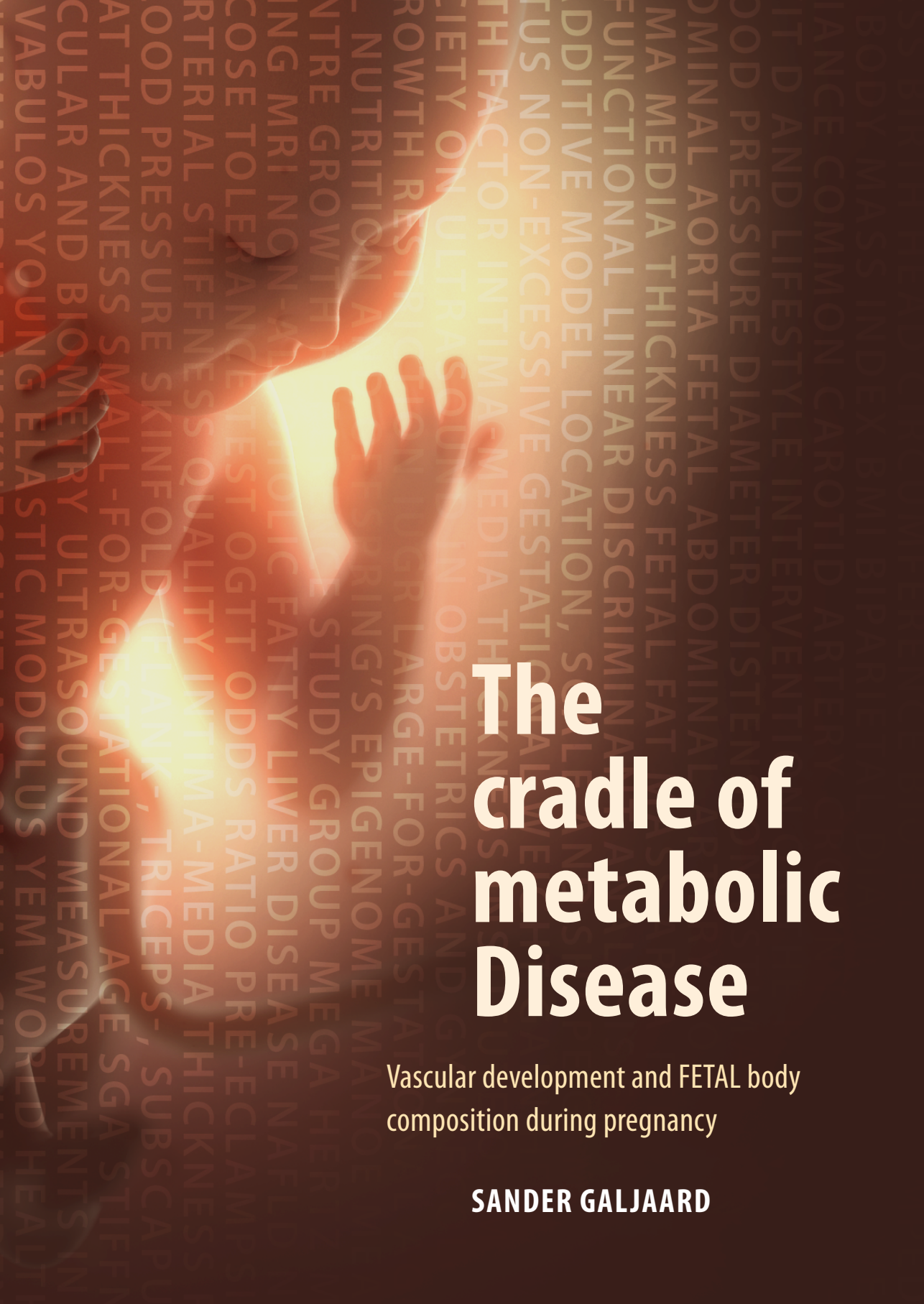

The cradle of metabolic Disease

Vascular development and FETAL body composition during pregnancy

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ULTRA-GELUIDSONDERZOEK

"…ultrageluid wordt het ook wel genoemd. Tegenwoordig zetten ze het in om overlast gevende jongeren te verjagen van hun hangplekken. In sommige gemeenten zweren ze bij die techniek. De Mosquito. Wie jonger is dan pakweg 25 jaar hoort een mateloos irritant geluid. Wie ouder is hoort niets. Voor de één klinkt er dus een hels kabaal, terwijl het voor de ander weldadig stil is. Gelijktijdig, op dezelfde plek! Wat er voor de één is, is er voor de ander niet. Wie beweert dat het er niet is heeft gelijk. Maar wie volhoudt dat het er wel is heeft ook gelijk.

Het is maar net hoe je het bekijkt. Niets is wat het lijkt'.

ULTRA-SOUND EXAMINATION

"…also known as ultrasound, it is often used as a deterrent against antisocial behaviour. With some borough councils it is the preferred method to make NEET[1] hangouts unpopular. It is nicknamed "The Mosquito" and causes people under 25 years of age to hear an intensely annoying sound. Those over 25 do not hear a thing. While NEETs complain about a bloody racket, older people experience peace and quiet in the same place, at the same time. You may argue that there is no sound, while you can equally maintain that there is.

It all depends on the perspective. Nothing is what it seems."

(translation (c) 2015 Marc Weide)

Uit 'Echo, prenataal onderzoek en keuzevrijheid', Maarten Slagboom, 2011 uitgeverij Augustus, p. 18, ISBN 9789045704586

[1] NEET - young people Not in Employment, Education or Training" (16-24 years, UK)

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http://www.e-pubs.nl?epub=s.galjaard

CONTENT

Chapter 1.	General aim, outline and introduction.	5
Chapter 2.	Fetal growth and developmental programming.	27
Chapter 3.	Sex differences in fetal growth and immediate birth outcomes in a low-risk Caucasian population.	39
Chapter 4.	The influence of weight gain patterns in pregnancy on fetal growth using cluster analysis in an obese and non-obese population	59
Chapter 5.	Fetal central adiposity *in utero* is affected by both excessive gestational weight gain and obesity: a prospective cohort study in normoglycemic women.	75
Chapter 6.	Neonatal adiposity is predicted by fetal central fat accumulation *in utero*, a prospective cohort study in normoglycemic women.	95
Chapter 7.	Intima-Media Thickness measurements in the fetus and the mother during pregnancy: a feasibility study.	115
Chapter 8.	Excessive gestational weight gain increases maternal common carotid artery stiffness in pregnancy.	133
Chapter 9.	Fetal abdominal aorta remodelling correlates independent of fetal size and maternal obesity, a longitudinal study *in utero*.	151
Chapter 10.	Summary and future perspectives.	169
Appendices	List of abbreviations	183
	Abstract of Research	187
	Nederlandse Samenvatting	191
	Supplement	209
	Collaborators' list	231
	Dankwoord	239
	CV & Publications	243


ABDOMINAL AORTA FETAL ABDOMINAL
INTIMA MEDIA THICKNESS FETAL FAT
FUNCTIONAL LINEAR DISCRIMINANT
ADDITIVE MODEL LOCATION, SCALE AND
MELLITUS NON-EXCESSIVE GESTATIONAL
GROWTH FACTOR INTIMA-MEDIA THICKNESS
SOCIETY ON OBSTETRICS AND
GROWTH LARGE-FOR-GESTA
NUTRITION EPIGENOME
GROWTH STUDY GROUP
IMAGING MRI FATTY LIVER DISEASE
GLUCOSE TOLERANCE TEST OGTT ODDS RATIO
ARTERIAL STIFFNESS QUALITY
BLOOD PRESSURE SKINFOLD TRICEPS
FAT THICKNESS SMALL-FOR-GESTATIONAL AGE
VASCULAR AND BIOMETRY ULTRASOUND
STUDY VABULOS YOUNG ELASTIC MODULUS

Chapter 1

General Introduction

GENERAL AIM

The research in this doctoral thesis is focused on the dynamic effects of pregnancy in the background of the obese women. Effects of maternal obesity on short term changes in outcomes of body and cardiovascular development during pregnancy are investigated. Obesity research in pregnancy is not focused on static relationships, but is a domain based on dynamic changes that occur during two transitions: from woman to mother and from fetus to newborn. Maternal weight for example can be unchanged while pregnant, but during most pregnancies weight changes are striking (both weight gain and weight loss). In addition, glucose intolerance increases and insulin sensitivity decreases during pregnancy. If these changes meet predefined criteria, it is regarded as a disease, known as gestational diabetes mellitus (GDM). Important cornerstones for metabolic research in pregnancy are the effects of maternal adiposity (body mass index -BMI-), nutrient intake and storage (gestational weight gain -GWG-) and abnormal glucose

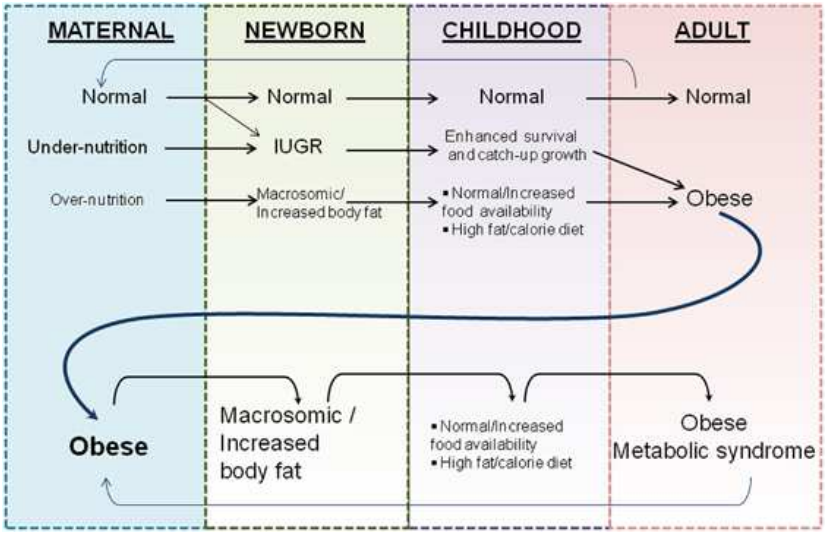

Figure 1. The role of 'gestational programming' in population shifts towards obesity and metabolic syndrome. Normal weight mothers usually give birth to normal-weight infants with normal adiposity. These offspring develop into normal adults with normal body fat content and a normal metabolic profile. The increased incidence of prematurity and intrauterine growth restriction (IUGR) resulting from undernutrition, among other factors, combined with improved neonatal survival, formula feeding and exposure to a Western postnatal diet has resulted in increased offspring obesity and metabolic syndrome. A small portion of obese mothers give birth to newborns with increased body fat, as a result of overnutrition (consumption of a high-fat diet). These processes may contribute to the population shift towards an obese phenotype, with second generation obese women at increased risk for giving birth to newborns with increased body fat content, and who in turn, are at risk of developing obesity and metabolic syndrome. (Reproduced with permission of the author and publisher, Desai et al., 2015)[2]

tolerance (GDM). These three contributors play an important role in the development of complications in the mother and the fetus, both short and long term.[1] Therefore the *in utero* development of the fetus is regarded as a time of vigorous growth and development.[2] The fetal period is often conveniently summarized by its resultant 'birthweight', an outcome parameter used to compare 'intra-uterine' effects on long- and short term complications (figure 1).

This thesis explores in detail how and why changes in obese pregnant women impact the development of the unborn child. Specifically it aims to elucidate the development of fetal body composition and vascular properties, with specific attention for the dynamic vascular changes which occur in the pregnant mother and which through the child's environment, impact the child. To provide insight into these processes and achieve the aims of this thesis requires extensive use of non-invasive techniques, like ultrasound, to examine both mother and her unborn child. It is therefore necessary to explain in more detail the background of this frequently used method and to provide a general background on the research domain of obesity. The introduction to this thesis will provide the reader with this background. The introduction will elaborate on how ultrasound has evolved (part 1) and on how obesity effects pregnancy (part 2). Subsequently we will discuss the maternal- and fetal vascular changes of the great arteries (part 3). We conclude the introduction with discussion of ultrasound limitations in the obese pregnant population (part 4).

The populations in chapter 5, 6, 8 and 9 are based on three clinical studies:

- **DALI** (vitamin D And Lifestyle Intervention) study to prevent GDM in the 'obese' (BMI ≥29kg/m^2) pregnant women. European, multicentre, randomized controlled trial. Three chronological sub-studies: pilot study, main (lifestyle) study and vitamin D-lifestyle study. FP7/2007–2013, 242187 (ISRCTN70595832). Local EC number: S52172. Pilot and lifestyle participants recruited in our centre, are investigated as sub-studies for this thesis. The vitamin D trial is currently ongoing.
- **MANOE** (MAternal Nutrition and Offspring's Epigenome). A prospective, monocentre, observational study. Relationship between intake of maternal methyl-group donors (methionine, food folate, choline, and betaine) during pregnancy and offspring DNA methylation. Local EC number: S54026. Currently ongoing.
- **VABULOS** (VAscular and Biometry Ultrasound measurements in LOw risk pregnancy Study). Prospective, observational cohort for maternal and fetal ultrasound measurements in low-risk pregnancies. Local EC number: S55325. Study completed.

