markers can contribute to the understanding of first trimester fetal growth and early developmental adaptation mechanisms.

We assessed the reproducibility of fetal proportion volumetric measurements in the late first trimester of pregnancy. Our measurements are an extension to already existing techniques for volumetric measurement of the embryo and embryonic head using V-scope software[12, 13]. To our knowledge we are the first to develop volumetric measurement that include proxies for first trimester thoracic and abdominal development. We achieved good intra- and interobserver reproducibility of volumetric measurements of the total fetus, head-trunk, head, trunk, thorax and abdomen. For volumetric measurement of the fetus and head we found similar reproducibility to previous VR studies[12, 13]. We found good ICCs, CVs and mean differences for volumetric measurements of thorax and abdomen, only the limits of agreement of ±10% were slightly exceeded for both intra- and interobserver agreement. The requirement of ±10% limits of agreement were arbitrarily determined in advance of the study to establish that the measurements are also useful for future association studies. Therefore, we still consider the reproducibility of volumetric measurement of thorax and abdomen as good. We could not prove good intra- and interreproducibility of the extremities volume measurements as the limits of agreement greatly exceeded the ±10% for both intra- and interobserver reproducibility. A possible explanation for the inability to reproducibly measure the volume of the extremities, is the relatively smaller volume and the frequent presence of acoustic shadowing compared to the other proportion measurements.

Thus, the volumetric measurements of the total fetus, head-trunk, head, trunk, thorax and abdomen will be specifically useful for first trimester growth assessment and for future association studies investigating developmental adaptation mechanisms.

Clinical implications of these novel measurements may include the early detection of asymmetrical growth restriction through the calculation of fetal proportion ratios. This might identify fetuses at risk for adverse birth outcomes[17]. More importantly, in large scale population-based research settings, like the Generation R *Next* cohort study, these measurements may be useful as a proxy to investigate associations of developmental adaptations in the first trimester with parental lifestyle characteristics during periconception.

Possible mechanisms behind the associations of impaired fetal growth and adverse health outcomes include developmental programming during fetal life[2]. Periconceptional exposures might trigger adaptive responses in organ development[2]. Thus, volumetric measurements of organs already during the embryonic phase, could contribute to the understanding of these adaptive responses in organ development and should be the focus of future studies.

Strengths and limitations

The technique we propose can be used on a large scale in clinical research settings, in either a immersive or desktop VR setting[10]. It is an easily comprehensible technique that can be conducted following a detailed protocol. As a possible limitation can be mentioned that the researcher requires to follow a training program of approximately 20 hours to ensure that the measurements are conducted according to protocol and in a reproducible fashion. To this end, the volumetric measurements are first practiced in a rehearsal setting. Furthermore, we compared the mean of the two measurements to achieve good interobserver reproducibility. This implies that in research settings, measurements have to be conducted twice by the same observer. The technique was time consuming with approximately 20 minutes needed to conduct the volumetric measurement of the total fetus and the fetal proportions in a single 3D dataset. The success percentage of the current study ranged from 92% for volumetric measurement of the total fetus to 80% for volumetric measurements of the thorax and abdomen. This can be explained as the fetal proportion volumetric measurements requires good quality 3D ultrasound dataset. The third ventricle in the brain and the diaphragm are used as anatomical reference points, of which the visualization is required to perform the measurements. This good quality cannot always be guaranteed, for example in case

of fetal movements or maternal adiposity. As the study was embedded in a population-based cohort and a high percentage of the pregnancies were conceived spontaneously with maternal BMI within the normal range, we expect the external validity to be high.

Conclusion

Fetal proportion volumetric measurements in the late first trimester using three-dimensional ultrasound in combination with a VR system are feasible and reproducible, except for volumetric measurements of the fetal extremities. These novel volumetric measurements enable detailed study on first trimester fetal development and growth, which may lead to better understanding of early fetal developmental adaptation mechanisms leading to adverse birth outcomes and unfavorable cardiovascular risk profiles in later life.

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SUPPLEMENTAL MATERIALS

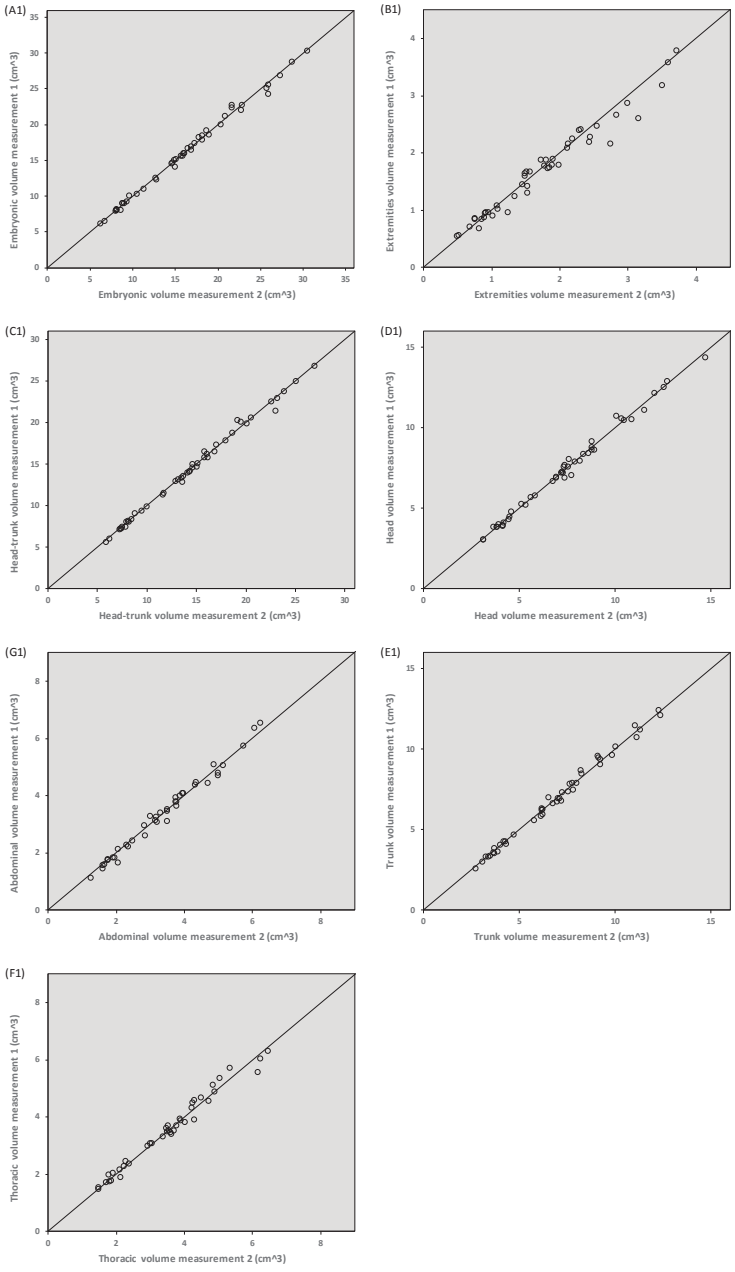


Figure S1A. Measurements of observer 1 plotted with line of equality for: A) Embryonic volume, B) Head-trunk volume, C) Extremities volume, D) Head volume, E) Trunk volume, F) Thoracic volume, G) Abdominal volume.

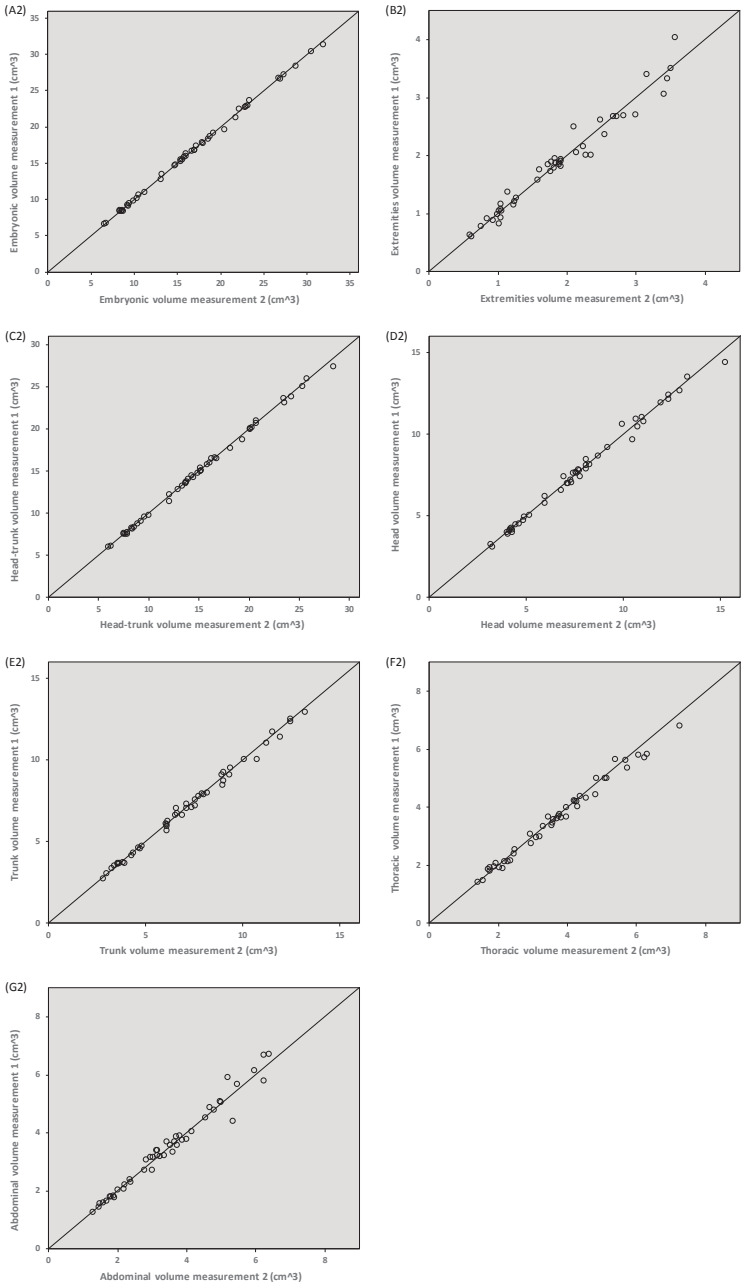


Figure S1B. Measurements of observer 2 plotted with line of equality for: A) Embryonic volume, B) Head-trunk volume, C) Extremities volume, D) Head volume, E) Trunk volume, F) Thoracic volume, G) Abdominal volume.

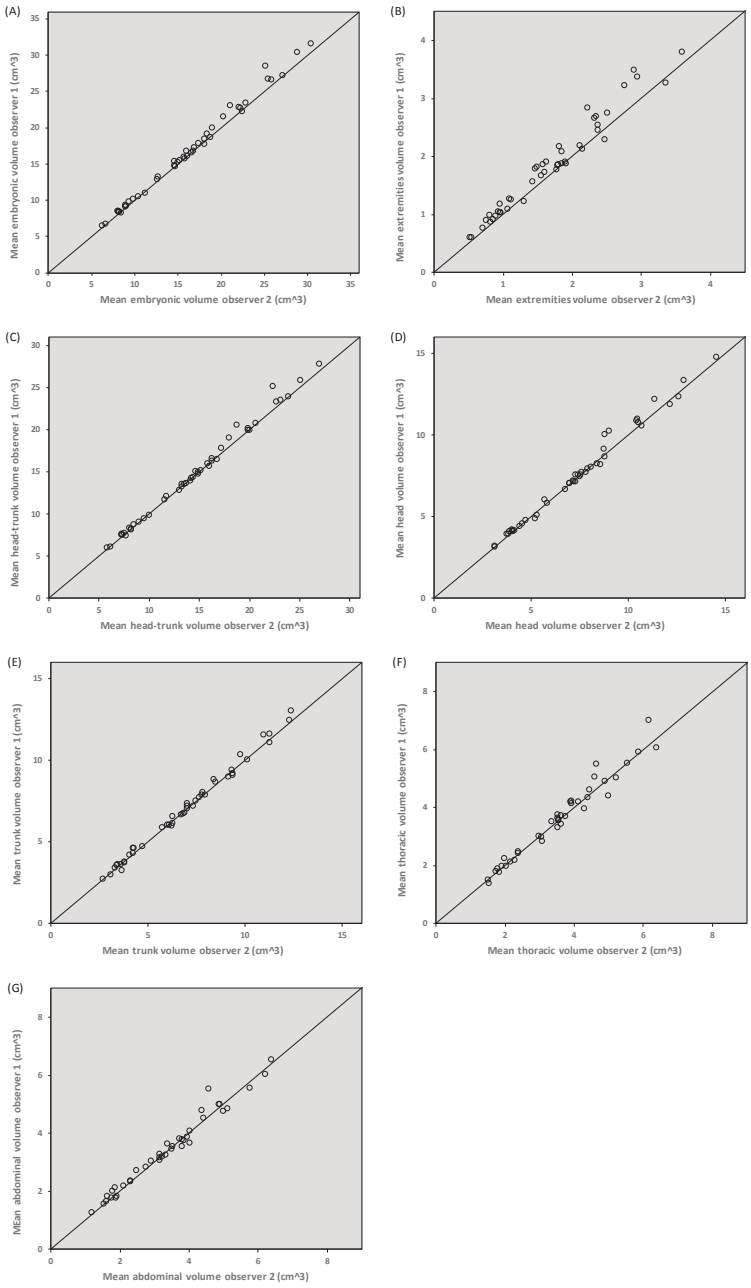


Figure S2. Measurements of observer 1 plotted against measurements of observer 2 with line of equality for: A) Embryonic volume, B) Head-trunk volume, C) Extremities volume, D) Head volume, E) Trunk volume, F) Thoracic volume, G) Abdominal volume.

SCREENING STUDIES

3.1: POPULATION SCREENING FOR GESTATIONAL HYPERTENSIVE DISORDERS USING MATERNAL, FETAL AND PLACENTAL CHARACTERISTICS: A POPULATION-BASED PROSPECTIVE COHORT STUDY

Prenat Diagn. 2020

Jan S. Erkamp
Vincent W.V. Jaddoe
Liesbeth Duijts
Irwin K.M. Reiss
Annemarie G.M.G.J. Mulders
Eric A.P. Steegers
Romy Gaillard

ABSTRACT

Objective

To determine screening performance of maternal, fetal and placental characteristics for selecting pregnancies at risk of gestational hypertension and preeclampsia in a low-risk multi-ethnic population.

Methods

In a prospective population-based cohort among 7,124 pregnant women, we collected maternal characteristics including body mass index, ethnicity, parity, smoking and blood pressure in early-pregnancy. Fetal characteristics included second and third trimester estimated fetal weight and sex determined by ultrasound. Placental characteristics included first and second trimester placental growth factor concentrations and second and third trimester uterine artery resistance indices.

Results

Maternal characteristics provided the best screening result for gestational hypertension (AUC 0.79(95%CI 0.76-0.81)) with 40% sensitivity at 90% specificity. For preeclampsia, the maternal characteristics model led to a screening performance of AUC 0.74(95%CI 0.70-0.78) with 33% sensitivity at 90% specificity. Addition of second and third trimester placental ultrasound characteristics only improved screening performance for preeclampsia (AUC 0.78(95%CI 0.75-0.82), with 48% sensitivity at 90% specificity).

Conclusions

Routinely measured maternal characteristics, known at the start of pregnancy, can be used in screening for pregnancies at risk of gestational hypertension or preeclampsia within a low-risk multi-ethnic population. Addition of combined second and third trimester placental ultrasound characteristics only improved screening for preeclampsia.

INTRODUCTION

Gestational hypertensive disorders (GHD) are common complications affecting 5% to 10% of pregnancies, and are major risk-factors for maternal and fetal mortality and morbidity[1-4]. Up to 50% of women with gestational hypertension will be diagnosed with preeclampsia[5]. Screening for women at risk of GHD may provide an opportunity for intensified monitoring, leading to earlier diagnosis and possible interventions before severe disease develops.

Several maternal, fetal and placental characteristics are associated with the risks of GHD[5-9]. First trimester screening models for GHD have been developed, using different screening parameters[10]. 'Simple' first trimester screening models mainly consist of maternal characteristics such as age, body mass index, parity, and medical or obstetric history[11]. More advanced first trimester screening models, which next to maternal characteristics often consist of biophysical and biochemical parameters, such as uterine artery pulsatility index (UtA-PI), mean arterial pressure (MAP) and biomarkers, report higher area-under-the-receiver-operating-characteristic (ROC) curves[6, 12]. Performances of these advanced parameters vary among studies, which may be partly explained by differences in population characteristics. Many studies have focused on Caucasian women and specifically selected high-risk women, including multiparous women with previous pregnancy complications, nulliparous women or women with a high body mass index (BMI). Restrictions to specific populations limit translation to clinical practice, and replication of obtained screening performance remains challenging.

We first assessed the potential of routinely measured maternal characteristics known in early-pregnancy, for screening of gestational hypertension and preeclampsia within a multi-ethnic population-based prospective cohort study among 7,124 low-risk pregnancies. Next, we further explored whether the addition of detailed fetal biometry measurements, placental Doppler vascular resistance indices and placental biomarkers, obtained throughout pregnancy, further improved screening of gestational hypertension and preeclampsia.

METHODS

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early-pregnancy onwards in Rotterdam, the Netherlands[13]. The study was approved by the local Medical Ethical Committee (MEC 198.782/2001/31). Written consent was obtained from participating women. Pregnant women were enrolled between 2001 and 2006. Response rate at birth was 61%. In total, 8,879 women were enrolled during pregnancy. We excluded non-singleton live-births, women with pre-existing hypertension and women without information on GHD, leading to 7,124 pregnant women (**Figure S1**).

Maternal, fetal and placental characteristics used for screening

Maternal characteristics in early-pregnancy

We selected maternal characteristics, known in early-pregnancy, associated with GHD[14]. Maternal age was assessed at enrolment and categorized; <25.0 years, 25.0-34.9 years, ≥35.0 years[15]. Maternal height and weight were measured without shoes and heavy clothing at enrolment. BMI was calculated and categorized: normal weight (BMI<25kg/m^2), overweight (BMI 25.0-30.0kg/m^2), obese (BMI≥30.0 kg/m^2)[16]. Information about ethnicity (categorized as previously described), parity (nulliparous or multiparous), and smoking status (non-smoking, early-pregnancy-only and continued smoking) was obtained at enrolment by questionnaire[14, 15, 17, 18]. Blood pressure was measured at a median 13.8 (IQR 12.4-16.1) weeks gestation using Omron-907 automated digital oscillometer sphyghmanometer (OMRON Healthcare Europe, Hoofddorp, the Netherlands). The mean value of two blood pressure readings over a 60-s interval was documented[19, 20]. As blood pressure is part of the diagnosis of GHD, only blood pressure measured < 20^{th} weeks gestation was used.

Fetal characteristics

Ultrasound examinations were carried out in two dedicated research centres in first (median 13.2 (IQR 12.2-14.7) weeks), second (median 20.5 (IQR 19.9-21.3) weeks) and third trimester (median 30.3 (IQR 29.8-30.9) weeks). We established gestational age from the first ultrasound examination[15]. Estimated fetal weight (EFW) was calculated according to Hadlock et al[21]. Gestational age-adjusted Standard-Deviation-Scores (SDS) for EFW was based on reference growth charts from the whole study population, and represent the equivalent of z-scores[15]. We defined screen-positive as EFW<10^{th} percentile in second or third trimester.

Placental characteristics

Uterine artery resistance index (UtA-RI) was derived from flow velocity waveforms in second and third trimester[22-25]. We defined screen-positive UtA-RI as UtA-RI SDS value in the highest decile. Placental growth factor (PlGF) was measured in first and second trimester maternal venous blood samples at a median 13.2 (IQR 12.2-14.9) and 20.3 (IQR 19.9-21.07), respectively[26, 27]. Gestational-age-adjusted multiples of the medians (MoM) were calculated[26, 27]. Screen-positive was defined as first or second trimester PlGF MoM in the lowest decile.

Gestational hypertensive disorders

Information about GHD was obtained from medical records[28]. Occurrence of hypertension and related complications were cross-validated using hospital registries, and defined using criteria of the International Society for the Study of Hypertension in Pregnancy[28, 29]. Gestational hypertension (n=273, 3.8%) was defined as de-novo hypertension (blood pressure ≥ 140/90 mmHg), appearing >20 weeks gestational age. Preeclampsia (n=149, 2.1%) was defined as de-novo hypertension (blood pressure ≥140/90 mmHg) after 20 weeks gestation with concurrent proteinuria. As secondary outcome, we defined early-onset preeclampsia (n=14, 0.2%) as preeclampsia with a delivery <34 weeks gestational age based on our available data[30]. Any gestational hypertensive disorder was defined as either gestational hypertension or preeclampsia.

Statistical analyses

Because our study is focused on screening for GHD in a low-risk population, we aimed to use maternal, fetal and placental characteristics which are obtained routinely or are simple and relatively cost-effective to obtain where possible, to enable simple translation of findings to clinical practice. Therefore, we first constructed a baseline model, consisting of maternal characteristics known in early-pregnancy and associated with GHD, including maternal age, BMI, ethnicity, parity and smoking to assess the screening potential of a simple maternal characteristics model. To evaluate the additional effect of first trimester blood pressure, we added first trimester MAP, per 10 mmHg, to the baseline model. Second, as fetal ultrasounds are routinely available in second trimester and often in third trimester, we added fetal parameters to the model: fetal sex and second trimester and/or third trimester EFW screening result. Next, we added placental parameters, which are not routinely available during pregnancy in low-risk populations; second and/or third trimester UtA-RI screening result, and first and/or second trimester screening result of PlGF. We assessed the variance explained for each logistic regression model. We obtained predicted values from these regression models and assessed model performance via ROC curves and calculation of Area-Under-the-Curve (AUC), along

with the sensitivity at different false-positive-rates (1-specificity). Positive predictive values (PPV) and negative predictive values (NPV) and Positive likelihood ratios (PLR) and negative likelihood ratios (NLR) at a 10% false-positive-rate (90% specificity) were calculated from the models. To compare model performance of different models, we assessed whether the change in effect size of obtained AUCs from the different models was clinically relevant and statistically significant. Based on previous studies focused on screening for similar adverse outcomes, we considered an approximate 4-5% change in AUC as clinically relevant, as this change is likely associated with a detectable increase in sensitivity[11, 31, 32]. When model comparison fulfilled this criterion, we tested whether this change was statistically significant using the test of DeLong et al[33]. When addition of a characteristic clinically and statistically significantly improved screening performance of the model, this characteristic was included and used as a new baseline model for further analyses. Screening models were developed for gestational hypertension and any pre-eclampsia, separately. As secondary outcome, we explored the screening performance of these characteristics for early-onset preeclampsia, separately. We performed two sensitivity analyses to assess the robustness of our findings: 1) we assessed model performance when we used 'any gestational hypertensive disorder' as outcome; 2) we explored if we obtained similar screening models if we added screening characteristics to the baseline model, in order of their occurrence during pregnancy, instead of based on clinical availability within a low risk population. Missing values were dealt with by adding a missing category for each maternal and fetal characteristic to the models, which resembles clinical practice. Analyses were performed using Statistical Package of Social Sciences version 24.0 for Windows (IBM Corp., Armonk, NY, USA).

RESULTS

Population characteristics

Table 1 shows population characteristics according to presence of gestational hypertension or preeclampsia. **Table S1** shows population characteristics according to presence of early-onset preeclampsia and any GHD.

Table 1. Characteristics of mothers and their children (n=7,124)

	No gestational hypertensive disorders n=6,702	Preeclampsia n=149	P-value[a]	Gestational hypertension n=273	P-value[b]
Maternal characteristics					
Age (years)			0.07		0.60
< 25, No. (%)	1,364(20.4)	30(20.1)		47(17.2)	
25-35, No. (%)	4,364(65.1)	102(68.5)		188(68.9)	
> 35, No. (%)	974(14.5)	17(11.4)		38(13.9)	
Height, mean (SD) (cm)	167.3(7.3)	165.9(7.2)	0.03	168.8(6.9)	<0.01
Weight, mean (SD) (kg)	68.3(12.5)	72(16.1)	<0.01	78.4(17.8)	<0.01
Body Mass Index, mean (SD) (kg/m^2)			<0.01		<0.01
Normal, No. (%)	4,298(64.1)	70(47.0)		115(42.1)	
Overweight, No. (%)	1,661(24.8)	47(31.5)		77(28.2)	
Obese, No. (%)	698(10.4)	30(20.1)		79(28.9)	
Education, No. higher education (%)	2,720(40.6)	41(27.5)	0.05	108(39.5)	0.19
Race / Ethnicity					
Dutch or European, No. (%)	3,232(58.2)	75(53.6)	0.18	198(73.6)	<0.01
Surinamese, No. (%)	561(8.8)	19(13.6)		23(8.6)	
Turkish, No. (%)	575(9.0)	11(7.9)		11(4.1)	
Moroccan, No. (%)	431(6.7)	5(3.6)		8(3.0)	
Cape Verdean or Dutch Antilles, No. (%)	469(7.3)	17(12.2)		12(4.5)	
Parity, No. nulliparous (%)	3,667(55.2)	117(79.1)	<0.01	208(76.2)	<0.01
Smoking, No. (%)			0.09		0.67
None, No. (%)	4,265(72.1)	97(74.0)		173(70.0)	
Early-pregnancy only, No. (%)	531(9.0)	17(13.0)		26(10.5)	
Continued, No. (%)	1,121(18.9)	17(13.0)		48(19.4)	
Mean systolic blood pressure, median (IQR), mmHg	114(107 to 122)	120 (112 to 128)	<0.01	124(116 to 132)	<0.01
Mean diastolic blood pressure, median (IQR), mmHg	67(61 to 73)	73(66 to 80)	<0.01	75(70 to 83)	<0.01

Table 1. Continued

	No gestational hypertensive disorders n=6,702	Preeclampsia n=149	P-value[a]	Gestational hypertension n=273	P-value[b]
Mean arterial pressure, median (IQR), mmHg	82.7(77.0 to 88.7)	88.3(81.4-95.3)	<0.01	91.3(85 to 98.5)	<0.01
Estimated fetal weight, mean (SD) (g)					
Second trimester, mean (SD), SDS	-0.15(0.96)	-0.20(0.87)	0.52	0.00(1.08)	0.03
Second trimester, mean (SD), (g)	371(86)	376(90)		383(92)	
Third trimester, mean (SD), SDS	0.00(0.98)	-0.19(1.17)	<0.01	0.15(1.18)	0.08
Third trimester, mean (SD), (g)	1612(255)	1550(249)		1639(255)	
Placental growth factor					
First trimester, median (IQR) MOM	1.01(0.76 to 1.35)	0.80(0.59 to 1.13)	<0.01	0.92(0.68 to 1.15)	0.07
First trimester, median (IQR), ng/ml	43.5(29.2 to 73.0)	35.5(23.2 to 57.58)		35.2(26.4 to 60.0)	
Second trimester, median (IQR), MOM	1.00(0.73 to 1.39)	0.71(0.50 to 1.11)	<0.01	0.86(0.65 to 1.17)	<0.01
Second trimester, median (IQR), ng/ml	199.3(145.4 to 286.9)	145.8(93.9 to 213.4)		174.0(131.6 to 244.1)	
Uterine artery resistance index					
Second trimester, mean (SD), SDS	-0.01(0.99)	0.53(1.27)	<0.01	0.02(1.10)	0.76
Second trimester, mean (SD)	0.54(0.09)	0.59(0.11)		0.54(0.10)	
Third trimester, mean (SD), SDS	-0.01(0.99)	0.75(1.43)	<0.01	-0.14(1.00)	0.14
Third trimester, mean (SD)	0.48(0.08)	0.54(0.11)		0.47(0.08)	

Abbreviation: IQR: inter quartile range. Values are observed data and represent means (SD), medians (IQR) or number of subjects (valid %). Differences in subject characteristics between participants with and without gestational hypertensive disorders were evaluated using one-way ANOVA tests for continuous variables and chi-square tests for categorical variables. [a] P-value for comparison of population characteristics among women without gestational hypertensive disorders and with pre-eclampsia. [b] P-value for comparison of population characteristics among women without gestational hypertensive disorders and with gestational hypertension.

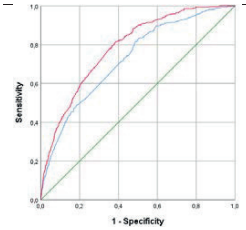
Screening for gestational hypertension using maternal, fetal and placental characteristics

Maternal early-pregnancy characteristics had a moderate screening performance for gestational hypertension (AUC 0.73 (95% Confidence interval (CI) 0.70-0.76)) (**Figure 1**). Model performance improved significantly when blood pressure was added (AUC 0.79 (95%CI 0.76-0.81), p-value for model comparison to maternal characteristics model: 0.003). Using this model led to a 40% sensitivity at 90% specificity with PPV of 0.14; NPV of 0.97; PLR of 4; NLR of 0.67). **Table S2** shows effect estimates for the maternal characteristics in this model for the risk of gestational hypertension. The maternal characteristics model (with blood pressure in early-pregnancy) was used as baseline model for further analyses. Addition of fetal or placental screening results to the maternal characteristics model did not improve screening performance. When adding screening characteristics in chronological order to the screening model for gestational hypertension, the best model did not change (**Figure S2**).

Screening for preeclampsia using maternal, fetal and placental characteristics

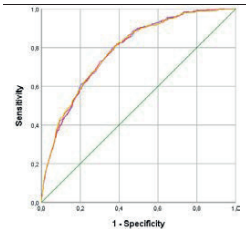
Maternal characteristics model had a moderate screening performance (AUC 0.70 (95%CI 0.66-0.74) for preeclampsia (**Figure 2**). Addition of blood pressure to the model led to a higher AUC (0.74 (95%CI 70-0.79)), but the difference was not statistically significant. Using this model, we obtained a sensitivity of 33% at 90% specificity. **Table S2** shows the effect estimates for the included maternal characteristics in this model for the risk of preeclampsia. Addition of fetal characteristics did not improve screening for preeclampsia in comparison to the maternal characteristics model including early-pregnancy blood pressure. Addition of both second and third trimester UtA-RI led to a clinically improved screening performance (AUC 0.78 (95% CI 0.75 to 0.82), sensitivity 48% at 90% specificity, PPV of 0.09; NPV of 0.99; PLR of 4.8; NLR of 0.58, p-value for comparison with maternal characteristics model including early-pregnancy blood pressure <0.01, **Figure 2**). Subsequent addition of first or second trimester PlGF did not further improve model performance. **Figure S2** shows that when adding screening characteristics in chronological order to the screening model for any preeclampsia, the addition of 2nd trimester PlGF clinically and significantly improved the maternal characteristics with blood pressure model. Subsequent further addition of 2nd and 3rd trimester UtA-RI improved model performance (AUC 0.80 (95% CI 0.77 to 0.84). This obtained model did not perform better in screening for preeclampsia than the obtained model based on clinical availability including maternal characteristics, blood pressure and second and third trimester UtA-RI only (p-value for comparison >0.05).

Models based on maternal characteristics



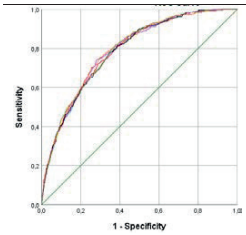
Models[†]	AUC (95% CI)	Sensitivity at specificity		
		70%	80%	90%
Maternal characteristics: (Blue line)	0.73 (0.70-0.76)			
Blood pressure: (Red line)	0.79 (0.76-0.81)	69%	58%	40%

Models based on maternal and fetal characteristics



Models[‡]	AUC (95% CI)	Sensitivity at specificity		
		70%	80%	90%
Baseline: (Blue line)	0.79 (0.76-0.81)	69%	58%	40%
Fetal sex: (Red line)	0.79 (0.76-0.81)			
2nd trimester EFW: (Purple line)	0.79 (0.76-0.81)			
3rd trimester EFW: (Orange line)	0.79 (0.76-0.81)			
2nd and 3rd trimester EFW: (Yellow line)	0.79 (0.76-0.81)			

Models based on maternal and placental characteristics



Models[§]	AUC (95% CI)	Sensitivity at specificity		
		70%	80%	90%
Baseline: (Blue line)	0.79 (0.76-0.81)	69%	58%	40%
2nd trimester UtA-RI: (Red line)	0.79 (0.76-0.81)			
3rd trimester UtA-RI: (Purple line)	0.79 (0.77-0.82)			
2nd and 3rd trimester UtA-RI: (Orange line)	0.79 (0.77-0.82)			
1st trimester PlGF: (Yellow line)	0.79 (0.77-0.82)			
2nd trimester PlGF: (Black line)	0.79 (0.76-0.81)			
1st and 2nd trimester PlGF: (Gray line)	0.79 (0.77-0.82)			

Figure 1. Screening performance for gestational hypertension based on maternal, fetal and placental characteristics

AUC: area under the curve; CI: confidence interval; EFW: Estimated fetal weight; PlGF: Placental Growth Factor; UtA-RI: Uterine artery resistance index. Values are AUC (95% CI), Sensitivity at 70%, 80% and 90% specificity.