The objective of this thesis was to investigate the effects of pregnancy dynamics (GWG and glucose intolerance) on the short term outcomes of infant body and cardiovascular development from the perspective of different prepregnancy BMI classes. These early

life developments impact the infants' start in life and it is thought that this start may potentially induce long-term metabolic effects.

The research conducted for this thesis is unique as it combines: very high quality ultrasonic sensing with prospective and longitudinal measurements performed with a substantial number of patients under highly controlled conditions. This allowed for a series of primary studies which advance the current state of knowledge on the dynamics of pregnancy and infant development. The thesis consists of the following chapters. Chapter one provides an overall overview and introduction of key history and concepts. Chapters two and three reveal general risk factors for adverse fetal growth and the role of fetal gender on separate intra-uterine development and (adverse) perinatal outcome. Chapter 4 highlights maternal influences on the timing of adverse fetal growth. Chapters 5 and 6 perform a fetal sequencing in order to find early predictors for neonatal adiposity. And chapters 7 to 9 discern with novel techniques interesting new information on the modulation of maternal and fetal great arteries during pregnancy.

OUTLINE AND AIMS OF THE DOCTORAL THESIS

Chapter 2. Review on the role and mechanisms of events during the antenatal, perinatal and postnatal period, resulting in later life diseases. Animal studies and clinical research.

Chapter 3. To develop fetal growth curves for a low risk Caucasian population according to WHO Multicentre Growth Reference Study Group recommendations. Further customize antenatal growth for fetal gender and compare immediate birth outcomes between boys and girls to align them with neonatal growth curves.

Chapter 4. Prospective cohort study to examine clustered gestational weight gain patterns in obese and non-obese population, related to fetal growth and fetal weight development.

Chapter 5. Prospective cohort study to determine distinctive fetal body composition features as a reflection of early fetal adiposity in normoglycemic mothers.

Chapter 6. Prospective cohort study to detect early fetal predictors for neonatal adiposity in normoglycemic mothers.

Chapter 7. Prospective cohort study to investigate the feasibility of vascular measurements (intima media thickness) at four pre-defined fetal and four pre-defined maternal arterial locations in order to determine vascular changes that could be associated with impaired vascular function.

Chapter 8. Prospective cohort study to detect modifications of the vascular properties in the maternal common carotid artery (CCA) with radio-frequency ultrasound in relation to maternal characteristics.

Chapter 9. Prospective cohort study to detect modifications in the fetal abdominal aorta (fAA) with radio-frequency ultrasound in relation to maternal characteristics and fetal growth and development.

Chapter 10. Summary and future perspectives

INTRODUCTION

Fetal growth: historical overview and growth charts

Historical overview

In the 1960's medical visualisation technology was introduced in practice to support diagnostics and therapy in pregnancy. The first fetal images were obtained as static Röntgen recordings, which resulted in snapshots of the intra-uterine life. Through these snapshots clinicians could gain insight onto the number of fetuses, the location of the baby and fetal skeleton abnormalities. As a supporting technique X-ray amniography was used. In this technique a water- and fat dissolvable contrast medium was injected into the amniotic sac to subsequently visualise the outlines of the skin and the fetus' bowels in Röntgen snapshots. Sir William Liley used the X-ray amniography to apply the first intra-uterine therapy, in 1963, a fetal blood transfusion for rhesus allo-immunisation. The first reports on the use of ultrasound instead of Rontgen for care during pregnancy appeared no earlier than the late sixties. The very first ultrasound used the A-mode technique in which only one-dimensional peaks were visible. The A-mode technique was quickly replaced by the superior B-mode technique. With B-mode ultrasound, two-dimensional images were obtained with some variation in contrast. In this way one could 'expose', for instance, the fetal head.[3]

Measuring fetal growth

Stuart Campbell, a British pioneer in obstetrical ultrasound, made the first steps towards a standardized fetal measurement. Campbell standardised the BiParietal Diameter (BPD): the head diameter of the fetus.[4] The 'BPD' was used to determine 'the age' (gestational age) of the fetus.[5] In addition to this first item, ultrasound was increasingly used to gain information on the fetal development in general.[6] The neural tube defect was one of the first prenatal diagnoses and the arrival of 'real-time' scanners in the late seventies, further increased the possibilities to assess fetal condition.[7] In the same period -and stimulated by improving imaging techniques-, growth charts were developed for femur length, abdominal- and head circumference. Finally, this has led to the guidelines of the International Society on Ultrasound in Obstetrics and Gynaecology (ISUOG), standardizing the fetal parameters, used for fetal growth and development.[8] Globally, ultrasound was only used in high risk pregnancies, but in Germany routine ultrasound became available for every pregnancy from the eighties onwards. This progress generated a lot more data on 'normal´ physiological fetal growth than before.

Growth charts and limitations

Routine ultrasounds in Germany initially also led to the release within short intervals of many different fetal curves. The diversity of reference curves was caused by the many differences in study population and methodology, that were used. The populations differed for example with respect to: ethnicity, fetal gender and biological and demographic determinants. Most importantly, methodological differences between the growth studies increased the risk of certain research biases. Examples of these differences include the use of cross-sectional or longitudinal design and the specific approach used to analyse and interpret the data. A review in 2012, found 83 growth curves, published between 1971-2008.[9] Only a few of the reviewed studies sufficiently controlled the inclusion of women, to widely exclude the influence of confounding factors. Underlying 'confounding' factors were not excluded or statistically corrected for in many of the studies, while it is demonstrated that they influence fetal growth. Examples of confounding factors were: maternal smoking, chronic maternal diseases (pre-existent hypertension), hypertension developed during pregnancy and congenital abnormalities or pre-term birth. Excluding these factors could be seen as an approach to establish a 'normal growth' trajectory and is regarded by some clinicians as a desired phenomenon since these curves would detect early growth deviation. However, this approach also presents a different type of problem as confounding factors may be associated with factors the study does not want to exclude. For instance, the prevalence of 'smoking' is associated with maternal age, -ethnicity and -parity. In an attempt to create a 'normal population' by excluding smokers, the examined population is now out of balance with respect to maternal age, ethnicity and parity. This is called *skewing* of the population, which leads to a highly sensitive, but less specific growth charts for abnormal fetal growth. Overall there is much scepticism on these 'supernormalised' study population. Therefore many current growth studies should be interpreted as studies with an observational character. These population based reference growth charts can show *how* a baby grows, but they are less equipped to predict how a baby *should* grow. Another limitation in the construction of growth curves is the lack of a clearly implemented protocol on pregnancy dating. A gestational age confirmation is essential in the development of both normative and descriptive growth curves.[9]

Developments in the use of growth charts

Developments in the area of growth monitoring and growth charts attempt to improve upon the shortcomings addressed above. So called 'customized growth charts' take account of influential factors such as ethnicity, smoking and maternal length.[10-12] These customised models of fetal growth should create the possibility for clinicians to obtain a better growth reference for a specific baby from specific parents with its specific features and background. It is based upon entering the required parameters in the software

which then produces a reference curve.[13] These curves have the opportunity to distinguish both sensitively, and specifically abnormal growth. In addition to customising growth charts with respect to parental or fetal characteristics, increased longitudinal measurements provide further possibilities for translation into postnatal growth curves. The development of longitudinal- instead of cross-sectional growth curves, provides the opportunity to discriminate a constitutionally small fetus from a real growth restricted fetus.[14] Statistically, the longitudinal growth curves are challenging to construct. In 2006, the World Health Organization (WHO), published their Multicentre Growth Reference Study Group results on the different statistical models for growth in children. They advised the GAMLSS (Generalized Additive Models for Location, Scale and Shape, www.gamlss.org) model for longitudinal growth studies and centile estimation.[15,16] It has already been applied by several study groups, one of which (Papageorghiou et al) has already published both postnatal and prenatal growth data.[17,18,19]

Third trimester scan

Regarding the longitudinal aspect of fetal growth, it should be noted that it is characterized by fast progressive growth, starting at 180 – 200 days (26 – 28 weeks) post menstrual age. Therefore fetal growth is not linear. This profile supports the rationale for a mid-third trimester (28-32 weeks) ultrasound scan. This scan could allow early detection of a high risk for growth restriction for a specific fetus. Furthermore, an ultrasound scan at this gestational age has the beneficial aspect that it provides information on the fetus' position (head-, breech- and transverse position), the location of the placenta and on the evolution of certain organ structures. Certain organ structures are susceptible for signs of abnormal development in the third trimester, such as the fetal heart, brain and kidneys. Despite these theoretical advantages, there doesn't seem to be much evidence in current literature for the implementation of a routine third trimester scan in the clinical care for the pregnant woman. A Cochrane review published in 2008 reports no improvements at all on perinatal outcome after an ultrasound carried out after 24 weeks pregnancy.[20] Therefore, in many countries there is no third trimester (growth) scan for low risk pregnancies within standard prenatal healthcare programs.[21,22] Until now, only one Norwegian study, using 20 year old data (but recently published), showed an improvement in the ability to detect aberrant growth by using a third trimester ultrasound.[23] This randomised prospective study compared an extra third trimester ultrasound scan with the existing routine of a second trimester ultrasound followed by a clinical diagnostic examination of the fundus-symphysis height in the third trimester. Detecting constitutional small growth (-2SD, -22%) had a better sensitivity of 80% (vs 46%) and a specificity of 87% vs 94% for the ultrasound group compared to the physical examination group. Detecting constitutional large growth (+2SD, +22%) had a sensitivity of 91% vs 36% and specificity of 70% vs 90% for ultrasound scans.When using third trimester ultrasounds

more anatomical disorders were found (especially from the urinary system), although this difference was not statistically significant.[23] Skrastad et al showed an improved detection of aberrant growth, but confirmed the Cochrane conclusion that no improvement was added by the third trimester scan with regard to the perinatal morbidity and mortality. In general, growth estimation (estimated fetal weight) by ultrasound has a variation of 6-12% of the actual fetal weight.[24]

In summary, ultrasound for fetal growth and development provides an estimate of the measured parameter. Currently there is an ISUOG guideline available, standardizing the separate fetal parameters, but no guidelines are available for the preference of a certain growth chart. Thus, there are mostly local charts available, which are geographically oriented, normative or descriptive, customized or population based and cross-sectional or longitudinal. Recently longitudinal growth curves are investigated with the WHO statistical model, the GAMLSS method. Finally, there is no evidence for the implementation of a routine third trimester scan in the absence of improved perinatal morbidity and mortality. On an individual basis, though, there seems to be some benefit in detecting fetal developmental abnormalities for heart, brain and kidney.

Maternal obesity and fetal growth & development

Obesity and Gestational Weight Gain (GWG)

In 2009 the Institute of Medicine (IOM) released new guidelines on weight gain during pregnancy.[25] The guidelines recommend lower and upper limits per BMI class and the BMI classes were synchronized with the cut-offs used in the WHO BMI classes (table 1). The IOM started in 1990 with weight gain recommendations per BMI class in order to prevent premature births and small for gestational age (SGA <2.5kg) children. There were signs that these adverse outcomes were associated with too low GWG. In 2009 the focus of the IOM shifted towards recommendations for the obese women and upper GWG limits related to worldwide rise of obesity. The IOM was concerned because of the association of crossing the upper limits of GWG in the obese women and the risks for macrosomia, caesarean section and postpartum weight retention.[25] Recently Bogaerts

Table 1. Recommended ranges of GWG per prepregnancy BMI, according to the IOM 2009 revised criteria.[25]

Prepregnancy BMI	Recommended GWG	Rates of GWG per week, 2nd & 3rd trimester, mean (range) kg/week
Underweight (<18.5kg/m^2)	12.5-18kg	0.51 (0.44-0.58)
Normal weight (18.5-24.9kg/m^2)	11.5-16kg	0.42 (0.35-0.50)
Overweight (25.0-29.9kg/m^2)	7-11.5kg	0.28 (0.23-0.33)
Obese (≥30kg/m^2)	5-9kg	0.22 (0.17-0.27)

et al. demonstrated a decreased adverse perinatal outcome for obese women who *lose* weight, without increasing the risks for low birth weight or SGA babies.[26] Around the same time, looking at obese and overweight women, Catalano et al. showed an increased incidence of SGA babies and decreased neonatal fat mass, when women had insufficient GWG according to the revised 2009 IOM guidelines.[27] In summary, the IOM guidelines on GWG for each BMI class are useful and easy to use in clinical studies and epidemiology, but are also constantly monitored and updated with new insights.

Influence of obesity on fetal growth

The determinants of fetal growth changes are traditionally categorized into three causality groups: pre-placental ('maternal'), placental and post-placental ('fetal'). Fetal abnormalities are regarded as post-placental causes and can be expressed in fetal growth restriction (chromosomal abnormality) or fetal overgrowth (fetal syndrome, e.g. Beckwith Wiedemann). A malformed placenta is often associated with early growth restriction and causes can be intrinsic (placental infarction or bleeding) or related to maternal characteristics (smoking, DM).

Maternal obesity is associated with both fetal overgrowth and fetal growth restriction. Therefore obesity shows a U-shaped fetal growth distribution, with an overrepresentation of obese mothers on both (extreme) growth sides. On the left side of the distribution we see a concentration of small fetal growth caused by underdevelopment of the placenta in obese mothers with bad metabolic function (hypertension or a unknown overt diabetes) and therefore resulting in bad blood vessel formation. On the right side of the U-shaped fetal growth distribution, obesity causes fetal overgrowth through an impaired glucose tolerance and higher insulin resistance.[28] Maternal obesity -and especially when associated with an android fat distribution ('central adiposity')- is regarded as a pre-diabetic state with an increased risk to develop insulin resistance and a reduced β-cell function in the maternal pancreas. This causes increased maternal blood sugar levels, which are transported trans-placental to the fetus. This in turn leads to increased β-cell activity within the fetal pancreas. The baby will produce more insulin and Insulin Growth Factors (IGF) and hereby increase its body volume. Research focused on IGF-1 and IGF-2 has found that IGF-2 is a very important prenatal growth stimulator for the fetus' organs in particular.[29] Animal studies again emphasize the regulatory function of the placental protein IGF-2, showing a central role for the protein in trans-placental transport of nutrients, resulting in fetal overgrowth.[30] These mechanisms explain why obesity is associated with an increased fetal growth, which manifests in the third trimester of pregnancy and results in the end in heavy (macrosomic) babies. Indeed, even when an official diagnosis of diabetes mellitus or gestational diabetes could not be made, a positive correlation between the glucose value in the mothers blood during third trimester and the birth weight can be observed.[31,32] In a study of 68 obese pregnant

women compared to 53 pregnant women with a normal BMI, a positive correlation was found between maternal insulin resistance and the neonatal fat mass. This relationship was based on the same principle of higher maternal blood-sugar levels, even though those women did not yet meet the (gestational) diabetes criteria.[32] Also, in the same study maternal BMI was correlated with neonatal insulin resistance as measured in umbilical cord-blood samples.[32]

Obesity and fetal-neonatal body composition

As mentioned above, the fetus' biggest weight gain is achieved during the second half of pregnancy caused by the growth of both lean- and fat mass. The fat mass increase is considered the main cause for the baby's growth acceleration in the third trimester. The increase of fat mass is strongly determined by intra-uterine nutrition, whereas the lean mass (muscle- and bone tissue) is more dependent on the parents' genetic potential.[33,34] The difference in the amount of fat deposition in the fetus and neonate is also reflected by the traditional classification for 'small-for-gestational age' (SGA <2.5kg), 'appropriate-for-gestational age' (AGA) and 'large-for-gestational age' (LGA >4.0kg) children.[35] The last definition is often referred to as 'macrosomia'. Although this classification is officially defined by birth weight, there is increasing awareness on the fact that the distribution of fat at birth contains more information about the preceding prenatal period and therefore also contains information about the risk profile for metabolic disorders of the neonate in the future.[36] Overall there is a difference in the body composition of a neonate born in normal glucose tolerant pregnant women and those babies born in mothers who were diagnosed with GDM. In this last group Catalano et al. found a significantly ($p = 0.002$) higher fat mass (436 ± 206g) in the neonates, when compared to newborns from mothers without GDM (362 ± 198g).[36] In obese/overweight women the neonates also showed an increased ($p = 0.003$) body fat mass (11.6 ± 4.7%, 420 ± 220g) at birth when compared to babies' from lean/normal BMI mother (9.7 ± 4.3%, 380 ± 170g).[37] The lean mass from the babies in this last study did not differ between the two BMI clusters. To assess neonatal body composition, different measurements methods are in use and guidelines for recommendation of a certain techniques are lacking. Also for children under the age of two years, there is no actual definition present of an aberrant body composition or of the 'neonatal obese infant' up until now. One of the suggestions in literature to define a 'baby BMI' is the Ponderal Index (PI). This is birth weight divided by height raised to the power of 3 (g/cm^3).[38] This measure could be used to discriminate more reliably after birth a tall proportioned baby and a smaller disproportioned baby of the same weight. The latter child being considered as 'adipose'. Body proportionality indexes such as birth weight/birth length and birth weight/birth $length^2$ have been proposed by the WHO multicentre growth study group, but they still have to prove their value as predictors of metabolic changes in later life.[17,39] Several studies have addressed

neonatal body composition by air displacement plethysmography (ADP), bio-electrical impedance analysis (BIA), anthropometry measurements or skinfold measurements.[34-40] Skinfolds measurements are practical and bed-side, but need training, and there have been concerns on the reproducibility. The reported coefficients of variation vary from: 2.6-6% for various skinfolds measurements in neonates.[31,34] A benefit of skinfold measurements, is that it informs on the distribution of neonatal fat (trunk or extremities), a quality that is not available in the alternatives (ADP or BIA). ADP/BIA give a general, but objective, fat- and lean-mass outcome and is therefore regarded to be the current normative for study purposes, but indeed no information is obtained on the location of the fat depositions.[40]

Fetal body composition and adiposity

Evaluation of the nutritional state *in utero*, by estimating fetal body composition, might improve the prediction of specific perinatal risks in (i.e. 'neonatal adiposity') conditions of under- and overgrowth of the fetus.[28,41] Anthropometric ultrasound measurements have shown a unique exponential pattern of the growth profile during the second half of gestation, both in lean- and in fat mass in the normal fetus. This suggests that the measurement of fetal fat could provide a more sensitive and specific marker of abnormal fetal growth (figure 2).[41,42] Larciprete et al. demonstrated this growth pattern with prenatal

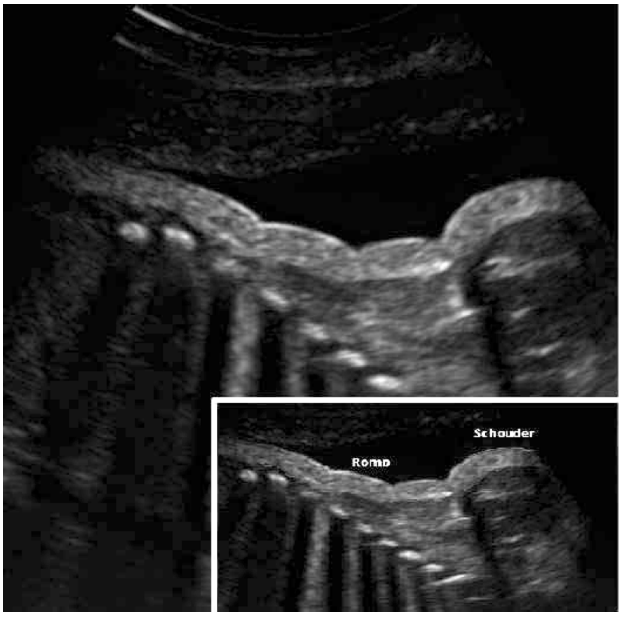

Figure 2. Typical presentation of a macrosomic fetus on 32 weeks of gestational age. There is clearly subcutaneous fat deposition visible around the shoulders ('schouder') and trunk ('romp'). Finally a healthy son was born of >4.0kg at 39 weeks. (Galjaard-Devlieger, thesis, 2015).

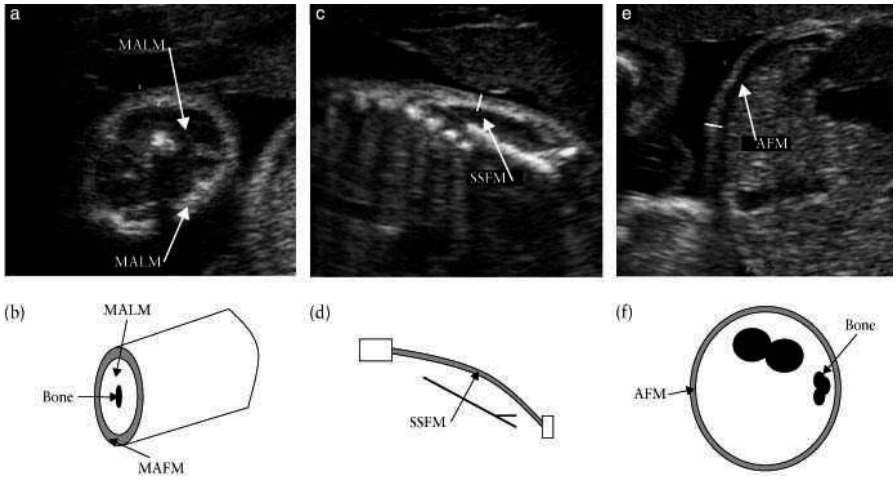

Figure 3. Fetal subcutaneous tissue thickness. Ultrasound images (a, c, e) and schematic representations (b, d, f) showing how the different subcutaneous tissue thickness measurements are obtained. (a,b) Axial view of the fetal arm showing mid-arm fat mass (MAFM) and mid-arm lean mass (MALM) evaluation; the process is similar for mid-thigh fat and lean mass. (c,d) Evaluation of the subscapular fat mass (SSFM) measurement. (e,f) Evaluation of the abdominal fat mass (AFM). (Reproduced with permission of the author and publisher, Larciprete et al., 2003)[42]

ultrasound measures of fetal body composition with skinfold thickness and by analysing the humerus (upper arm) and femur (upper leg) extremities (figure 3).[42] In addition to these fat measurements, specific 'metabolic' organs can be examined like the fetal liver.[43] Partially correlated to the fetal liver size, is the fetal abdominal circumference (AC), which appears to be a good predictor for the neonatal PI, in the third trimester.[44] First cautious steps are taken to include fetal fat measures into antenatal weight estimates although the number of cases included in this study is relatively small.[45,46] It has to be mentioned, though, that these measurements are still controversial and strongly dependent on the examiners' expertise, which is why they are not yet part of routine clinical care.[41] Better detection of the high risk fetus may help to break the vicious circle in which prenatal abnormal development occurs under influence of maternal environmental factors, with the aim to prevent neonatal and eventually adult metabolic diseases.[1,2]

Maternal and fetal vascular development

The evaluation of fetal body composition and adiposity *in utero*, might improve the prediction of the intra-uterine development as a risk factor for cardiovascular disease.[28] Low birth weight or intra-uterine growth restriction (IUGR) is associated with cardiovascular disease (CVD), non-insulin-dependent diabetes and hypertension in later life.[47] Some authors have suggested that fetal undernourishment causes adaptations that involve cardiovascular structures and may even cause early vessel abnormalities. This has been

called the 'fetal origin' theory where adults, who were small at birth, show higher blood pressure and increased risk of cardiovascular diseases.[48,49] In addition, although studies are still limited, there is data showing that being born large for gestational age (LGA) is associated with an increased aortic intima media thickness in childhood.[50-52] Hemodynamic changes in pregnancy have been well described, but vascular research has focused mainly on hyperdynamic changes (preeclampsia) in the mother.[53,54] Information on wall thickness (intima-media thickness, IMT) is available, but data on stiffness changes in the great arteries is scarce.[55,56] Vascular changes have also been demonstrated in newborns with intra-uterine growth restriction through measurement of the abdominal aortic IMT, which is a sensitive marker for the development of atherosclerosis in later life.[57-59] Histology of the abdominal aorta wall of a stillborn infant with prenatally increased IMT, revealed inflammation of the thickened intima layer.[60] Post-mortem studies performed on humans in early adulthood (15-34 years) showed the abdominal aorta to be the first site involved in the process of atherosclerosis[61] However few studies have measured IMT in the fetus during pregnancy, and up to now only a single technique has been used to investigate the IMT in the fetus.[62-64] More functional vascular properties (distension, stiffness and elasticity) have not yet been investigated in the fetus.

In mothers there is inconsistency on whether IMT is increased in the Common Carotid Artery (CCA) during pregnancy.[65,66] A gestational age-dependent factor has been proposed.[66,67] Recently pre-eclamptic women were found to have increased IMT measurements of the CCA in late third trimester pregnancy compared to normotensive women (459±95μm vs. 351±85μm).[68] A difference remained between the two groups after 18 months. This was the first report of the maternal CCA with high resolution ultrasound, based on Radio Frequency (RF ultrasound). No longitudinal study until now has reported on fetal or the maternal great arteries for functional vascular properties (elasticity and stiffness) during pregnancy with RF ultrasound.