[†] Maternal characteristics model: maternal age, BMI, ethnicity, parity and smoking;

Blood pressure model: Maternal characteristics model and first trimester mean arterial pressure per 10 mm Hg.

[‡] Baseline model: maternal age, BMI, ethnicity, parity and smoking, and first trimester MAP per 10 mm Hg;

Fetal sex model: Baseline model + fetal sex;

2nd trimester EFW model: Baseline model and 2nd trimester estimated fetal weight <10th percentile;

3rd trimester EFW model: Baseline model and 3rd trimester estimated fetal weight <10th percentile;

2nd and 3rd trimester EFW model: Baseline model, 2nd and 3rd trimester estimated fetal weight <10th percentile.

[§] Baseline model: maternal age, BMI, ethnicity, parity, smoking, and first trimester MAP per 10 mm Hg;

2nd trimester UtA-RI model: Baseline model, 2nd trimester uterine artery resistance index >90th percentile;

3rd trimester UtA-RI model: Baseline model, 3rd trimester uterine artery resistance index >90th percentile;

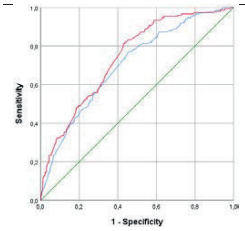
2nd and 3rd trimester UtA-RI model: Baseline model, 2nd and 3rd trimester uterine artery resistance index >90th percentile.

1st trimester PlGF model: Baseline model, 1st trimester placental growth factor < 10th percentile;

2nd trimester PlGF model: Baseline model, 2nd trimester placental growth factor < 10th percentile;

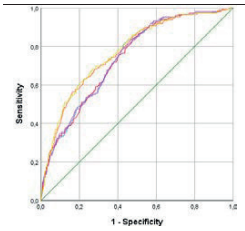
1st and 2nd trimester PlGF model: Baseline model, 1st and 2nd trimester placental growth factor < 10th percentile.

Models based on maternal characteristics



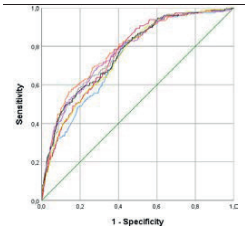
Models†	AUC (95% CI)	Sensitivity at specificity		
		70%	80%	90%
Maternal characteristics: (Blue line)	0.70 (0.66-0.74)	55%	45%	28%
Blood pressure: (Red line)	0.74 (0.70-0.78)	58%	49%	33%

Models based on maternal and fetal characteristics



Models‡	AUC (95% CI)	Sensitivity at specificity		
		70%	80%	90%
Baseline: (Blue line)	0.74 (0.70-0.78)	58%	49%	33%
Fetal sex: (Red line)	0.74 (0.70-0.76)			
2nd trimester EFW: (Purple)	0.74 (0.70-0.78)			
3rd trimester EFW: (Orange line)	0.77 (0.73-0.81)			
2nd and 3rd trimester EFW: (Yellow line)	0.77 (0.74-0.81)			

Models based on maternal and placental characteristics



Models§	AUC (95% CI)	Sensitivity at specificity		
		70%	80%	90%
Baseline: (Blue line)	0.74 (0.70-0.78)	58%	49%	33%
2nd trimester UtA-RI: (Red line)	0.76 (0.73-0.80)			
3rd trimester UtA-RI: (Purple line)	0.77 (0.74-0.81)			
2nd and 3rd trimester UtA-RI: (Orange line)	0.78 (0.75-0.82)	71%	61%	48%
2nd and 3rd trimester UtA-RI and 1st trimester PlGF: (Yellow line)	0.79 (0.75-0.83			
2nd and 3rd trimester UtA-RI and 2nd trimester PlGF: (Black line)	0.80 (0.77-0.84)			
2nd and 3rd trimester UtA-RI and 1st and 2nd trimester PlGF: (Gray line)	0.80 (0.77-0.84)			

Figure 2. Screening performance for preeclampsia based on maternal, fetal and placental characteristics

AUC: area under the curve; CI: confidence interval; EFW: Estimated fetal weight; PlGF: Placental Growth Factor; UtA-RI: Uterine artery resistance index. Values are AUC (95% CI), Sensitivity at 70%, 80% and 90% specificity.

† Maternal characteristics model: maternal age, BMI, ethnicity, parity and smoking;

Blood pressure model: Maternal characteristics model and first trimester mean arterial pressure per 10 mm Hg.

‡ Baseline model: maternal age, BMI, ethnicity, parity and smoking, and first trimester MAP per 10 mm Hg;

Fetal sex model: Baseline model + fetal sex;

2nd trimester EFW model: Baseline model and 2nd trimester estimated fetal weight <10th percentile;

3rd trimester EFW model: Baseline model and 3rd trimester estimated fetal weight <10th percentile;

2nd and 3rd trimester EFW model: Baseline model, 2nd and 3rd trimester estimated fetal weight <10th percentile.

§ Baseline model: maternal age, BMI, ethnicity, parity, smoking, and first trimester MAP per 10 mm Hg;

2nd trimester UtA-RI model: Baseline model, 2nd trimester uterine artery resistance index >90th percentile;

3rd trimester UtA-RI model: Baseline model, 3rd trimester uterine artery resistance index >90th percentile;

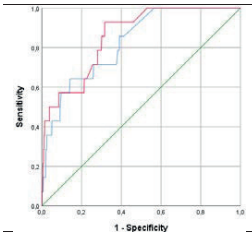
2nd and 3rd trimester UtA-RI model: Baseline model, 2nd and 3rd trimester uterine artery resistance index >90th percentile.

2nd and 3rd trimester UtA-RI model and 1st trimester PlGF model: 2nd and 3rd trimester UtA-RI model, 1st trimester placental growth factor < 10th percentile;

2nd and 3rd trimester UtA-RI model and 2nd trimester PlGF model: 2nd and 3rd trimester UtA-RI model , 2nd trimester placental growth factor < 10th percentile;

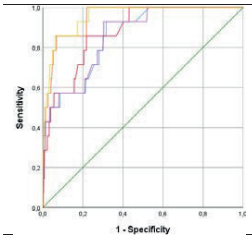
2nd and 3rd trimester UtA-RI model and 1st and 2nd trimester PlGF model: 2nd and 3rd trimester UtA-RI model, 1st and 2nd trimester placental growth factor < 10th percentile.

Models based on maternal characteristics



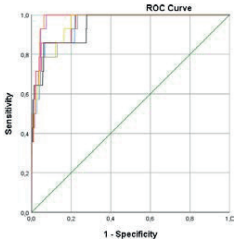
Models[†]	AUC (95% CI)	Sensitivity at specificity		
		70%	80%	90%
Maternal characteristics: (Blue line)	0.83 (0.73-0.92)			
Blood pressure: (Red line)	0.86 (0.78-0.94)	79%	57%	57%

Models based on maternal and fetal characteristics



Models[‡]	AUC (95% CI)	Sensitivity at specificity		
		70%	80%	90%
Baseline: (Blue line)	0.86 (0.78-0.94)			
Fetal sex: (Red line)	0.88 (0.80-0.95)			
2nd trimester EFW: (Green line)	0.86 (0.76-0.94)			
3rd trimester EFW: (Orange line)	0.95 (0.91-0.99)	100%	86%	86%
2nd and 3rd trimester EFW: (Yellow line)	0.96 (0.92-0.99)			

Models based on maternal characteristics, fetal and placental characteristics



Models[§]	AUC (95% CI)	Sensitivity at specificity		
		70%	80%	90%
Baseline: (Blue line)	0.95 (0.91-0.99)	100%	86%	86%
2nd trimester UtA-RI: (Red line)	0.96 (0.92-0.99)			
3rd trimester UtA-RI: (Green line)	0.98 (0.97-0.99)			
2nd and 3rd trimester UtA-RI: (Orange line)	0.98 (0.97-0.99)			
1st trimester PlGF: (Red line)	0.95 (0.92-0.99)			
2nd trimester PlGF: (Green line)	0.95 (0.90-0.99)			
1st and 2nd trimester PlGF: (Orange line)	0.95 (0.91-0.99)			

Figure 3. Screening performance for secondary outcome early-onset preeclampsia based on maternal, fetal and placental characteristics

AUC: area under the curve; CI: confidence interval; EFW: Estimated fetal weight; PlGF: Placental Growth Factor; UtA-RI: Uterine artery resistance index. Values are AUC (95% CI), Sensitivity at 70%, 80% and 90% specificity.

[†] Maternal characteristics model: maternal age, BMI, ethnicity, parity and smoking;

Blood pressure model: Maternal characteristics model and first trimester mean arterial pressure per 10 mm Hg.

[‡] Baseline model: maternal age, BMI, ethnicity, parity and smoking, and first trimester MAP per 10 mm Hg;

Fetal sex model: Baseline model + fetal sex;

2nd trimester EFW model: Baseline model and 2nd trimester estimated fetal weight <10th percentile;

3rd trimester EFW model: Baseline model and 3rd trimester estimated fetal weight <10th percentile;

2nd and 3rd trimester EFW model: Baseline model, 2nd and 3rd trimester estimated fetal weight <10th percentile.

[§] Baseline model: maternal age, BMI, ethnicity, parity, smoking, and first trimester MAP per 10 mm Hg, and 3rd trimester EFW;

2nd trimester UtA-RI model: Baseline model, 2nd trimester uterine artery resistance index >90th percentile;

3rd trimester UtA-RI model: Baseline model, 3rd trimester uterine artery resistance index >90th percentile;

2nd and 3rd trimester UtA-RI model: Baseline model, 2nd and 3rd trimester uterine artery resistance index >90th percentile.

1st trimester PlGF model: Baseline model, 1st trimester placental growth factor < 10th percentile;

2nd trimester PlGF model: Baseline model, 2nd trimester placental growth factor < 10th percentile;

1st and 2nd trimester PlGF model: Baseline model, 1st and 2nd trimester placental growth factor < 10th percentile.

Maternal characteristics with blood pressure achieved a good performance for the secondary outcome early-onset preeclampsia (AUC 0.86 (95%CI 0.78-0.94) with a sensitivity 57% at 90% specificity), which was better than screening for preeclampsia at any gestational age (**Figure 3**). Addition of third trimester EFW screening result, but not other fetal or placental characteristics, clinically significantly improved model performance (AUC 0.95 (95%CI 0.91-0.99), sensitivity 86% at 90% specificity, p-value for comparison to the maternal characteristics model including early-pregnancy blood pressure: 0.039). When adding screening characteristics in chronological order to the screening model for early-onset preeclampsia, the best model did not change (**Figure S3**).

When we assessed screening for any GHD we observed a moderate model performance for maternal characteristics including blood pressure (AUC 0.77 (95%CI 0.74-0.79), **Figure S4**). Addition of fetal or placental characteristics did not improve screening. When adding screening characteristics in chronological order to the screening model for any GHD, the best model did not change (**Figure S3**).

DISCUSSION

Main findings

Maternal characteristics including age, body mass index, ethnicity, parity, smoking and blood pressure known in early-pregnancy have a moderate screening performance for pregnancies at risk of gestational hypertension and preeclampsia in a low risk multi-ethnic population. Addition of combined second and third trimester placental ultrasound screening results only improved screening performance for preeclampsia, in addition to simple maternal characteristics.

Interpretation of main findings

GHD are a major cause of maternal, fetal and neonatal morbidity and mortality[2, 3, 5, 7]. It is well-known that maternal, fetal and placental characteristics are associated with GHD, but the strength of associations of different factors varies across studies and populations[11, 34, 35]. Based on these associations screening models for GHD can be developed, using groups of screening parameters, which may aid earlier identification of women at risk of GHD. However, screening of the general population of pregnant women for GHD in clinical practice remains highly challenging.

Previous studies have focused on using maternal characteristics for the prediction of preeclampsia, whereas fewer studies have focused on prediction of gestational

hypertension[11, 34, 36-38]. A previous systematic review among 29 studies, reporting on 70 models for prediction of preeclampsia using routinely collected maternal characteristics, showed that screening performance for preeclampsia at any gestational age ranges from moderate (AUC 0.67 (95%CI 0.59-0.76)) to good (AUC 0.81 (95%CI 0.80-0.82))[11]. The study with the highest screening performance, was conducted among 6,015 mainly Caucasian (74%) women, and developed a screening model for preeclampsia at any gestational age using maternal characteristics including ethnic origin, BMI, previous preeclampsia and family history of preeclampsia[37]. A review among 92 studies, examining 25,356,688 pregnancies, assessed risk of preeclampsia among women with and without individual clinical risk-factors determined <16 weeks gestation, to select risk-factors for future development of prediction models[36]. The authors noted that for selection of pregnancies at risk of preeclampsia on a population level, the use of common risk-factors may be more useful than the use of rare but strongly associated risk-factors. These rare but strong risk-factors could be more useful on an individual level[36]. Fewer studies developed models for prediction of gestational hypertension using only maternal characteristics[37, 38]. The previously mentioned study among 6,015 women showed moderate screening performance for gestational hypertension (AUC 0.69 (95%CI 0.68-0.70), using a model consisting of maternal characteristics including ethnic origin, BMI, previous preeclampsia and family history of preeclampsia[37].

We observed that routinely measured maternal characteristics in early-pregnancy can be used in screening for risk of both gestational hypertension and preeclampsia with sensitivity ranging from 33-40% at 90% specificity. Screening performance improved to 57% at 90% specificity, when we only focused on early-onset preeclampsia. Screening performance of our screening model based on maternal characteristics is quite comparable to previously developed models[11]. The strength of our maternal characteristics model is that, in contrast with most models which use previous preeclampsia, pre-existing conditions or family history, we only used maternal characteristics routinely collected in clinical care, and as such are available early in pregnancy. These characteristics can be used in low-resource settings, and are applicable for both nulliparous and parous women. Furthermore, in contrast to studies using tertiary or infertility care populations, we fitted our maternal characteristics model on a low-risk multi-ethnic population, which is more representative of the general obstetric population. We used a similar model for prediction of gestational hypertension and preeclampsia, which may be easier to use in clinical practice. Taken together, the current study shows that one model consisting of routinely measured maternal characteristics including blood pressure in early-pregnancy may be used to detect pregnancies at risk of gestational hypertension and preeclampsia

in a low-risk multi-ethnic population and leads to a moderate screening performance. This screening performance seems comparable to screening models using more specific and rare maternal characteristics, which may only be applicable in specific populations.

The value of fetal and placental characteristics in addition to maternal characteristics for screening for GHD is debated. EFW has been associated with GHD, and newborns born from pregnancies complicated by preeclampsia have a 5%-23% lower birth weight compared newborns born after uncomplicated pregnancies[39-41]. To our knowledge, EFW has not been studied as predictor for gestational hypertension or preeclampsia[39]. In our study, addition of second or third trimester EFW to maternal characteristics did not lead to better screening performance for gestational hypertension and preeclampsia. For early-onset preeclampsia, sensitivity did improve to 86% at 90% specificity. This positive effect on screening performance may be explained by reversed causation as fetal ultrasound was performed around 30 weeks gestation. Although increased UtA impedance and low PlGF have been described as predictors for GHD, evidence on added value of these parameters is conflicting[11, 12, 42, 43]. A prospective screening study for gestational hypertension and preeclampsia in a low-risk multi-ethnic population among 8,366 women created first trimester models consisting of maternal history, blood pressure, PAPP-A, and UtA-PI, and reported that there was a small contribution of placental measurements. Model performance was better for screening for preeclampsia than for gestational hypertension[12]. A recent study among 4,212 nulliparous singleton pregnancies reported that in addition to maternal characteristics, blood pressure, 20 week UtA-PI, PAPP-A and PlGF did not improve screening performance for early-onset preeclampsia[43]. In our low-risk multi-ethnic population, single addition of more advanced fetal and placental screening parameters did not improve screening for gestational hypertension and preeclampsia. Only when both second and third trimester UtA-Doppler screening results were added simultaneously, screening performance improved for preeclampsia only. The presence of screening benefit of repeated UtA-RI results for preeclampsia but not for gestational hypertension is likely due to a larger role of the placenta in the pathophysiology of preeclampsia[4]. Because our study was specifically focused on screening for GHD in low-risk pregnant populations, we used routinely measured or easily available characteristics as much as possible to enable translation of our findings to clinical practice and low-resource settings. We therefore added screening characteristics to the screening model based on clinical availability. However, earlier screening for GHD allows potential earlier interventions. We further explored whether addition of placental and fetal screening characteristics in a chronological order led to different screening models. However, this did not affect our findings. We hypothesize that differences between screening performances of parameters in different models may be due to the fact that maternal characteristics are strongly correlated to GHD,

but also with fetal and placental parameters, possibly reducing screening potential of these more advanced fetal and placental measurements[16, 18, 44]. Thus, our results suggest that the additional screening benefit of fetal and placental parameters for screening for gestational hypertension and preeclampsia is limited. Only when both second and third trimester UtA-Doppler screening results were added simultaneously, screening performance improved for preeclampsia only. Use of fetal and placental measurements may have a larger contribution to screening for GHD among higher risk populations.

Our study adds to existing evidence that maternal characteristics, routinely measured in clinical practice, known early in any pregnancy, can be used in screening for risk of gestational hypertension and preeclampsia in low-risk multi-ethnic populations. It should be further explored if maternal characteristics and MAP collected before pregnancy yield similar screening results to enable early risk selection and modify risk-factors even before conception[45, 46]. Before any screening model for GHD can be implemented in clinical practice, further research is necessary. To date, aggregated analysis of screening models was not possible due to large heterogeneity of studies. Future large studies should test promising models in diverse populations, utilizing maternal characteristics as much as possible, before adding more advanced fetal and placental measurements, as maternal characteristics are more easy and cost-effective to use in clinical practice and can also be implemented in low-resource settings, in which GHD lead to the poorest outcomes[47]. In these studies, benefits due to identification of true-positives versus harm caused by false-positives should be evaluated in contemporary low-risk populations. For current clinical practice, this study shows that maternal characteristics in early-pregnancy contain valuable information for assessment of risk of GHD, and should be considered in routine care.

Strengths and limitations

We collected prospective data from early-pregnancy onwards in 7,124 women with information regarding GHD. We defined diagnosis of preeclampsia according to the 2001 ISSHP criteria[28, 29]. As no exact gestational age at diagnosis of GHD was available, misclassification of the onset of preeclampsia may have occurred. We considered it important to specifically assess screening performance of our models for early-onset preeclampsia, as this is often regarded as a different entity with a higher risk of adverse outcomes. Our findings of a better screening performance for early-onset preeclampsia are in line with previous studies[11]. However, as misclassification of gestational age at diagnosis of preeclampsia may have occurred, early-onset preeclampsia needs to be considered as secondary outcome and our models for screening for early-onset preeclampsia need to be interpreted with more caution and replicated among other

study populations. Measurements of blood pressure, ultrasound examinations and blood samples collection were performed according to the study protocol and blinded with regard to pregnancy outcomes due to the prospective nature of the study[48]. Research findings were reported to healthcare providers, which may have led to intensified monitoring or interventions influencing the outcome and possibly effecting screening performance. Current guidelines recommend low-dose Aspirin-prophylaxis for women at higher risk of developing preeclampsia[49, 50]. Use of prophylaxis likely influences occurrence of preeclampsia, and could therefore influence obtained model performance of the screening models. However, as our study participants were pregnant between 2001 and 2006, low-dose aspirin prophylaxis was not yet part of obstetric guidelines. Thus, our screening models were not affected by low-dose aspirin-prophylaxis among women at higher risk of preeclampsia. The current population is not a clinical population, but a low-risk population, which may have influenced screening performance.

Conclusion

Routinely measured maternal characteristics and blood pressure in early-pregnancy have a moderate screening performance for pregnancies at risk of gestational hypertension and preeclampsia in a contemporary multi-ethnic, low-risk population. Addition of combined second and third trimester placental ultrasound screening results only improved screening performance for preeclampsia, in addition to simple maternal characteristics.

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SUPPLEMENTARY MATERIALS

Table S1. Characteristics of mothers and their children with early-onset preeclampsia and any gestational hypertensive disorder

Characteristics	No gestational hypertensive disorders n=6,702	Early-onset preeclampsia n=14	P-value[a]	Any gestational hypertensive disorder n=422	P-value[b]
Maternal characteristics					
Age, years			0.23		0.52
< 25, No. (%)	1,364(20.4)	4(29)		77(18.2)	
25-35, No. (%)	4,364(65.1)	9(64)		290(6.7)	
> 35, No. (%)	974(14.5)	1(7)		55(13.0)	
Height, mean (SD) (cm)	167.3(7.3)	136.6(5.8)	0.06	167.7(7.2)	0.17
Weight, mean (SD) (kg)	68.3(12.5)	70.9(10.5)	0.56	76.2(17.4)	<0.01
Body Mass Index, mean (SD) (kg/m^2)			0.11		<0.01
Normal, No. (%)	4,298(64.1)	5(35.7)		185(43.8)	
Overweight, No. (%)	1,661(24.8)	5(35.7)		124(29.4)	
Obese, No. (%)	698(10.4)	3(21.4)		109(25.8)	
Education, No. higher education (%)	2,720(40.6)	4(33.3)	0.24	149(36.3)	0.04
Race / Ethnicity			0.39		<0.01
Dutch or European, No. (%)	3,232(58.2)	6(50.0)		273(66.7)	
Surinamese, No. (%)	561(8.8)	3(25.0)		42(10.3)	
Turkish, No. (%)	575(9.0)	0(0)		22(5.4)	
Moroccan, No. (%)	431(6.7)	0(0)		13(3.2)	
Cape Verdean or Dutch Antilles, No. (%)	469(7.3)	3(25.0)		29(7.0)	
Parity, No. nulliparous (%)	3,667(55.2)	11(78.6)	0.68	325(77.2)	<0.01
Smoking			0.27		0.24
None, No. (%)	4,265(72.1)	5(50.0)		270(71.4)	
Early-pregnancy only, No. (%)	531(9.0)	2(20.0)		43911.4)	
Continued, No. (%)	1,121(18.9)	3(30.0)		65(17.2)	
Mean systolic blood pressure, median (IQR), mmHg	114(107 to 122)	120(108 to 125)	0.31	122(115 to 130)	<0.01
Mean diastolic blood pressure, median (IQR), mmHg	67(61 to 73)	71(62 to 80)	0.03	74(69 to 82)	<0.01
Mean arterial pressure, median (IQR), mmHg	82.7(77.0 to 88.7)	90(82.5 to 95.3)	0.06	90.3(84.3 to 96.7)	<0.01
Estimated fetal weight					
Second trimester, mean (SD), SDS	-0.15(0.96)	-0.40(0.82)	0.19	-0.07(1.02)	0.04

Table S1. Continued

Characteristics	No gestational hypertensive disorders n=6,702	Early-onset preeclampsia n=14	P-value[a]	Any gestational hypertensive disorder n=422	P-value[b]
Second trimester, mean (SD), (g)	371(86)	340(73)		381(91)	
Third trimester, mean (SD), SDS	0.00(0.98)	-1.48(0.70)	<0.01	0.04(1.16)	0.86
Third trimester, mean (SD), (g)	1612(255)	1217(112)		1609(256)	
Placental growth factor					
First trimester, median (IQR) MoM	1.01(0.76 to 1.35)	0.76(0.39 to 1.28)	0.24	0.87(0.63 to 1.14)	<0.01
First trimester, median (IQR), ng/ml	43.5(29.2 to 73.0)	26.3(17.2 to 51.9)		35.4(24.6 to 59.8)	
Second trimester, median (IQR), MoM	1.00(0.73 to 1.39)	0.52(0.21 to 0.64)	0.08	0.81(0.57 to 1.14)	<0.01
Second trimester, median (IQR), ng/ml	199.3(145.4 to 286.9)	93.0(42.7 to 133.0)		165.3(116.2 to 237.9)	
Uterine artery resistance index					
Second trimester, mean (SD), SDS	-0.01(0.99)	1.81(1.09)	<0.01	0.22(1.20)	<0.01
Second trimester, mean (SD)	0.54(0.09)	0.70(0.10)		0.56(0.11)	
Third trimester, mean (SD), SDS	-0.01(0.99)	2.92(1.76)	<0.01	0.16(1.24)	0.02
Third trimester, mean (SD)	0.48(0.08)	0.71(0.14)		0.50(0.10)	

Abbreviation: IQR: inter quartile range. Values are observed data and represent means (SD), medians (IQR) or number of subjects (valid %). P-values for comparison with "no gestational hypertensive disorders" group, using ANOVA for continuous variables, and Chi² test for categorical variables. [a] *P-value for comparison of population characteristics among women without gestational hypertensive disorders and with early-onset pre-eclampsia.* [b] *P-value for comparison of population characteristics among women without gestational hypertensive disorders and with gestational hypertensive disorders.*

Table S2. Odds of gestational hypertensive disorders given the characteristics in the maternal characteristics model

	Gestational hypertension	Preeclampsia	Any gestational hypertensive disorder
Maternal characteristics			
Age, years			
< 25, No. (%)	0.85(0.59-1.22)	0.69(0.44-1.08)	0.78(0.58-1.04)
25-35, No. (%)	*ref*	*ref*	*ref*
> 35, No. (%)	1.18(0.82-1.72	1.00(0.28-1.69)	1.12(0.82-1.53)
Missing			
Body Mass Index			
Normal	*ref*	*ref*	*ref*
Overweight	1.50(1.10-2.04)	1.59(1.08-2.35)	1.57(1.23-2.00)
Obese	3.00(2.14-4.21)	2.05(1.08-2.35)	2.80(2.11-3.73)
Missing			
Race / Ethnicity			
Dutch or European	*ref*	*ref*	*ref*
Surinamese	0.87(0.55-1.37)	1.94(1.15-3.27)	1.19(0.83-1.69)
Turkish	0.42(0.22-0.80)	1.26(0.65-2.44)	0.64(0.40-1.01)
Moroccan	0.50(0.24-1.04)	0.82(0.32-2.09)	0.58(0.32-1.04)
Cape Verdean or Dutch Antilles	0.53(0.28-0.97)	2.11(1.20-3.70)	0.96(0.63-1.46)
Missing			
Parity			
Nulliparous	*ref*	*ref*	*ref*
Parous	0.39(0.29-0.53	0.30(0.20-0.46)	0.34(0.27-0.44)
Missing			
Smoking			
None	*ref*	*ref*	*ref*
Early-pregnancy only	1.17(0.76-1.81)	1.27(0.74-2.15)	1.22(0.86-1.72)
Continued	1.16(0.82-1.64)	0.68(0.40-1.16)	0.98(0.72-1.31)
Missing			
Mean arterial pressure			
40-49.9 mmHg	.	.	.
50-59.9 mmHg	.	9.57(2.07-44.25)	3.2(0.71-14.47)
60-69.9 mmHg	0.09(0.01-0.67)	0.37 (0.09-1.54)	0.18(0.06-0.58)
70-79.9 mmHg	0.33(0.21-0.54)	0.87(0.54-1.41)	0.51(0.36-0.71)

Table S2. Continued

	Gestational hypertension	Preeclampsia	Any gestational hypertensive disorder
80-89.9 mmHg	*ref*	*ref*	*ref*
90-99.9 mmHg	1.76(1.31-2.37)	1.88(1.24-2.84)	1.85(1.44-2.36)
100-109.9 mmHg	3.34(2.24-5.00)	3.46(8.00-5.99)	3.73(2.66-5.25)
110-119.9 mmHg	5.49(2.13-14.18)	.	4.06(1.59-10.33)
120-129.9 mmHg	9.89(1.28-76.33)	.	7.34(0.99-55.53)
130-139.9 mmHg	11.66(0.64-211.52)	.	9.81(0.55-175.73)
Missing			

Values are odds ratios (95% confidence intervals). The maternal characteristics model consists of maternal age, BMI, ethnicity, parity, smoking, and first trimester MAP per 10 mm Hg;

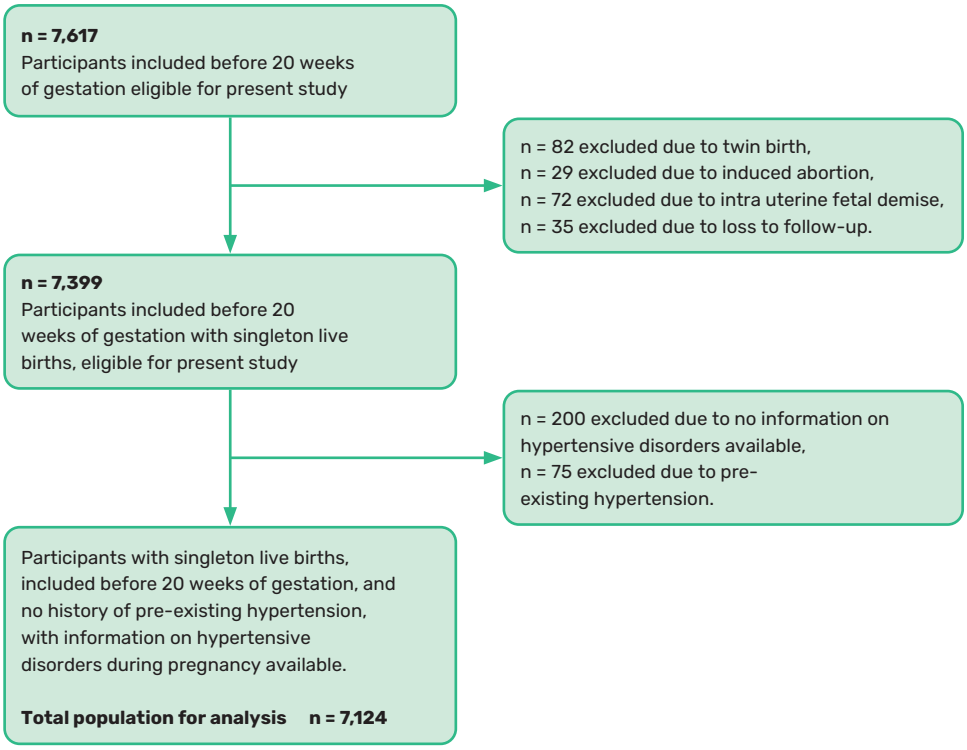


Figure S1. Flowchart population for analysis

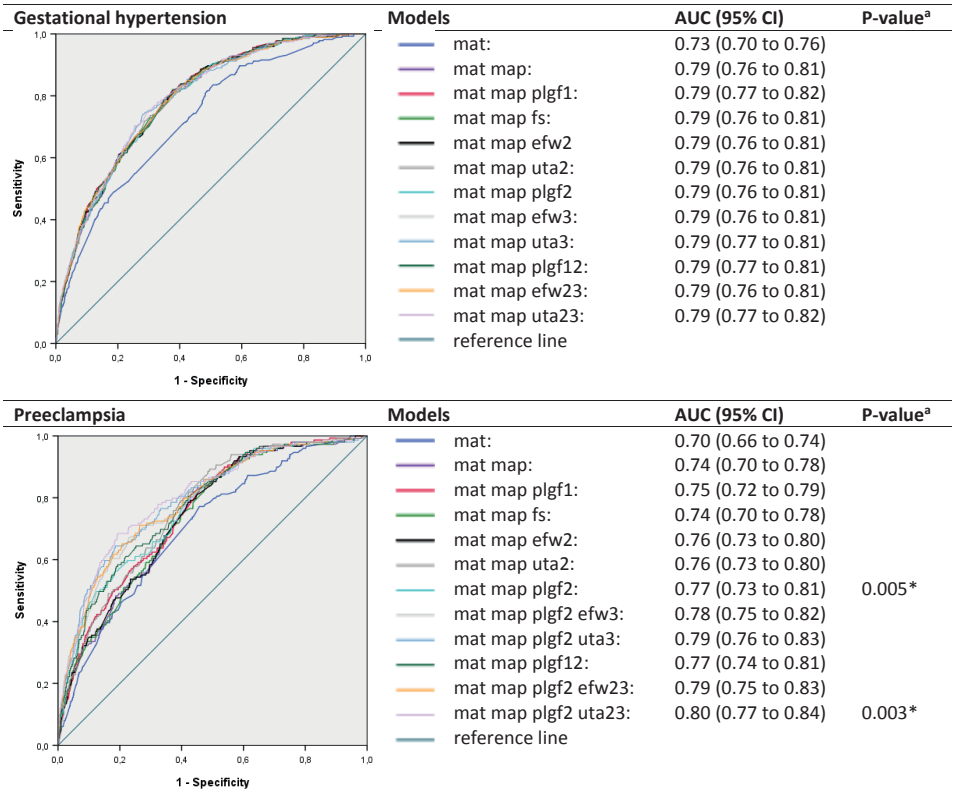


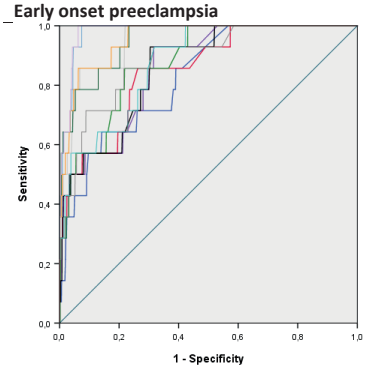
Figure S2. Performance of screening models for gestational hypertension and preeclampsia, with parameters added in order of measurement during pregnancy

Abbreviations: AUC: Area under the curve; CI: Confidence Interval; Mat: maternal characteristics; Map: mean arterial pressure; Plgf1: 1st trimester placental growth factor; Fs: fetal sex; EFW2: 2nd trimester estimated fetal weight; Uta2: 2nd trimester uterine artery resistance index; Plgf2: 2nd trimester placental growth factor; Efw3: 3rd trimester estimated fetal weight; Uta3: 3rd trimester uterine artery resistance index; Plgf12: 1st and 2nd trimester placental growth factor; Efw23: 2nd and 3rd trimester estimated fetal weight; Uta23: 2nd and 3rd trimester uterine artery resistance index.