Maternal obesity and visualisation

It can be challenging to achieve a high quality ultrasound result for fetal growth and development when examining an overweight or obese pregnant woman. This is mainly caused by the additional fat tissue located between the ultrasound probe and the fetus.[69] The end result is practically always inferior when compared to an ultrasound conducted on a pregnant woman with normal BMI. The fat tissue absorbs and scatters the ultrasound waves, causing a negative correlation between the distance of the ultrasound probe to the fetus and the image quality. Not even the extensive technical improvements of the ultrasound equipment achieved in these last decennia have been able to undo these limitations. In clinical practice when mothers are obese, repeated sonograms are often needed before a somewhat satisfactory imaging result can be obtained.[70] Furthermore, obese pregnant women have a higher incidence of fetal

Table 2. Risk of congenital disorders during pregnancy for mothers with obesity compared to normal weight. (From: Stothard et al., 2009)[71]

Malformation	OR (95% CI)	P- Value
Neural tube defects	1.87 (1.62-2.15)	0.001
Cardiac defects	1.30 (1.12-1.51)	0.001
Anal atresia	1.48 (1.12-1.97)	0.006
Limb disorders (-reduction)	1.34 (1.03-1.73)	0.03

malformations. These include mainly neural tube defects ('spina bifida'), heart defects, anal atresia and limb disorders (Table 2).[71] The combination of increased suspicion of malformations and a poorer imaging, requires particular attention for the clinical caretakers. Uhden et al. reported that fetal heart defects are more common with overweight and obese women (relative risk = 2.04), while the percentage qualitatively *in*complete studies increased from 6.4% in normal weight women to 17.4% in obese women, at the 20 weeks 'morphology' ultrasound scan.[72] Hendler et. al scored the percentage of 'insufficient / poor visualisation' at the 20 weeks ultrasound scan in the obese women. They showed that the percentage of poor visualisations in obese women was 37% for cardiac structures and 43% for cranio-spinal structures, compared to 19% and 30% respectively in normal weight women. A repetition of the ultrasound examination two weeks later reduced the proportion of insufficient visualisation of the fetal heart down to 20% in the obese women.[73] It still demonstrates an inadequate ultrasound examination of the fetal heart in 1 out of 5 obese pregnant women. The FaSTER trial, an ultrasound study on the identification of Down syndrome, reported that the chance of detecting general fetal abnormalities decreased by 30% under the influence of maternal obesity (OR van 0.7; 95%CI 0.6-0.9; p = 0.001).[74]

The limited visualisation and the necessity to repeat the sonographic study in obesity is not only stressful for the women, but also forces extra pressure on the organisation and economics of the prenatal care system.

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ABDOMINAL AORTA FETAL ABDOMINAL
INTIMA MEDIA THICKNESS FETAL FAT
FUNCTIONAL LINEAR DISCRIMINANT
ADDITIVE MODEL LOCATION, SCALE AND
MELLITUS NON-EXCESSIVE GESTATIONAL
GROWTH FACTOR INTIMA-MEDIA THICKNESS
SOCIETY ON OBSTETRICS AND
GROWTH LARGE-FOR-GESTA
NUTRITION EPIGENOME
GROWTH STUDY GROUP
IMAGING MRI FATTY LIVER DISEASE
GLUCOSE TOLERANCE TEST OGTT ODDS RATIO
ARTERIAL STIFFNESS QUALITY
BLOOD PRESSURE SKINFOLD TRICEPS
FAT THICKNESS SMALL-FOR-GESTATIONAL AGE
VASCULAR AND BIOMETRY ULTRASOUND
STUDY VABULOS YOUNG ELASTIC MODULUS

Chapter 2

Fetal growth and developmental programming, Review

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ABSTRACT

The environment in utero and in early neonatal life may induce a permanent response in the fetus and the newborn, leading to enhanced susceptibility to later diseases. This review concentrates on the role and mechanisms of events during the antenatal and immediate postnatal period resulting in later life diseases, concentrating on abnormal growth patterns of the fetus.

Fetal overgrowth is related to exposure to a diabetic intra uterine environment, increasing the vulnerability to transgenerational obesity and hence an increased sensitivity to more diabetic mothers. This effect has been supported by animal data.

Fetal growth restriction is more complex with a role of malnutrition in utero and catch up growth due to a high caloric intake and low physical activity in later life. Metabolic changes and a transgenerational effect of intra uterine malnutrition have been supported by animal data.

In recent years the discovery of alterations of the genome due to different influences during embryonic life, called epigenetics, has led to the phenomenon of fetal programming resulting in changing transgenerational metabolic effects.

INTRODUCTION

It is known that environmental factors influence health and the development of diseases. Certainly the environment in utero and in early neonatal life may induce a permanent response in the fetus and the newborn, leading to enhanced susceptibility to later diseases. These effects can be transgenerational and are probably due to an epigenetic transmission mediated by the mother (Aerts and Van Assche, 2006; Poston, 2010).

Abnormal environmental factors during pregnancy may lead to increased fetal growth, due to increased transplacental nutrients or may lead to fetal growth restriction, due to decreased transplacental transfer. Both extremes of fetal growth can induce developmental programming through epigenetic adaptations.

The present review will concentrate on the role and mechanisms of events during the antenatal and immediate postnatal period resulting in diseases in later life, concentrating on abnormal growth patterns in the fetus.

FETAL ENVIRONMENT AND INCREASED FETAL GROWTH

Human data

Even before the discovery of insulin, Dubreuil and Anderodias (1920) described giant islets of Langerhans in the pancreas of a newborn of a diabetic mother. Pedersen proposed that increased transplacental transfer of glucose leads to increased insulin secretion by the fetus, inducing macrosomia (Pedersen et al, 1954). It was confirmed that fetal hyperinsulinism was due to B cell hyperplasia and the hypothesis was put forward that hyperactivity of the fetal B cells may result in reduced secretory capacity in later life. Furthermore, an intact hypothalamic structure was needed to induce B cell hyperplasia, predicting the role of the hypothalamus in perinatal programming (Van Assche and Gepts, 1971).

Dörner was among the first to provide evidence that exposure to a diabetic intra-uterine environment increases the risk of developing diabetes in the offspring. Moreover he showed a transgenerational effect and the importance of good metabolic control in the prevention of long-term consequences (Dörner et al, 1973, 1984). These observations were confirmed by several epidemiologic studies and recently summarized: the excess of maternal transmission of diabetes is consistent with an epigenetic effect of hyperglycaemia in pregnancy acting in addition to genetic factors that induce diabetes in next generations. Individuals with genetic susceptibilities may be the most vulnerable to the in utero influences of maternal diabetes (Mclean et al, 2006; Poston, 2010).

Offspring of diabetic mothers also have an increased risk for obesity related to the degree of fetal hyperinsulinism (Silverman et al, 1991). Furthermore impaired glucose

tolerance and obesity in adults due to a diabetic intra-uterine environment is independent of the genetic background (Clausen et al, 2008; Dabelea et al, 2000; Plagemann et al, 1997).

Maternal obesity has gained an epidemic proportion in Europe and the developed world (EU document A6-450/2006). Maternal obesity and increased weight gain during pregnancy are associated with increased birth weight independent of genetic factors. It is also evident that maternal obesity induces higher transplacental transfer of nutrients resulting in fetal hyperinsulinism, increased fetal growth and adiposity (Ludwig and Currie, 2010). Maternal obesity therefore has a transgenerational effect inducing obesity and diabetes (Dabelea and Crume, 2011).

Animal data

Animal models of diabetes and obesity during pregnancy may unravel the specific effects of an exposure to an abnormal intra-uterine environment independent of inherited trails. In the rat, mild diabetes during pregnancy can be induced with a low dose of streptozotocin, stimulating the insulin producing B cell, hyperinsulinism and increased weight of the fetus. (Over-) Stimulation of the B cells in utero leads to a reduced insulin secretion during later life in conditions of increased demands such as obesity and pregnancy (Aerts and Van Assche, 1977; Devlieger et al, 2008). In experimental induced gestational diabetes a transgenerational effect was demonstrated (Aerts and Van Assche, 1979). Moreover, normalization of the maternal glycaemia by islet transplantation prevents deleterious effects for the fetus and offspring (Aerts and Van Assche, 1992). It seems clear that (over-) stimulation of the fetal B cell reduces the function of this cell in the offspring (Aerts and Van Assche, 2006).

However it may be possible that fetal hyperinsulinism itself may induce malprogramming (Plagemann et al, 1998b). Indeed another pathophysiologic mechanism may be related to the hypothalamic control of food intake, body weight and glucose metabolism (Plagemann et al, 1998a). The ventromedial hypothalamic nucleus (VMN), the lateral hypothalamic area (LHA) and the arcuate hypothalamic nucleus (ARC) of the medio-basal hypothalamus play an important role in this context. Hyperinsulinism in the fetus of pregnant rats with diabetes is associated with increased insulin content in the hypothalamus leading to an impaired organization of the VMN, the antagonistic LHA being unaltered (Plagemann et al, 1998b). Hypotrophy and hypoplasia of the VMN may result in a diminished function of the satiety center, together with a relative overactivity of the antagonistic LHA, stimulating appetite and weight gain (Plagemann et al, 2009). Neuropeptides in the ARC play an important role in the regulation of food intake, body weight and metabolism. In particular neuropeptide Y (NPY) is the most potent orexigenic neuropeptide and is regulated via the circulating satiety factors leptin and insulin, both suppressing NPY expression (Plagemann et al 1998b). Perinatal hyperinsulinism

induces resistance of the NPYergic system against the regulatory signals leptin and insulin, leading to hyperphagia and overweight in adult offspring (Plagemann and Harder, 2009). Islet transplantation in induced maternal diabetes prevents the effects in VMN and ARC (Franke et al, 2005; Harder et al, 2001). Furthermore malprogramming of the hypothalamic structures is also operational in the diabetic lactating period, suggesting the importance of the milk composition (Fahrenkrog et al, 2004; Plagemann et al, 2002).

Recently, several experimental models in rat and mice of diet induced obesity have been reported inducing fetal hyperinsulinism and increased body weight, leading to glucose intolerance, insulin resistance, obesity, hyperphagia and hypertension in the offspring (Holemans et al, 2004; Nivoit et al, 2009; Samuelsson et al, 2008;). These effects are transgenerational (Holemans et al, 2004). It is clear that diet induced obesity during pregnancy in animals show comparable changes in the fetus and the offspring as seen in experimental diabetes. Fetal hyperinsulinism is the result of B cell (over-) stimulation and induces B cell dysfunction in later life. Hyperleptinaemia in the early neonatal period induces overweight, hyperphagia, diabetic tendency and cardiovascular problems in later life. Hypothalamic alterations, induced by fetal hyperinsulinism are associated with neonatal leptin resistance with long term consequences (Plagemann and Harder, 2009). The important role of induced leptin resistance is confirmed in maternal obesity: young offspring of obese dams are leptin resistant, and remain leptin resistant and hyperphagic when adult (Kirk et al, 2009).

Insulin is also known as a potent mitogenic factor, which is another reason to decrease fetal hyperinsulinism to lower the risk for cancer in later life (Van Assche et al, 2010).

FETAL ENVIRONMENT AND INTRA-UTERINE GROWTH RESTRICTION

Human data

The fetus with intra-uterine growth restriction shows a reduced amount of B cells related to low insulin and glucose levels (Van Assche et al, 2001). Studies in England and Wales, demonstrating the association between low birth weight and adult impairment of glucose tolerance, obesity, type 2 diabetes and cardiovascular diseases have led to the Barker hypothesis of fetal origin of adult diseases (Barker and Osmond, 1986; Barker et al, 1993). Consequent population studies have confirmed the relation between low birth weight and adult diseases (Leon et al, 1996). The most vulnerable period in pregnancy is shown in studies of the maternal malnutrition during the Dutch hunger winter in 1944. This malnutrition resulted in a higher incidence of impaired glucose tolerance, type 2 diabetes and cardiovascular risks in the offspring especially when deprivation occurred in the third trimester (Ravelli et al, 1998).

The consequences in later life are also determined by caloric intake. A population subjected to poor nutrition in the perinatal period can keep a normal glucose tolerance as long as they remain on a low caloric diet. A change to a higher caloric intake and low physical activity increases the incidence of impaired glucose tolerance and type 2 diabetes (Aerts and Van Assche, 2006). In this respect, a high caloric intake in the postnatal period inducing catch up growth will aggravate the consequences in later life (Gluckman et al, 2008; Hales and Barker, 1992). Breastfeeding may prevent accelerated caloric intake in the postnatal period (Rodekamp et al, 2009).

It is accepted that the underdevelopment of the fetal B cells may lead to reduced B cell function in later life, certainly in periods of increased demands. Leptin may also play an important role. High caloric intake in the newborn after a period of fetal growth restriction may induce a leptin charge. Hyperleptinaemia in the early neonatal period induces hyperphagia, overweight, diabetic tendency and cardiovascular problems in later life (Plagemann and Harder, 2009), certainly in situations of previous fetal undernutrition (Vickers et al, 2008).

Animal data

Several animal models mainly in the rat have studied the impact of reduced fetal nutrition on long term consequences. Global food restriction by semi starvation or by unilateral uterine artery ligation during pregnancy results in underdevelopment of the fetal B cells, low insulin levels and intra-uterine growth restriction (De Prins and Van Assche, 1982; Garofano et al, 1998). In adult rats, insulin resistance and subtle changes in vascular function are found (Holemans et al, 1996, 1999). However hypertension did *not* seem to be a consequence (Holemans et al, 1999; Simmons et al, 2001).

An isocaloric, but low protein diet has been frequently used for the induction of intra-uterine growth restriction. A reduced B cell mass and reduced insulin secretion in the fetus was correlated with the occurrence of gestational diabetes in the offspring (Dahri et al, 1991; Dahri et al, 1995). Offspring from pregnancies with low protein diet *do* seem to have an increased blood pressure (Langley and Jackson, 1994). Here, also the deficiency in the total islet mass and specifically in the B cell mass during fetal life, results in a reduced adaptation in adult life leading to gestational diabetes with macrosomic fetuses, indicating a transgenerational effect (Blondeau et al, 1999; Boloker et al, 2002).