[a] P-values for comparison of area under the curves using the DeLong method for comparison of 2 correlated areas under the curve. AUCs were statistically tested when a clinically relevant (at least 4%) improvement in AUC was observed.

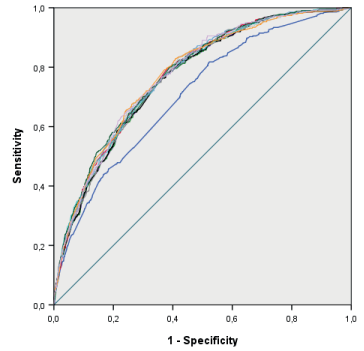
** Indicates a statistically significantly improved area under the curve, compared to the previous model with an asterisk(*), or compared to the model consisting of maternal characteristics and MAP.*

Early onset preeclampsia



Models	AUC (95% CI)	P-value[a]
mat:	0.83 (0.73 to 0.92)	
mat map:	0.86 (0.78 to 0.94)	
mat map plgf1:	0.85 (0.76 to 0.94)	
mat map fs:	0.88 (0.80 to 0.95)	
mat map efw2	0.86 (0.78 to 0.94)	
mat map uta2:	0.89 (0.80 to 0.97)	0.26
mat map plgf2:	0.88 (0.80 to 0.95)	
mat map efw3:	0.95 (0.91 to 0.99)	0.03*
mat map efw3 uta3:	0.98 (0.97 to 0.99)	
mat map efw3 plgf12:	0.95 (0.91 to 0.99)	
mat map efw23:	0.96 (0.92 to 0.99)	
mat map efw3 uta23:	0.96 (0.92 to 0.99)	
reference line		

Any gestational hypertensive disorder



Models	AUC (95% CI)	P-value[a]
mat:	0.71 (0.68 to 0.73)	
mat map:	0.77 (0.74 to 0.79)	
mat map plgf1:	0.77 (0.75 to 0.79)	
mat map fs:	0.76 (0.75 to 0.79)	
mat map efw2:	0.76 (0.75 to 0.79)	
mat map uta2:	0.77 (0.75 to 0.79)	
mat map plgf2:	0.77 (0.75 to 0.79)	
mat map efw3:	0.77 (0.75 to 0.79)	
mat map uta3:	0.77 (0.75 to 0.79)	
mat map plgf12:	0.78 (0.76 to 0.80)	
mat map efw23:	0.77 (0.75 to 0.80)	
mat map uta23:	0.78 (0.75 to 0.80)	
reference line		

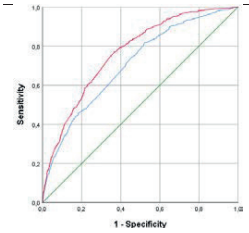
Figure S3. Performance of screening models for early onset preeclampsia and any gestational hypertensive disorder, with parameters added in order of measurement during pregnancy

Abbreviations: AUC: Area under the curve; CI: Confidence Interval; Mat: maternal characteristics; Map: mean arterial pressure; Plgf1: 1st trimester placental growth factor; Fs: fetal sex; EFW2: 2nd trimester estimated fetal weight; Uta2: 2nd trimester uterine artery resistance index; Plgf2: 2nd trimester placental growth factor; Efw3: 3rd trimester estimated fetal weight; Uta3: 3rd trimester uterine artery resistance index; Plgf12: 1st and 2nd trimester placental growth factor; Efw23: 2nd and 3rd trimester estimated fetal weight; Uta23: 2nd and 3rd trimester uterine artery resistance index.

[a] P-values for comparison of area under the curves using the DeLong method for comparison of 2 correlated areas under the curve. AUCs were statistically tested when a clinically relevant (at least 4%) improvement in AUC was observed.

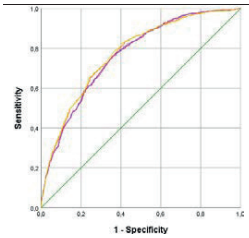
** Indicates a statistically significantly improved area under the curve, compared to the previous model with an asterisk(*), or compared to the model consisting of maternal characteristics and MAP.*

Models based on maternal characteristics



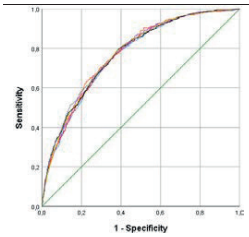
Models†	AUC (95% CI)	Sensitivity at specificity		
		70%	80%	90%
Maternal characteristics: (Blue line)	0.71 (0.68-0.73)			
Blood pressure: (Red line)	0.77 (0.74-0.79)	79%	57%	57%

Models based on maternal and fetal characteristics



Models‡	AUC (95% CI)	Sensitivity at specificity		
		70%	80%	90%
Baseline: (Blue line)	0.77 (0.74-0.79)	79%	57%	57%
Fetal sex: (Red line)	0.76 (0.74-0.79)			
2nd trimester EFW: (Green line)	0.76 (0.74-0.79)			
3rd trimester EFW: (Orange line)	0.77 (0.75-0.79)			
2nd and 3rd trimester EFW: (Yellow line)	0.77 (0.75-0.80)			

Models based on maternal and placental characteristics



Models§	AUC (95% CI)	Sensitivity at specificity		
		70%	80%	90%
Baseline: (Blue line)	0.77 (0.74-0.79)	79%	57%	57%
2nd trimester UtA-RI: (Red line)	0.77 (0.75-0.79)			
3rd trimester UtA-RI: (Green line)	0.77 (0.74-0.79)			
2nd and 3rd trimester UtA-RI: (Orange line)	0.77 (0.75-0.79)			
1st trimester PlGF: (Red line)	0.77 (0.75-0.79)			
2nd trimester PlGF: (Green line)	0.77 (0.75-0.79)			
1st and 2nd trimester PlGF: (Orange line)	0.78 (0.76-0.80)			

Figure S4. Screening performance for any gestational hypertensive disorder based on maternal, fetal and placental characteristics

AUC: area under the curve; CI: confidence interval; EFW: Estimated fetal weight; PlGF: Placental Growth Factor; UtA-RI: Uterine artery resistance index. Values are AUC (95% CI), Sensitivity at 70%, 80% and 90% specificity.

† Maternal characteristics model: maternal age, BMI, ethnicity, parity and smoking;

Blood pressure model: Maternal characteristics model and first trimester mean arterial pressure per 10 mm Hg.

‡ Baseline model: maternal age, BMI, ethnicity, parity and smoking, and first trimester MAP per 10 mm Hg;

Fetal sex model: Baseline model + fetal sex;

2nd trimester EFW model: Baseline model and 2nd trimester estimated fetal weight <10th percentile;

3rd trimester EFW model: Baseline model and 3rd trimester estimated fetal weight <10th percentile;

2nd and 3rd trimester EFW model: Baseline model, 2nd and 3rd trimester estimated fetal weight <10th percentile.

§ Baseline model: maternal age, BMI, ethnicity, parity, smoking, and first trimester MAP per 10 mm Hg;

2nd trimester UtA-RI model: Baseline model, 2nd trimester uterine artery resistance index >90th percentile;

3rd trimester UtA-RI model: Baseline model, 3rd trimester uterine artery resistance index >90th percentile;

2nd and 3rd trimester UtA-RI model: Baseline model, 2nd and 3rd trimester uterine artery resistance index >90th percentile.

1st trimester PlGF model: Baseline model, 1st trimester placental growth factor < 10th percentile;

2nd trimester PlGF model: Baseline model, 2nd trimester placental growth factor < 10th percentile;

1st and 2nd trimester PlGF model: Baseline model, 1st and 2nd trimester placental growth factor < 10th percentile.

SCREENING STUDIES

3.2: SECOND AND THIRD TRIMESTER FETAL ULTRASOUND POPULATION SCREENING FOR RISKS OF PRETERM BIRTH AND SMALL-SIZE AND LARGE-SIZE FOR GESTATIONAL AGE AT BIRTH: A POPULATION-BASED PROSPECTIVE COHORT STUDY

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Jan S. Erkamp
Ellis Voerman
Eric A.P. Steegers
Annemarie G.M.G.J. Mulders
Irwin K.M. Reiss
Liesbeth Duijts
Vincent W.V. Jaddoe
Romy Gaillard

ABSTRACT

Background

Preterm birth, small-size for gestational age (SGA) and large-size for gestational age (LGA) at birth are major risk factors for neonatal and long-term morbidity and mortality. It is unclear which periods of pregnancy are optimal for ultrasound screening to identify fetuses at risk of preterm birth, SGA or LGA at birth. We aimed to examine whether single or combined second and third trimester ultrasound in addition to maternal characteristics at the start of pregnancy, are optimal to detect fetuses at risk for preterm birth, SGA and LGA.

Methods

In a prospective population-based cohort among 7,677 pregnant women, we measured second and third trimester estimated fetal weight (EFW), and uterine artery pulsatility and umbilical artery resistance indices as placenta flow measures. Screen positive was considered as EFW or placenta flow measure <10th or >90th percentile. Information about maternal age, body mass index, ethnicity, parity, smoking, fetal sex and birth outcomes was available from questionnaires and medical records. Screening performance was assessed via Receiver Operating Characteristic (ROC)-Curves and Area Under the Curve (AUC) along with sensitivity at different false positive rates.

Results

Maternal characteristics only and in combination with second trimester EFW had a moderate performance for screening for each adverse birth outcome. Screening performance improved by adding third trimester EFW to the maternal characteristics (AUCs for preterm birth 0.64 (95%CI 0.61 to 0.67); SGA 0.79 (95%CI 0.78 to 0.81); LGA 0.76 (95%CI 0.75; 0.78)). Adding third trimester placenta measures to this model improved only screening for risk of preterm birth (AUC 0.72 (95%CI 0.66 to 0.77) with sensitivity 37% at specificity 90%) and SGA (AUC 0.83 (95%CI 0.81 to 0.86) with sensitivity 55% at specificity 90%). Combining second and third trimester fetal and placental ultrasound did not lead to a better performance as compared to using only third trimester results.

Conclusions

Combining single third trimester fetal and placental ultrasound results with maternal characteristics has the best screening performance for risks of preterm birth, SGA and LGA. As compared to second trimester screening, third trimester screening may double the detection of fetuses at risk of common adverse birth outcomes.

BACKGROUND

Preterm birth, small-size for gestational age (SGA) and large-size for gestational age (LGA) at birth explain up to 30% of neonatal death, and are strong risk factors for short-term and long-term morbidity[1, 2]. The majority of newborns who experience abnormal fetal growth are unidentified until birth[3-6]. SGA or LGA newborns who have not been identified antenatally have strongly increased risks of morbidity and mortality, compared to those who have been identified antenatally[6-9]. Abnormal fetal growth is an important reason for induction of labour, and is therefore a common cause of induced preterm birth[3, 10]. However, studies have shown that spontaneous preterm birth is often preceded by impaired or accelerated fetal growth[3, 9]. Current pregnancy care protocols include dating ultrasounds and detailed structural ultrasounds at 20 weeks gestational age (GA) to assess congenital anomalies and fetal size[11, 12]. Third trimester ultrasound screening is mostly used in selected populations. Technological developments in obstetric ultrasounds may lead to future changes in ultrasound screening protocols, such as early-pregnancy size and congenital anomalies assessment and third trimester growth assessment. The performance of routine third trimester ultrasound screening, independent of other maternal and fetal characteristics, is not clear. A review of eight controlled trials did not suggest consistent benefits of ultrasound after 24 weeks GA on pregnancy outcomes[13]. A prospective observational cohort study among 3,977 nulliparous women suggested that third trimester ultrasound, in addition to second trimester ultrasound, tripled the detection of fetuses subsequently born SGA compared to selective third trimester ultrasound[14].

We used data from a population-based observational study among 7,670 pregnant women to examine whether single or combined second or third trimester fetal and placental ultrasound examinations, in addition to maternal characteristics, are optimal to detect fetuses at risk for preterm birth, SGA and LGA.

METHODS

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards in Rotterdam, the Netherlands[15]. The study has been approved by the local Medical Ethical Committee (MEC 198.782/2001/31). Written consent was obtained from all women. All pregnant women were enrolled between 2001 and 2005. The response rate at birth was 61%, which was calculated by dividing the number of participating live born children by the total number of live born

children born in the study area during the inclusion period[16]. 8,879 women were enrolled during pregnancy. We excluded women without second and third trimester ultrasound data (n=1,130), non-singleton-live-births (n=33), and women with outcome data missing (n=46). This led to a population for analysis of 7,670 pregnant women (**Figure S1** shows the flowchart for the population for analysis).

Maternal characteristics at the start of pregnancy

We selected maternal characteristics known at the start of pregnancy, which are important determinants of adverse birth outcomes[3, 17-19]. Maternal age was assessed at enrolment and categorized; <25.0 years, 25.0–34.9 years, ≥35.0 years[3]. Maternal height (cm) and weight (kg) were measured without shoes and heavy clothing at enrolment and BMI (kg/m^2) was calculated and categorized for clinical purposes: normal weight (BMI<25 kg/m^2), overweight (BMI 25.0-30.0 kg/m^2) and obese (BMI≥30.0 kg/m^2)[18]. Information about ethnicity and parity and smoking was obtained by questionnaire and categorized as previously described[3, 17].

Second and third trimester fetal and placental ultrasounds

Ultrasound examinations were carried out in two dedicated research centers in first (median 13.2 weeks GA, interquartile range (IQR) 12.2 to 14.7), second (median 20.5 weeks GA, IQR 19.9 to 21.3) and third trimester (median 30.4 weeks GA, IQR 29.8 to 30.9)[3]. We established GA by using data from the first ultrasound[3]. In second and third trimester, we measured fetal head circumference, abdominal circumference (AC) and femur length to the nearest millimeter using standardized procedures[20]. Estimated fetal weight (EFW) was calculated using the formula of Hadlock et al, in line with clinical practice[21]. GA-adjusted SDS for growth measures were based on reference growth charts from the whole study population[3]. In line with clinical practice, we defined screen-positive as EFW or AC in the lowest or highest decile in second or third trimester [5, 8, 14, 22, 23]. Both extremes of EFW and AC are associated with a higher risk of common adverse birth outcomes and perinatal morbidity and mortality[3, 14]. This approach leads to one screening test for all adverse birth outcomes, which strongly improves ease-of-use in clinical practice. However, EFW>90th percentile is not associated with an increased risk of delivering a SGA newborn. Similarly, EFW<10th percentile is not associated with an increased risk of delivering a LGA newborn. Thus, defining screen positive as EFW <10th percentile and >90th percentile for all adverse birth outcomes in our screening models may reduce the observed screening performance. The performance of the screening model may be improved when we define screen positive separately for SGA (as EFW <10th percentile), and for LGA (as EFW >90th percentile). We consider one combined screening test for all adverse birth outcomes more applicable for clinical practice, but to assess whether this

affects the observed screening performance we also evaluated screening performance of models in which we defined "screen-positive" separately for SGA (EFW<p10) and LGA (EFW>p90). Second-to-third trimester EFW or AC change was classified screen-positive if the change was in the lowest or highest decile.

Uterine artery resistance indices (UtA-RI) and Umbilical artery pulsatility indices (UA-PI) are measures of vascular resistance in the uterine and umbilical arteries, respectively. Increased UtA-RI and UA-PI are associated with impaired placental vascular development and increased risks of abnormal intra-uterine growth and adverse perinatal outcomes[22, 24-28]. These parameters may therefore be of additional value in clinical screening models. These parameters were derived from flow velocity waveforms in second and third trimester[29]. We defined screen-positive UtA-RI or UA-PI or second-to-third trimester change as a value in the highest decile.

Birth outcomes

Information about offspring sex, GA and weight at birth, gestational hypertensive disorders, assisted vaginal delivery and caesarean delivery was obtained from medical records[15]. GA-adjusted SDS for birth weight were constructed using North European growth standards[30]. Preterm birth was defined as GA <37 weeks at birth. Spontaneous preterm birth was defined as spontaneous preterm labour or preterm premature rupture of membranes resulting in birth <37 weeks' GA. According to clinical standards, SGA and LGA at birth were defined as a GA-adjusted birth weight <10th and >90th percentile in the study cohort, respectively.

Statistical analyses

First, we calculated the absolute percentages of screen positive second and third trimester fetal ultrasounds among newborns born preterm, SGA and LGA. Second, we aimed to assess screening performance for preterm birth, SGA and LGA based on different predefined screening models. We constructed five predefined logistic regression models for screening of preterm birth, SGA and LGA, respectively. Preterm birth, SGA and LGA were the dependent variables in these different predefined logistic regression models. For each logistic regression model, we assessed the variance explained of the model. We obtained predicted values from these regression models and further assessed model performance via Receiver Operating Characteristic (ROC)-Curves and calculation of the Area Under the Curve (AUC), along with the sensitivity at different false-positive-rates (1-specificity). The five predefined logistic regression models for screening of preterm birth, SGA and LGA were: 1) maternal-characteristics-model including maternal age, BMI, ethnicity, parity and smoking and fetal sex; 2) second-trimester-model (model 1

plus screening result based on second trimester EFW); 3) third-trimester-model (model 1 plus screening result based on third trimester EFW); 4) combined second-and-third-trimester-model (model 1 plus screening result based on second and third trimester EFW); 5) second-to–third trimester fetal growth model (model 4 plus second-to-third trimester EFW change). To compare model performance of the different predefined models, we assessed the change in effect size of the obtained AUCs from the different models. If the change in effect size was considered clinically relevant, we used the method by DeLong et. al for assessing whether the AUCs for two or more correlated receiver operating characteristic curves were statistically significantly different[31]. Positive and negative predictive values (PPV, NPV), and positive and negative likelihood ratios (PLR, NLR) at a 10% false-positive-rate (90% specificity) were calculated for our best model. Third, in a subsample of women with placenta flow measures available, we assessed the additional screening performance of placenta measures by adding second and third trimester UA-PI and UtA-RI screening results to the five models using a similar approach. To test the robustness of our findings, we performed 8 formal sensitivity analyses. We assessed: 1) whether screening performance for spontaneous preterm birth was similar to screening performance for any preterm birth; 2) whether using stricter cut-off values to define screen-positive results improved screening performance (EFW<5th percentile or EFW>95th percentile; 3) whether our models improved when we used AC instead of EFW; 4) whether using only UA-PI or UtA-RI screening results leads to comparable screening performance as using both measurements combined; 6) whether defining "screen-positive" for individual outcomes separately (screen positive as EFW<10th percentile only for SGA and screen positive as EFW>90th percentile only for LGA), instead of defining screen-positive as either EFW<10th or EFW>90th for all adverse birth outcomes, affects screening performance; 7) whether the screening performance changed when the outcome SGA was defined as moderate or extreme SGA (gestational-age-adjusted birth weight<5th or <3rd percentile, respectively) or defined as moderate or extreme LGA (gestational-age-adjusted birth weight>95th or >97th percentile, respectively); 8) whether performance of our model was similar for selecting SGA or LGA newborns with adverse outcomes (SGA pregnancies complicated by gestational hypertensive disorders and LGA pregnancies resulting in delivery using assisted vaginal delivery or caesarean section). Finally, to assess how maternal characteristics affect our obtained screening performance of the different screening models, we assessed the screening performance of second and third trimester ultrasound without incorporating maternal characteristics in the models. To deal with missing values, we added a missing category for each maternal and fetal characteristic to the models. This approach resembles clinical practice. Analyses were performed using the Statistical Package of Social Sciences version 24.0 for Windows (IBM Corp., Armonk, NY, USA).

RESULTS

Participants characteristics

Table 1 shows that 345 (4.5%) newborns were born preterm, 768 (10%) were SGA, and 767 (10%) were LGA at birth. **Table S1** gives all fetal and placental characteristics. Non-response analyses showed that women without placental measurements were more likely to have a higher BMI, and lower educational level (**Table S2**). Of all newborns with a second trimester EFW<10^{th} percentile or >90^{th} percentile, 91 (5.9%) were born preterm, 214 (13.9%) were born SGA, and 179 (11.7%) were born LGA. Of all newborns with a third trimester EFW<10^{th} or >90^{th} percentile, 110 (7.2%) were born preterm, 335 (21.8%) were born SGA, and 277 (18.1%) were born LGA (**Table 2**). In univariate logistic regression analyses, all maternal exposures were associated with at least one of the adverse birth outcomes, whereas EFW was associated with all three adverse birth outcomes (results available upon request).

Screening for risks of preterm birth

Figure 1 shows that the maternal-characteristics-model had a moderate performance for the detection of preterm birth (AUC 0.60 (95% CI 0.57 to 0.63), which did not improve by adding second trimester EFW (AUC 0.61 (95% CI 0.58 to 0.64)). Screening improved by adding third trimester EFW (AUC 0.64 (95% CI 0.61 to 0.67) to the maternal-characteristics-model, p-value for AUC comparison to the maternal-characteristics-model <0.01, **Table S3**). AUCs effect estimates did not further improve by combining second and third trimester EFW results or using EFW-change. Adding placenta flow measures to the third-trimester EFW model strongly improved detection of preterm birth (AUC of 0.72 (95% CI 0.66 to 0.77), p-value for model comparison to the third trimester EFW model <0.01, **Table S3**). Compared to the second-trimester-model, the third-trimester-model with placenta flow measures nearly doubled detection of fetuses at risk of preterm birth, as sensitivity increases from 19% for the second-trimester-model to 38% for the third-trimester-model with placenta flow measures (PLR: 3.8; NLR: 0.69; PPV: 15%; NPV: 97%) at 90% specificity (**Figure 1**, **Table S4**).

Table 1. Characteristics of mothers and their children (N = 7,670)

Characteristics	Value[a]
Maternal characteristics	
Age, median (IQR), years	30.3(25.9 to 33.4)
< 25, No. (%)	1,573(20.5)
25-35, No. (%)	4,972(64.8)
> 35, No. (%)	1,125(14.7)
Height, mean (SD) (cm)	167.3(7.4)
Weight, mean (SD) (kg)	69.3(13.2)
Body Mass Index, mean (SD) (kg/m^2)	24.8(4.5)
Normal, No. (%)	4,709(61.8)
Overweight, No. (%)	1,979(26.0)
Obese, No. (%)	932(12.2)
Education, No. higher education (%)	3,055(42.9)
Race / Ethnicity, No. (%)	
Dutch or European, No. (%)	4,289(58.2)
Surinamese, No. (%)	655(8.9)
Turkish, No. (%)	673(9.1)
Moroccan, No. (%)	473(6.4)
Cape Verdian or Dutch Antilles, No. (%)	560(7.6)
Parity, No. nulliparous (%)	4,308(56.6)
Smoking, No. (%)	
None, No. (%)	4,967(72.8)
Early-pregnancy only, No. (%)	595(8.7)
Continued, No. (%)	1261(18.5)
Birth characteristics	
Males, No. (%)	3,861(50.3%)
Gestational age, median (IQR), weeks	40.1(39.1 to 41.0)
Birth weight, mean (SD) grams	3,423(544)
Preterm birth, No. (%)	345(4.5)
Spontaneous preterm birth, No. (%)	294(3.0)
Small-size for gestational age[1] <10 birth centile (<-1.4SDS), No. (%)	768(10)
Large-size for gestational age[1] >90 birth centile (>1.18SDS), No. (%)	767(10)
Caesarean delivery, No. (%)	836(11.9)
Assisted vaginal delivery, No. (%)	964(13.8)
Apgar score below 7 at 5 minutes, No. (%)	78(1.0)

Values are observed data and represent means (SD), medians (IQR) or number of subjects (valid %). Abbreviations: IQR: inter quartile range; SD: standard deviation;

[1]Body mass index is defined as normal (BMI <25), overweight (BMI 25 – 30), obese (BMI>30).

[2]Preterm birth is defined as birth <37 weeks of gestational age.

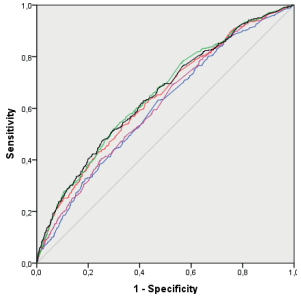
[3]SGA is defined as <10th percentile of gestational age-and sex-adjusted birth weight; LGA is defined as >90th percentile of gestational age-and sex-adjusted birth weight.

Table 2. Adverse birth outcomes by second and third trimester estimated fetal weight screening results (N=7,670) a

	Preterm birth			Small-size for gestational age at birth			Large-size for gestational age at birth		
	Yes	No	Total	Yes	No	Total	Yes	No	Total
2nd trimester									
Estimated fetal weight <10th percentile (screen-positive)	41 (5.3%)	726 (94.7%)	767	192 (25.0%)	575 (75.0%)	767	30 (3.9%)	737 (96.1%)	767
Estimated fetal weight 10 – 90th percentile (screen negative)	254 (4.1%)	5.882 (95.9%)	6136	554 (9.0%)	5.582 (91.0%)	6.136	588 (9.6%)	5.548 (90.4%)	6.136
Estimated fetal weight >90th percentile (screen-positive)	50 (6.5%)	717 (93.5)	767	22 (2.9%)	745 (97.1%)	767	149 (19.4%)	618 (80.6%)	767
Total	345	7.325	7.670	768	6,902	7,670	767	6,903	7,670
3rd trimester	Yes	No	Total	Yes	No	Total	Yes	No	Total
Estimated fetal weight <10th percentile (screen-positive)	75 (9.8%)	692 (90.2%)	767	331 (43.2%)	436 (58%)	767	4 (0.5%)	763 (99.5%)	767
Estimated fetal weight 10 – 90th percentile (screen negative)	235 (3.8%)	5.901 (96.2%)	6136	433 (7.1%)	5.703 (92.9%)	6.136	490 (8%)	5.646 (92%)	6.136
Estimated fetal weight >90th percentile (screen-positive)	35 (4.6%)	732 (95.4%)	767	4 (0.5%)	763 (99.5%)	767	273 (35.6%)	494 (64.4%)	767
Total	345	7.325	7.670	768	6.902	7.670	767	6.903	7.670

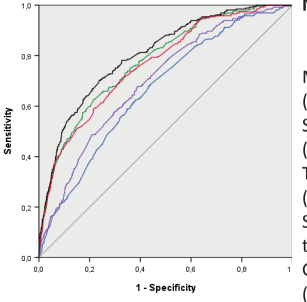
[a] *Values are absolute numbers (% of total within the corresponding screening category)*

Screening performance for preterm birth based on maternal characteristics and estimated fetal weight[b]



Models	AUC (95% Confidence Interval)
Maternal characteristics: (Blue line)	0.60 (0.57 to 0.63)
Second trimester: (Purple line)	0.61 (0.58 to 0.64)
Third trimester: (Red line)	0.64 (0.61 to 0.67)
Second and third trimester: (Green line)	0.65 (0.62 to 0.68)
Growth: (Black line)	0.65 (0.62 to 0.68)

Screening performance for preterm birth based on maternal characteristics, estimated fetal weight, and placenta flow measures[c]



Models	AUC (95% Confidence Interval)
Maternal characteristics: (Blue line)	0.64 (0.59 to 0.69)
Second trimester: (Purple line)	0.68 (0.62 to 0.73)
Third trimester: (Red line)	0.72 (0.66 to 0.77)
Second and third trimester: (Green line)	0.75 (0.69 to 0.80)
Growth: (Black line)	0.76 (0.71 to 0.82)

Sensitivity of the third-trimester-models based on estimated fetal weight at different diagnostic thresholds and specificities[d]

Specificity	Estimated fetal weight percentile diagnostic threshold on <p10 or >p90	Estimated fetal weight percentile diagnostic threshold on <p5 or >p95
70%	48%	49%
80%	37%	38%
90%	24%	25%

Sensitivity of the third-trimester-models based on estimated fetal weight and placenta flow measures at different diagnostic thresholds and specificities[d]

Specificity	Estimated fetal weight percentile diagnostic threshold on <p10 or >p90	Estimated fetal weight percentile diagnostic threshold on <p5 or >p95
70%	61%	54%
80%	52%	46%
90%	38%	34%

Figure 1. Screening performance for preterm birth[a]

AUC: area under the curve; CI: Confidence interval; EFW: Estimated fetal weight;

[a] Receiver operating characteristic curve for the detection of fetuses at risk for preterm birth based on second and third trimester fetal ultrasound and placenta measures, and derived sensitivity and specificity of the third-trimester-model.

[b] Model characteristics: Maternal characteristics; Second-trimester-model: Maternal characteristics and 2nd trimester EFW; Third-trimester-model: Maternal characteristics and 3rd trimester EFW; Combined model: Maternal characteristics, 2nd and 3rd trimester EFW; Growth model: Maternal characteristics, 2nd and 3rd trimester EFW and 2nd to 3rd trimester EFW change.

[c] AUCs based on a sample of 2705 participants with all placenta measures available. EFW models as described under [b] with additional placenta measures. Second-trimester-model: 2nd trimester UtA-RI and 2nd trimester UA-PI. Third-trimester-model; 3rd trimester UtA-RI and 3rd trimester UA-PI; Second and third-trimester-model: 2nd and 3rd trimester UtA-RI, and 2nd and 3rd trimester UA-PI; Growth model: 2nd and 3rd trimester UtA-RI and 2nd and 3rd trimester UA-PI, 2nd to 3rd trimester change of UtA-RI and UA-PI.

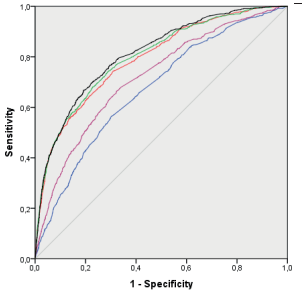
[d] Effects of changing the diagnostic threshold on the sensitivity of the third-trimester-model used for screening of preterm birth.

We observed similar model performances when we only took spontaneous preterm birth into account (**Figure S2**). Using stricter diagnostic cut-offs led to similar AUCs and sensitivities (**Figure 1**, **Figure S3**). We did not observe differences in results when we used AC instead of EFW (**Figure S4**). Overall, combined use of UtA-RI and UA-PI tended to be better than separate use (**Figure S5**). **Figure S6** shows that without maternal characteristics, screening performance of the third-trimester-model with placenta flow measures for preterm birth was considerably lower.