Intra-uterine growth restriction as such, does not program the hypothalamus towards increased appetite and obesity. However, high caloric intake and increased leptin concentration in the neonatal period may induce hyperphagia and obesity in later life (Plagemann and Harder, 2009).

EPIGENETIC ADAPTATIONS

Exposure to environmental changes in utero has been investigated as epigenetic events resulting in transgenerational effects, such as macrosomia, obesity and diabetes. The alterations on the genome, without changes of the DNA are characterized as epigenetics. This is a slow process of changing methyl groups on cytosine bases in DNA by methyltransferases. Modifications are identified as de-methylation, histone deacetylation and increased histone acetylation, independent from replication (Feng et al 2010). Therefore, DNA methylation appears to occur before cellular differentiation, as no de novo methyltransferases are present after cellular differentiation. This suggests a role for environmental influence, such as excessive nutrition, nutritional restriction or low folic acid and vitamin B12 levels, on DNA methylation in early life development (Hanson and Gluckman, 2008; McLean et al, 2006).

Especially during periconception and embryonic life the rate of DNA synthesis is high and therefore susceptible for alterations in DNA methylation, leading to metabolic imprinting for leptine, SOCS3 and glucose transporter mechanisms for energy homeostasis (Dabelea and Crume, 2011). Whether these changes are irreversible is not yet clear, although animal studies on developmental programming indicate that an obese phenotype is not transmitted to their offspring by mating, but merely by uterine environmental influence. This stresses the importance of embryonic environment on epigenetic imprinting for metabolic mechanisms on the future generations (Bouchard et al, 2010; Dabelea and Crume, 2011; Waterland et al, 2007).

CONCLUSIONS

It seems clear that events in utero induce a response in the fetus and newborn, leading to enhanced susceptibility for later diseases.

In maternal diabetes and in maternal obesity increased transplacental nutrient supply to the fetus induces B cell hyperactivity, hyperinsulinism and increased fetal growth. Fetal hyperinsulinism is responsible for changes in the hypothalamus resulting in malprogramming of food intake, body weight and glucose metabolism. Insulin is also known as a potent mitogenic factor. From the clinical point of view it is therefore evident that in maternal diabetes and in maternal obesity fetal hyperinsulinism should be avoided. Early detection of gestational diabetes and strict metabolic control are essential. Obesity needs to be prevented preferably at adolescence and certainly before pregnancy.

Intra-uterine growth restriction is associated with a reduced B cell mass and low insulin secretion, the underdeveloped fetal B cell is responsible for a reduced B cell activity in later life. Neuroendocrine mechanisms, such as leptin charge, are involved through

malprogramming leading to excessive catch up growth. Therefore, efforts should be done to prevent intra-uterine growth restriction by early detection and management of hypertensive disorders in pregnancy and by optimal nutrition of the pregnant women.

Not every overweight newborn and not every newborn with intra-uterine growth restriction will develop problems in later life. It depends on bad or good adaptation and to the plasticity of his adaptation later on (Gluckman et al, 2008). This is called epigenetics, which means that by different environmental influences, alterations are made during early intra-uterine life on gene expressions, leading to aberrant metabolic phenotypes, like obesity. More profound understanding of the effect of fetal programming and the transference to future generations will be possible with further studies exploring epigenetic mechanisms. (Waterland et al, 2007).

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ABDOMINAL AORTA FETAL ABDOMINAL
INTIMA MEDIA THICKNESS FETAL FAT
FUNCTIONAL LINEAR DISCRIMINANT
ADDITIVE MODEL LOCATION, SCALE AND
MELLITUS NON-EXCESSIVE GESTATIONAL
GROWTH FACTOR INTIMA-MEDIA THICKNESS
SOCIETY ON OBSTETRICS AND
GROWTH LARGE-FOR-GESTA
NUTRITION EPIGENOME
GROWTH STUDY GROUP
IMAGING MRI FATTY LIVER DISEASE
GLUCOSE TOLERANCE TEST OGTT ODDS RATIO
ARTERIAL STIFFNESS QUALITY
BLOOD PRESSURE SKINFOLD TRICEPS
FAT THICKNESS SMALL-FOR-GESTATIONAL AGE
VASCULAR AND BIOMETRY ULTRASOUND
STUDY VABULOS YOUNG ELASTIC MODULUS

Chapter 3

Sex differences in fetal growth and immediate birth outcomes in a low-risk Caucasian population

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Submitted

ABSTRACT

Importance: Gender is recognized as a determining factor for differences in outcome in adult medicine. Little information is available on fetal gender influence in the prenatal period, when development is fastest.

Objective: To develop gender specific fetal growth curves for a low risk population according to WHO Multicentre Growth Reference Study Group recommendations and to compare immediate birth outcomes between boys and girls.

Design: Population based retrospective longitudinal cohort study.

Setting: Academic teaching hospital Leuven, Belgium

Participants: First-, second-, and third trimester fetal ultrasound examinations conducted `2002-2012. The data was selected using the following criteria: routine examinations in uncomplicated singleton pregnancies, Caucasian ethnicity, confirmation of gestational age by a crown rump length (CRL) measurement in the first trimester. Longitudinal examinations were only considered if containing all four routine fetal measurements: Biparietal Diameter (BPD), Head Circumference (HC), Abdominal Circumference (AC) and Femur Length (FL). GAMLSS (Generalized Additive Model Location, Scale and Shape) was used to align the timeframes of measurements.

Main outcome measures: Gender specific longitudinal growth curves for the four fetal measurements BPD, HC, AC, FL from 12 to 40 weeks for boys, girls and the total group; estimated fetal weight compared to birth weight; immediate birth outcomes for boys and girls.

Results: A total of 27680 complete scans were selected from the astraia© ultrasound database representing 12368 pregnancies. Fetal growth curves for BPD, HC, AC and FL were derived. The HC and BPD were significantly larger in boys compared to girls from 20 weeks' gestation onwards ($p<0.001$) equating to a 3 day difference at 20-24 weeks. Boys were significantly heavier, longer and had greater head circumference than girls ($p<0.001$) at birth. The Apgar score at one minute ($p=0.01$) and arterial cord pH ($p<0.001$) were lower in boys.

Conclusions: These longitudinal fetal growth curves for the first time allow integration with neonatal and paediatric WHO gender specific growth curves. Immediate birth outcomes are worse in boys than girls, and gender differences in intra uterine growth is sufficiently distinct to have a clinically important effect on fetal weight estimation and second trimester dating. Gender differences might play a role in obstetric or immediate neonatal viability management.

INTRODUCTION

Ultrasound has been an indispensable tool for diagnosis in obstetrics and fetal growth assessment for at least 4 decades.[1-3] Clinical management in pregnancies is increasingly based on ultrasound measurements derived in the first trimester and on the recognition of pathological fetal growth, which depends on reliable, standardized growth curves.[4] Although it is widely known that boys are slightly larger than girls in the first trimester and at birth, there has been no consideration of fetal gender in the development and interpretation of fetal growth curves.[5-8] This gender dichotomy seems important since there is clear evidence that gestation specific neonatal outcomes are worse in boys, indicating the vulnerability of the male embryo and fetus. [9,10]

Many charts have been published on fetal growth using different methodologies from the early 1990s until early in this decade, after which new (dating-) protocols emerged.[11] Most normal ranges were designed from cross sectional data,[12-19] which by their nature may represent fetal size at a given point but do not directly infer growth. To derive information on fetal growth, statistical strategies using repeat measurements are required but longitudinal methodologies are utilized more rarely.[20,21] Given these complexities the World Health Organization (WHO) Multicentre Growth Reference Study Group (MGRS) recommended GAMLSS (Generalized Additive Model Location, Scale and Shape) for the construction of the WHO Growth Standards.[22,23] Most recently growth charts have been developed in regions of Europe and the United States and customization based on ethnicity reported.[11,12,18,19,24]

Our aim was to develop gender specific longitudinal first-, second-, and third trimester normal growth reference curves within a low-risk Caucasian population with a robust WHO endorsed longitudinal statistical methodology. Further, we aimed to test the validity of these curves by comparing the estimated fetal weights derived from these charts to actual birth weight, and determine whether there was a gender specific difference in immediate birth outcome.

METHODS

This was a retrospective longitudinal cohort study of first-, second-, and third trimester fetal biometry ultrasound examinations performed during the period 2002-2012 in the University Hospital Leuven. The study was approved by the ethics committee of the University Hospitals KU Leuven. The data was selected from the astraia© ultrasound database with all four routine biometry measurements (BPD, HC, AC and FL) present, with the following criteria (figure 1): indication 'routine fetal growth' (level 1 and 2 excluded),

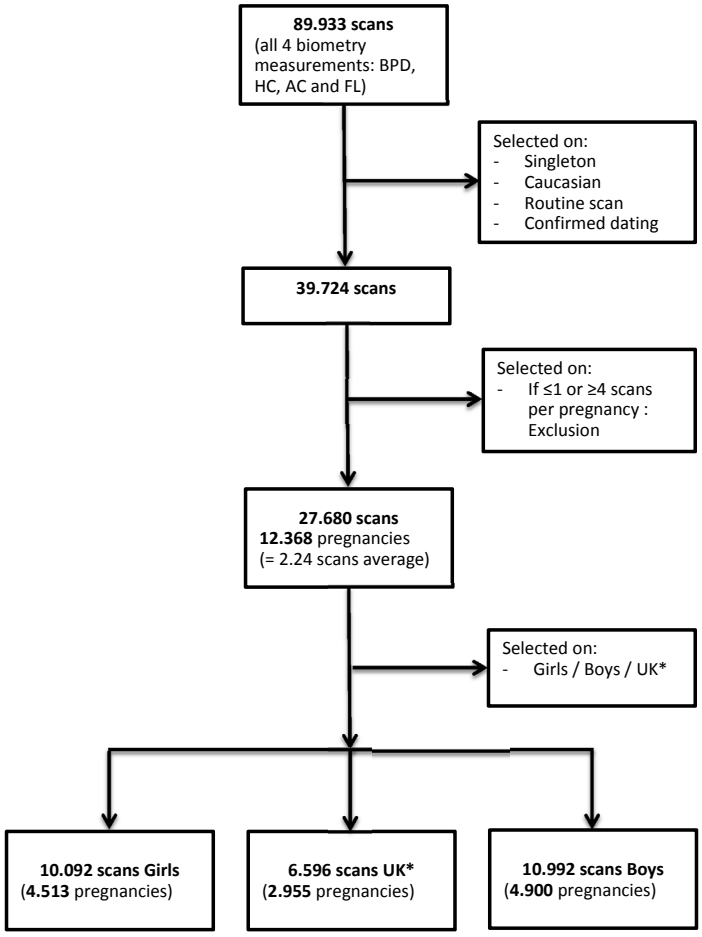

Figure 1. Flowchart on selection procedure for normal routine fetal ultrasound scans between 2002-2012. *UK = Unknown gender.

singleton pregnancy, ethnicity 'Caucasian' and gestational age confirmed by a crown rump length (CRL) measurement (3-83mm) in the first trimester.[25] Only pregnancies with at least 2 or maximum 3 scans (first-, second-, and third trimester) were selected, representing a routine of care scheme for a low risk-population. The measurements were performed with the following ultrasound machines (with time period of usage): Kretz Voluson 730 (2002-2006), ESAOTE Technos (2002-2006), Acuson Sequoia (2002-2007), General Electric Voluson® 730 Expert (GE healthcare Medical systems, Kretztechnik, Zipf, Austria 2007-2012), General Electric Voluson E8 (GE healthcare Medical systems, Kretztechnik, Zipf, Austria 2007-2012). The first three devices were equipped with a 4-8 MHz curved linear array probe. The GE Voluson E730 and GE Voluson E8 used a curved

4-8 MHz volumetric 3D abdominal probe. All growth data were immediately stored in an electronic database (astraia© Software Inc., Munich, Germany). Fetal measurements were based on the following two-dimensional biometric parameters: Biparietal Diameter (BPD), Head Circumference (HC), Abdominal Circumference (AC) and Femur Length (FL), as designated in the guidelines descriptions (Supplement, e-text 3.1).[26] Only complete fetal data-sets (all 4 measurements) were analyzed. Neonatal data from the included patients were extracted from their birth-files for gestational age at delivery, gender, birth-weight, -length, head-circumference, Apgar scores (AS) for 1st and 5th minute after birth, umbilical cord arterial pH and base excess (BE) measurement. Only gender specific neonatal data-sets were analyzed.

Statistical analysis

Outliers in BPD, HC, AC or FL were removed from the data. GAMLSS (Generalized Additive Models for Location, Scale and Shape, www.gamlss.org) was applied to construct the growth curves for all four fetal routine fetal biometry measurements: BPD, HC, AC and FL, by use of the R-package GAMLSS.[22,23] We assessed several distributions: Box-Cox-t, Box-Cox Cole and Green, and Box-Cox Power Exponential. Goodness-of-fit of the models was assessed with QQ plots, AIC (Akaike Information Criterium) and worm plots. The goodness-of-fit was investigated covering the gestational age 12-40 weeks period, and for substrata of this period. GAMLSS smoothed the ante-natal growth curves for BPD, HC, AC, FL and EFW. For the EFW, the Hadlock-3 formula [Log_{10} EFW = 1.3350.0034(AC) (FL) + 0.0316(BPD) + 0.0457(AC) + 0.1623(FL)] was used.[11] The 5th, 10th, 50th, 90th and 95th percentile were plotted with grid lines. The whole analysis was done three times: for all pregnancies, for boys and for girls. SAS 9.4 was used for merging the fetal database with the neonatal database, and analyzing the neonatal data.

RESULTS

Between 2002 and 2012 89933 scans were selected. After restricting to a low-risk population a total of 27680 scans remained representing 12368 pregnancies (figure 1). The mean maternal BMI was 23.8 kg/m^2 (std. 4.8), 7% of the women smoked. Gender specific birth data-sets could be ascertained in 76.1% of the cases and are outlined in table 1. In total we had 4900 boys and 4513 girls, representing respectively 10992 and 10092 scans. The mean birth-weight, -length and head-circumference was significantly ($p<0.001$) different for boys (3450g, 50.9cm, 34.9cm) as compared to girls (3329g, 50.1cm, 34.3cm). A low one minute AS (≤5) was more common in boys (3.8%) as compared to girls (2.9%) ($p=0.01$) as was a low five minute AS (≤7) for boys (3.2%) compared to girls (2.3%, table 1) ($p=0.009$). The arterial umbilical cord pH was lower in boys compared to girls ($p<0.001$).

Table 1. Neonatal data for boys, girls and combined in term and preterm pregnancies.

	Total (N= 9413)	Boys (N= 4900, 52.1%)	Girls (N= 4513, 47.9%)	P-value*
GA at birth (wks,0/7d), mean ± std	39w2/7 ± 1w5d	39w2/7 ± 1w5d	39w3/7 ± 1w5d	0.02
Birth weight (g), mean ± std	3392 ± 512	3450 ± 515	3329 ± 502	<0.001
Length (cm), mean ± std	50.5 ± 2.4	50.9 ± 2.4	50.1 ± 2.4	<0.001
HC (cm), mean ± std	34.6 ± 1.6	34.9 ± 1.6	34.3 ± 1.5	<0.001
1 minute AS ≤5	3.4%	3.8%	2.9%	0.01
5 minute AS ≤7	2.8%	3.2%	2.3%	0.009
pH Umb Art, mean ± std	7.27 ± 0.07	7.269 ± 0.072	7.274 ± 0.075	<0.001
pH < 7.10 **mean ± std**	1.9% (177) 7.04 ± 0.06	0.9% (82) 7.041 ± 0.059	1.0% (95) 7.042 ± 0.062	0.90
BE <-10 mEq/L **mean ± std**	1.4% (132) -13.46 ± 4.01	0.8% (71) -13.8 ± 4.2	0.6% (61) -13.1 ± 3.7	0.30

Neonatal demographic data available in 9413 (76%) of the selected cases '2002-2012. GA = gestational age; wks = weeks; d = days; g = gram; cm = centimeter; HC = Head Circumference; AS = Apgar Scores; pH = pondus Hydrogenium; Umb Art = Umbilical artery; BE = Base Excess; Std. = Standard Deviation. * P-value represents difference Boys vs Girls.

There was no difference in asphyxia, defined as a pH<7.10, in boys (0.9%) compared to girls (1.0%, p = 0.90) and abnormal BE (<-10mEq/L) was the same for both sexes. There was no difference in preterm birth (<37 weeks) for girls (5.7%) and boys (6.5%, table 2) (p=0.14) which occurred in 6% of the pregnancies overall. In the pre-term group boys were heavier (P=0.003), longer (p=0.005) and had larger head-circumferences (p=0.006). The immediate outcome of AS and pH were also differently in boys and girls, although not statistically different due to the smaller group (table 2). The term-group is outlined separately in the supplement (e-table 3.1).

GAMLSS longitudinal fetal antenatal growth curves for BPD, HC, AC, FL from 12-40 weeks were developed for boys, girls and combined (Supplement, e-figures 3.1-3.4). For each parameter the 5th, 10th, 50th, 90th and 95th centiles were constructed. Actual values for these centiles and grid-curves are also outlined in the supplement (e-tables 3.3-3.12). Comparing the two gender growth trajectories and their percentiles, for BPD,

Table 2. Neonatal data for boys, girls and combined in preterm (<37weeks) pregnancies.

	Total (N= 576)	Boys (N= 317, 55.0%)	Girls (N= 259, 45.0%)	P-value*
GA at birth (wks,0/7d), mean ± std	34w6/7 ± 2w2/7	35w0/7 ± 2w1/7	34w5/7 ± 2w3/7	0.19
Birth weight (g), mean ± std	2481 ± 637	2553 ± 604	2392 ± 665	0.003
Length (cm), mean ± std	46.3 ± 4.2	46.8 ± 4.1	45.7 ± 4.3	0.005
HC (cm), mean ± std	32.5 ± 2.5	32.8 ± 2.4	32.2 ± 2.6	0.006
1 minute AS ≤5	8.1%	8.6%	7.4%	0.62
5 minute AS ≤7	7.9%	8.9%	6.7%	0.32
pH Umb Art, mean ± std	7.29 ± 0.07	7.285 ± 0.070	7.289 ± 0.076	0.50

Neonatal demographic data available in 576 pre-term cases '2002-2012. GA = gestational age; wks = weeks; d = days; g = gram; cm = centimeter; HC = Head Circumference; AS = Apgar Scores; pH = pondus Hydrogenium; Umb Art = Umbilical artery; Std. = Standard Deviation. * P-value represents difference Boys vs Girls.

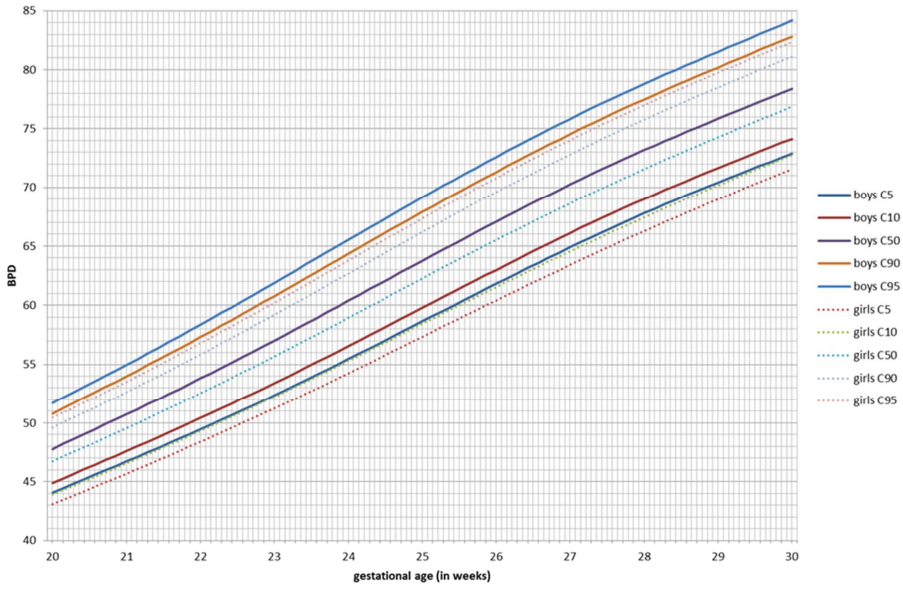

Figure 2. Biparietaldiameter (BPD) in mm for boys and girls from 20-30 weeks of gestation for percentiles 5, 10, 50, 90 and 95.

there was a significant (p<0.001) difference for all percentiles in boys having higher BPD measurements (figure 2, table 3). At 24 weeks the 50th percentile BPD for boys (60.4mm) is significant higher as compared to girls (58.9mm, p<0.001, e-table 3.8, 3.13). This corresponds to a difference of three gestational days. The boys 5th percentile aligns with the 10th percentile of the girls, and the 90th percentile aligns with the 95th percentile of

Table 3. BPD reference values for boys and girls from 12-40weeks.

GA	Boys (BPD)					Girls (BPD)				
	C5	C10	C50	C90	C95	C5	C10	C50	C90	C95
12	16,7	17,3	19,4	21,6	22,3	16,5	17,1	19,2	21,3	21,9
13	20,4	21,0	23,2	25,6	26,3	20,1	20,7	22,9	25,1	25,7
14	24,3	25,0	27,3	29,7	30,4	24,0	24,6	26,8	29,1	29,7
15	28,2	28,9	31,3	33,8	34,5	27,8	28,5	30,8	33,1	33,7
16	32,0	32,6	35,1	37,6	38,3	31,4	32,1	34,5	36,8	37,5
17	35,3	36,0	38,5	41,0	41,8	34,7	35,4	37,9	40,3	41,1
18	38,3	39,0	41,6	44,3	45,1	37,8	38,5	41,0	43,6	44,4
19	41,3	42,0	44,7	47,6	48,4	40,5	41,3	44,0	46,7	47,5
20	44,0	44,9	47,8	50,8	51,7	43,1	43,9	46,7	49,6	50,5
21	46,7	47,6	50,7	54,0	55,0	45,7	46,5	49,6	52,6	53,5
22	49,5	50,4	53,8	57,3	58,4	48,4	49,3	52,5	55,8	56,8
23	52,4	53,4	57,0	60,8	61,9	51,2	52,2	55,7	59,2	60,2
24	55,5	56,6	60,4	64,3	65,5	54,2	55,3	58,9	62,7	63,8
25	58,6	59,8	63,8	67,9	69,1	57,3	58,4	62,3	66,2	67,3
26	61,8	63,0	67,1	71,3	72,6	60,4	61,5	65,5	69,6	70,8
27	64,9	66,1	70,2	74,5	75,8	63,4	64,6	68,6	72,8	74,0
28	67,8	69,0	73,2	77,5	78,8	66,3	67,4	71,5	75,8	77,0
29	70,4	71,6	75,8	80,2	81,5	69,0	70,2	74,3	78,5	79,7
30	72,9	74,1	78,4	82,8	84,2	71,6	72,7	76,9	81,1	82,4
31	75,3	76,6	81,0	85,5	86,9	74,0	75,2	79,4	83,6	84,9
32	77,6	78,9	83,3	87,9	89,4	76,2	77,4	81,7	86,0	87,3
33	79,5	80,9	85,4	90,1	91,6	78,1	79,4	83,7	88,2	89,5
34	81,2	82,6	87,3	92,1	93,7	79,8	81,1	85,6	90,2	91,6
35	82,5	84,0	88,9	93,9	95,5	81,3	82,7	87,3	92,1	93,5
36	83,6	85,1	90,3	95,6	97,3	82,7	84,1	88,9	93,9	95,3
37	84,6	86,2	91,7	97,4	99,2	84,1	85,5	90,5	95,6	97,2
38	85,7	87,4	93,2	99,3	101,2	85,5	87,0	92,1	97,4	99,0
39	87,0	88,8	94,9	101,2	103,3	86,9	88,4	93,7	99,3	100,9
40	88,4	90,3	96,6	103,2	105,3	88,2	89,8	95,3	101,1	102,8

Reference values in mm for fetal biparietal diameter (BPD) for boys and girls for each gestational week for the median and 5th, 10th, 90th and 95th centiles. GA = gestational age.

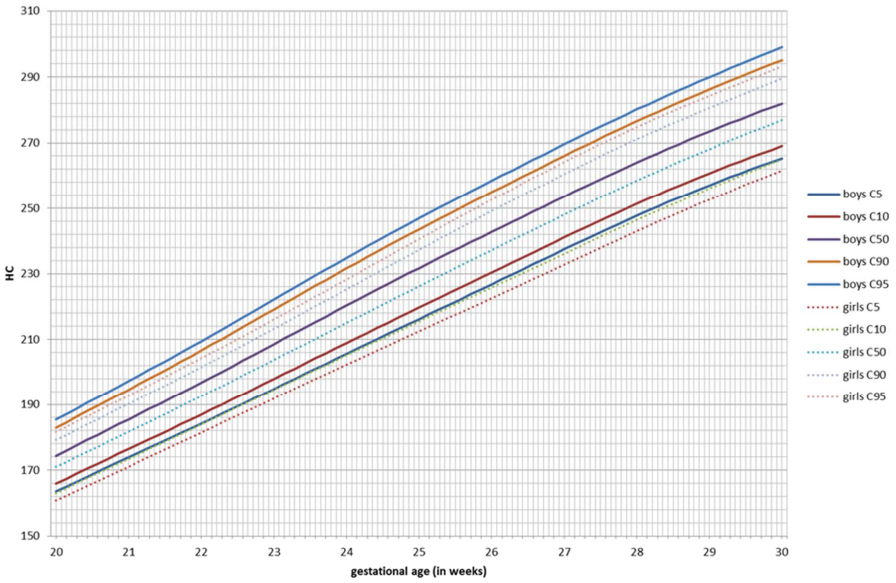

Figure 3. Headcircumference (HC) in mm for boys and girls from 20-30 weeks of gestation for percentiles 5, 10, 50, 90 and 95.

Table 4. HC reference values for boys and girls from 12-40weeks.