Screening for risks of small-size and large-size for gestational age at birth

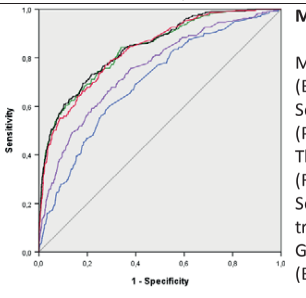
The maternal-characteristics-model and second-trimester-model had a moderate screening performance for detection of SGA at birth (AUCs 0.67 (95% CI 0.65 to 0.69) and 0.72 (95% CI 0.70 to 0.74), respectively) (**Figure 2**). Compared to these models, the third-trimester-model significantly improved detection (AUC 0.79 (95% CI 0.78 to 0.81) with a sensitivity of 50% at 90% specificity (p-value for AUC comparison to the maternal-characteristics-model and second-trimester-model <0.01, **Table S3**). Compared to the second-trimester-model, the third-trimester-model increased detection of fetuses at risk of SGA by a third, as sensitivity increases from 33% for the second-trimester-model to 50% for the third-trimester-model at 90% specificity (**Figure 2, Table S4**). Effect estimates of the AUCs did no further clinically improve by combining second and third trimester EFW results or using EFW-change. Adding placenta flow measures to the third-trimester-model did slightly improve screening performance for SGA at birth (AUC 0.83 (95% CI 0.81 to 0.86) p-value for AUC comparison to the third-trimester-model<0.01, **Figure 2**, **Table S3**) leading to a sensitivity of 55% at 90% specificity (PLR: 5,5; NLR: 0.5; PPV: 38%; NPV: 95%). The third-trimester-model had the best screening performance for detecting LGA with an AUC of 0.76 (95% CI 0.75 to 0.78) and corresponding sensitivity of 43% at 90% specificity (**Figure 3**). Compared to the second-trimester-model, the third-trimester-model increased the detection of fetuses at risk of LGA by a third, as the sensitivity increases from 28% for the second-trimester-model to 43% for the third-trimester-model (PLR: 4.3; NLR: 0.63; PPV: 32%; NPV: 93%) at 90% specificity (**Figure 3, Table S4**). Adding placenta flow measures to the third-trimester-model did not improve LGA screening performance.

Screening performance for small-size for gestational age based on maternal characteristics and estimated fetal weight[b]



Models	AUC (95% Confidence Interval)
Maternal characteristics: (Blue line)	0.67 (0.65 to 0.69)
Second trimester: (Purple line)	0.72 (0.70 to 0.74)
Third trimester: (Red line)	0.79 (0.78 to 0.81)
Second and third trimester: (Green line)	0.80 (0.78 to 0.82)
Growth: (Black line)	0.81 (0.80 to 0.83)

Screening performance for small -size for gestational age based on maternal characteristics, estimated fetal weight, and placenta flow measures[c]



Models	AUC (95% Confidence Interval)
Maternal characteristics: (Blue line)	0.70 (0.67 to 0.74)
Second trimester: (Purple line)	0.75 (0.72 to 0.79)
Third trimester: (Red line)	0.83 (0.81 to 0.86)
Second and third trimester: (Green line)	0.84 (0.81 to 0.86)
Growth: (Black line)	0.84 (0.82 to 0.87)

Sensitivity of the third-trimester-models based on estimated fetal weight at different diagnostic thresholds and specificities[d]

Specificity	Estimated fetal weight percentile diagnostic threshold on <p10 or >p90	Estimated fetal weight percentile diagnostic threshold on <p5 or >p95
70%	73%	67%
80%	63%	57%
90%	50%	42%

Sensitivity of the third-trimester-models based on estimated fetal weight and placenta flow measures at different diagnostic thresholds and specificities[d]

Specificity	Estimated fetal weight percentile diagnostic threshold on <p10 or >p90	Estimated fetal weight percentile diagnostic threshold on <p5 or >p95
70%	79%	70%
80%	68%	60%
90%	55%	46%

Figure 2. Screening performance for small-size for gestational age[a]

AUC: area under the curve; CI: Confidence interval; EFW: Estimated fetal weight;

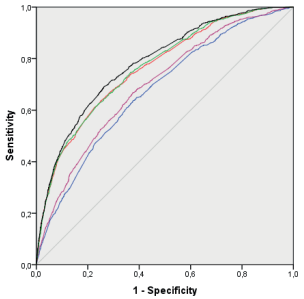
[a] Receiver operating characteristic curve for the detection of fetuses at risk for small-size for gestational age based on second and third trimester fetal ultrasound and placenta measures, and derived sensitivity and specificity of the third-trimester-model.

[b] Model characteristics: Maternal characteristics; Second-trimester-model: Maternal characteristics and 2nd trimester EFW; Third-trimester-model: Maternal characteristics and 3rd trimester EFW; Combined model: Maternal characteristics, 2nd and 3rd trimester EFW; Growth model: Maternal characteristics, 2nd and 3rd trimester EFW and 2nd to 3rd trimester EFW change.

[c] AUCs based on a sample of 2705 participants with all placenta measures available. EFW models as described under [b] with additional placenta measures. Second-trimester-model: 2nd trimester UtA-RI and 2nd trimester UA-PI. Third-trimester-model; 3rd trimester UtA-RI and 3rd trimester UA-PI; Second and third-trimester-model: 2nd and 3rd trimester UtA-RI, and 2nd and 3rd trimester UA-PI; Growth model: 2nd and 3rd trimester UtA-RI and 2nd and 3rd trimester UA-PI, 2nd to 3rd trimester change of UtA-RI and UA-PI.

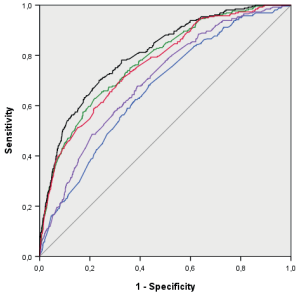
[d] Effects of changing the diagnostic threshold on the sensitivity of the third-trimester-model used for screening for small-size for gestational age.

Screening performance for large-size for gestational age based on maternal characteristics and estimated fetal weight[b]



Models	AUC (95% Confidence Interval)
Maternal characteristics: (Blue line)	0.68 (0.66 to 0.70)
Second trimester: (Purple line)	0.70 (0.68 to 0.72)
Third trimester: (Red line)	0.76 (0.75 to 0.78)
Second and third trimester: (Green line)	0.77 (0.75 to 0.78)
Growth: (Black line)	0.78 (0.77 to 0.80)

Screening performance for large-size for gestational age based on maternal characteristics, estimated fetal weight, and placenta flow measures[c]



Models	AUC (95% Confidence Interval)
Maternal characteristics: (Blue line)	0.67 (0.63 to 0.70)
Second trimester: (Purple line)	0.70 (0.67 to 0.73)
Third trimester: (Red line)	0.77 (0.74 to 0.80)
Second and third trimester: (Green line)	0.78 (0.75 to 0.81)
Growth: (Black line)	0.81 (0.79 to 0.83)

Sensitivity of the third-trimester-models based on estimated fetal weight at different diagnostic thresholds and specificities[d]

Specificity	Estimated fetal weight percentile diagnostic threshold on <p10 or >p90	Estimated fetal weight percentile diagnostic threshold on <p5 or >p95
70%	68%	62%
80%	57%	48%
90%	43%	37%

Sensitivity of the third-trimester-models based on estimated fetal weight and placenta flow measures at different diagnostic thresholds and specificities[d]

Specificity	Estimated fetal weight percentile diagnostic threshold on <p10 or >p90	Estimated fetal weight percentile diagnostic threshold on <p5 or >p95
70%	67%	62%
80%	55%	51%
90%	44%	35%

Figure 3. Screening performance for large-size for gestational age[a]

AUC: area under the curve; CI: Confidence interval; EFW: Estimated fetal weight;

[a] Receiver operating characteristic curve for the detection of fetuses at risk for large-size for gestational age based on second and third trimester fetal ultrasound and placenta measures, and derived sensitivity and specificity of the third-trimester-model.

[b] Model characteristics: Maternal characteristics; Second-trimester-model: Maternal characteristics and 2nd trimester EFW; Third-trimester-model: Maternal characteristics and 3rd trimester EFW; Combined model: Maternal characteristics, 2nd and 3rd trimester EFW; Growth model: Maternal characteristics, 2nd and 3rd trimester EFW and 2nd to 3rd trimester EFW change.

[c] AUCs based on a sample of 2705 participants with all placenta measures available. EFW models as described under [b] with additional placenta measures. Second-trimester-model: 2nd trimester UtA-RI and 2nd trimester UA-PI. Third-trimester-model; 3rd trimester UtA-RI and 3rd trimester UA-PI; Second and third-trimester-model: 2nd and 3rd trimester UtA-RI, and 2nd and 3rd trimester UA-PI; Growth model: 2nd and 3rd trimester UtA-RI and 2nd and 3rd trimester UA-PI, 2nd to 3rd trimester change of UtA-RI and UA-PI.

[d] Effects of changing the diagnostic threshold on the sensitivity of the third-trimester-model used for screening for large-size for gestational age.

Model performance was the same when screen-positive was defined separately for SGA and LGA, as when screen-positive was defined as one screening test for both SGA and LGA (**Figure S7**). When we used stricter diagnostic cut-offs (screen-positive defined as EFW<5th or >95th percentile), the sensitivities for detection of SGA and LGA slightly decreased (**Figure 2** and **3** respectively, ROCs and AUCs in **Figure S3**). When we used stricter outcome cut-offs (extreme SGA and LGA defined as gestational-age-adjusted birth weight <3rd or >97th percentile, and moderate SGA or LGA defined as gestational-age-adjusted birth weight <5th or >95th percentile, respectively), the model performance slightly improved as compared to our main analysis (Sensitivities, ROCs and AUCs in **Figure S8** and **Figure S9**). When we assessed screening performance for SGA newborns with pregnancies complicated by gestational hypertensive disorders and LGA newborns with pregnancies resulting in assisted vaginal delivery or caesarean section, we observed similar model performance as our main analysis (**Figure S10**). We did not observe differences in results when we used AC instead of EFW (**Figure S4**). When we excluded maternal smoking from the models, results were similar (findings not shown). Without incorporating maternal characteristics in the screening models, screening performance of the third-trimester-model for SGA and LGA was considerably lower (**Figure S6**).

DISCUSSION

Our results suggest that third trimester ultrasound examination in addition to maternal characteristics has the best screening performance for detecting fetuses at risk for preterm birth, SGA and LGA, compared to second trimester ultrasound or combined second and third trimester ultrasound. Compared to second trimester ultrasound screening, third trimester ultrasound screening would nearly double detection of fetuses at risk of these adverse birth outcomes in a low risk population.

Interpretation of main findings

Preterm birth, SGA and LGA are strongly related to perinatal morbidity and mortality and have long-term consequences for disease risk[2, 4, 32]. Abnormal fetal growth and impaired placental function are important risk factors for adverse birth outcomes, with the strongest associations observed for third trimester fetal and placental measures[3-5, 28]. Despite these observed associations, the additional clinical value for third trimester screening for fetuses at risk for common adverse birth outcomes remains unclear. A review of 13 controlled trials showed no beneficial effects of routinely performed ultrasound after 24 weeks GA on pregnancy outcomes[13]. These trials were mainly performed in the early nineties. Recent developments in ultrasound techniques and treatment protocols, and changes in prevalence of women at risk of abnormal fetal

growth limit the applicability of these results to current clinical practice. Technological ultrasound advancements in obstetrics may lead to implementation of fetal size and anomaly scans in first trimester and fetal growth assessment later in pregnancy. Further insight into the optimal period for ultrasound screening for adverse birth outcomes is therefore urgently needed.

Despite reported associations of suboptimal fetal growth and impaired placental function with preterm birth, no previous studies assessed the screening performance of second and third trimester ultrasound for preterm birth risk[3, 33]. We observed that third trimester fetal and placental ultrasound together with maternal characteristics had the best screening performance for preterm birth. We did not find a benefit of second to third trimester EFW change for screening for preterm birth, although previously published work from our cohort showed that second to third trimester EFW change was associated with the risk of preterm birth[3]. In this previous analysis, we only assessed the association of second to third trimester EFW change with the risk of preterm birth and did not consider second or third trimester fetal size in the analysis. Contrary, in our current analysis, we assessed the screening performance for preterm birth of the addition of second to third trimester EFW change to second and third trimester fetal size, and observed it did not further improve screening performance. Thus, it seems that an association between second to third trimester EFW change with the risk of preterm birth is present, but that this does not add to screening performance for preterm birth when we also consider second and third trimester fetal size. The additional value of placenta measures to the screening model may be explained by the role of placental dysfunction in preterm birth[1]. We observed the strongest screening performance for using a combination of umbilical and uterine artery resistance indices. However, differences compared to single use of either measurement were small. As the umbilical artery pulsatility indices are technically easier to measure, this measure might be most appropriate for use in clinical practice. Overall, in our relatively healthy low-risk population, the combination of third trimester fetal and placental ultrasound with maternal characteristics led to a doubling of antenatally identified newborns at risk for preterm birth compared to second trimester ultrasound or maternal characteristics only. A limited number of previous studies explored screening performance by single and combined second and third trimester EFW or AC measurements for prediction of SGA or LGA, taking into account maternal characteristics. A retrospective study among 3,520 women reported a moderate screening performance for SGA with a sensitivity of 41.8% at 90% specificity using a combination of maternal factors, first trimester chemistry results and second trimester EFW and placenta measures[34]. Another retrospective cohort study among 1,979 women reported that adding maternal characteristics and third trimester fetal and placental ultrasound to second trimester ultrasound results

improved sensitivity from 51.3% to 69.7% for SGA and from 44.1% to 59.4% for LGA at 90% specificity[35]. A recent cohort study among 3,440 pregnancies assessed the screening value of single versus serial fetal biometry at 28, 32 and 36 weeks GA for SGA and LGA[36]. This study observed that single fetal biometry at 32 weeks had a higher sensitivity than longitudinal analysis from more observations projecting EFW at 40 weeks[36]. In our study, the third trimester ultrasound was performed at an average of 30 weeks of gestation, as compared to an average 34 to 36 weeks of gestation in other studies[36, 37]. Although screening performance of third trimester ultrasound may improve when performed later in third trimester, third trimester ultrasound screening around 30 weeks' gestation is valuable as it offers a larger window for interventions. In our study we observed that third trimester fetal and placental ultrasound together with maternal characteristics had the best screening performance for SGA and LGA. Already in our low-risk population, this approach led to a third increase in detection of fetuses at risk of SGA or LGA compared to second trimester ultrasound screening. We did not observe additional screening benefit for combining second and third trimester ultrasounds or for using AC instead of EFW.

It is well-established that newborns born SGA or LGA may be both constitutionally or pathologically small or large for their gestational age[38]. It has been suggested that newborns who are pathologically small or large for their gestational age due to abnormal fetal growth have increased risks of morbidity and mortality, as compared to those newborns who are constitutionally small or large for their gestational age[38]. To better distinguish potential pathological SGA and LGA newborns from constitutional SGA and LGA newborns, we also assessed the screening performance of our screening models for moderate and extreme SGA and LGA, and for SGA and LGA complicated by pregnancy or delivery complications. We found that the screening performance was similar. This suggests our third trimester screening model may aid in the identification of newborns who are pathologically small or large for their gestational age. We did not use customized birth weight centiles for classification of abnormal size at birth as a method to distinguish potential pathological SGA and LGA newborns from constitutional SGA or LGA newborns, as previous studies have not shown strong results regarding the use of customized charts to identify SGA or LGA newborns at higher risk of mortality and adverse short-term and long-term outcomes[39, 40]. A limitation of our cohort is that we do not have extensive information available on neonatal morbidity. Further studies are needed to replicate our findings and to assess whether our screening model identifies SGA and LGA born newborns at risk of morbidity and mortality, considering more detailed measures of neonatal morbidity.

Overall, we observed slightly lower sensitivities for screening for SGA and LGA than previous studies, which could be explained by taking into account maternal

characteristics, our relatively healthy low-risk population and the earlier timing of third trimester ultrasound[36, 37]. As maternal characteristics are simple and cost-effective measurements, easily available within clinical practice, we specifically aimed to assess their screening performance for screening of adverse birth outcomes within low-risk populations and the subsequent additional screening performance of more expensive and time-consuming fetal and placental ultrasound measurements. We found that in absence of maternal characteristics, the screening models had an inferior screening performance compared to when maternal characteristics were taken into account but the third trimester fetal and placental ultrasound still had the best screening performance for adverse birth outcomes. Thus, our findings underline the importance of considering maternal characteristics within low-risk populations for screening of adverse birth outcomes and the potential value of third trimester ultrasound.

Our findings suggest that implementation of third trimester fetal and placental ultrasound, combined with common maternal characteristics, would nearly double detection of fetuses at risk for common adverse birth outcomes compared to second trimester ultrasound and provides further evidence for critical evaluation of current obstetric care guidelines. Improved detection of fetuses at risk of preterm birth, SGA and LGA provides the clinician the opportunity to optimize monitoring and interventions[41]. Monitoring could be intensified by additional assessments of fetal size, cervical length and umbilical artery waveforms using (Doppler) ultrasound and fetal wellbeing using cardiotocography, which might further improve detection of fetuses at risk of adverse outcomes whom may benefit from interventions, such as administering steroids for fetal lung maturation if preterm birth is imminent or termination of pregnancy if signs of placental insufficiency occur. Previous studies have shown that SGA or LGA newborns who were identified antenatally have lower risks of morbidity and mortality, compared to those who were unidentified antenatally[6-9]. However, it has also been suggested that prenatal diagnosis of abnormal fetal growth may lead to poorer outcomes due to subsequent interventions[42]. Benefits due to identification of true positives versus harm caused by false positives and interventions should be evaluated. Future well-designed randomized controlled trials are needed to confirm our results, and to assess whether the advantages of screening outweigh the potential harm from parental anxiety and iatrogenic morbidity, in contemporary low-risk populations.

Strengths and limitations

We had a prospective data collection from early pregnancy onwards and a large sample of 7,670 women with fetal growth measurements available. The non-response at baseline might have led to selection of a more healthy population, which might affect

the generalizability of results to high-risk populations. We also had a relatively small number of cases of adverse birth outcomes, which might further indicate a selection towards a low-risk population. To assess whether a screening model improved by adding additional maternal, fetal or placental characteristics, we assessed if changes in AUCs effect estimates of different screening models were clinically relevant and whether the differences in AUCs of two different models were statistically significant. What is considered clinically relevant may be arbitrary. Based on previous studies focused on screening for similar adverse birth outcomes, we considered an approximate 4-5% change in effect estimate of the AUC as clinically relevant, as this change is likely associated with a detectable increase in sensitivity[14, 27, 43]. Next, when model comparison fulfilled this criterion, we used a statistical test by DeLong et al. to see if this change was statistically significant[31]. This method takes into account two correlated AUCs, which is necessary as two curves are constructed based on the same individuals. We included common maternal characteristics, easily available within all pregnant women and applicable to low-risk pregnant populations, within our maternal screening model. Another predictor for preterm birth, SGA or LGA at birth is occurrence of either of these outcomes in a previous pregnancy. We did not use this maternal characteristic for screening in our models, as women with a previous preterm birth, SGA or LGA newborn are already considered higher risk pregnant women and often intensified monitoring and additional ultrasounds for fetal growth are indicated. Among higher risk populations, a different third trimester ultrasound screening model including other maternal characteristics may be more applicable or even a separate screening model for nulliparous and multiparous women may be needed. Further studies assessing screening performance for adverse birth outcomes of third trimester fetal and placental ultrasound, in combination with more maternal characteristics such as previous pregnancy complications, among high-risker populations are needed. All ultrasound measurements were performed according to the study protocol and blinded with regard to pregnancy outcomes due to the prospective nature of the study. Abnormal research ultrasound results were reported to healthcare providers and some participants might have been treated as a consequence of abnormal (research) ultrasound findings, which might have affected the screening performance. For example, if an abnormal EFW in a research ultrasound was found, this may have led to induction of labour before 37 weeks of gestation, which is considered iatrogenic preterm birth. However, when we restricted our analyses to spontaneous preterm birth only we found similar screening performance. Thus, the performance of our model screening for preterm birth does not seem to be driven by iatrogenic preterm birth.

Conclusion

Maternal characteristics together with single third trimester fetal and placental ultrasound has the best screening performance for preterm birth, and SGA and LGA at birth, compared to using only second trimester ultrasound or combined second and third trimester ultrasound. Compared to second trimester ultrasound screening, third trimester ultrasound screening would nearly double detection of fetuses at risk of these common adverse birth outcomes in low risk populations.

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SUPPLEMENTARY MATERIALS

Table S1. Fetal and placental characteristics (N = 7670)

Second trimester fetal and placental characteristics	
Gestational age in weeks, median (IQR)	20.5(19.9 to 21.3)
Head circumference, mean (SD) (mm)	179.5(14.7)
Abdominal circumference, mean (SD) (mm)	157(15)
Femur length, mean (SD) (mm)	33.5(3.6)
Estimated fetal weight, mean (SD) (g)	382.3(95.5)
Estimated fetal weight < 10th percentile (<-1.32SDS) No. (%)	767(10)
Estimated fetal weight > 90th percentile (>1.19SDS) No. (%)	767(10)
Uterine artery resistance index, mean (SD)	0.54(0.09)
Umbilical artery pulsatility index, mean (SD)	1.2(0.19)
Third trimester fetal and placental characteristics	
Gestational age in weeks, median (IQR)	30.4(29.8 to 30.9)
Head circumference, mean (SD) (mm)	284.9(12.4)
Abdominal circumference, mean (SD) (mm)	263.8(16.5)
Femur length, mean (SD) (mm)	57.5(3)
Estimated fetal weight, mean (SD) (g)	1615.9(256.2)
Estimated fetal weight < 10th percentile (<-1.20SDS), No. (%)	767(10)
Estimated fetal weight > 90th percentile (>1.35SDS) No. (%)	767(10)
Uterine artery resistance index	0.48(0.08)
Umbilical artery pulsatility index	0.98(0.17)
Second to third trimester estimated fetal weight change <10th percentile (<1.10SDS) No (%)	767(10)
Second to third trimester estimated fetal weight change >90th percentile (>1.35SDS) No (%)	767(10)

Abbreviations: IQR: inter quartile range; SD: Standard deviation;
Values are observed data and represent means (SD), medians (IQR) or number of subjects (valid %).

Table S2. Characteristics of mothers and their children with and without placenta measures available[a]

	Placenta measures available N=7014[b]	No placenta measures available N=656[c]	P-value[d]
Maternal characteristics			
Age			0.13
< 25, No (%)	1419(20.5)	154(23.5)	
25-35, No (%)	4559(65)	413(63)	
> 35, No (%)	1036(14.8)	89(13.6)	
Height, mean (SD) (cm)	167.3(7.36)	166.6(7.4)	0.013
Weight, mean (SD) (kg)	69(13)	721(14.4)	<0.01
Body Mass Index, mean (SD) (kg/m^2)			<0.01
Normal, No (%)	4359(62.5)	350(54.2)	
Overweight, No (%)	1809(25.9)	170(26.3)	
Obese, No (%)	806(11.6)	126(19.5)	
Education, No. higher education (%)	2837(43.6)	218(36.2)	<0.01
Race / Ethnicity, No. (%)			0.11
Dutch or European, No (%)	3949(58.5)	340(55)	
Surinamese , No (%)	587(8.7)	68(11)	
Turkish, No (%)	622(9.2)	51(8.3)	
Moroccan , No (%)	428(6.3)	45(7.3)	
Cape Verdian or Dutch Antilles, No (%)	506(7.5)	54(8.8)	
Parity, No. nulliparous (%)	3951(56.8)	357(54.7)	0.06
Smoking, No. (%)			0.19
None, No (%)	4532(72.6)	435(75)	
Early-pregnancy only, No (%)	556(8.9)	39(6.7)	
Continued, No (%)	1155(18.5)	106(18.3)	
Birth characteristics			
Males, No. (%)	3549(50.6)	312(47.6)	0.14
Gestational age, median (IQR), weeks	40.1(39.1 to 41.0)	40(39.0 to 41.0)	0.07
Birth weight, mean (SD) grams	3424(542)	3419(567)	0.83
Preterm birth[e], No. (%)	307(4.4)	38(5.8)	0.09
Small for gestational age[e], No. (%)	693(9.9)	75(11.4)	0.08
Large for gestational age[e], No. (%)	689(9.8)	78(11.9)	0.08
Caesarean delivery, No. (%)	740(11.6)	82(13.6)	0.67
Assisted vaginal delivery, No. (%)	889(13.9)	75(12.5)	0.67
Apgar score below 7 at 5 minutes, No. (%)	73(0.8)	5(0.8)	0.75

Abbreviations: IQR: inter quartile range; SD: Standard deviation.

[a] Because these measurements were only performed in one of two research centers, second and third trimester uterine artery resistance indices were available in subgroups of n=4,361 and n=4,193 women, respectively. Second and third trimester UA-PI were available in n=5,831 and n=6,224 women, respectively.

[b] Participants with any of the following placenta measures available: 2nd or 3rd trimester umbilical artery pulsatility index or 2nd or 3rd trimester uterine artery resistance index. Values are observed data and represent means (SD), medians (IQR) or number of subjects (valid %).

[c] Participants without any of the following placenta measures available: 2nd or 3rd trimester umbilical artery pulsatility index or 2nd or 3rd trimester uterine artery resistance index. Values are observed data and represent means (SD), medians (IQR) or number of subjects (valid %).

[d] P-value for difference between groups.

[e] Preterm birth is defined as birth before 37 weeks; Small-size for gestational age is defined as < 10th percentile (<-1.4 SDS) of gestational age-and sex-adjusted birth weight; Large-size for gestational age is defined as > 90th percentile (>1.18 SDS) of gestational age-and sex-adjusted birth weight.

Table S3. Statistical significance level of comparison of screening models with and without placenta measures, for screening for preterm birth, small-size and large-size for gestational age

	Preterm birth	Small-size for gestational age	Large-size for gestational age
Maternal characteristics vs. third trimester model	P<0.01	P<0.01	P<0.01
Second trimester model vs. third trimester model	P<0.01	P<0.01	P<0.01
Third trimester model vs. Third trimester model and placenta measures*	P<0.01	P<0.01	NA

*Models are based on maternal characteristics, estimated fetal weight, and placenta measures. NA: Not applicable as no comparison was made. Values are p-values for comparison of models, using the method of DeLong et. al for comparison of two correlated AUCs. *Analyses performed in a subsample with all placenta measures available.*

Table S4. Estimated sensitivity at different levels of specificity for models screening for preterm birth, small-size and large-size for gestational age

		Estimated sensitivities	
	Specificity	Maternal characteristics model	Second trimester model[a]
Preterm birth	70%	44%	44%
	80%	33%	32%
	90%	18%	19%
Small-size for gestational age	70%	56%	65%
	80%	43%	51%
	90%	25%	33%
Large-size for gestational age	70%	53%	58%
	80%	43%	44%
	90%	25%	28%

Values are estimated sensitivity at different levels of specificity. Derived from receiver operating characteristic curve.
[a]Second trimester model: Maternal characteristics and 2nd trimester estimated fetal weight.

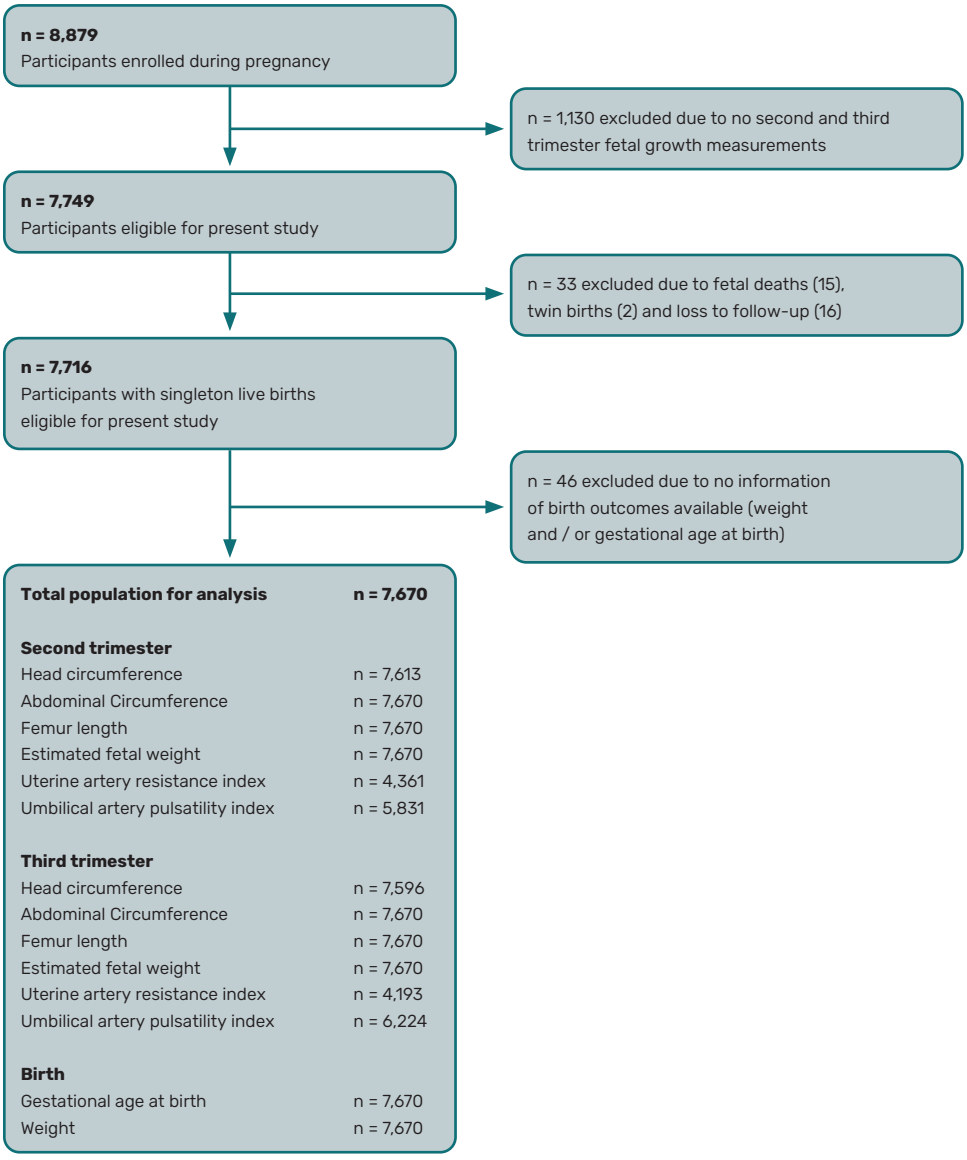
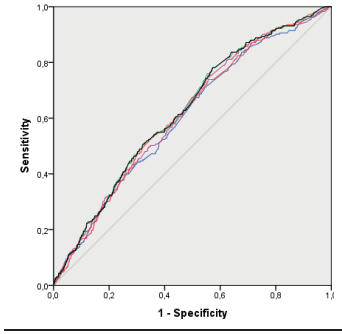


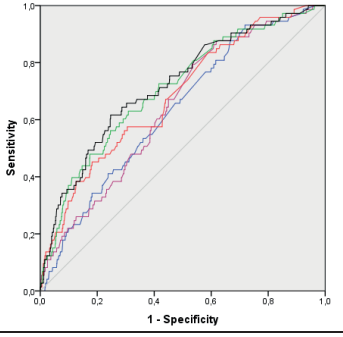
Figure S1. Flowchart population for analysis

Screening performance for spontaneous preterm birth based on maternal and fetal characteristics[b]



Models	AUC (95% Confidence Interval)
Maternal characteristics: (Blue line)	0.60 (0.57 to 0.64)
Second trimester: (Purple line)	0.62 (0.58 to 0.65)
Third trimester: (Red line)	0.61 (0.58 to 0.65)
Second and third trimester: (Green line)	0.63 (0.59 to 0.66)
Growth: (Black line)	0.63 (0.59 to 0.66)

Screening performance for spontaneous preterm birth based on maternal, fetal and placental characteristics[c]



Models	AUC (95% Confidence Interval)
Maternal characteristics: (Blue line)	0.63 (0.57 to 0.69)
Second trimester: (Purple line)	0.64 (0.59 to 0.70)
Third trimester: (Red line)	0.68 (0.62 to 0.74)
Second and third trimester: (Green line)	0.71 (0.65 to 0.77)
Growth: (Black line)	0.72 (0.66 to 0.78)

Figure S2. Receiver operating characteristic curves for models screening for spontaneous preterm birth[a]

[a] Receiver operating characteristic curves and corresponding area under the curve (95% confidence interval) of models based on predicted values from the five models for screening for spontaneous preterm birth. Spontaneous preterm birth was defined as birth before 37th week of gestation, after non-medically induced labor.

[b] Model characteristics: Maternal characteristics; Second trimester model: Maternal characteristics and 2nd trimester EFW; Third trimester model: Maternal characteristics and 3rd trimester EFW; Second and third trimester model: Maternal characteristics, 2nd and 3rd trimester EFW; Growth model: Maternal characteristics, 2nd and 3rd trimester EFW and 2nd to 3rd trimester EFW change.

[c] AUCs based on a sample of 2705 participants with all placenta measures available. EFW models as described under [b] with additional placenta measures. Second trimester model: 2nd trimester uterine artery resistance index and 2nd trimester umbilical artery pulsatility index. Third trimester model; 3rd trimester uterine artery resistance index and 3rd trimester umbilical artery pulsatility index; Second and third trimester model: 2nd and 3rd trimester uterine artery resistance index, and 2nd and 3rd trimester umbilical artery pulsatility index; Growth model: 2nd and 3rd trimester uterine artery resistance index and 2nd and 3rd trimester umbilical artery pulsatility index, 2nd to 3rd trimester change of uterine artery resistance index and umbilical artery pulsatility index.

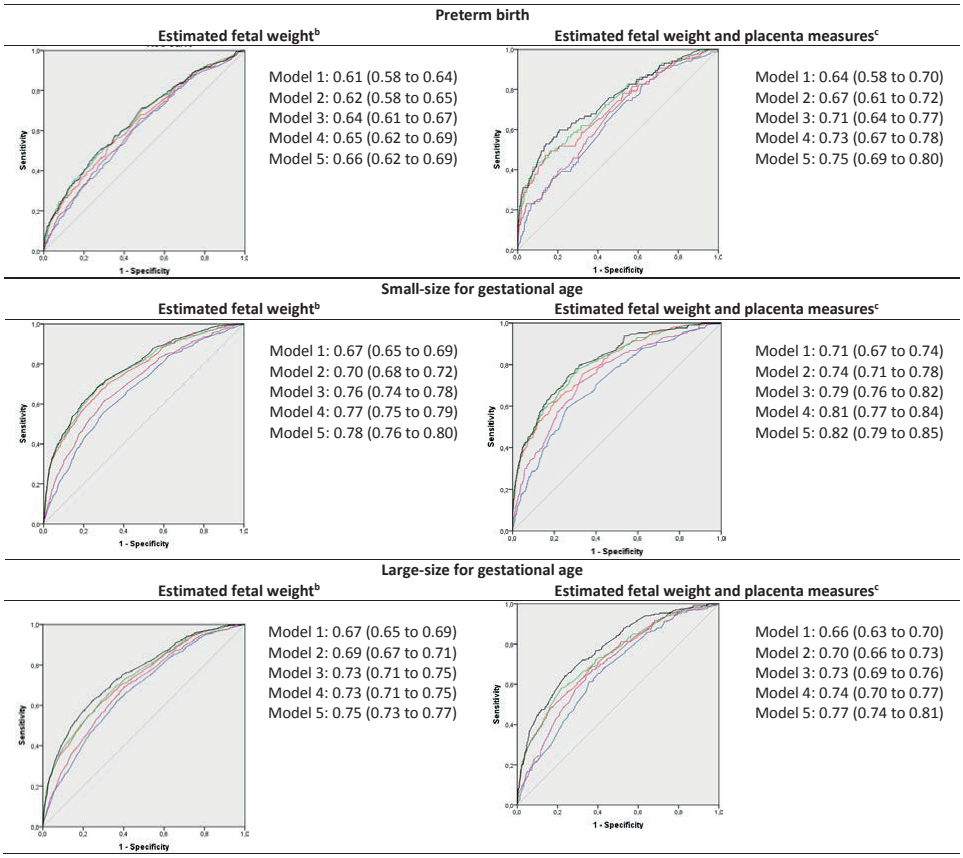


Figure S3. Receiver operating characteristic curves for models screening for preterm birth, small-size and large-size for gestational age, with screen positive defined as estimated fetal weight <5th percentile, or >95th percentile[a]

[a] Receiver operating characteristic curves and corresponding area under the curve (95% CI) based on predicted values from five models for screening for preterm birth, small- and large-size for gestational age. Models are based on maternal characteristics, second and third trimester fetal and placental ultrasound.

[b] Model 1: Maternal characteristics; Model 2: Maternal characteristics and 2nd trimester EFW; Model 3: Maternal characteristics and 3rd trimester EFW; Model 4: Maternal characteristics, 2nd +3rd trimester EFW; Model 5: Maternal characteristics, 2nd + 3rd trimester EFW and 2nd to 3rd trimester EFW change.

[c] AUCs based on a sample of 2705 participants with all placenta measures available. Models as EFW models under [b], adding the following placenta measures. Model 2: 2nd trimester uterine artery resistance index, and 2nd trimester umbilical artery pulsatility index. Model 3: 3rd trimester uterine artery resistance index, and 3rd trimester umbilical artery pulsatility index; Model 4: 2nd and 3rd trimester uterine artery resistance index, and 2nd and 3rd trimester umbilical artery pulsatility index; Model 5: 2nd and 3rd trimester uterine artery resistance index, and 2nd and 3rd trimester umbilical artery pulsatility index, 2nd to 3rd trimester change of uterine artery resistance index and umbilical artery pulsatility index.

Grey line: Reference line;

Blue line: Model 1: Maternal characteristics model;

Purple line: Model 2: Second trimester model;

Red line: Model 3: Third trimester model;

Green line: Model 4: Second and third trimester model;

Black line: Model 5: Growth model.

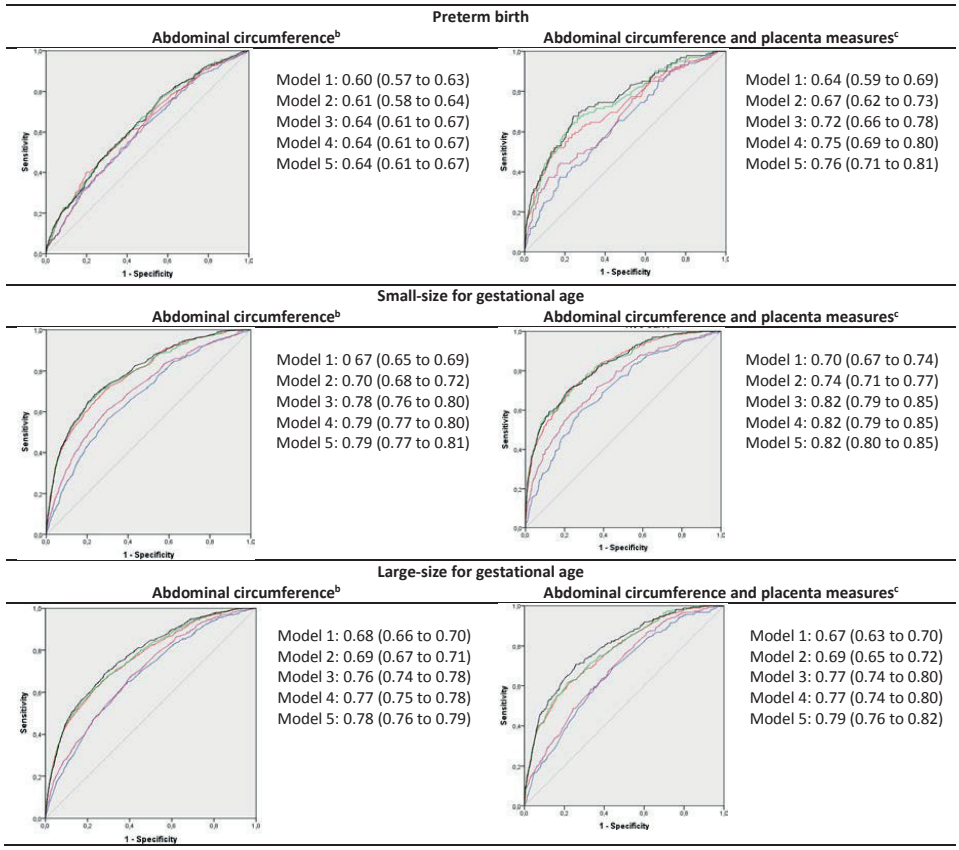


Figure S4. Receiver operating characteristic curves for models screening for preterm birth, small-size and large-size for gestational age using abdominal circumference instead of estimated fetal weight[a]

[a] Receiver operating characteristic curves of models based on predicted values from five models for preterm birth, small- and large-size for gestational age. Models based on fetal abdominal circumference, and placenta measures.

[b] Model 1: Maternal characteristics; Model 2: Maternal characteristics and 2nd trimester AC; Model 3: Maternal characteristics and 3rd trimester AC; Model 4: Maternal characteristics, 2nd +3rd trimester AC; Model 5: Maternal characteristics, 2nd + 3rd trimester AC and 2nd to 3rd trimester AC change.

[c] AUCs based on a sample of 2705 participants with all placenta measures available. Models as AC models under [b], adding the following placenta measures: Model 2: 2nd trimester uterine artery resistance index and 2nd trimester umbilical artery pulsatility index. Model 3: 3rd trimester uterine artery resistance index and 3rd trimester umbilical artery pulsatility index; Model 4: 2nd and 3rd trimester uterine artery resistance index, and 2nd and 3rd trimester umbilical artery pulsatility index; Model 5: 2nd and 3rd trimester uterine artery resistance index and 2nd and 3rd trimester umbilical artery pulsatility index, 2nd to 3rd trimester change of uterine artery resistance index and umbilical artery pulsatility index.

Grey line: Reference line;

Blue line: Model 1: Maternal characteristics model;

Purple line: Model 2: Second trimester model;

Red line: Model 3: Third trimester model;

Green line: Model 4: Second and third trimester model;

Black line: Model 5: Growth model

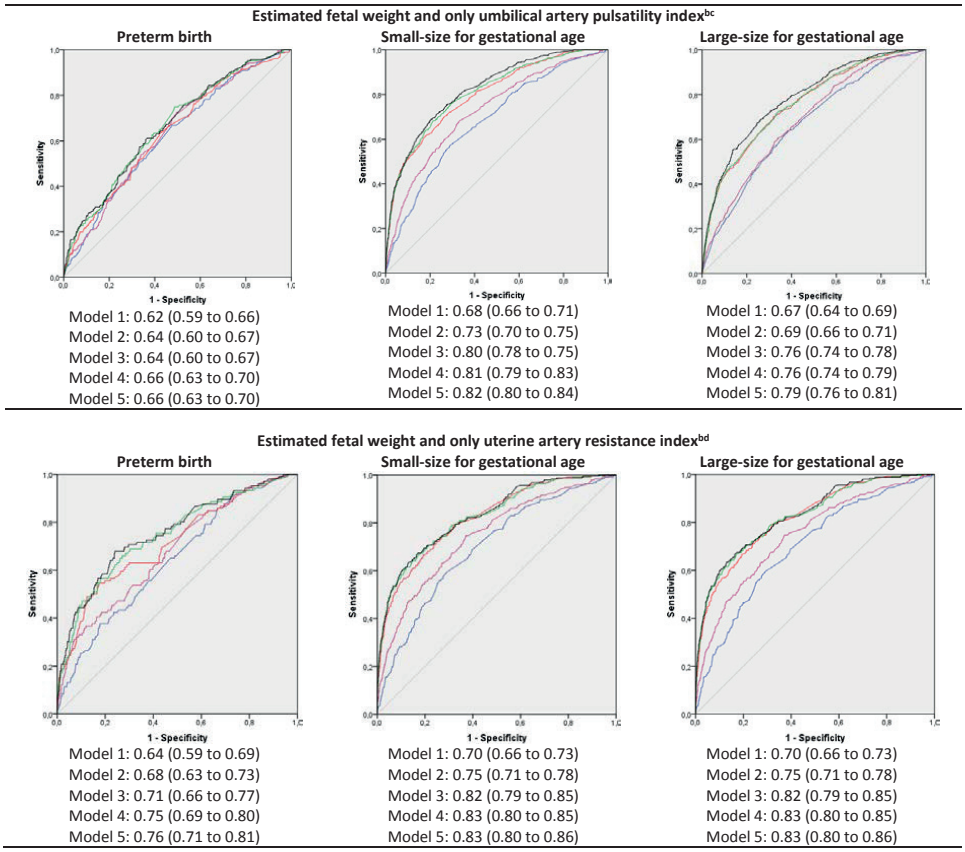


Figure S5. Receiver operating characteristic curves for screening for preterm birth, small-size and large-size for gestational age, with separate umbilical and uterine artery indices[a]

[a] Receiver operating characteristic curves of models based on EFW combined with umbilical artery pulsatility index or uterine artery pulsatility index. Values are area under the curve (95% confidence interval).

[b] Model 1: Maternal characteristics; Model 2: Maternal characteristics and 2nd trimester EFW; Model 3: Maternal characteristics and 3rd trimester EFW; Model 4: Maternal characteristics, 2nd +3rd trimester EFW; Model 5: Maternal characteristics, 2nd + 3rd trimester EFW and 2nd to 3rd trimester EFW change.

[c] EFW models as described under [b] with additional placenta measures. Second trimester model: 2nd trimester umbilical artery pulsatility index. Third trimester model; 3rd trimester umbilical artery pulsatility index; Second and third trimester model: 2nd and 3rd trimester umbilical artery pulsatility index; 2nd and 3rd trimester umbilical artery pulsatility index, 2nd to 3rd trimester change of umbilical artery pulsatility index.

[d] EFW models as described under [b] with additional placenta measures. Second trimester model: 2nd trimester uterine artery resistance index; Third trimester model; 3rd trimester uterine artery resistance index; Second and third trimester model: 2nd and 3rd trimester uterine artery resistance index; Growth model: 2nd and 3rd trimester uterine artery resistance index and 2nd to 3rd trimester change of uterine artery resistance index.

Grey line: Reference line;

Blue line: Model 1: Maternal characteristics model;

Purple line: Model 2: Second trimester model;

Red line: Model 3: Third trimester model;

Green line: Model 4: Second and third trimester model;

Black line: Model 5: Growth model.

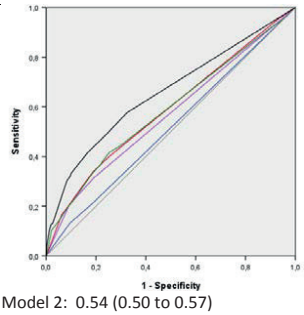
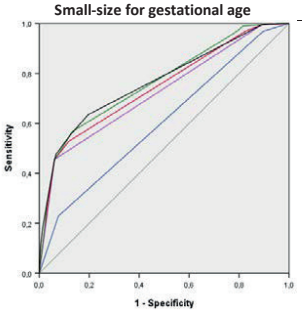
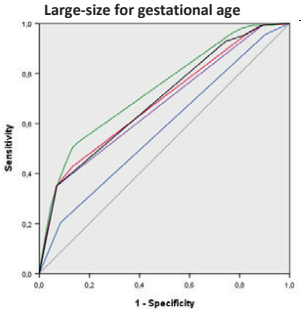
Preterm birth	Small-size for gestational age	Large-size for gestational age
		
Model 2: 0.54 (0.50 to 0.57) (blue) Model 3: 0.57 (0.54 to 0.60) (purple) Model 3[c]: 0.66 (0.61 to 0.70) (black) Model 4: 0.59 (0.56 to 0.63) (red) Model 5: 0.60 (0.57 to 0.63) (green)	Model 2: 0.61 (0.59 to 0.63) (blue) Model 3: 0.71 (0.69 to 0.73) (purple) Model 3 [c]: 0.77 (0.74 to 0.80) (black) Model 4: 0.74 (0.72 to 0.76) (red) Model 5: 0.76 (0.74 to 0.78) (green)	Model 2: 0.58 (0.56 to 0.60) (blue) Model 3: 0.68 (0.65 to 0.70) (purple) Model 3 [c]: 0.69 (0.67 to 0.72) (black) Model 4: 0.69 (0.67 to 0.71) (red) Model 5: 0.73 (0.71 to 0.75) (green)

Figure S6. Receiver operating characteristic curves for models screening for preterm birth, small-size and large-size for gestational age based on estimated fetal weight, without maternal characteristics[ab]

[a] Receiver operating characteristic curves and corresponding area under the curve (95% CI) based on predicted values from four models for preterm birth, small- and large-size for gestational age. Models are based on second and third trimester fetal ultrasound.

[b] Model 2: 2nd trimester EFW; Model 3: 3rd trimester EFW; Model 4: 2nd +3rd trimester EFW; Model 5: 2nd + 3rd trimester EFW and 2nd to 3rd trimester EFW change.

[c] AUCs based on a sample of 2705 participants with all placenta measures available. Models 3[c] 3rd trimester EFW + 3rd trimester uterine artery resistance index and 3rd trimester umbilical artery pulsatility index;

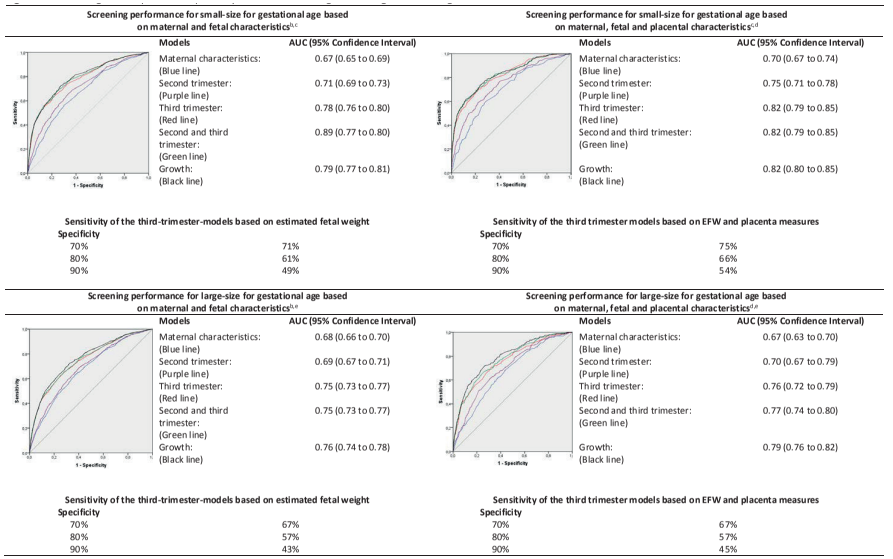


Figure S7. Defining screen positive separately for small- and large-size for gestational age[a]

AUC: area under the curve; CI: Confidence interval; EFW: Estimated fetal weight;

[a] Receiver operating characteristic curve for the detection of fetuses at risk for small-size and large-size for gestational age based on second and third trimester fetal ultrasound and placenta measures, and derived sensitivity and specificity of the third trimester model.

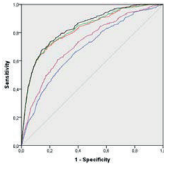
[b] Model characteristics: Maternal characteristics; Second trimester model: Maternal characteristics and 2nd trimester EFW; Third trimester model: Maternal characteristics and 3rd trimester EFW; Combined model: Maternal characteristics, 2nd and 3rd trimester EFW; Growth model: Maternal characteristics, 2nd and 3rd trimester EFW and 2nd to 3rd trimester EFW change.

[c] Screen positive is defined as EFW <10th percentile.

[d] AUCs based on a sample of 2705 participants with all placenta measures available. Models are EFW models as described under [b] with additional placenta measures. Second trimester model: 2nd trimester uterine artery resistance index and 2nd trimester umbilical artery pulsatility index. Third trimester model; 3rd trimester uterine artery resistance index and 3rd trimester umbilical artery pulsatility index; Second and third trimester model: 2nd and 3rd trimester uterine artery resistance index, and 2nd and 3rd trimester umbilical artery pulsatility index; Growth model: 2nd and 3rd trimester uterine artery resistance index and 2nd and 3rd trimester umbilical artery pulsatility index, 2nd to 3rd trimester change of uterine artery resistance index and umbilical artery pulsatility index.

[e] Screen positive is defined as EFW >90th percentile.

Screening performance for small-size for gestational age based on maternal and fetal characteristics[b]

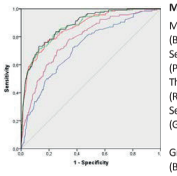


Models	AUC (95% Confidence Interval)
Maternal characteristics: (Blue line)	0.69 (0.66 to 0.71)
Second trimester: (Purple line)	0.73 (0.70 to 0.75)
Third trimester: (Red line)	0.82 (0.80 to 0.85)
Second and third trimester: (Green line)	0.83 (0.81 to 0.85)
Growth: (Black line)	0.84 (0.82 to 0.86)

Sensitivity of the third trimester models based on EFW[d]

Specificity	SGA if gestational age adjusted birthweight <p5
70%	77%
80%	71%
90%	59%

Screening performance for small-size for gestational age based on maternal, fetal and placental characteristics[c]

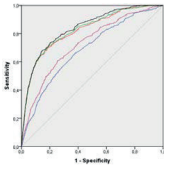


Models	AUC (95% Confidence Interval)
Maternal characteristics: (Blue line)	0.72 (0.68 to 0.77)
Second trimester: (Purple line)	0.78 (0.74 to 0.82)
Third trimester: (Red line)	0.87 (0.84 to 0.90)
Second and third trimester: (Green line)	0.88 (0.85 to 0.91)
Growth: (Black line)	0.88 (0.85 to 0.91)

Sensitivity of the third trimester models based on EFW and placenta measures[d]

Specificity	SGA if gestational age adjusted birthweight <p5
70%	84%
80%	78%
90%	63%

Screening performance for large-size for gestational age based on maternal and fetal characteristics[b]

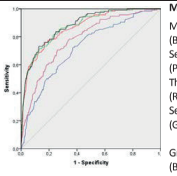


Models	AUC (95% Confidence Interval)
Maternal characteristics: (Blue line)	0.69 (0.67 to 0.72)
Second trimester: (Purple line)	0.72 (0.69 to 0.74)
Third trimester: (Red line)	0.79 (0.76 to 0.81)
Second and third trimester: (Green line)	0.79 (0.77 to 0.81)
Growth: (Black line)	0.81 (0.79 to 0.83)

Sensitivity of the third trimester models based on EFW[d]

Specificity	LGA if gestational age adjusted birthweight >p95
70%	72%
80%	63%
90%	48%

Screening performance for large-size for gestational age based on maternal, fetal and placental characteristics[c]



Models	AUC (95% Confidence Interval)
Maternal characteristics: (Blue line)	0.67 (0.62 to 0.72)
Second trimester: (Purple line)	0.72 (0.68 to 0.77)
Third trimester: (Red line)	0.78 (0.74 to 0.82)
Second and third trimester: (Green line)	0.80 (0.76 to 0.84)
Growth: (Black line)	0.82 (0.79 to 0.86)

Sensitivity of the third trimester models based on EFW and placenta measures[d]

Specificity	LGA if gestational age adjusted birthweight >p95
70%	67%
80%	61%
90%	43%

Figure S8. Receiver operating characteristic curves for models screening for small-size and large-size for gestational age according to size at birth <5th percentile, or >95th percentile[a]

[a] Receiver operating characteristic curves and corresponding area under the curve (95% confidence interval) of models based on predicted values from the five models for small- and large-size for gestational age. Small-size for gestational age defined as gestational age adjusted birth weight <5th percentile. Large-size for gestational age defined as gestational age adjusted birth weight >95th percentile.

[b] Model 1: Maternal characteristics; Model 2: Maternal characteristics and 2nd trimester EFW; Model 3: Maternal characteristics and 3rd trimester EFW; Model 4: Maternal characteristics, 2nd +3rd trimester EFW; Model 5: Maternal characteristics, 2nd + 3rd trimester EFW and 2nd to 3rd trimester EFW change.

[c] AUCs based on a sample of 2705 participants with all placenta measures available. Models as EFW models under [b], adding the following placenta measures. Model 2: 2nd trimester uterine artery resistance index, and 2nd trimester umbilical artery pulsatility index. Model 3; 3rd trimester uterine artery resistance index, and 3rd trimester umbilical artery pulsatility index; Model 4: 2nd and 3rd trimester uterine artery resistance index, and 2nd and 3rd trimester umbilical artery pulsatility index; Model 5: 2nd and 3rd trimester uterine artery resistance index, and 2nd and 3rd trimester umbilical artery pulsatility index, 2nd to 3rd trimester change of uterine artery resistance index and umbilical artery pulsatility index.

[d] Effects of changing the outcome threshold on the sensitivity of the third trimester model used for screening of fetuses at risk for small-size and large-size for gestational age.

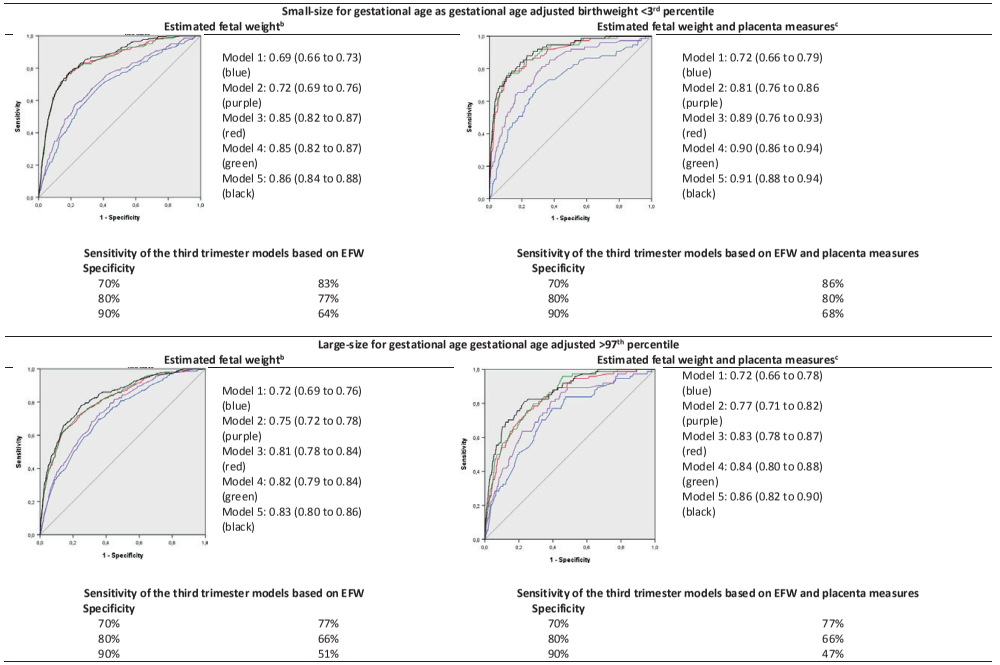


Figure S9. Receiver operating characteristic curves for models screening for small-size and large-size for gestational age at birth defined as birth weight <3rd and >97th percentile, respectively[a]

[a] Receiver operating characteristic curves and corresponding area under the curve (95% CI) based on predicted values from five models for small- and large-size for gestational age. Models are based on maternal characteristics, second and third trimester fetal and placental ultrasound.

[b] Model 1: Maternal characteristics; Model 2: Maternal characteristics and 2nd trimester EFW; Model 3: Maternal characteristics and 3rd trimester EFW; Model 4: Maternal characteristics, 2nd +3rd trimester EFW; Model 5: Maternal characteristics, 2nd + 3rd trimester EFW and 2nd to 3rd trimester EFW change.

[c] AUCs based on a sample of 2705 participants with all placenta measures available. Models as EFW models under [b], adding the following placenta measures. Model 2: 2nd trimester uterine artery resistance index, and 2nd trimester umbilical artery pulsatility index. Model 3; 3rd trimester uterine artery resistance index, and 3rd trimester umbilical artery pulsatility index; Model 4: 2nd and 3rd trimester uterine artery resistance index, and 2nd and 3rd trimester umbilical artery pulsatility index; Model 5: 2nd and 3rd trimester uterine artery resistance index, and 2nd and 3rd trimester umbilical artery pulsatility index, 2nd to 3rd trimester change of uterine artery resistance index and umbilical artery pulsatility index.

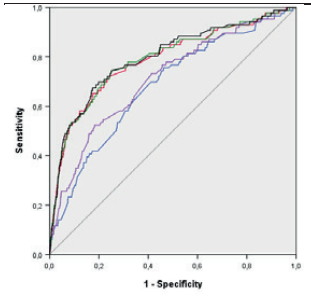
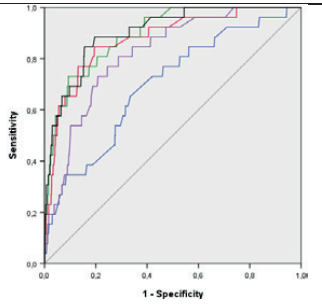
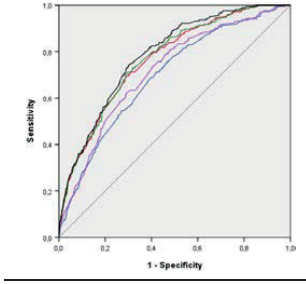
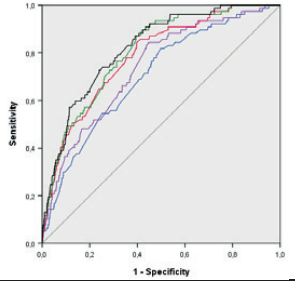
Small-size for gestational age and gestational hypertensive disorders	
Estimated fetal weight[b]	**Estimated fetal weight and placenta measures[c]**
 Model 1: 0.68 (0.63 to 0.74) (blue) Model 2: 0.71 (0.66 to 0.77) (purple) Model 3: 0.79 (0.73 to 0.84) (red) Model 4: 0.79 (0.74 to 0.85) (green) Model 5: 0.80 (0.74 to 0.85)	 Model 1: 0.70 (0.60 to 0.80) (blue) Model 2: 0.82 (0.75 to 0.89) (purple) Model 3: 0.88 (0.81 to 0.94) (red) Model 4: 0.90 (0.84 to 0.95) (green) Model 5: 0.90 (0.85 to 0.96) (black)
Large-size for gestational age and assisted vaginal delivery or cesarean section	
Estimated fetal weight[b]	**Estimated fetal weight and placenta measures[c]**
 Model 1: 0.69 (0.86 to 0.73) (blue) Model 2: 0.72 (0.68 to 0.75) (purple) Model 3: 0.77 (0.73 to 0.80) (red) Model 4: 0.77 (0.74 to 0.80) (green) Model 5:0.78 (0.76 to 0.81) (black)	 Model 1: 0.70 (0.65 to 0.76) (blue) Model 2: 0.74 (0.68 to 0.79 (purple) Model 3: 0.79 (0.74 to 0.83) (red) Model 4: 0.80 (0.76 to 0.85) (green) Model 5: 0.82 (0.78 to 0.86) (black)

Figure S10. Receiver operating characteristic curves for models screening for small-size and large-size for gestational age at birth with adverse outcomes[a]

[a] Receiver operating characteristic curves and corresponding area under the curve (95% CI) based on predicted values from five models for small- and large-size for gestational age. Models are based on maternal characteristics, second and third trimester fetal and placental ultrasound.

[b] Model 1: Maternal characteristics; Model 2: Maternal characteristics and 2nd trimester EFW; Model 3: Maternal characteristics and 3rd trimester EFW; Model 4: Maternal characteristics, 2nd +3rd trimester EFW; Model 5: Maternal characteristics, 2nd + 3rd trimester EFW and 2nd to 3rd trimester EFW change.

[c] AUCs based on a sample of 2705 participants with all placenta measures available. Models as EFW models under [b], adding the following placenta measures. Model 2: 2nd trimester uterine artery resistance index, and 2nd trimester umbilical artery pulsatility index. Model 3; 3rd trimester uterine artery resistance index, and 3rd trimester umbilical artery pulsatility index; Model 4: 2nd and 3rd trimester uterine artery resistance index, and 2nd and 3rd trimester umbilical artery pulsatility index; Model 5: 2nd and 3rd trimester uterine artery resistance index, and 2nd and 3rd trimester umbilical artery pulsatility index, 2nd to 3rd trimester change of uterine artery resistance index and umbilical artery pulsatility index.

SCREENING STUDIES

3.3: CUSTOMIZED VERSUS POPULATION BIRTH WEIGHT CHARTS FOR IDENTIFICATION OF NEWBORNS AT RISK OF LONG-TERM ADVERSE CARDIO-METABOLIC AND RESPIRATORY OUTCOMES: A POPULATION-BASED PROSPECTIVE COHORT STUDY

BMC Medicine 2019

Jan S. Erkamp
Vincent W.V. Jaddoe
Annemarie G.M.G.J. Mulders
Eric A.P. Steegers
Irwin K.M. Reiss
Liesbeth Duijts
Romy Gaillard

ABSTRACT

Background

Customized birth weight charts take into account physiological maternal characteristics that are known to influence fetal growth to differentiate between physiological and pathological abnormal size at birth. It is unknown whether customized birth weight charts better identify newborns at risk of long-term adverse outcomes than population birth weight charts. We aimed to examine whether birth weight classification according to customized charts is superior to population charts at identification of newborns at risk of adverse cardio-metabolic and respiratory health outcomes.