GA	Boys (HC)					Girls (HC)				
	C5	C10	C50	C90	C95	C5	C10	C50	C90	C95
12	61,3	63,3	70,3	77,4	79,4	61,9	63,6	69,7	76,1	78,1
13	74,4	76,5	83,8	91,1	93,2	74,3	76,1	82,6	89,5	91,6
14	88,2	90,3	97,8	105,3	107,5	87,3	89,2	96,1	103,3	105,4
15	101,9	104,1	111,6	119,2	121,4	100,6	102,6	109,6	117,0	119,2
16	115,2	117,4	124,9	132,6	134,8	113,6	115,6	122,8	130,4	132,6
17	128,0	130,2	137,8	145,6	147,8	126,3	128,3	135,7	143,3	145,6
18	140,3	142,5	150,3	158,2	160,5	138,5	140,7	148,2	156,0	158,3
19	152,2	154,5	162,6	170,7	173,1	150,1	152,3	160,0	168,0	170,4
20	163,4	165,8	174,3	182,9	185,4	160,7	163,0	171,0	179,2	181,6
21	173,9	176,4	185,5	194,6	197,3	171,1	173,4	181,7	190,2	192,8
22	184,2	187,0	196,8	206,6	209,5	181,5	183,9	192,6	201,5	204,2
23	194,8	197,9	208,5	219,1	222,2	191,9	194,5	203,7	213,2	216,0
24	205,6	208,9	220,3	231,6	234,8	202,3	205,1	215,0	225,2	228,3
25	216,2	219,7	231,7	243,6	247,0	212,5	215,5	226,2	237,3	240,6
26	226,8	230,4	242,8	255,0	258,5	222,5	225,8	237,2	249,1	252,7
27	237,4	241,1	253,6	266,0	269,6	232,7	236,1	248,0	260,5	264,2
28	247,7	251,4	264,0	276,5	280,2	243,0	246,4	258,5	271,1	274,8

Table 4. HC reference values for boys and girls from 12-40weeks. (continued)

GA	Boys (HC)					Girls (HC)				
	C5	C10	C50	C90	C95	C5	C10	C50	C90	C95
29	257,1	260,7	273,4	286,2	290,0	252,7	256,1	268,1	280,6	284,3
30	265,3	269,0	282,0	295,1	299,0	261,6	265,0	277,0	289,5	293,2
31	273,0	276,8	290,0	303,7	307,7	269,6	273,1	285,5	298,3	302,1
32	280,2	284,0	297,6	311,9	316,3	276,5	280,2	293,2	306,7	310,8
33	286,5	290,4	304,4	319,4	324,1	282,6	286,4	300,1	314,4	318,7
34	292,1	296,1	310,3	326,0	331,0	288,0	292,0	306,2	321,1	325,5
35	297,7	301,6	315,9	332,2	337,5	292,6	296,7	311,3	326,5	331,0
36	302,9	306,8	321,2	338,2	343,9	296,6	300,7	315,5	330,9	335,5
37	307,3	311,3	326,1	344,1	350,4	301,0	305,2	320,1	335,8	340,4
38	311,9	316,0	331,1	350,4	357,5	306,5	310,7	325,9	341,7	346,4
39	317,3	321,4	336,7	357,1	365,2	312,8	317,1	332,4	348,5	353,3
40	323,5	327,6	342,9	364,2	373,1	319,4	323,7	339,2	355,4	360,2

Reference values in mm for fetal head-circumference (HC) for boys and girls for each gestational week for the median and 5th, 10th, 90th and 95th centiles. GA = gestational age.

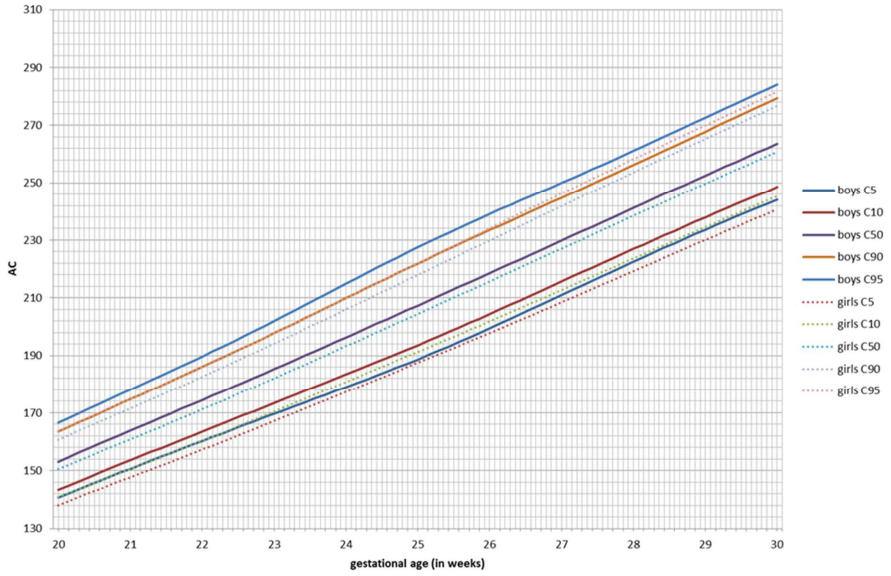

Figure 4. Abdominal circumference (AC) in mm for boys and girls from 20-30 weeks of gestation for percentiles 5, 10, 50, 90 and 95.

the girls. For HC these differences were even more pronounced (p<0.001; e-figure 3.1, e-table 3.14). The prenatal difference of HC of boys at the 95th percentile increases to +6.5mm at 35 weeks, but it is already present at twenty weeks' gestation (+3.8mm, fig-

ure 3, table 4). The neonatal head-circumference confirmed this difference of +6mm as being significant between boys and girls (p<0.001; table 1). Generally, prenatal AC measurements were significantly higher in boys than in girls, but less demonstrably across the total gestational period than for BPD and HC (figure 4, e-table 3.15). For FL there was no significant difference between boys and girls in their antenatal growth percentiles (figure 5, e-table 3.16). The EFW was different in boys throughout the gestational age at different percentiles compared to girls, except for the 40 weeks measurement (table 5, e-table 3.17). Girls reach the 500g EFW one day later (22w3/7) as compared to the boys (22w2/7, e-table 3.12). At the 50th percentile at 24 weeks boys are estimated 21gram heavier compared to girls (p=0.02, e-table 3.12).

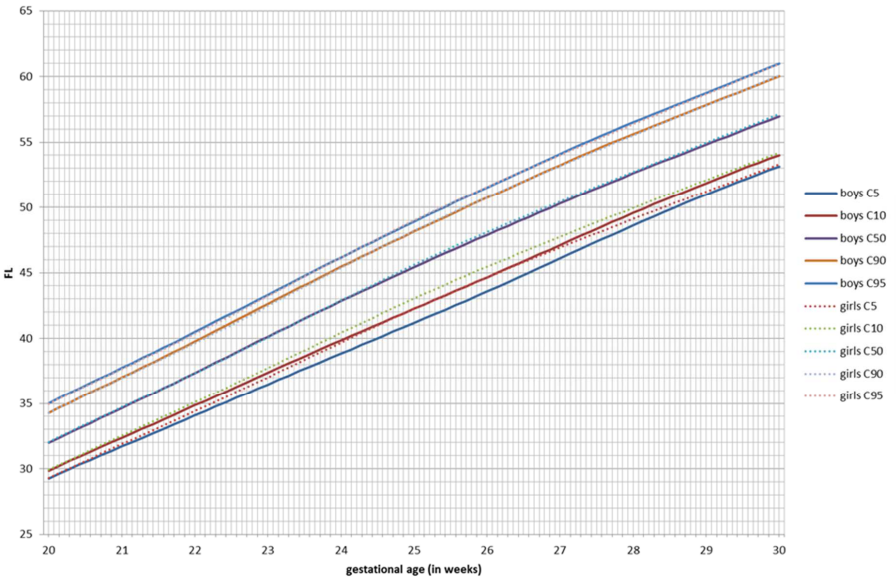

Figure 5. Femur length (FL) in mm for boys and girls from 20-30 weeks of gestation for percentiles 5, 10, 50, 90 and 95.

Table 5. EFW reference values for boys and girls from 12-40weeks.

GA	Boys (EFW)					Girls (EFW)				
	C5	C10	C50	C90	C95	C5	C10	C50	C90	C95
12	50	51	57	63	65	49	50	55	61	63
13	60	62	69	78	81	61	62	69	76	79
14	77	80	89	101	105	79	82	90	101	104
15	104	107	120	136	142	103	106	117	131	136
16	131	136	152	172	179	128	132	147	165	171
17	159	165	185	208	216	159	164	183	206	213

Table 5. EFW reference values for boys and girls from 12-40weeks. (continued)

GA	Boys (EFW)					Girls (EFW)				
	C5	C10	C50	C90	C95	C5	C10	C50	C90	C95
18	199	205	230	260	269	198	204	229	257	266
19	248	256	287	324	336	242	250	281	316	328
20	298	308	346	391	406	290	299	337	380	394
21	352	364	410	465	482	342	354	398	450	466
22	414	429	486	552	574	402	416	470	531	551
23	488	506	576	658	685	475	493	557	631	655
24	574	596	681	781	813	561	582	660	750	779
25	671	698	799	917	955	658	684	777	884	919
26	779	810	928	1065	1110	765	795	905	1032	1073
27	901	938	1073	1230	1280	883	918	1046	1193	1241
28	1038	1080	1233	1410	1467	1014	1054	1201	1369	1424
29	1188	1235	1407	1605	1669	1158	1204	1369	1558	1620
30	1348	1400	1593	1814	1886	1314	1366	1550	1761	1831
31	1511	1571	1788	2035	2116	1478	1536	1743	1979	2059
32	1672	1739	1985	2265	2357	1638	1704	1937	2206	2298
33	1825	1902	2182	2504	2610	1788	1863	2128	2437	2544
34	1970	2058	2380	2752	2876	1927	2012	2314	2671	2796
35	2108	2209	2577	3007	3152	2060	2156	2500	2912	3059
36	2244	2358	2775	3267	3434	2193	2301	2689	3163	3336
37	2379	2506	2974	3530	3720	2330	2452	2887	3428	3629
38	2513	2655	3174	3795	4009	2475	2610	3095	3707	3940
39	2647	2802	3373	4060	4299	2626	2774	3310	3999	4265
40	2776	2946	3570	4325	4591	2780	2943	3531	4300	4605

Reference values in g for Estimated Fetal Weight (EFW) for boys and girls for each gestational week for the median and 5th, 10th, 90th and 95th centiles. GA = gestational age.

DISCUSSION

In this study we have constructed antenatal growth and estimated fetal weight charts, with a strict and clearly defined selection protocol in a normal Caucasian population and in boys and girls. Boys have significantly larger late-second and third trimester HC, BPD and AC measurements than girls. For FL there are no differences. The implication of these findings is that a boy and a girl at exactly 24weeks gestation might, based on the current late second trimester dating protocols with head measurements, be assigned a gestation as much as 3 days different and a EFW difference of 21gram at 24weeks favoring the boys. These antenatal differences were confirmed at birth with boys being significant heavier, longer and having larger head-circumferences as compared to girls.

The one and five minute AS and cord pH were lower in boys. The dating and weight estimation differences could potentially play an important part in determining prenatal and immediate perinatal viability management in terms of timing the administration of maternal steroids for fetal lung maturation, decisions for delivery and possible resuscitation. Also in the post-term period management, these gender differences could influence decisions including the timing for labor-inductions, affecting an even larger population and potentially putting girls at risk of stillbirth by assuming that their gestational maturity to be less than it is, if second trimester dating of the pregnancy has been undertaken.[27]

In one cross-sectional study a difference has been shown between fetal head measurements for both boys and girls, although the curves were constructed with the older linear regression models.[28] They also confirmed the birth-weight difference, but did not report information on neonatal head circumference or other outcomes (AS, cord pH). Another unselected multi-ethnic combined cross-sectional and longitudinal population study also found differences in fetal head and abdomen measurements using statistical methods current at that time, however no birth outcomes were available[29].

Whilst it has been demonstrated that gestation specific neonatal outcomes are worse in boys than in girls,[9,10] what had not been previously appreciated in a routine population is that boys both have lower Apgar scores at both 1 and 5 minutes and lower cord pH values at delivery than girls. These results underline male vulnerability in the perinatal period. In a recent published elegant report on neonatal outcome in appropriately grown term babies, gender differences were demonstrated in terms of lower Apgar scores at 5 minutes and higher rates of instrumental deliveries for failure to progress in labor for boys.[30] This concerned a multi-ethnic retrospective cohort from one center and birth data for specified for both genders. They demonstrated a birth-weight difference of 135 g at term, comparing closely with the 121 g that we report), but their data lacked other anthropometric data (birth-length and head-circumference) and ante-natal growth data. It is of course possible that neonatal outcomes are worse because immediate birth outcomes are worse. Whether this is an attribute of being male *per se*, or some effect of fetal size on delivery, cannot be explained from their results or ours. We can demonstrate that the gender differences in fetal anthropometry starting from 20 weeks onwards affect fetal dating and the estimated fetal weight. In our preterm sub-analysis the birth weight differences between boys and girls are also present in absolute mean differences (Δ birth-weight 161 g, Δ birth-length 0.8cm, Δ HC 0.6cm), and there are noticeable differences between AS and umbilical cord pH (table 2), although not statistically significant due to smaller numbers. One hypothesis is that either the differences in biometry are relatively more important in the (full grown) male fetus interacting with maternal pelvic limitations causing more labor dystocia for boys, and hence lower AS. Alternatively, other fetal gender-specific factors can influence the

birth process and compromising the immediate birth outcomes. Gender-specific body composition at birth has been reported, where the male infant has more fat mass and lean body mass than the female infant, especially in well-nourished mothers.[31] This phenomenon has been associated with gender different intra-uterine physical adaptations to an enhanced nutrient supply from the mother. The male infant body composition has been more subject to maternal influences as higher pre-gestational BMI and excessive gestational weight gain.[32] Lastly, the lung maturation of the male fetus proceeds slower than in the female fetus, possibly contributing to a higher rate of low AS in the term grown fetus. In animal studies lung fluid secretion is inhibited and the lung fluid absorption initiated by adrenalin infusions at birth.[33] And preterm asphyxiated male infants have lower adrenaline levels than female infants, again putting the boys at higher risk.[34] Whether in the term infant this will be similar, is unknown.