Methods

In a population-based prospective cohort study among 6,052 pregnant women and their children, we measured infant catch-up growth, overweight, high blood pressure, hyperlipidaemia, liver steatosis, clustering of cardio-metabolic risk factors and asthma at age 10. Small-size and large-size for gestational age at birth was defined as birth weight in the lowest or highest decile, respectively, of population or customized charts. Association with birth weight classification was assessed using logistic regression models.

Results

Of the total of 605 newborns classified small-size for gestational age by population charts, 150 (24.8%) were reclassified appropriate-size for gestational age by customized charts, whereas of the total of 605 newborns classified large-size for gestational age by population charts, 129 (21.3%) cases were reclassified appropriate-size for gestational age by customized charts. Compared to newborns born appropriate-size for gestational age, newborns born small-size for gestational age according to customized charts had increased risks of infant catch-up-growth (Odds Ratio (OR) 5.15 (95% Confidence Interval (CI) 4.22 to 6.29), high blood pressure (OR 2.05 (95%CI 1.55 to 2.72)), and clustering of cardio-metabolic risk factors at 10 years (OR 1.66 (95% CI 1.18 to 2.34)). No associations were observed for overweight, hyperlipidaemia, liver steatosis or asthma. Newborns born large-size for gestational age according to customized charts had higher risk of catch-down-growth only (OR 3.84 (95% CI 3.22 to 4.59). The direction and strength of the observed associations were largely similar when we used classification according to population charts.

Conclusions

Small-size for gestational age newborns seem to be at risk of long-term adverse cardio-metabolic health outcomes, irrespective of use of customized or population birth weight charts.

BACKGROUND

Small-size for gestational age (SGA) or large-size for gestational age (LGA) are important risk factors for adverse perinatal outcomes and death[1]. Children born SGA or LGA also have increased risks of suboptimal growth, cardio-metabolic and respiratory development throughout childhood, leading to increased risks of obesity, coronary heart disease, type 2 diabetes and obstructive respiratory disease in later life[2, 3]. Usually, population birth weight charts, which take into account gestational age at birth and sex, are used to discriminate between SGA, appropriate-size for gestational age (AGA) or LGA newborns[4]. Newborns classified SGA or LGA by these population charts include those who have grown according to their physiological growth potential and end up constitutionally small or large at birth, and those who have fetal growth restriction or acceleration and end up pathologically small or large at birth. Maternal characteristics, such as age, height, body mass index (BMI), ethnicity and parity and fetal sex are important determinants of fetal growth and cause non-pathological variation in birth weight[5, 6]. Customized charts take these physiological maternal and fetal characteristics into account for classification of normal and abnormal weight at birth[7, 8]. Customized charts may therefore be better able to distinguish constitutionally from pathologically small or large-size for gestational age at birth newborns[9]. Previous studies assessing the superiority of customized over population charts to identify SGA and LGA newborns at risk of short-term adverse outcomes are scarce and show conflicting results[10-13].

We hypothesized that compared to population charts, customized charts can better identify newborns at risk of long-term adverse health outcomes. We examined in a population-based prospective cohort study among 6,052 newborns the associations of SGA and LGA based on both customized and population charts for identification of newborns at risk of adverse growth patterns, cardio-metabolic and respiratory risk factors in childhood.

METHODS

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards in Rotterdam, the Netherlands[14]. The study has been approved by the local Medical Ethical Committee (MEC 198.782/2001/31). Written consent was obtained from all participating women. All pregnant women were enrolled between 2001 and 2005. Response rate at birth was 61%. 8,879 women enrolled during pregnancy. We excluded non-singleton live births (n=246), participants without

information on weight and gestational age at birth or maternal characteristics needed to generate customized charts (n=2,004), and children without long-term outcomes available (n=577). The population for analysis comprised 6,052 mothers and their children (**Figure S1**).

Classification of birth weight by customized and population charts

Customized charts have been developed within our study cohort as described previously, and include gestational age, fetal sex, maternal parity, age, height, weight, and ethnicity[7]. The pathological determinant maternal smoking was also used for the development of the customized charts because it has a substantial effect on fetal growth and birth weight and led to a more accurate regression model[7]. For the construction of a customized growth chart, the term for smoking was set to zero, whether the pregnant woman smoked or not. Hereby non-smoking was used as reference category within our customized models. To calculate the customized birth weight percentile, we entered the maternal characteristics, fetal sex and gestational age at birth for each newborn within our customized charts model and compared actual birth weight to the expected weight. For the population charts, we used gestational age adjusted weight charts modelled on the same population[7]. We calculated the birth weight percentile, by entering gestational age at birth for each newborn within our population charts model and compared actual birth weight to the expected weight. The population chart only included gestational age and no other characteristics, which allows for the optimal comparison between the population charts and customized charts in which any difference in outcome would only be explained by the process of customization. The formulas for both the customized charts and population charts have been published previously[7]. If the observed birth weight for gestational age was <10th or >90th percentile of the customized or population chart, the newborn was classified SGA or LGA respectively, otherwise AGA. We compared classifications according to customized and populations charts and further defined customized and population only SGA and LGA newborns. "Customized only" SGA or LGA newborns are classified AGA by population charts but reclassified SGA or LGA by customized charts. "Population only" SGA or LGA newborns are classified AGA by customized charts but reclassified SGA or LGA by population charts. Mode of delivery, offspring sex, gestational age, weight and APGAR score were obtained from medical records[15]. Preterm birth was defined as a gestational age of <37 weeks at birth.

Childhood growth, cardio-metabolic and respiratory outcomes

Well-trained staff in the Community Health Centers obtained postnatal growth characteristics at the age of 12 months, and was available for 4,205 (69.5%) participants.

Catch-up and catch-down-growth for weight were defined as an increase or decrease of >0.67 SD of weight from birth to 12 months of age, respectively[16]. This change represents the width of each percentile band on standard growth charts.

At the age of 10 years, children were invited for detailed measurements. We measured height and weight without shoes and heavy clothing. We calculated sex- and age-adjusted childhood BMI SDS based on Dutch reference growth charts (Growth Analyzer 4.0 Dutch Growth Research Foundation), and categorized BMI into normal, overweight and obesity using the definition of Cole et al[17, 18]. Total body fat and lean mass were measured with a dual-energy X-ray absorptiometry (DXA) scanner (iDXA, Ge-Lunar, 2008, Madison, WI, USA) using encore software version 13.6. Fat mass index (FMI) was calculated: fat mass(kg)/height(m)^2. Children were scanned using a 3.0 Tesla MRI (Discovery MR750w, GE Healthcare, Milwaukee, WI, USA) using standard protocols[19]. Visceral fat volumes were generated by summing volumes and multiplying by the gravity of adipose tissue, 0.9 g/ml. Liver fat fraction was determined by the average mean signal intensities from four samples of at least 4 cm^2 from the central portion of the hepatic volume. Liver steatosis was defined as liver fat fraction >=5.0%. Blood pressure was measured four times in supine position, with one-minute intervals at the right brachial artery using the automatic sphygmomanometer Datascope Accutor Plus (Paramus, NK)[20]. The mean of the last three measurements was calculated to determine blood pressure. High blood pressure was defined as systolic or diastolic blood pressure >90th percentile, using sex-, age- and height-specific cut-points[21]. Non-fasting venous blood samples were collected to measure total cholesterol, high-density lipoprotein (HDL)-cholesterol, triglycerides and insulin concentrations using Cobas 8000 analyzer (Roche, Almere, the Netherlands). Recommendations from National Cholesterol Education Program for children age 2-9 were used to define adverse levels of total cholesterol (>5.1 mmol/l)[22]. For clustering of cardio-metabolic risk factors, we used the definition of childhood metabolic syndrome phenotype, which is having three or more of the following components: visceral fat mass >75th percentile; systolic or diastolic blood pressure >75th percentile; HDL-cholesterol <25th percentile or triglycerides >75th percentile; and insulin level >75th percentile of our study population[23].

Forced Expiratory Volume in the first second (FEV_1), Forced Vital Capacity (FVC), FEV1:FVC and Forced Expiratory Flow after expiring 75% of FVC (FEF_{75}) were measured by spirometry (MasterScreen-Pneumo, Jaeger Toennies (Viasys) CareFusion Netherlands)[24]. Measures were converted into sex-, height-, age-, and ethnicity-adjusted SDS according to the Global Lung Initiative reference data[25]. Asthma was defined as ever physician-diagnosed asthma at age 10, obtained by parental reported questionnaires.

Statistical analyses

First, each newborn was classified into birth weight categories using both customized and population classifications. Descriptive data of birth weight categories were compared. Second, the percentages of newborns reclassified as SGA or LGA by the customized charts only or population charts only were assessed and population characteristics were compared using one-way ANOVA for continuous and chi-square test for categorical variables. Third, we assessed the associations of SGA and LGA at birth according to both customized and population charts with adverse outcomes using linear and logistic regression models for continuous and categorical outcomes, respectively. Non-normally distributed variables were log-transformed and SDS were calculated. For categorical outcomes, we calculated prevalences of adverse outcomes among SGA, AGA and LGA newborns, by dividing the number of cases by the number of newborns in each birth weight category. Finally, we assessed the predictive performance of both classifications for the prediction of the risk of long term adverse health outcomes among SGA and LGA newborns by calculating Receiver Operating Characteristic(ROC)-curves, the corresponding Area Under the Curve and sensitivity at a 90% specificity. We did not adjust our analyses for potentially confounding maternal characteristics, as customized classification already considers maternal characteristics and we were interested in comparing the classifications. All analyses were performed using the Statistical Package of Social Sciences version 24.0 for Windows (IBM Corp., Armonk, NY, USA).

RESULTS

Population characteristics

Table 1 shows population characteristics. Compared to newborns classified AGA by customized charts, newborns classified SGA by customized charts more often had heavier mothers and their mothers more often smoked throughout pregnancy. They were more often born premature or with a low APGAR score. Newborns classified LGA by customized charts more often had multiparous mothers, compared to newborns classified AGA by customized charts. Compared to newborns classified AGA by population charts, newborns classified SGA by population charts more often had nulliparous mothers, mothers with a lower weight and their mothers more often smoked throughout pregnancy. They were also more often born premature or with a low APGAR score. Newborns classified LGA by population charts had heavier mothers and mothers who were multiparous, compared to newborns classified AGA by population charts.

Table 1. Maternal and birth characteristics of newborns classified as SGA, AGA or LGA by customized and population birth weight classifications

	Customized classification[a]			Population classification[b]		
	Small-size for gestational age n=605	Appropriate-size for gestational age n=4,842	Large-size for gestational age n=605	Small-size for gestational age n=605	Appropriate-size for gestational age n=4,842	Large-size for gestational age n=605
Maternal characteristics						
Age, median (95% range), years	30.5(19.7 to 39.8)	30.6(19.6 to 39.1)	30.7(20.1 to 39.3)	29.7(19.0 to 39.6)	30.5(19.8 to 39.0)	31.6(21.5 to 39.8)
Height, mean (SD) (cm)	166.2(7.4)	167.7(7.4)	169.3(7.3)	164.3(7.1)	167.7(7.3)	170.6(7.2)
Weight, mean (SD) (kg)	71.8(16.3)	68.7(12.3)	70.4(12.9)	64.8(12.5)	68.8(12.4)	76.0(14.6)
Body Mass Index, mean (SD) (kg/m^2)	26(5.6)	24.4(4.1)	24.6(4.4)	24.0(4.4)	24.5(4.2)	26.2(5.1)
Obesity	123(20.3)	485(10.0)	60(9.9)	58(9.6)	491(10.1)	119(19.7)
Education, No. Higher (%)	217(37)	2,137(45)	306(52)	206(34.8)	2,141(45.2)	313(52.8)
Ethnicity, No. (%) Dutch/European	332(54.9)	2,899(59.9)	395(65.3)	283(46.8)	2911(60.1)	432(71.4)
Parity, No. Nulliparous (%)	348(57.5)	2,792(57.7)	330(54.5)	434(71.7)	2,787(57.6)	249(41.2)
Smoking, No. (%)						
-None	440(66.3)	3,755(73.9)	510(79.6)	379(65.2)	3,466(74.4)	462(79.4)
-Early-pregnancy only	50(7.5)	453(8.9)	61(9.5)	45(7.7)	418(9.0)	55(9.5)
-Continued	174(26.2)	875(17.2)	70(10.9)	157(27.0)	773(16.6)	65(11.2)
Birth characteristics						
Males, No. (%)	295(42.8)	2,665(50.5)	390(59.2)	242(40.0)	2,435(50.3)	362(59.8)
Gestational age, median (95% range) weeks	39.7(32.0 to 42.3)	40.3(36.3 to 42.3)	39.9(36.2 to 42.1)	40.3(36.3 to 42.4)	40.3(36.3 to 42.4)	39.9(36.0 to 42.0)
Birth weight, mean (SD) grams	2,622(483)	3,440(425)	4,176(396)	2581(421)	3,442(416)	4,230(399)
Preterm birth, No. (%)	74(12.2)	184(3.8)	21(3.5)	66(10.9)	187(3.9)	26(4.3)
Caesarean delivery, No. (%)	107(18.9)	467(10.5)	94(17.1)	102(18.2)	478(10.8)	88(16.0)
Assisted delivery, No. (%)	75(13.3)	644(14.5)	75(13.6)	59(15.9)	651(14.7)	54(9.9)
APGAR score below 7 at 5 minutes, No. (%)	16(2.8)	40(0.9)	4(0.9)	12(2.1)	44(0.9)	5(0.9)

Abbreviations: SD: Standard deviation. Values are median (95% range), mean (SD) and absolute numbers (%)

[a] SGA was defined as gestational age adjusted birth weight < 10th percentile of the customized chart. AGA is defined as gestational age adjusted birth weight >10th and <90th percentile according to the customized chart. LGA was defined as gestational age adjusted birth weight >90th percentile of the customized chart.

[b] SGA was defined as gestational age adjusted birth weight < 10th percentile of the population chart. AGA is defined as gestational age adjusted birth weight >10th and <90th percentile of the population chart. LGA was defined as gestational age adjusted birth weight >90th percentile of the population chart.

Table 2. Agreement of classification of gestational age adjusted birth weight by customized and population birth weight classifications

		Customized classification[a]		
		Small-size for gestational age n=605	Appropriate-size for gestational age n=4,842	Large-size for gestational age n=605
Population classification[b]	Small-size for gestational age n=605	455 (75.2)	150 (24.8)	0 (0)
	Appropriate-size for gestational age n= 4,842	150 (24.8)	4,563 (94.2)	129 (21.3)
	Large-size for gestational age n=605	0 (0)	129 (21.3)	476 (78.7)

Values are absolute cases (%).

[a] SGA was defined as gestational age adjusted birth weight <10th percentile of the customized chart. AGA is defined as gestational age adjusted birth weight >10th and <90th percentile according to the customized chart. LGA was defined as gestational age adjusted birth weight >90th percentile of the customized chart.

[b] SGA was defined as gestational age adjusted birth weight < 10th percentile of the population chart. AGA is defined as gestational age adjusted birth weight >10th and <90th percentile of the population chart. LGA was defined as gestational age adjusted birth weight > 90th percentile of the population chart.

Characteristics of newborns classified SGA or LGA by customized or population charts only

Table 2 shows that of 605 newborns classified SGA using population charts, 150 (24.8%) were reclassified AGA using customized charts, whereas of 605 newborns classified LGA using population charts, 129 (21.3%) cases were reclassified AGA using customized charts. Mothers of newborns who were classified SGA by customized charts only were likely to have higher BMIs, to be of Dutch or European ethnicity, and were more often multiparous compared to mothers of newborns classified SGA by both customized and population charts (**Table 3**). Newborns classified SGA by customized charts only, had a higher birth weight, and were less likely to be born preterm and after assisted delivery compared to newborns classified SGA by both customized and population charts. Mothers of newborns classified LGA by customized charts only, had lower age and BMI, were more often of Dutch or European ethnicity, and were more often nulliparous, compared to mothers of newborns classified LGA by customized and population charts. Newborns classified LGA by customized charts only, had lower birth weight and were more likely born after assisted delivery compared to newborns classified LGA by both charts. Mothers of newborns who were classified SGA by population charts only were younger, less likely to be obese, of Dutch or European ethnicity and to be nulliparous

Table 3. Maternal and birth characteristics of SGA or LGA newborns by customized charts only compared to newborns classified SGA or LGA by both classifications

	Small-size for gestational age			Large-size for gestational age		
	Customized only[a] n=150	Customized and population[b] n=455	p-value	Customized only[a] n=129	Customized and population[b] n=476	p-value
Maternal characteristics						
Age, median (95% range), years	30.5(20.4 to 39.0)	30.5(19.6 to 40.3)	0.215	27.7(19.9 to 36.0)	31.2(20.7 to 40.0)	<0.001
Height, mean (SD) (cm)	169.0(7.8)	165.3(7.0)	<0.001	165.7(6.4)	170.3(7.2)	<0.001
Weight, mean (SD) (kg)	85.4(18.1)	67.3(12.8)	<0.001	60.8(7.6)	72.9(12.8)	<0.001
Body Mass Index, mean (SD) (kg/m2)	30.0(6.5)	24.6(4.6)	<0.001	22.2(2.5)	25.2(4.6)	<0.001
Obesity, No. (%)	67(44.7)	56(12.3)	<0.001	2(1.6)	58(12.2)	<0.001
Education, No Higher (%)	51(34.0)	166(36.5)	0.582	57(44.2)	249(52.3)	0.102
Race / Ethnicity, No. (%) Dutch or European	101(67.3)	253(55.6)	0.011	56(43.4)	327(68.7)	<0.001
Parity, No. nulliparous (%)	50(33.3)	298(65.5)	<0.001	108(83.7)	222(46.6)	<0.001
Smoking, No. (%)			0.055			0.711
-None	109(75.2)	282(64.5)		100(80.0)	366(79.2)	
-Early-pregnancy only	9(6.2)	33(7.6)		14(11.2)	45(9.7)	
-Continued	27(18.6)	122(27.9)		11(8.8)	51(11.0)	
Birth characteristics						
Males, No. (%)	67(44.7)	185(40.7)	0.388	72(55.8)	281(59.0)	0.511
Gestational age, median (95% range), weeks	40.1(32.6 to 42.4)	39.6(31.9 to 42.3)	0.007	40.0(36.4 to 42.4)	39.9(36.1 to 42.0)	0.058
Birth weight, mean (SD) grams	2,981(412)	2,503(445)	<0.001	3,922(319)	4,245(386)	<0.001
Preterm birth, No. (%)	12(8.0)	62(13.6)	0.068	3(2.3)	18(3.8)	0.423
Caesarean delivery, No. (%)	19(13.4)	88(20.8)	0.051	20(16.7)	74(17.2)	0.897
Assisted delivery, No. (%)	9(6.3)	66(15.6)	0.005	27(22.5)	48(11.1)	0.001
APGAR score below 7 at 5 minutes, No. (%)	5(3.4)	11(2.5)	0.549	0(0)	5(1.1)	0.237

Abbreviations: SD: Standard deviation; Values are median (95% range), mean (SD) and absolute numbers (%), and p-values for comparison between population only and customized and population classification. Continuous variables were tested using ANOVA, categorical variables were tested using Chi² tests.

[a] As defined by the population birth weight classification, but appropriately sized according to customized birth weight classification.

[b] As defined by the both customized and population birth weight classification.

compared to mothers of newborns classified SGA by both charts (**Table S1**). Their newborns showed similar patterns to newborns classified SGA by customized charts only. Mothers of newborns classified LGA by population charts only were older, had higher BMI, were more likely multiparous, and their newborns had lower birth weight compared to newborns classified LGA using both charts.

Customized and population birth weight classification and childhood outcomes

Based on customized charts, newborns classified SGA had a higher risk of infant catch-up-growth compared to newborns classified AGA (Odds ratio (OR) 5.15 (95% confidence interval (CI) 4.22 to 6.29), **Figure 1A**). Risk of catch-down-growth was higher among newborns classified LGA using customized charts, compared to newborns classified AGA (OR 3.84 (95% CI 3.22 to 4.59), **Figure 1B**). We observed similar associations when birth weight was classified using population charts.

Compared to newborns classified AGA, newborns classified SGA using customized charts had higher risks of high childhood blood pressure (OR 2.05 (95%CI 1.55 to 2.72)) and clustering of cardio-metabolic risk factors (OR 1.66 (95% CI 1.18 to 2.34)). They also tended to have higher risk of childhood overweight (OR 1.24 (95% CI 0.95 to 1.60)), hyperlipidaemia (OR 1.25 (95% CI 0.88 to 1.79)) and liver steatosis (OR 1.77 (95% CI 0.88 to 3.54)), but these findings did not reach statistical significance (**Figure 1C-G**). We observed similar associations when we used the population classification. Newborns classified LGA using customized charts did not have increased risks of any adverse cardio-metabolic outcome. Newborns classified LGA using population charts had higher risk of overweight (OR 1.29 (95% CI 1.00 to 1.67), and a lower risk of hyperlipidaemia (OR 0.57 (95%CI 0.36 to 0.90) compared newborns classified AGA, but the differences in effect estimates compared to customized charts were very small. No associations of newborns classified SGA or LGA using either classification with asthma was found. When we repeated the analyses among newborns classified SGA or LGA by customized or population charts only, largely similar findings were observed. We only observed a slightly higher risk of high childhood blood pressure (OR 2.17 (95% CI 1.31 to 3.58) among newborns classified SGA by customized charts only compared to those classified SGA by population charts only (**Figure 2**). **Table S2** shows AUCs and derived sensitivities at a 90% specificity for both classifications for the risk of each long-term adverse health outcome. Both classifications had a poor to moderate ability to discriminate between those with and those without long-term adverse health outcomes with AUCs (95% CI)

ranging from 0.51 (95% CI 0.48-0.54) and 0.51 (95% CI 0.48-0.54) for risk of childhood asthma diagnosis to 0.66 (95% CI 0.64-0.69) and 0.63 (95% CI 0.61-0.65) for risk of infant catch up growth for customized and population charts, respectively.

Results presented in the supplementary materials show associations of birth weight using both customized and population classifications with continuously measured blood pressure, lipid, glucose and insulin concentrations, and lung function (**Table S3-5**). Altogether, no differences in associations were observed between effect estimates based on customized or population birth weight classifications.

DISCUSSION

Our findings suggest that newborns born SGA have increased risks of an adverse cardio-metabolic profile at school-age. Newborns born LGA have an increased risk of catch-down growth. Similar associations were present for classifications using customized charts and population charts, which suggests that customized charts are not superior to population charts at identification of SGA newborns at increased risk of adverse cardio-metabolic and respiratory outcomes at later age.

Interpretation of main findings

Birth weight is a strong determinant of neonatal health and health in later life[3]. Both experimental studies as well as large population studies have suggested that newborns born SGA or LGA as a results of adverse fetal exposures, experience developmental adaptations which put them at increased risks of adverse health outcomes in later life[3, 26]. Thus, identification of newborns with abnormal size at birth is important to identify individuals who might benefit from preventive strategies from early life onwards to prevent chronic diseases throughout the life course. Customized charts have been a topic of research for several decades as these charts may identify a higher proportion of newborns that are pathologically SGA or LGA and at increased risk of adverse outcomes, compared to population charts which may identify both constitutionally and pathologically SGA or LGA newborns[8, 9].

Previous studies mainly focused on the effects of customization on selecting newborns at risk for adverse perinatal outcomes. A meta-analysis including 20 studies comparing the effectiveness of customized versus population charts for prediction of adverse perinatal outcomes has shown similar effect estimates for associations of abnormal size at birth with intra-uterine fetal demise, neonatal intensive care unit admission, and neonatal and perinatal death[10]. A recent population-based linkage study among 979,912

singleton pregnancies in the United Kingdom between 1992 and 2010 assessed the predictive ability of non-customized versus partially customized birth weight centiles for the prediction of the risks of stillbirth, infant death and neonatal morbidity. This study showed that partial customization of birth weight charts does not improve prediction of these perinatal complications[27]. For the partial customization, maternal height, parity and fetal sex were used. Contrary, analysis of data on live births and stillbirths in England and Wales between 2007 and 2012 from the Office of National Statistics, suggested in areas that implemented customized charts, a decline in stillbirths rates of 19% occurred, while stillbirth rates remained the same in areas that did not implement customized charts[8, 28]. However, these findings need to be interpreted carefully and causality cannot be established from these observational studies. Recently, a study across different countries in Europe, including the UK, performed between 2004 and 2010 showed that rates of stillbirths declined by an average of 17%. A large number of these countries did not implement the use of customized charts. Thus, in comparison by the overall decline in stillbirth rates in Europe, the difference in decline in stillbirth rates in areas with and without implementation of customized charts may be relatively small[28, 29]. To date, no studies compared the use of customized and population charts to identify newborns at risk of long-term adverse health outcomes. We observed that customized charts were not better at selecting newborns at risk of adverse long-term cardio-metabolic or respiratory outcomes compared to population charts. As the majority of SGA newborns are classified as such by both charts, the benefit of the customized classification would mainly be present among the small group of newborns reclassified as having a normal or abnormal size for gestational age at birth by the customized charts. Within our study, newborns classified SGA by customized charts only did have higher risk of high blood pressure compared to AGA newborns and all SGA newborns, but these effects were not large enough to lead to a significant benefit of the use of customized over population charts. Thus, overall our study does not provide strong evidence for the use of customized charts to better identify newborns at risk of long-term adverse health outcomes. When we determined the accuracy of both classification methods for the prediction of individual risk of adverse outcomes, we observed a poor to moderate performance for both customized and population charts. This suggests that neither classification can be used for individual prediction of the risk for long-term adverse health outcomes based on classifying size at birth. However, the apparent increased risk of long-term adverse health outcomes among the group of SGA newborns, classified using either classification, suggest that on a population level this characteristic can be used for screening or prevention strategies, especially in combination with other prognostic factors.

There are several reasons why we might not observe strong differences in risks of long-term adverse outcomes between birth weights classified using customized or population charts. First, current customized birth weight charts have been criticized as they might not yet capture growth potential well enough to truly differentiate between pathologically and constitutionally SGA and LGA newborns[11, 30]. This would explain why we did not observe a clear benefit of customized charts over population charts for the identification of newborns at risk of adverse outcomes in later life. Future studies should determine whether customized charts can be improved by removal or addition of other parameters associated with fetal size and birth outcomes, such as parameters of placental vascular resistance or biomarkers[31, 32]. Second, it has been hypothesized that the observed stronger associations of abnormal fetal size or size at birth for gestational age based on customized charts with adverse perinatal outcomes could be explained by confounding by for example preterm birth and maternal obesity[11]. In a previous study among 4,095 women and their offspring, obesity and preterm birth were more prevalent among mothers of newborns classified SGA using customized charts[11]. Associations with adverse outcomes attenuated after adjustment for maternal obesity and preterm birth. In our study, prevalence of obesity among mothers of newborns classified SGA using customized charts was twice that of mothers of newborns classified SGA using population charts. Among newborns classified SGA using customized charts only, maternal obesity was even four-fold higher, which might explain a tendency for larger effect sizes for risk of childhood overweight, high blood pressure and clustering of cardio-metabolic risk factors. Thus, small differences in effect estimates between associations of SGA classified by customized and population charts might be explained by confounding factors. Finally, we might not have found strong differences in risk of adverse outcomes between customized and population charts, because our population is relatively healthy. We did not have extreme cases of SGA or LGA and the prevalence of long-term adverse health outcomes is low within our cohort. The potential advantage of the use of customized charts might be stronger among higher-risk populations. Further studies in these populations are needed.

Based on the findings in our study and the fact that population charts are easier to use and widely implemented, we would not recommend implementation of customized birth weight charts for identification of newborns at risk of long-term adverse health outcomes.

Strengths and limitations

We had a prospective data collection from early pregnancy onwards and a large sample of 6,052 newborns available with detailed childhood growth, cardio-metabolic and

respiratory measurements. Loss to follow-up could have reduced statistical power and led to biased effect estimates if associations differ between children included and not included in the analysis. We do not think this poses a problem within our study, as the aim of our study was to compare two classification methods. The non-response at baseline might have led to selection of a healthier population, which might affect the generalizability of our results to higher-risk populations. In clinical practice, often sex-specific population charts are used to classify abnormal size at birth weight. Given the aim of our study to specifically assess the effect of customization by major determinants of fetal growth, we constructed a population chart which included gestational age only to enable the most optimal comparison. By including fetal sex in the population chart, we could underestimate the effect of customized charts, as fetal sex is one of the major physiological determinants of fetal growth. If we had included fetal sex in our population charts, we expect similar or even weaker differences between the associations of abnormal size at birth with the risk of long-term adverse outcomes according to customized charts and population charts. Which maternal factors should be included in the customized charts also remains debatable. We included the pathological variable maternal smoking in the construction of the model to obtain a better fitted model. For the construction of a customized growth chart the term for smoking was set to zero, whether the pregnant woman smoked or not, and thereby non-smoking was used as reference category within our customized model for all women. This approach still allowed us to detect pathological fetal growth restriction due to maternal smoking during pregnancy. A similar approach may also be used for other pathological variables and further improve customized charts. Further studies are needed to explore whether customized charts which consider more maternal factors improve the classification of size at birth. Blood sample collection was performed in a non-fasting state at different time-points in the day. Since glucose and insulin levels are sensitive towards carbohydrate intake and vary during the day, this may have led to non-differential misclassification and an underestimation of the observed effect estimates.

Conclusion

SGA newborns seem to be at risk of long-term adverse cardio-metabolic health outcomes, irrespective of use of customized or population birth weight charts. Our results suggest that customized charts are not superior to population charts at selecting newborns at risk of adverse childhood growth, cardio-metabolic and respiratory outcomes. Based on these findings, we do not recommend implementation of customized charts for selection of newborns at risk of long-term adverse outcomes.

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SUPPLEMENTARY MATERIALS

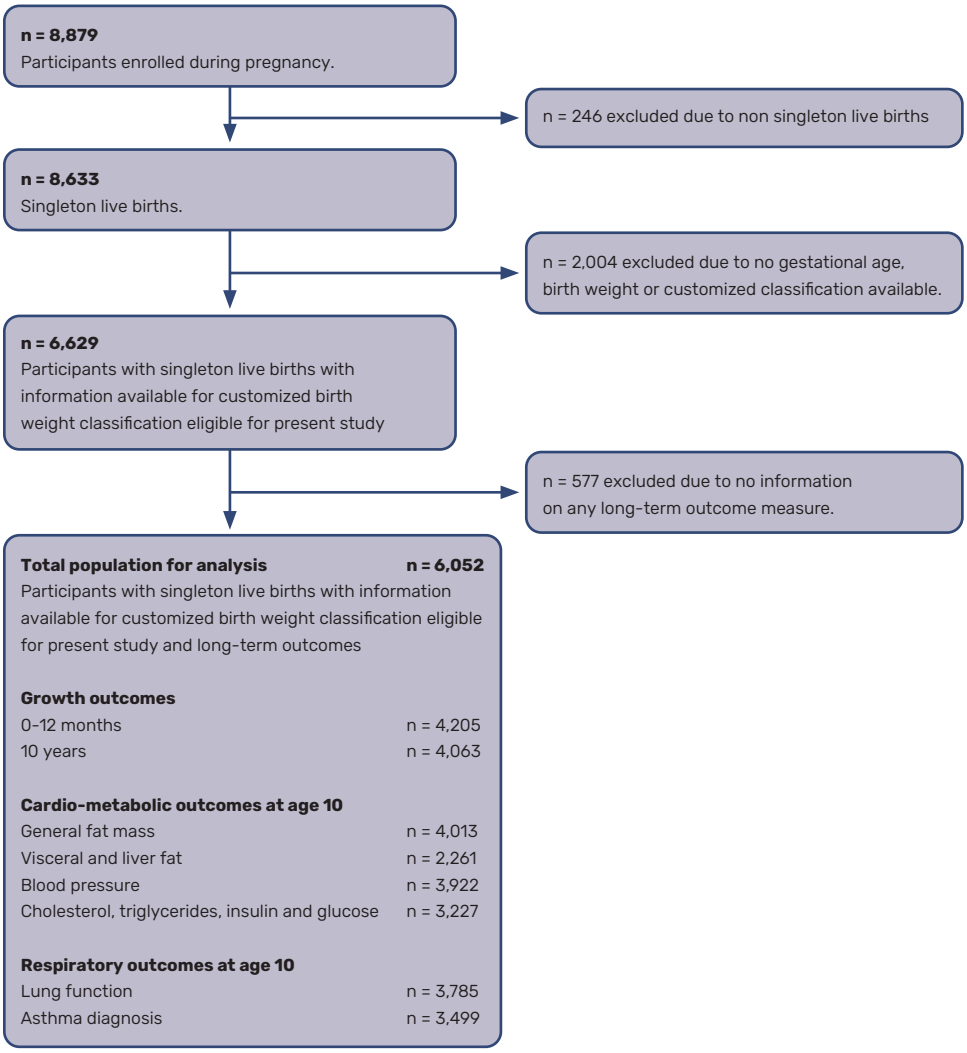

Figure S1. Flowchart population for analysis

Table S1. Maternal and birth characteristics of SGA and LGA newborns by population charts only compared to newborns classified SGA or LGA by both classifications

	Small-size for gestational age			Large-size for gestational age		
	Population only[a] n=150	Customized and population[b] n=455	p-value	Population only[a] n=129	Customized and population[b] n=476	p-value
Maternal characteristics						
Age, median (95% range), years	26.8(17.3 to 38.6)	30.5(19.6 to 40.3)	<0.001	32.9(21.7 to 39.6)	31.2(20.7 to 40.0)	0.002
Height, mean (SD) (cm)	161.4(6.4)	165.3(7.0)	<0.001	171.6(7.3)	170.3(7.2)	0.064
Weight, mean (SD) (kg)	57.2(7.4)	67.3(12.9)	<0.001	87.5(15.2)	72.9(12.8)	<0.001
Body Mass Index, mean (SD) (kg/m2)	22.0(2.6)	24.6(4.6)	<0.001	29.8(5.5)	25.2(4.6)	<0.001
Obesity, No. (%)	2(1.3)	56(12.3)	<0.001	61(47.3)	58(12.2)	<0.001
Education, No Higher (%)	40(26.7)	166(36.5)	0.028	64(49.6)	249(52.3)	0.586
Race / Ethnicity, No. (%) Dutch or European	46(30.7)	253(55.6)	<0.001	99(76.7)	327(68.7)	0.076
Parity, No. nulliparous (%)	136(90.7)	298(65.5)	<0.001	27(20.9)	222(46.6)	<0.001
Smoking, No. (%)			0.690			0.887
-None	97(67.4)	282(64.5)		96(80.0)	366(79.2)	
-Early-pregnancy only	12(8.3)	33(7.6)		10(8.3)	45(9.7)	
-Continued	35(24.3)	122(27.9)		14(11.7)	51(11.0)	
Birth characteristics						
Males, No. (%)	57(38.0)	185(40.7)	0.564	81(62.8)	281(59.0)	0.440
Gestational age, median (95% range), weeks	40.1(36.5 to 42.6)	39.6(31.9 to 42.3)	<0.001	40.1(34.1 to 42.0)	39.9(36.1 to 42.0)	0.940
Birth weight, mean (SD) grams	2816(201)	2504(445)	<0.001	4060(411)	4245(386)	<0.001
Preterm birth, No. (%)	4(2.7)	62(13.6)	<0.001	8(6.2)	18(3.8)	0.229
Caesarean delivery, No. (%)	14(10.3)	88(20.8)	0.006	14(11.9)	74(17.2)	0.164
Assisted delivery, No. (%)	23(16.9)	66(15.6)	0.717	6(5.1)	48(11.1)	0.050
Apgar score below 7 at 5 minutes, No. (%)	1(0.7)	11(2.5)	0.175	0	5(1.1)	0.243

Abbreviations: SD: Standard deviation; Values are median (95% range), mean (SD) and absolute numbers (%), and p-values for comparison between population only and customized and population classification. Continuous variables were tested using ANOVA, categorical variables were tested using Chi² tests.

[a] As defined by the population birth weight classification, but appropriately sized according to customized birth weight classification.

[b] As defined by the both customized and population birth weight classification.

Table S2. Predictive power of customized and population charts for long-term adverse outcomes

		Customized charts	Population charts
Catch-up growth	AUC (95% CI)	0.66 (0.64 to 0.69)	0.63 (0.61 to 0.65)
	Sensitivity	32.4	24.1
Catch-down growth	AUC (95% CI)	0.62 (0.61 to 0.64)	0.63 (0.61 to 0.64)
	Sensitivity	19.8	20.7
Overweight	AUC (95% CI)	0.52 (0.49 to 0.54)	0.52 (0.49 to 0.54)
	Sensitivity	11.2	11.6
High blood pressure	AUC (95% CI)	0.55 (0.52 to 0.58)	0.53 (0.50 to 0.56)
	Sensitivity	16.9	14.1
Hyperlipidemia	AUC (95% CI)	0.53 (0.49 to 0.56)	0.54 (0.50 to 0.57)
	Sensitivity	12.0	12.6
Liver steatosis	AUC (95% CI)	0.56 (0.49 to 0.63)	0.55 (0.48 to 0.63)
	Sensitivity	16.1	16.1
Clustering of cardio-metabolic riskfactors[a]	AUC (95% CI)	0.53 (0.49 to 0.56)	0.54 (0.50 to 0.57)
	Sensitivity	15.2	13.0
Asthma diagnosis	AUC (95% CI)	0.51 (0.48 to 0.54)	0.51 (0.48 to 0.54)
	Sensitivity	10.3	10.6

Values are area under the receiver operating characteristic curve (95% Confidence Intervals), sensitivity(%) at 90% specificity.

[a]Clustering of cardio-metabolic risk factors is defined as having three or more of the following components: visceral fat mass >75th percentile; systolic or diastolic blood pressure >75th percentile; HDL-cholesterol <25th percentile or triglycerides >75th percentile; and insulin level >75th percentile of our study population.

Table S3. Body composition and blood pressure at 10 years among newborns classified SGA or LGA by customized and population charts

	Fat mass index	n=	Systolic blood pressure	n=	Diastolic blood pressure	n=
Customized[a]						
Small-size for gestational age	0.23 (0.12 to 0.33)	387	0.21 (0.10 to 0.31)	379	0.15 (0.04 to 0.26)	379
Appropriate-size for gestational age	*ref*	3238	*ref*	3164	*ref*	3164
Large-size for gestational age	-0.07 (-0.18 to 0.04)	388	-0.07 (-0.17 to 0.04)	379	-0.08 (-0.19 to 0.03)	379
Population[b]						
Small-size for gestational age	0.22 (0.12 to 0.33)	388	0.05 (-0.05 to 0.16)	382	0.05 (-0.06 to 0.16)	382
Appropriate-size for gestational age	*ref*	3236	*ref*	3157	*ref*	3157
Large-size for gestational age	-0.03 (-0.13 to 0.08)	389	-0.01 (-0.12 to 0.09)	383	-0.03 (-0.14 to 0.08)	383
Customized only[c]						
Small-size for gestational age	0.22(0.02 to 0.42)	100	0.38(0.18 to 0.58)	98	0.34(0.14 to 0.54)	98
Appropriate-size for gestational age	*ref*	3053	*ref*	2979	*ref*	2979
Large-size for gestational age	-0.05(-0.27 to 0.17)	83	-0.18 (-0.40 to 0.04)	80	-0.10(-0.32 to 0.13)	80
Population only[d]						
Small-size for gestational age	0.19(-0.01 to 0.39)	101	-0.18(-0.38 to 0.02)	101	-0.03(-0.22 to 0.17)	101
Appropriate-size for gestational age	*ref*	3053	*ref*	2979	*ref*	2979
Large-size for gestational age	0.14(-0.08 to 0.35)	84	0.10(-0.12 to 0.31)	84	0.16(-0.06 to 0.37)	84

Values are SDS (95% CI). N = number of measurements available.

[a] SGA was defined as gestational age adjusted birth weight <10th percentile of the customized chart. AGA is defined as gestational age adjusted birth weight >10th and <90th percentile according to the customized chart. LGA was defined as gestational age adjusted birth weight >90th percentile of the customized chart.

[b] SGA was defined as gestational age adjusted birth weight <10th percentile of the population chart. AGA is defined as gestational age adjusted birth weight >10th and <90th percentile of the population chart. LGA was defined as gestational age adjusted birth weight >90th percentile of the population chart.

[c] SGA was defined as gestational age adjusted birth weight <10th percentile of the customized chart, but >10th percentile according to the population chart. AGA is defined as gestational age adjusted birth weight >10th and <90th percentile of both the customized and population chart. LGA was defined as gestational age adjusted birth weight >90th percentile of the customized chart, but not <90th percentile according to the population chart.

[d] SGA was defined as gestational age adjusted birth weight <10th percentile of the population birth weight chart, but >10th percentile according to the customized chart. AGA is defined as gestational age adjusted birth weight >10th and <90th percentile of both the population and customized chart. LGA was defined as gestational age adjusted birth weight >90th percentile of the population chart, but not <90th percentile of the customized chart.

Table S4. Cholesterol, triglycerides, insulin and glucose at 10 years among newborns classified SGA or LGA by customized and population charts

	Total cholesterol	n=	HDL cholesterol	n=	Triglycerides	n=	Insulin	n=	Glucose	n=
Customized[a]										
SGA	0.04 (-0.08 to 0.17)	277	-0.07 (-0.20 to 0.05)	276	0.17 (0.04 to 0.29)	274	0.15 (0.03 to 0.27)	277	-0.06 (-0.19 to 0.06)	277
AGA	*ref*	2274	*ref*	2275	*ref*	2267	*ref*	2270	*ref*	2275
LGA	-0.13 (-0.26 to -0.01)	284	0.03 (-0.09 to 0.15)	284	-0.04 (-0.17 to 0.08)	284	-0.05 (-0.17 to 0.08)	283	0.05 (-0.18 to 0.07)	283
Population[b]										
SGA	0.10 (-0.03 to 0.23)	271	-0.05 (-0.17 to 0.08)	271	0.22 (0.10 to 0.35)	267	0.17 (0.04 to 0.29)	271	-0.08 (-0.21 to 0.05)	271
AGA	*ref*	2277	*ref*	2277	*ref*	2271	ref	2273	*ref*	2278
LGA	-0.20 (-0.32 to 0.08)	287	0.00 (-0.11 to 0.14)	287	-0.03 (-0.15 to 0.09)	287	-0.01 (-0.14 to 0.11)	286	-0.01 (-0.14 to 0.11)	286
Customized only[c]										
SGA	-0.09 (-0.32 to 0.15)	73	-0.03 (-0.26 to 0.21)	72	0.04 (-0.20 to 0.27)	72	-0.03 (-0.26 to 0.20)	73	-0.12 (-0.35 to 0.11)	73
AGA	*ref*	2147	*ref*	2148	*ref*	2142	ref	2143	*ref*	2148
LGA	0.06 (-0.20 to 0.33)	57	0.14 (-0.26 to 0.21)	57	-0.04 (-0.30 to 0.22)	57	-0.11 (-0.37 to 0.15)	57	-0.23 (-0.49 to 0.03)	57
Population only[d]										
SGA	0.13 (-0.11 to 0.38)	67	0.08 (-0.16 to 0.32)	67	0.24 (-0.00 to 0.49)	65	0.02 (-0.23 to 0.26)	67	-0.21 (-0.46 to 0.03)	67
AGA	*ref*	2147	*ref*	2148	*ref*	2142	ref	2143	*ref*	2148
LGA	-0.25 (-0.51 to 0.01)	60	0.01 (-0.25 to 0.27)	60	0.01 (-0.25 to 0.26)	60	0.03 (-0.23 to 0.29)	60	*-0.05 (-0.30 to 0.21)*	60

Abbreviations: SGA: Small-size for gestational age; AGA: Appropriate-size for gestational age; LGA: Large-size for gestational age.

Values are SDS (95% CI). N = number of measurements available.

[a] SGA was defined as gestational age adjusted birth weight <10th percentile of the customized chart. AGA is defined as gestational age adjusted birth weight >10th and <90th percentile according to the customized chart. LGA was defined as gestational age adjusted birth weight >90th percentile of the customized chart.

[b] SGA was defined as gestational age adjusted birth weight <10th percentile of the population chart. AGA is defined as gestational age adjusted birth weight >10th and <90th percentile of the population chart. LGA was defined as gestational age adjusted birth weight >90th percentile of the population chart.

[c] SGA was defined as gestational age adjusted birth weight <10th percentile of the customized chart, but >10th percentile according to the population chart. AGA is defined as gestational age adjusted birth weight >10th and <90th percentile of both the customized and population chart. LGA was defined as gestational age adjusted birth weight >90th percentile of the customized chart, but not <90th percentile according to the population chart.

[d] SGA was defined as gestational age adjusted birth weight <10th percentile of the population birth weight chart, but >10th percentile according to the customized chart. AGA is defined as gestational age adjusted birth weight >10th and <90th percentile of both the population and customized chart. LGA was defined as gestational age adjusted birth weight >90th percentile of the population chart, but not <90th percentile of the customized chart.

Table S5. Measures of lung function at 10 years among newborns classified SGA or LGA by customized and population charts

	FEV1	n=	FEV1:FVC	n=	FEF75	n=
Customized[a]						
Small-size for gestational age	-0.19 (-0.30 to -0.09)	364	0.02 (-0.09 to 0.13)	364	-0.02 (-0.12 to 0.08)	365
Appropriate-size for gestational age	*ref*	3048	*ref*	3048	*ref*	3051
Large-size for gestational age	0.13 (0.02 to 0.24)	368	-0.02 (-0.12 to 0.09)	368	0.04 (-0.06 to 0.14)	368
Population[b]						
Small-size for gestational age	-0.18 (-0.29 to -0.07)	364	-0.06 (-0.17 to 0.05)	364	-0.06 (-0.16 to 0.04)	364
Appropriate-size for gestational age	*ref*	3052	*ref*	3052	*ref*	3055
Large-size for gestational age	0.07 (-0.03 to 0.18)	364	-0.04 (-0.14 to 0.07)	364	0.01 (-0.11 to 0.09)	365
Customized only[c]						
Small-size for gestational age	-0.20 (-0.41 to 0.00)	93	0.15 (-0.05 to 0.35)	93	0.11 (-0.08 to 0.30)	94
Appropriate-size for gestational age	*ref*	2880	*ref*	2880	*ref*	2882
Large-size for gestational age	0.31 (0.09 to 0.53)	79	0.16 (-0.05 to 0.38)	79	0.24 (0.03 to 0.45)	79
Population only[d]						
Small-size for gestational age	-0.13 (-0.34 to 0.07)	93	-0.13 (-0.33 to 0.07)	93	-0.01 (-0.21 to 0.18)	93
Appropriate-size for gestational age	*ref*	2880	*ref*	2880	*ref*	2880
Large-size for gestational age	0.06 (-0.17 to 0.29)	75	0.12 (-0.11 to 0.34)	75	0.04 (-0.17 to 0.25)	76

Abbreviations: FEV1: Forced expiratory volume in the first second; FVC: Forced vital capacity; FEF75: Forced expiratory flow at 75% of FVC.
Values are SDS (95% CI). N = number of measurements available.
[a] SGA was defined as gestational age adjusted birth weight <10th percentile of the customized chart. AGA is defined as gestational age adjusted birth weight >10th and <90th percentile according to the customized chart. LGA was defined as gestational age adjusted birth weight >90th percentile of the customized chart.
[b] SGA was defined as gestational age adjusted birth weight <10th percentile of the population chart. AGA is defined as gestational age adjusted birth weight >10th and <90th percentile of the population chart. LGA was defined as gestational age adjusted birth weight >90th percentile of the population chart.
[c] SGA was defined as gestational age adjusted birth weight <10th percentile of the customized chart, but >10th percentile according to the population chart. AGA is defined as gestational age adjusted birth weight >10th and <90th percentile of both the customized and population chart. LGA was defined as gestational age adjusted birth weight >90th percentile of the customized chart, but not <90th percentile according to the population chart.
[d] SGA was defined as gestational age adjusted birth weight <10th percentile of the population birth weight chart, but >10th percentile according to the customized chart. AGA is defined as gestational age adjusted birth weight >10th and <90th percentile of both the population and customized chart. LGA was defined as gestational age adjusted birth weight >90th percentile of the population chart, but not <90th percentile of the customized chart.

GENERAL DISCUSSION

GENERAL DISCUSSION

Introduction

Pregnancy complications are a major public health problem worldwide. In the Netherlands, the perinatal mortality rate calculated as the sum of fetal deaths after 28 weeks of pregnancy, and neonatal deaths before 7 days after birth is 10.45 per 1000 births[1]. In Rotterdam, the percentages of complications are even higher[2]. Treatment of gestational hypertensive disorders and abnormal fetal growth consists of intensified monitoring and management of symptoms where possible. Screening for, and early identification of women and their offspring at risk of pregnancy and childhood complications with subsequent monitoring and management may prevent adverse pregnancy outcomes, and improve later life health.

The aim of the studies presented in this thesis was to identify which maternal, fetal and placental parameters can be used for screening for common pregnancy complications with implications for short-term and long-term neonatal and childhood health outcomes in a healthy, low-risk, multi-ethnic population.

Maternal characteristics

Various maternal characteristics are well known to be associated with risk of pregnancy complications. Physical characteristics, such as body mass index and age, lifestyle characteristics, such as smoking habits and folic acid intake, and socio-demographic characteristics, such as ethnicity and household income are all associated with pregnancy outcomes[3-7]. The exact mechanisms underlying these associations are not always clear. Understanding the mechanisms underlying these associations is important. It might lead to improved selection of women at risk of adverse outcomes, improved monitoring, and could even contribute towards better management and treatment strategies. In this thesis, we focus specifically on the potential influence of young and advanced maternal age and impaired maternal early-pregnancy glucose metabolism on pregnancy outcomes and the potential underlying mechanisms. These factors are important in current clinical practice, due to a changing population of patients. The prevalence of advanced maternal age has been rising in the past decades, due to a larger population of women aged 35-45, more reliable contraception, more opportunities for education and career, and the advent and improvement of assisted reproductive technologies[8]. Similarly, due to changing diets and lifestyle habits, but also higher maternal age, the prevalence of obesity, diabetes mellitus type 2 and gestational diabetes have been increasing. Maternal age and glucose concentrations in early pregnancy may be responsible for alterations in placental hemodynamics and maternal blood pressure.

These alterations in placental hemodynamics and maternal blood pressure may likely contribute to subsequent increased risks of adverse pregnancy outcomes among women with young or advanced maternal age or hyperglycemia during pregnancy.

Maternal age

Young maternal age is defined as childbearing in women aged <20 years. It is associated with fetal growth restriction, preterm birth and high risk of fetal and neonatal death[9, 10]. Advanced maternal age is defined as childbearing in women aged ≥40 years. It is associated with fetal growth restriction, preeclampsia, placental abruption, preterm birth and fetal death[9, 11, 12]. The mechanism underlying these adverse pregnancy outcomes are not fully understood, but they likely involve the placenta and placental hemodynamics. Previous studies within the Generation R Study have already shown that maternal age impacts the maternal circulation[13]. For example, advanced maternal age is associated with blood pressure in the third trimester, which is an important parameter for the diagnosis of gestational hypertensive disorders and is associated with adverse pregnancy outcomes[13]. Maternal age, among other maternal socio-demographic characteristics, has also been shown to be associated with the resistance in the uterine arteries in the third trimester[14]. This may possibly be due to impaired placentation or general reduced vascular compliance among older women. Increased uterine artery resistance indices in the second and third trimesters are associated with increased risks of adverse pregnancy outcomes[14]. These suboptimal parameters of placental function and maternal circulation could, at least in part, be responsible for the observed higher risk of adverse pregnancy outcomes in the extremes of the maternal age spectrum[15].

In this thesis, we observed that compared to women aged 25-29.9 years, women younger than 20 years had an increased risk of third trimester uterine artery notching. Third trimester uterine artery notching is associated with risk of pregnancy complications, and in the case of young maternal age may be a result of biologic immaturity[16]. Simultaneously, young maternal age was not associated with increased utero-placental or feto-placental resistance, placental weight at birth, birth weight or their ratio. It might be that among young mothers, due to overall adequate vascular quality and hemodynamics, small changes in utero-placental flow and resistance can be more easily compensated, and that therefore no associations were found. Additionally, the mean maternal age among the maternal age <20 years group was relatively high (19 years), so biologic immaturity may be less pronounced and the group was relatively small, so we may have been underpowered to detect small changes in placental flow measures.

Compared to women aged 25-29.9, higher maternal age was associated with a higher third trimester uterine artery resistance index. This effect was already present at age

30-34.9 years, but was even more pronounced among women aged ≥40. Although the effect estimates from our study were small and within the normal range, on a population level, these higher indices may partly explain the higher risk of adverse pregnancy outcomes among higher maternal age groups. Maternal age between 35-39.9 years was associated with a lower placental weight at birth, indicating impaired placental function. In the maternal age group ≥ 40 years, placental weight was not significantly lower compared to the reference group of 25-29.9 years, likely due to a lack of power in this small group. Although the direction of the associations of maternal age with placental flow measures were similar in second trimester compared to third trimester, only associations in third trimester were statistically significant. Possibly, the effects of maternal age on utero-placental and feto-placental vascular resistance become more pronounced later in pregnancy, when the fetus and placenta are larger and demands to adequately supply the fetus with oxygen and nutrients are higher.

Underlying mechanisms

Normally in early pregnancy, trophoblast invasion and spiral artery remodeling takes place[17]. These processes are necessary to ensure adequate flow to the placenta, resulting in larger vessels with lower resistance and increased end diastolic flow[17]. In the case of young maternal age, these changes may not have taken place adequately, which may have contributed to the association of young maternal age with third trimester uterine artery notching[16]. Another possible explanation for higher risk of adverse pregnancy outcomes among young maternal age pregnancies, is the competition for nutrients. As the mother herself is still growing and developing, she might compete for nutrients with the fetus growing in her uterus, possibly leading to a suboptimal or adverse intra uterine environment. Other explanations may be a higher prevalence of risk factors and adverse lifestyles among adolescent pregnancies, such as lower educational attainment, higher prevalence of smoking and lower folic acid intake.

The mechanisms underlying increased risk of adverse pregnancy outcomes among advanced maternal age pregnancies may be different from those underlying risks among young maternal age pregnancies. Previous studies have shown that ageing leads to diminished blood flow and reduced compliance of the uterine vessels and altered endothelium function[18-20]. Our findings suggest that in the case of advanced maternal age, not impaired placentation explains these observed associations, but rather overall reduced vascular function and structure of the macro- and microvasculature due to advanced maternal age may lead to increased risk of adverse pregnancy outcomes. Finally, we observed that higher maternal age was associated with a lower birth weight, and that this association attenuated after taking into account third trimester uterine artery vascular resistance. Thus, even a small difference in third trimester utero-

placental vascular function among older women may play a pathophysiological role in the established associations of advanced maternal age with an increased risk of pregnancy complications, such as an abnormal birth weight.

Maternal early-pregnancy glucose concentrations

Maternal early-pregnancy glucose concentrations are associated with birth outcomes. A previous study from the Generation R Study Group has shown that maternal early-pregnancy glucose concentrations are associated with fetal growth[21]. A higher maternal early-pregnancy non-fasting glucose level was associated with decreased fetal growth rates in mid-pregnancy, and increased fetal growth rates from late pregnancy onwards[21]. Possibly, this may be due to adverse effects of higher glucose concentrations on placentation, resulting in placental insufficiency and growth restriction. The exact mechanism by which early-pregnancy hyperglycaemia impacts placentation and placental function is unknown. We hypothesized that higher early-pregnancy non-fasting glucose concentrations negatively influence placental flow measures, blood pressure and risk of gestational hypertensive disorders. We did not observe associations of early-pregnancy non-fasting glucose concentrations with blood pressure in mid or late pregnancy, placental hemodynamics or gestational hypertensive disorders. A previous study has shown that women with gestational diabetes mellitus have strongly increased risk of gestational hypertensive disorders[22]. The lack of associations in the current study of early-pregnancy glucose with gestational hypertensive disorders may be due to several factors. First, a high random non-fasting glucose measurement may not be as strongly correlated with gestational diabetes mellitus as a positive oral glucose tolerance test, which is the gold standard for diagnosis of gestational diabetes mellitus. Second, the timing of the measurement may be too early, as impaired glucose metabolism and associated hyperglycaemia may only become more apparent later in pregnancy. Third, the prevalence of gestational hypertensive disorders in our study population was much lower compared to that in other studies, suggesting a healthy population and decreasing statistical power to detect associations of glucose concentrations with gestational hypertensive disorders. Finally, as participants of the Generation R Study mostly had glucose concentrations within the normal range, maternal early-pregnancy non-fasting glucose concentrations may not be a valuable predictor for maternal pregnancy complications. Possibly, in higher-risk populations stronger associations of early pregnancy glucose with adverse pregnancy outcomes may be present.

Underlying mechanisms

In the current study, except for early pregnancy blood pressure, we did not find associations of maternal early-pregnancy non-fasting glucose concentrations with

placental flow measures, blood pressure or gestational hypertension in mid or late pregnancy. However, studies have shown a higher risk of gestational hypertensive disorders among women with gestational diabetes mellitus[23, 24]. Simultaneously, normalization of risk among women with gestational diabetes mellitus who have received treatment for the condition has been observed[25]. Hyperglycemia may, already in early pregnancy, lead to suboptimal early placental development. Hyperglycemia has been shown to promote a pro-inflammatory environment and cytokine derangements, which may act on the endothelium, inducing placental vascular changes[26, 27]. Further studies have shown associations of hyperglycemia during pregnancy with reduced invasiveness of the trophoblast, increased oxidative stress in the maternal and fetal milieu, disrupted vasculogenesis and macroscopically and histologically altered placentae[27-32]. Finally, insulin may have a direct toxic effect on the placenta[26, 27]. The combination of adverse effects of (prolonged) exposure to hyperglycemia and insulin on the maternal and fetal milieu, and presence of common risk factors associated with both diabetes and gestational hypertensive disorders may lead to increased risks of adverse pregnancy outcomes.

Summary

- Both young and advanced maternal age are associated with impaired third trimester utero-placental vascular function, which may predispose to an increased risk of pregnancy complications.
- Maternal early pregnancy non-fasting glucose concentrations are associated with blood pressure in early pregnancy, but not in later pregnancy, and are not associated with placental flow measures and gestational hypertensive disorders among low-risk pregnant women.

Screening

In the research described in this thesis, we examined the role of maternal, fetal and placental characteristics in screening for risk of short-term adverse outcomes, including gestational hypertensive disorders, abnormal fetal growth and long-term adverse childhood health outcomes. We additionally aimed to identify the feasibility and reproducibility of novel early pregnancy 3-dimensional ultrasound markers, as a proxy of actual fetal body proportions, which may be used in future screening efforts.

A large number of screening models using maternal, fetal and placental characteristics have been developed in the past decades for several adverse pregnancy complications[33-37]. Implementation of screening models in clinical practice remains a challenge for several reasons. First, different screening models have been developed using different maternal, placental and fetal characteristics[33]. As associations of these characteristics with the outcome of interest differ among different populations, comparison of screening models is difficult. Similarly, timing of screening and definitions of the exposures and outcomes vary strongly among screening studies, hindering comparison. Second, only a few screening models have been validated internally and even fewer have been validated externally[34, 38]. External validation is necessary to assess model performance among other population than the index population. Often, validation studies of promising screening models showed inferior model performance. In this thesis, we observed that among a low risk multi-ethnic population, maternal characteristics known in early-pregnancy can be used for screening for gestational hypertension and preeclampsia at any gestational age. Addition of combined second and third trimester placental ultrasound characteristics only improved screening for preeclampsia.

As compared to using maternal characteristics or second trimester fetal ultrasound only, combining maternal characteristics with third trimester fetal ultrasound, strongly improved screening for preterm birth, small-size for gestational age, and large-size for gestational age. Screening for preterm birth was further improved by addition of third trimester placental flow measures to the third trimester fetal ultrasound. We hypothesized that differences between screening performances of parameters in different models may be due to the fact that maternal characteristics, such as ethnicity, parity, smoking status and body mass index (BMI) are strongly correlated to the risk of adverse pregnancy outcomes, but also with fetal and placental parameters, possibly reducing the screening potential of these more advanced fetal and placental measurements[39-41]. Recently, a Dutch pragmatic, multicenter, stepped wedge cluster randomized controlled trial, among 13,046 low-risk women, compared routine third trimester ultrasound to usual care with regards to its effect on a composite of maternal outcomes and obstetric interventions[42]. This study concluded that routine third trimester ultrasound was indeed associated with a higher antenatal detection of small-size for gestational age fetuses, but not with a reduced incidence of severe adverse perinatal outcomes compared to usual care. However, based on a power-calculation, the study did not achieve the required sample size to reach the statistical power to definitively determine if routine third trimester ultrasound has beneficial or harmful effects on perinatal outcomes compared to usual care. Future randomized controlled studies are necessary to determine the value of maternal characteristics for screening for adverse pregnancy outcomes.

In current clinical practice population birth weight charts are used, which take into account gestational age at birth and sex, to discriminate between small-size for gestational age, appropriate-size for gestational age or large-size for gestational age newborns[43]. Newborns classified small-size or large-size for gestational age by these population charts include those who have grown according to their physiological growth potential and end up constitutionally small or large at birth, and those who have fetal growth restriction or acceleration and end up pathologically small or large at birth. Maternal characteristics, such as age, height, body mass index (BMI), ethnicity and parity and fetal sex are important determinants of fetal growth and cause non-pathological variation in birth weight[41, 44]. Customized charts take these physiological maternal and fetal characteristics into account for classification of normal and abnormal weight at birth[45, 46]. Customized charts may therefore be better able to distinguish constitutionally from pathologically small or large-size for gestational age at birth newborns[47]. A previous study reported that between 2007 and 2012, in areas that implemented customized charts, a decline in stillbirths rates of 19% occurred, while stillbirth rates remained the same in areas that did not implement customized charts. In comparison with the overall decline in stillbirth rates in Europe, the difference in decline between areas with and without implementation of customized charts is small. No studies have compared the use of customized and population charts to identify newborns at risk of long-term adverse health outcomes. Thus, the superiority of customized over population birth weight charts for screening for short-term and long-term adverse outcomes remains unknown. We observed that, irrespective of which classification was used, newborns classified small-size for gestational age had a higher risk of catch-up growth and adverse cardio-metabolic outcomes, compared to newborns classified appropriate-size for gestational age. Large-size for gestational age newborns had higher risk of catch-down growth, compared to newborns that were classified appropriate size for gestational age. We observed that customized charts were not superior to population charts at selecting small-size or large-size for gestational age newborns at risk of adverse childhood growth, cardio-metabolic and respiratory outcomes at the age of 10. Previous experimental and population studies have suggested that newborns born small-size for gestational age, as a result of adverse fetal exposures, experience developmental adaptations which may put them at increased risk of adverse health outcomes in later life[48, 49]. The apparent increased risk of long-term adverse health outcomes among small-size for gestational age newborns, suggest that on a population level, this characteristic can be used for screening of prevention strategies.

Finally, new measurements may aid in improved screening for adverse pregnancy outcomes. Novel markers need to be identified to further improve screening for adverse outcomes that are responsible for a vast amount of morbidity and mortality in the

general population; such as gestational hypertensive disorders, abnormal fetal growth, and long-term adverse health outcomes. Therefore, we focused on the feasibility and reproducibility of novel volume measurement of the fetus. Using 3-dimensional ultrasound and Virtual Reality, we found good feasibility, intra-observer reproducibility and inter-observer reproducibility of fetal body proportion measurement. These measurements enable detailed study on first trimester fetal development and growth, which may lead to better understanding of early developmental adaptation mechanisms leading to adverse birth outcomes and adverse cardiovascular risk profile in later life. Possibly, these new measurements can be used in future screening models. Well-designed screening and validation studies should be performed in low-risk, multi-ethnic populations, to determine optimal markers, and optimal timing for screening for common adverse health outcomes in the general population.

Summary

- Maternal characteristics can be used for screening for gestational hypertensive disorders in the general population.
- Third trimester ultrasound, in addition to maternal characteristics, has the best screening performance for detecting fetuses at risk of preterm birth, small-size and large-size for gestational age.
- Small-size for gestational age newborns have higher risk of adverse cardio-metabolic outcomes at age 10, irrespective of whether population-based or customized birth weight classification is used.
- We identified novel body proportion measurements using 3-dimensional ultrasound and Virtual Reality, and observed good intra- and inter-observer reproducibility.

Methodological considerations

The studies in this thesis were conducted in the Generation R Study and the Generation R *Next* Study. In the respective chapters, the strengths and limitations have been discussed. General methodological considerations will be discussed in the following paragraphs.

Selection bias

Selection bias may occur if the association between exposure and outcome are not equal between a group of women that participated and a group of women that was eligible to participate, but did not participate. Otherwise, selection bias may be introduced when selective loss to follow-up occurs. As previously described, of all children that were eligible to participate at birth in the inclusion period, 61% participated in the Generation R Study. Compared to what can be expected from the general population of Rotterdam, the percentages of women from lower socio-economic status and from ethnic minority groups were lower. Furthermore, participants had less pregnancy complications than can be expected which likely indicates selection of a healthier population. Because of the lower incidence of adverse pregnancy outcomes, this may have reduced statistical power, and it may have reduced the generalizability to the general population which is likely to be a less healthy population. As the Generation R Study is a prospective study, selection on the outcome at baseline is not an issue. Previous studies have shown that it is unlikely that results in prospective cohort studies are influenced due to selection bias by non-response at baseline, but more likely by selective loss to follow-up. Loss to follow up can lead to selection bias if associations differed between the participants in the study and the participants that left the study. In the studies described in this thesis, the loss to follow-up was low. For the study embedded in the Generation R *Next* Study, ultrasound data from a small random sample of participants was used. This random sample was drawn from women participating early during the inclusion period, when inclusion was still ongoing. As is known in population studies, characteristics of participants who respond early on may be different from characteristics of participants who need more persuasion before they are interested in participating, or from those who are not participating at all[50]. For our research question, studying the feasibility and reproducibility of novel fetal ultrasound markers, we do not think this poses a problem.

Information bias

Information bias may be introduced when misclassification of the exposure or the outcome occurs. Two types of misclassification exist; Differential and non-differential misclassification. If the exposure is related to the outcome, and vice versa, information bias due to differential misclassification may arise. Differential misclassification may lead to overestimation or underestimation of the results. If the exposure is unrelated to the outcome, information due to non-differential misclassification may arise. Non-differential misclassification may lead to underestimation or dilution of the results. The determinants in this thesis were collected prospectively using questionnaires. No information on the outcomes was known at the time of collection of the questionnaires used to gather information on the determinants. Neither the women nor the researchers

collecting the data were aware of the research questions in this manuscript at the time of the questionnaires. This makes differential misclassification of the exposure unlikely. Still, misclassification of the exposure may have occurred due to for example socially acceptable behavior, which may lead to and underreporting of for example smoking habits or over reporting of folic acid supplementation during pregnancy. The ultrasound data on fetal growth was collected using standardized protocols. High maternal BMI is known to be associated with larger fetuses, but simultaneously high maternal BMI impairs correct estimation of fetal weight using ultrasound. Therefore, misclassification of the estimated fetal weight, used as an exposure in the screening studies in this thesis, may have occurred. This may have led to a dilution or an overestimation of the effect. Differential misclassification of the outcome is unlikely, as the outcome data, such as birth weight and biological samples, were collected using standard operating procedures and using hospital registries, and the researchers responsible for collecting the data were blinded to the exposure.

In this thesis, we were specifically interested in screening for adverse outcomes. Screening may introduce different types of information bias, namely lead time bias, length bias and detection bias. When evaluating the effectiveness of a specific test, lead time bias may occur. Lead time bias is the amount of time between the detection of a disease based on new or experimental criteria, such as a novel screening method, and the time in which the disease would have been diagnosed without screening. The earlier detection may be perceived as prolonged survival. As survival time (lead time) is not an outcome measure in the studies described in the current thesis, we do not believe lead time bias occurred. Length bias may occur when some diseases are more aggressive than others. If the aggressiveness of a disease is such that these individuals have to leave the study before the screening takes place (e.g. have delivered before the third trimester ultrasound took place, due to severe early-onset preeclampsia), this may influence the results. In our study we focused on early-pregnancy screening, but also third trimester screening ultrasounds took place. In the case of severe early-onset preeclampsia, these women may have dropped out before the screening occurred, and may therefore have influenced the screening result. Detection bias occurs when a screening test may perform differently according to characteristics of the participants. Obesity is known to influence the accuracy of fetal ultrasound biometry, which means that fetuses of obese women are less likely to be accurately diagnosed as small-size or large-size for gestational age. Thus, an association between obesity and abnormal fetal size may be underestimated. Correction for maternal body mass index likely reduced or eliminated this detection bias.

Confounding

Confounding can occur if a factor is associated with both the exposure and the outcome, but the factor is not in the causal pathway between the exposure and the outcome. Bias may arise when this factor is not considered in the analyses. In the studies included in this thesis, confounding was eliminated as much as possible by correction for confounding variables on which data was available. Confounders can be identified using information on confounding factors from previous studies and literature on the subject, or by assessment of associations of the potential confounder in question with both the exposure or outcome, or a change in the effect estimate of more than 10 percent. After correction for confounders, residual confounding, which may be caused by unmeasured factors, may still result in biased effect estimates. Residual confounding always needs to be considered when interpreting effect estimates of observational studies.

Future research

Maternal characteristics

In this thesis, we first described associations of maternal characteristics with placental function to explore potential underlying mechanisms for well-known associations of maternal characteristics with pregnancy complications and identify novel maternal characteristics which may be used in future screening models. Further research is necessary to replicate our findings, and to explore further determinants of adverse pregnancy and long-term childhood outcomes.

First, observational studies are not designed to establish causality of the associations. To prove causality, randomized controlled trials are necessary. However, not all exposures studied in the current thesis can be further investigated using randomized controlled trials. For these exposures, which cannot be randomized, more advanced observational study designs are needed, such as the use of sibling comparison studies to assess the effect of maternal age on placental function. This way, comparison of altered placental development in response to maternal age can be assessed, with control for other family based socio-demographic and lifestyle factors shared among siblings. However, using this study design, the effect of parity may influence results and should be controlled for, as parity is known to be associated with placental function. To test the causality of maternal hyperglycemia during pregnancy on potential impaired blood pressure development and placental function during pregnancy and the risk of pregnancy complications, randomized controlled trials could be performed. The effect of hyperglycemia on the outcomes of interest could be studied by randomizing one group to strictly regulate glucose concentrations early in pregnancy, while the other group

would be left untreated or receives a placebo intervention. Results from the studies in this thesis should be considered when designing studies in which causality could be established.

Second, the underlying mechanisms of associations of maternal age and glucose concentrations with adverse outcomes should be further researched among both low and higher risk populations. Depending on the presence or absence of adverse exposures, such as the effect of young or advanced maternal age, or high glucose concentrations, early placentation may be negatively impacted. After placentation in early pregnancy has been completed, the placenta becomes responsible for oxygen and nutrient supply to the fetus. Suboptimal placentation or placental adaptations to adverse exposures may lead to long-term adverse outcomes. Future studies should focus on the effect of common adverse maternal exposures on early pregnancy placental parameters such as placental bed volume and flow, using novel techniques like power Doppler and 3-dimensional ultrasound. At birth, placental biopsies could be collected to see if histological alterations have taken place in exposed versus unexposed placentae.

Through impaired placental development or through direct effects, common adverse maternal exposures, such as young and advanced maternal age or hyperglycemia, could impact growth and development of the embryo and fetus, and subsequently, negatively impact pregnancy outcomes and long-term offspring health outcomes. This may already happen in the periconception phase, which is defined as a period 14 weeks prior to conception until 10 weeks after conception[51]. During this period, in the ovary of the mother, the oocyte develops and is exposed to the maternal milieu. Depending on the presence or absence of adverse maternal exposures, the oocytes may undergo molecular events like epigenetic modifications to the DNA. The higher prevalence of adverse cardio-metabolic outcomes at age 10 among newborns classified as small-size for gestational age may possibly be due to embryonic or fetal adaptations to an adverse maternal milieu. The embryo may adopt survival strategies that are beneficial in the presence this adverse maternal milieu, but these adaptations may be harmful in later life. Thus, adverse exposures to the maternal and embryonic milieu may impact placentation, embryonic and fetal growth and development, pregnancy outcomes and long-term maternal and child health. More research into this critical phase, using previously discussed ultrasound and histological placental parameters, may contribute to a better understanding of the interplay of genetic and environmental factors that impact placentation and early human development, and subsequently, short-term and long-term health outcomes.

Screening

Second, in this thesis we described that maternal, fetal and placental characteristics can be used for early screening for adverse pregnancy, birth and childhood outcomes. Also, we assessed the feasibility and reproducibility of novel ultrasound markers using 3-dimensional ultrasound and Virtual Reality in a healthy low-risk population. Further research is needed focused on replication of screening models and identification of the optimal period and optimal characteristics included in screening models.

First, we assessed the role of maternal, fetal and placental characteristics for screening for adverse pregnancy outcomes and adverse-long term childhood outcomes in a low risk multi-ethnic population. Our findings should be replicated among other low-risk multi-ethnic populations, which also include women and their offspring from different ethnic backgrounds as compared to our study population. These validation studies are needed to assess generalizability to other populations than the index population. These further studies should also focus on more detailed outcome assessment, to allow the assessment of screening performance for adverse common adverse pregnancy and birth outcomes with severe fetal or neonatal morbidity. For example; What is the screening performance of the models for selecting women at risk of severe early-onset preeclampsia, with offspring at high risk of neonatal morbidity or mortality. To address the power-issue that arises for these more rare outcome measures, meta-analyses should be performed. Although we did not find a benefit of customized birth weight charts over population birth weight charts at selecting newborns at risk of childhood outcomes, longer follow-up studies are necessary to assess whether either classification is better at selecting newborns at risk of adverse outcomes at later ages, such as adolescence or adulthood. Furthermore, customized charts might perform better in higher-risk populations, as the prevalence of adverse lifestyle and socio-demographic characteristics and adverse outcomes are likely higher among these populations. Therefore, replication studies in higher-risk populations with longer follow-up, are necessary.

Second, it should be studied if not maternal characteristics at the start of pregnancy, but already in the preconception period may help screening for adverse pregnancy, birth and childhood outcomes. This could help making a shift “upstream” from secondary to primary prevention. Secondary prevention, in the context of screening, aims to reduce the impact from current adverse maternal characteristics on pregnancy and pregnancy outcomes by selecting women that would benefit of intensified monitoring or intervention. Primary prevention, in the context of screening, would aim to reduce the prevalence of risk factors that predispose women to adverse pregnancy, birth and offspring outcomes by trying to modify them before they can cause harm. Benefits due to identification of true positives versus harm caused by false positives should

be evaluated. Future well-designed randomized controlled trials are needed to assess whether the advantages of screening outweigh the potential harm from parental anxiety and unnecessary interventions, in contemporary low-risk populations.

Third, a better understanding of the periconception phase may aid in identification of novel markers that can be used for screening for adverse pregnancy, birth and childhood outcomes. Close collaboration with neighbouring specialisms such as bioinformatics and medical imaging, may facilitate these processes. The advent of new technologies in recent years, such as 3-dimensional ultrasound and the use of Virtual Reality has already lead to novel preconception and early embryonic measurements. This has been giving insights into in-vivo early human development in fertility care populations. Further research into modern determinants of short-term and long-term health outcomes using new technologies in a low-risk population may aid in improvement of population screening. Further screening studies should determine the optimal combination of these determinants, utilizing routinely collected maternal characteristics as much as possible. These characteristics are freely available, which is especially important for low-resource settings, where pregnancy complications such as gestational hypertensive disorders, abnormal growth and preterm birth lead to a high morbidity and mortality.

Finally, integrated periconceptional population intervention studies, focusing on mother and her partner aiming to improve pregnancy and long-term health outcomes are necessary. In the manuscripts from the current thesis, we showed that maternal characteristics at the start of pregnancy are associated with placental function and we also showed that they can be used for screening for adverse pregnancy and birth outcomes. Some of the maternal characteristics, such as smoking habits, and folic acid intake, are modifiable. Integrated intervention studies could be used to see if intervention strategies may positively alter modifiable risk-factors in or before pregnancy. An integrated intervention could entail targeting of diet, physical activity, stress reduction, supplementation of micronutrients, and cessation of smoking, alcohol and drug use. Studies with long-term follow-up are necessary to determine if modification of these risk-factors leads to substantial improvements in short-term and long-term health outcomes.

Summary future research

- Randomized trials are necessary to prove causality of the observed associations between common adverse maternal characteristics and placental function.
- Further research is needed, focused on replication of screening models and identification of the optimal period and optimal characteristics included in screening models for short-term and long-term adverse outcomes among diverse populations.
- The screening value of novel determinants should be explored, such as early embryonic, fetal and placental determinants collected using 3-dimensional ultrasound and Virtual Reality.
- Future randomized controlled trials should assess the role of intervention strategies for modification of risk factors in the preconception phase and early pregnancy.

Clinical implications

In this thesis, we described associations of maternal characteristics with placental function. These effects of age on placental function could be a possible contributing factor to the previously observed higher risk of adverse outcomes among mothers with young or advanced maternal age. Knowing more about the underlying mechanisms that explain the associations of certain demographic or lifestyle factors with adverse pregnancy outcomes, could help the clinician to give better advice to mothers to be and their partners. Although the effect sizes of associations of maternal characteristics with pregnancy outcomes, and the effect sizes of the associations of birth weight with long-term cardiometabolic outcomes may be small, on a population level they could contribute to reduction in morbidity. Furthermore, we found that maternal, fetal and placental characteristics can be used for screening for important adverse pregnancy, birth and childhood outcomes. Before introduction in to clinical practice can take place, future well-designed randomized controlled trials are needed to confirm our results as described previously. Implementation of screening for adverse outcomes using the characteristics described in this thesis, could provide the clinician an opportunity to improve monitoring and interventions before severe disease develops.

Conclusion

Findings from this thesis suggest that maternal, fetal and placental characteristics can be used for early screening for adverse pregnancy, birth and childhood outcomes in a low-risk multi-ethnic population. Further studies should determine the role of novel determinants such as early pregnancy 3D ultrasound markers, in screening for short-term and long-term adverse outcomes in low-risk multi-ethnic populations.

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SUMMARY

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Chapter 1 describes the background and hypothesis for the studies presented in this thesis. Pregnancy complications are a major public health problem in the general population. Pregnancy complications, including gestational hypertensive disorders, abnormal fetal growth and birth weight have far reaching consequences for the health of both mother and child. Certain maternal, fetal and placental characteristics are associated with these adverse outcomes. The presence or absence of these characteristics during pregnancy, or possibly even before pregnancy, could help selecting women at higher risk of developing these pregnancy complications. This could lead to improved monitoring, and possibly intervention, before severe disease develops. In recent years, research has shown that, although pregnancy complications often manifest in third trimester, they likely find their cause in earlier pregnancy. Abnormal placentation in early pregnancy may play an important role. Screening for, and early identification of women and their offspring at risk of pregnancy and childhood complications with subsequent monitoring and management may prevent adverse pregnancy outcomes, and improve later life health. The general aim of this thesis is to identify which maternal, fetal and placental parameters can be used for screening for common pregnancy complications with implications for neonatal and childhood health outcomes in a healthy, low-risk, multi-ethnic population. The studies presented in this thesis were embedded in the Generation R Study and the Generation R *Next* study. These are population-based prospective cohort studies from fetal life, and from preconception onwards, respectively. Both studies are conducted in Rotterdam, The Netherlands. The studies are designed to identify early environmental and genetic determinants of growth, development and health in preconception, fetal life and childhood.

In **Chapter 2** studies on associations of early-pregnancy maternal characteristics with placental function are described. In **Chapter 2.1** we found that young and advanced maternal age are associated with placental function. Young maternal age, defined as maternal age below 20 years, was associated with increased risk of third trimester uterine artery notching. Advanced maternal age, defined as maternal age of 35 or higher, was associated with increased third trimester uterine artery resistance indices. Impaired placental function due to effects of young or advanced maternal age may explain the previously observed higher risk of adverse pregnancy outcomes among these women. In **Chapter 2.2** we found that maternal early-pregnancy non-fasting glucose concentrations were associated with higher early pregnancy blood pressure, but not with mid or late pregnancy blood pressure. No associations of early-pregnancy non-fasting glucose concentrations with placental flow measures and occurrence of gestational hypertensive disorders were found. In **Chapter 2.3** we identified novel

ultrasound parameters of fetal body proportion measurements using 3-dimensional ultrasound. We observed good intra- and inter-observer reproducibility of fetal body proportion measurements using 3-dimensional ultrasound and Virtual Reality offline volume measurements. These volume measurements may enable detailed study on first trimester fetal development and growth, which may lead to better understanding of early developmental adaptation mechanisms, and might be useful for screening for short-term and long-term adverse health outcomes in the general low-risk population.

In **Chapter 3** we describe studies that focus on screening for adverse pregnancy and childhood outcomes using maternal, fetal and placental characteristics. In **Chapter 3.1** we found that routinely measured maternal characteristics, including age, body mass index, ethnicity, parity, smoking and blood pressure known in early pregnancy have the best screening performance for pregnancies at risk of gestational hypertension and preeclampsia in a low risk multi-ethnic population. Addition of fetal or placental characteristics obtained throughout pregnancy did not improve screening performance in a low-risk multi-ethnic population, in addition to these simple maternal characteristics. In **Chapter 3.2** we found that third trimester ultrasound examination in addition to maternal characteristics has the best screening performance for detecting fetuses at risk for preterm birth, small-size for gestational age and large-size for gestational age, compared to second trimester ultrasound or combined second and third trimester ultrasound. Compared to second trimester ultrasound screening, third trimester ultrasound screening would nearly double detection of fetuses at risk of these adverse birth outcomes in a low risk population. When we added third trimester placental flow measures, only the screening performance for preterm birth significantly improved. In **Chapter 3.3** we found that newborns born small-size for gestational age have increased risks of an adverse cardio-metabolic profile at school-age. Newborns born large-size for gestational age have an increased risk of catch-down growth. Different classifications of birth weight have been described. Customized charts take physiological maternal and fetal characteristics into account for classification of normal and abnormal weight at birth, whereas population charts only take into account gestational age and sex. We found that similar associations were present for classifications using customized charts and population charts, which suggests that customized charts are not superior to population charts at identification of small-size for gestational age newborns at increased risk of adverse cardio-metabolic and respiratory outcomes at a later age.

In **Chapter 4** we provide a general discussion, in which we discuss the studies described in this thesis in a broader context. Furthermore, we describe implications and make suggestions for future research.

In conclusion, the studies in this thesis suggest that maternal characteristics can influence placental function. Furthermore, we found that maternal, fetal and placental characteristics can be used for early screening for adverse pregnancy and childhood outcomes. Finally, we proved the feasibility and reproducibility of novel ultrasound markers using 3-dimensional ultrasound and Virtual Reality in a healthy low-risk population. Future studies should focus on identification of novel early-pregnancy and preconception markers of adverse pregnancy and health outcomes. Integrated intervention studies could be used to see if intervention strategies may positively alter modifiable risk-factors in or even before pregnancy. Long-term follow-up is necessary to determine if modification of these risk-factors leads to substantial improvements in short-term and long-term health outcomes on a population level.

SAMENVATTING

Hoofdstuk 1 beschrijft de achtergronden en hypotheses die de basis vormen van de studies in dit proefschrift. Zwangerschapscomplicaties zijn een belangrijk probleem voor de volksgezondheid. Zwangerschapscomplicaties, waaronder hypertensieve aandoeningen van de zwangerschap, maar ook abnormale foetale groei en abnormaal geboortegewicht hebben verreikende consequenties voor de gezondheid van de moeder en haar kind. Specifieke maternale, foetale en placenta karakteristieken zijn geassocieerd met deze ongunstige uitkomsten. De aan of afwezigheid van deze karakteristieken gedurende de zwangerschap, of zelf al voor de zwangerschap, zouden kunnen helpen bij het selecteren van vrouwen die een hoger risico hebben op het ontwikkelen van zwangerschap complicaties. Dit zou kunnen leiden tot verbeterde monitoring en zelfs interventies, voordat ernstige ziekte kan ontstaan. Recent onderzoek heeft laten zien dat, hoewel zwangerschapscomplicaties zich vaak pas in het derde trimester van de zwangerschap manifesteren, de oorzaak van deze zwangerschapscomplicaties zich vaak eerder in de zwangerschap bevindt. Abnormale placentatie vroeg in de zwangerschap zou hierin een belangrijke rol kunnen spelen. Het screenen en het vroeg identificeren van vrouwen en hun kinderen die verhoogd risico hebben op complicaties tijden hun zwangerschap en kindertijd, en vervolgens monitoring en behandeling zou bij kunnen dragen aan het voorkomen van negatieve zwangerschapsuitkomsten, en het verbeteren van de gezondheid later in het leven.

Het doel van dit proefschrift is om te identificeren welke maternale, foetale en placenta parameters gebruikt kunnen worden bij het screenen naar deze veelvoorkomende zwangerschapscomplicaties, welke belangrijke gevolgen hebben voor de gezondheid tijdens de gehele kindertijd, in een gezonde, laag-risico, multi-etnische populatie. De studies in dit proefschrift werden uitgevoerd in de Generation R Studie en de Generation R *Next* studie. Dit zijn populatie-gebaseerde, prospectieve cohort studies, van respectievelijk de foetale en preconceptionele fase, tot aan de jong-volwassenheid. Beide studies zijn uitgevoerd in Rotterdam, Nederland. De studies zijn ontworpen voor het identificeren van vroege omgevings- en genetische determinanten van groei, ontwikkeling en gezondheid van een mens, voor en tijdens de zwangerschap en gedurende de jeugd.

In **Hoofdstuk 2** bestuderen we associaties van maternale karakteristieken, vroeg in de zwangerschap, met placenta functie. In **hoofdstuk 2.1** vonden we dat jonge en oudere maternale leeftijd zijn geassocieerd met placenta functie. Jonge maternale leeftijd, gedefinieerd als maternale leeftijd onder 20 jaar, was geassocieerd met verhoogd risico op notching van de arteria uterina in het derde trimester. Oudere maternale

leeftijd, gedefinieerd als maternale leeftijd van 35 of hoger, was geassocieerd met een verhoogde weerstandsindex van de arteria uterina in het derde trimester. Suboptimale placenta functie ten gevolge van jonge of oude maternale leeftijd kan mogelijk gedeeltelijk het verhoogde risico op zwangerschap complicaties in deze groepen vrouwen verklaren. In **hoofdstuk 2.2** vonden we dat verhoogde maternale niet-nuchtere glucose concentraties vroeg in de zwangerschap geassocieerd waren met een hogere bloeddruk vroeg in de zwangerschap, maar niet later in de zwangerschap. Er werden geen associaties waargenomen van deze glucose concentraties met maten van doorbloeding van de placenta, of met het voorkomen van hypertensieve complicaties van de zwangerschap. In **hoofdstuk 2.3** identificeerden we nieuwe echoscopische parameters van foetale lichaamsproporties, met behulp van 3-dimensionale echoscopie. We observeerden goede intra- en inter-observer reproduceerbaarheid van metingen van foetale lichaamsproporties middels 3-dimensionale echoscopie en Virtual Reality. Deze volume metingen zouden het mogelijk kunnen maken om gedetailleerd onderzoek te doen naar foetale ontwikkeling en groei in het eerste trimester, wat mogelijk kan leiden tot een beter begrip van vroege ontwikkeling en aanpassingsmechanismen, en zouden bruikbaar kunnen zijn bij screening naar negatieve gezondheidsuitkomsten op korte en lange termijn in de algemene, laag-risico populatie.

In **hoofdstuk 3** beschrijven we studies die gericht zijn op screening naar negatieve zwangerschapsuitkomsten en gezondheidsuitkomsten op de kinderleeftijd, met behulp van maternale, foetale en placenta karakteristieken. In **hoofdstuk 3.1** vonden we dat routinematig gemeten maternale karakteristieken, onder meer leeftijd, body mass index, etniciteit, pariteit, roken en bloeddruk, welke reeds aan het begin van de zwangerschap bekend zijn, het beste screeningsresultaat opleverden voor zwangerschappen die een verhoogd risico hebben op zwangerschapshypertensie en preeclampsie, in een laag risico multi-etnische populatie. Het toevoegen van foetale of placenta karakteristieken aan maternale karakteristieken leidde niet tot verbetering van het screeningresultaat in een laag-risico populatie. In **hoofdstuk 3.2** vonden we dat een echoscopisch onderzoek in het derde trimester, samen met het gebruik van maternale karakteristieken, het beste screeningsresultaat oplevert voor het detecteren van foetussen die een verhoogd risico hebben op vroeggeboorte, een laag geboortegewicht of een hoog geboortegewicht, in vergelijking met alleen tweede trimester of tweede en derde trimester echoscopie gecombineerd. In vergelijking met een tweede trimester echo, leidt een derde trimester echoscopische screening tot nagenoeg een verdubbeling van het aantal gedetecteerde foetussen die risico hebben op deze negatieve zwangerschapsuitkomsten in een laag-risico populatie. Wanneer we maten van placenta doorbloeding in het derde trimester toevoegden aan de screeningsmodellen, verbeterde alleen het screeningsresultaat voor vroeggeboorte. In **hoofdstuk 3.3** vonden we dat neonaten die te klein waren voor

de zwangerschapsduur een verhoogd risico hadden op een negatief cardio-metabool profiel op de schoolleeftijd. Neonaten die groot waren voor de zwangerschapsduur hebben een verhoogd risico op catch-down groei. Verschillende classificaties van geboortegewicht zijn omschreven. Customized classificaties houden rekening met fysiologische maternale en foetale karakteristieken voor de classificatie van abnormaal en normaal geboortegewicht. Populatie classificaties houden alleen rekening met de zwangerschapsduur en het geslacht van de neonaat. Wij vonden dat de customized en populatie classificaties dezelfde associaties met uitkomsten op de schoolleeftijd opleverden, wat suggereert dat de customized classificatie niet beter is dan de populatie classificatie in het identificeren van te kleine of te grote neonaten die een verhoogd risico hebben op negatieve cardio-metabole en respiratoire gezondheidsuitkomsten op latere leeftijd.

In **hoofdstuk 4** bediscussiëren we de studies uit dit proefschrift in een bredere context. Bovendien beschrijven we implicaties en doen we suggesties voor toekomstig onderzoek. Concluderend, de studies in dit proefschrift suggereren dat maternale karakteristieken de placenta functie kunnen beïnvloeden. Bovendien vonden we dat maternale, foetale en placenta karakteristieken vroeg in de zwangerschap gebruikt kunnen worden voor screening voor negatieve zwangerschapsuitkomsten en negatieve gezondheidsuitkomsten in de kindertijd. Tot slot bewezen we de haalbaarheid en reproduceerbaarheid van nieuwe echoscopische markers, gebruik makend van 3-dimensionale echoscopie en Virtual Reality, in een gezonde laag-risico populatie. Toekomstige studies zouden zich kunnen focussen op het identificeren van nieuwe markers van negatieve zwangerschaps- en gezondheidsuitkomsten. Geïntegreerde interventiestudies zouden kunnen worden benut om te onderzoeken of interventie strategieën in staat zijn om veranderbare negatieve maternale risicofactoren te veranderen, zelfs al voor de zwangerschap. Lange-termijn follow-up is nodig om te bepalen of het veranderen van deze risicofactoren leidt tot substantiële verbeteringen in de gezondheid op korte termijn en op lange termijn op populatieniveau.

ADDENDUM

AUTHORS AFFILIATIONS
PUBLICATION LIST
ABOUT THE AUTHOR
PHD PORTFOLIO
DANKWOORD

AUTHORS' AFFILIATIONS

Department of Obstetrics and Gynecology, Erasmus MC, Rotterdam, The Netherlands
AGMGJ Mulders, EAP Steegers

Department of Pediatrics, Erasmus MC, Rotterdam, The Netherlands
L Duijts, JS Erkamp, R Gaillard, VWV Jaddoe, IKM Reiss

The Generation R Study Group, Erasmus MC, Rotterdam, The Netherlands
JS Erkamp, R Gaillard, ML Geurtsen, VWV Jaddoe, E Voerman, C Wiertsema

PUBLICATION LIST

First Author

1. **Erkamp JS**, Cornette J. Contraception and Cardiovascular Disease. Pregnancy and Congenital Heart Disease: Springer; 2017. p. 23-33.

2. **Erkamp JS**, ten Kate-Booij MJ, Ewing PC, Schoenmakers S. Classical symptoms and risks associated with an ongoing molar pregnancy in case of a coexisting twin. Journal of Reproductive Medicine. 2018, Volume 63 - Issue 3 p. 249- 253

3. **Erkamp JS**, Jaddoe VWV, Mulders A, Steegers EAP, Reiss IKM, Duijts L, Gaillard R. Customized versus population birth weight charts for identification of newborns at risk of long-term adverse cardio-metabolic and respiratory outcomes: a population-based prospective cohort study. *BMC Med* 2019;17: 186.

4. **Erkamp JS**, Geurtsen ML, Duijts L, Reiss IKM, Mulders A, Steegers EAP, Gaillard R, Jaddoe VWV. Associations of maternal early-pregnancy glucose concentrations with placental hemodynamics, blood pressure and gestational hypertensive disorders. *Am J Hypertens* 2020.

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Co-Author

8. Cornette JMJ, **Erkamp JS**. Internal Podalic Version and Breech Extraction: Enhancing Realistic Sensations in a Simulation Model. *Obstet Gynecol* 2018;131: 360-363.

9. Wiertsema C, **Erkamp JS,** Mulders A, Duijts L, Gaillard R, Steegers EAP, de Koning A, Jaddoe VWV. Reproducibility of first trimester embryonic volume and fetal body proportion measurements in a population-based sample. *In preparation*

ABOUT THE AUTHOR

Jan Steven Erkamp was born in Wayne, United States of America, on the 27th of November 1989. Jan applied to medical school at the Erasmus University Rotterdam, obtained a MSc in Medicine in 2015, and decided to pursue a career in Obstetrics and Gynaecology. After a year of clinical work at the department of Obstetrics and Gynaecology of Franciscus Gasthuis, he became a PhD candidate at the Generation R Study Group. Jan played a role in the startup of a new prospective population-based cohort study called Generation R *Next*. During his PhD, he obtained a MSc in Clinical Epidemiology. Jan's research focused on screening for adverse pregnancy and childhood outcomes. He finished his PhD under supervision of Prof.dr. Vincent Jaddoe, Prof.dr. Irwin Reiss and dr. Romy Gaillard. In Januari of 2021, Jan will start his specialization to become a Gynaecologist. Jan lives in Rotterdam with two cats.

PHD PORTFOLIO

Summary PhD training and teaching activities

Name PhD student:	Jan S. Erkamp
Erasmus MC Department:	The Generation R Study Group
Research School:	Netherlands Institute for Health Sciences
PhD period:	May 2016 - April 2020
Promotors:	Prof. dr. V.W.V. Jaddoe, Prof. dr. I.K.M Reiss
Co-promotor:	Dr. R. Gaillard

1. PhD training	Year	Workload (ECTS)
Master of Science in Health Sciences (Research), specialization Epidemiology, NIHES, Erasmus University Rotterdam, the Netherlands	**2016-2019**	
Core courses		
Study Design		4.3
Biostatistical Methods I: Basic Principles		5.7
Biostatistical Methods II: Classical Regression Models		4.3
Research		32.6
Required courses		
Principles in Causal Inference		1.4
Principles of Research in Medicine and Epidemiology		0.7
Methods of Clinical Research		0.7
Clinical Trials		0.7
Health Economics		0.7
The Practice of Epidemiologic Analysis		0.7
Fundamentals of Medical Decision Making		0.7
Clinical Translation to Epidemiology		2.0
Clinical Epidemiology		3.7
Elective courses		
Genomics in Molecular Medicine		1.4
Logistic Regression		1.4
Repeated Measurements in Clinical Studies		1.4
Missing Values in Clinical Research		0.7
Women's Health		0.9

1. PhD training	Year	Workload (ECTS)
Principles of Epidemiologic Data-analysis		0.7
Maternal and Child Health		0.9
Quality of Life Measurement		0.9
Other courses		
Research Integrity, Erasmus MC, the Netherlands	2017	0.3
Seminars, congresses and presentations		
Generation R Research meetings, Erasmus MC, the Netherlands	2016-2020	1.0
Maternal and Child Health meetings, Erasmus MC, the Netherlands	2016-2020	1.0
Developmental Origins of Health and Disease (DOHaD), Rotterdam, the Netherlands. *Poster presentation*	2017	1.4
International Society of Ultrasound in Obstetrics and Gynaecology, World Congress, Berlin, Germany. *Electronic poster*	2019	1.4

2. Teaching	Year	Workload (ECTS)
Teaching ultrasound protocols and skills to PhD and Ultrasound personnel	2017-2018	2.0
Molar pregnancy: Teaching Residents Obstetrics & Gynaecology of Rotterdam and Leiden.	2018	1.0