Strength and weakness

Our antenatal growth curves are unique in that all four fetal growth parameters (BPD, HC, AC and FL) were measured in standardized circumstances in accordance with international guidelines.[26] Longitudinal growth charts were constructed for each parameter, with the WHO advocated GAMLSS method used.[22,23] GAMLSS can combine longitudinal data with a cross-sectional component and can construct centiles in a way that they are constrained and do not cross. Further, in using the GAMLSS analysis statistics, we could by synchronizing the statistical methods of the WHO, align the biometry measurements with the neonatal and pediatric charts.[22,23] With the available neonatal data, we could discriminate different growth curves for boys and girls for all four fetal growth parameters and hence the EFW. Since the introduction of ultrasound in antenatal care, many reports on fetal growth curves have been published.[11-21] To recognize pathological fetal growth depends on reliable, standardized growth curves.[35] Discrepancies between curves have often been attributed to differences in methodology and population selection.[36] A recent report reviewed fetal growth charts, demonstrating the wide variations of methodologies on how these charts have been constructed concluding that there were many grounds for bias in the growth curves that are currently used.[37] Particularly in 'inclusion/exclusion criteria', 'ultrasound quality control measures' and 'gestational dating protocols', many ambiguities existed. Standardization of the methodologies with a check-list was recommended to define a high quality study.[37] When we compare our growth charts to the requirements, these would be compliant for the combination of a high quality control score, longitudinal design, sample size and the fact that all four parameters (BPD, HC, AC and FL) were examined (e-table 3.2). All growth measurements were reviewed by certified staff members, judging all scanned images as to whether they adhered to the protocol described. We also incorporated a strict protocol on pregnancy dating. Only pregnancies that had a first trimester confirmation scan on

gestational age were included: Crown Rump Length (CRL) measurement between 3 and 83mm (gestational age $\geq 5^{+0}$ and $< 14^{+0}$ weeks).[4,25] In Belgium in routine obstetrical care every pregnant woman will be offered a first, second and third trimester ultrasound scan with fetal growth measurements. In many countries the third trimester scan is not part of routine care for low-risk pregnancies.[38] Also, to measure the four fetal growth parameters in the first trimester is not routine care and allowed us to define 'fetal growth' through serial measurements, instead of 'fetal size', as defined through cross-sectional measurements.[12-19,39] Furthermore we were able to eliminate aberrant fetal growth and extreme maternal influences by excluding fetal anomalies (level 1 and 2 indications) and including only mothers enrolled to a routine obstetric care scheme.[40] Finally a population based cohort was generated with a significant sample size over a period of eleven years. The description of a routine population could also be supported by our neonatal data. Neonatal data was complete for 76% in our cohort. The rate of premature birth was 6%, which is consistent with European nationally accepted norms. In our population selection we further customized the charts for one maternal and one fetal factor. We selected on ethnicity 'Caucasian' and the fetal gender. Other ethnicity derived customized growth curves have arisen in response to the early reference charts from mainly Europe and the United States.[18,19] Ethnicity seem to have a discriminative influence on fetal growth.[24,41] Girls and boys both have different neonatal growth curves, assuming there is a discriminative effect of the gender on their growth trajectories. In more than three quarters of our cohort complete neonatal data was registered, including gender registration. Therefore we focused on developing two separate fetal growth charts: both for boys and girls. Fetal gender, unlike maternal ethnicity, is not commonly known in the first trimester but it is from the 20 weeks' scan onwards ('anomaly' scan). From a clinical point of view it seemed therefore relevant to start discriminating these curves from 20 weeks' gestation onwards.

Some limitations on constructing these charts have to be addressed. The study was performed in a University teaching Hospital, a large tertiary referral center, not necessarily reflecting a routine setting. This center, on the other hand, also has a regional remit for routine obstetric care for low-risk pregnancies. Some maternal characteristics were not excluded in the selected cohort, deliberately to prevent 'super-normalization' of the cohort: smoking (occurred in 7%) and artificial conception was excluded for intracytoplasmatic-sperm-injection, since this is a level-1 indication. Finally, it is expected that within this large time-period some women with subsequent pregnancies were included more than once for this cohort.

Implications for clinical practice

Our fetal growth curves for the Caucasian population resemble predictive growth curves with the gender specified which can discern aberrant from normal fetal growth.

The longitudinal aspect and large cohort, covering the full trimesters have not been reported before in the Caucasian population. The neonatal data gave us the opportunity to customize for the fetal gender. There was a marked difference between fetal boys and girls in their growth trajectory for fetal head measurements and to a lesser extent the abdominal circumferences. Also for the estimated fetal weight there was a difference. This gender differentiation is important in ante-natal and perinatal care. Prenatal ultrasound is used not only to define fetal growth, but also gestational age. Both growth and fetal age are important in defining the time-point of fetal viability and the optimization of the timing of obstetrical interventions: e.g. medical elective birth or administration of corticosteroids for fetal lung-maturation in cases of threatened premature birth. Second trimester dating depends on fetal growth parameters and particular on the fetal head measurement. Our results suggest a gender specific approach in counseling future parents on important issues when fetal viability starts and when is the best time point to start obstetrical interventions.

The gender differences are further demonstrated by the immediate birth outcomes for males: different anthropometry (heavier, longer and bigger heads) and lower AS and lower cord pH. The significant lower AS and umbilical cord pH in boys underline the fetal male vulnerability, although in the asphyxia group (pH<7.10) there was no predominance by males, stating that boys do not have a higher risk of acidemia at birth in a routine population. Therefore the clinical importance of the pH findings (and perhaps also the AS) in our study can perhaps be considered as not clinically relevant.

In summary, we present fetal growth curves with the latest statistical tools in a large, routine pregnant population with state of the art ultrasound technology. The data covers the pregnancy period from 12 weeks onwards and there were differences between boys and girls for the fetal head, fetal abdomen measurements and the estimated fetal weight. Also the immediate neonatal outcome demonstrated gender differences favoring the girls. This could give caretakers the opportunity to take into account a gender tailored approach in life-decision care both at the margins of viability and post-term.

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ABDOMINAL AORTA FETAL ABDOMINAL
INTIMA MEDIA THICKNESS FETAL FAT
FUNCTIONAL LINEAR DISCRIMINANT
ADDITIVE MODEL LOCATION, SCALE AND
MELLITUS NON-EXCESSIVE GESTATIONAL
GROWTH FACTOR INTIMA-MEDIA THICKNESS
SOCIETY ON OBSTETRICS AND
GROWTH LARGE-FOR-GESTA
NUTRITION EPIGENOME
GROWTH STUDY GROUP
IMAGING MRI FATTY LIVER DISEASE
GLUCOSE TOLERANCE TEST OGTT ODDS RATIO
ARTERIAL STIFFNESS QUALITY
BLOOD PRESSURE SKINFOLD TRICEPS
FAT THICKNESS SMALL-FOR-GESTATIONAL AGE
VASCULAR AND BIOMETRY ULTRASOUND
STUDY VABULOS YOUNG ELASTIC MODULUS

Chapter 4

The influence of weight gain patterns in pregnancy on fetal growth using cluster analysis in an obese and non-obese population

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ABSTRACT

Objective

Excessive weight gain during pregnancy has an important influence on fetal growth and on weight development in future generations.

Design and Methods

A prospective cohort study of 325 obese and nonobese Caucasian women with naturally conceived, singleton pregnancies. They were followed up until delivery for maternal weight gain and for fetal growth with ultrasound-based weight estimations and final birth weight. Using cluster analysis distinct profiles of maternal weight gain during pregnancy were obtained. Longitudinal regression analysis was performed to investigate the relationship of the maternal weight gain profile and body mass index (BMI) on fetal growth and final birth weight.

Results

Cluster analysis revealed four discernable maternal weight gain profiles: twelve cases (3.7%) ended up at their starting weight or decreased in weight (cluster 1), 16 cases (4.9%) who slightly increased in weight (maximum 4 kg) as compared to their initial weight (cluster 2), 114 cases (35.1%) who gained between 4 and 12 kg in weight (cluster 3) and 183 cases (56.3%) who showed the largest weight gain: more than 12 kg (cluster 4).

There were statistically significant differences in fetal growth associated with weight gain cluster, which became apparent late in the second trimester and increased toward the end of pregnancy. Maternal BMI and maternal weight gain profile were independent predictors of fetal growth and birth weight.

Conclusions

Therefore, the conclusion is that cluster analysis permits to discern four gestational weight gain (GWG) patterns in obese and nonobese subjects and that both maternal BMI and maternal weight gain pattern during pregnancy positively influence fetal growth and birth weight.

INTRODUCTION

Maternal weight gain shows a wide variation, even in a low-risk homogeneous Caucasian population with normal pregnancy outcome (1). Despite this, the importance of adequate gestational weight gain (GWG) is well documented (2,3).

Excessive weight gain, based on the 2009 IOM guidelines, has an incidence of up to 50% in pregnancy and currently represents an important problem in Western populations (4-7). It is associated with gestational diabetes mellitus (GDM), an abnormal increase in fetal growth, neonatal macrosomia, emergency caesarean section and postpartum weight retention (8). This in turn feeds into the pandemic of obese pregnant mothers with its resultant deleterious influence on future generations through the effect of fetal programming (9-14).

Besides GWG, preconception body mass index (BMI) also affects fetal growth and pregnancy outcome: an abnormally high or low maternal BMI prior to gestation has been shown to influence fetal growth in the second and third trimester of pregnancy and is associated with a variety of adverse outcomes (15-17). However, maternal BMI does not seem to significantly affect growth in embryonic crown rump length (CRL) during the first trimester (18).

BMI groups are defined by the World Health Organization (WHO): BMI $\leq$ 18.5 kg/m^2 underweight; BMI ranging from 18.5-24.9 kg/m^2 normal weight; BMI ranging from 25.0-29.9 kg/m^2 overweight and $\geq$ 30.0 kg/m^2 obese. In recognition of the dual influence on fetal growth, current guidelines refer to an appropriate GWG range that is best adhered to for each of the BMI groups, with the lowest gain being advised in the obese group (7,19).

This is based on epidemiologic data looking at a total maternal weight gain and its relationship to healthy pregnancy outcome (8*)*. Few studies, however, have looked longitudinally at maternal weight during pregnancy (20-23). Women's individual weight gain pattern, influenced by nutrition and life-style during pregnancy, can be expected to have a greater impact on embryonic or fetal growth than preconception BMI (24,25). Because maternal weight gain pattern is being monitored at sparse set of time points, cluster analysis is particularly useful by using a discriminant function to determine the greatest separation between the clusters (26).

The aim of this study was to investigate whether the maternal weight gain profile in pregnancy and preconception BMI independently influence fetal growth.

DESIGN AND METHODS

This prospective study was performed at the Department of Obstetrics and Gynecology of the University Hospitals Leuven (Belgium), with approval of the Ethics Committee for Human Experimentation of the Faculty of Medicine, Katholieke Universiteit Leuven. All women signed informed written consent to take part in the study.

Between April 2007 and April 2008, the nutritional intake and GWG in 605 pregnancies were prospectively recorded by a single clinician (IG). From this cohort there was a well documented subgroup of 373 healthy Caucasian women, who underwent ultrasound examinations to measure fetal size in the University Hospital Leuven and delivered in this centre. They were recruited before 15 weeks gestation at the time of their first antenatal clinic visit and pregestational BMI was documented at this time. All the pregnancies were conceived naturally and showed normal evolution at the time of inclusion.

The endpoint of the pregnancies was the date of delivery, which ranged from 177 to 291 days gestation, on which the last maternal weight and the actual neonatal weight was measured. Exclusion criteria were: inadequate knowledge of the Dutch language, multiple pregnancies and underlying or development of significant maternal or fetal pathology. The incidence of premature delivery was 21/325 (6,4%), in accordance to earlier studies in the general obstetrical population (27).

From the 373 pregnancies initially recruited, 48 pregnancies were excluded because of missing complete longitudinal data from either weight measurements of the mother or from estimated weight measurements of the fetus. This was because of either drop

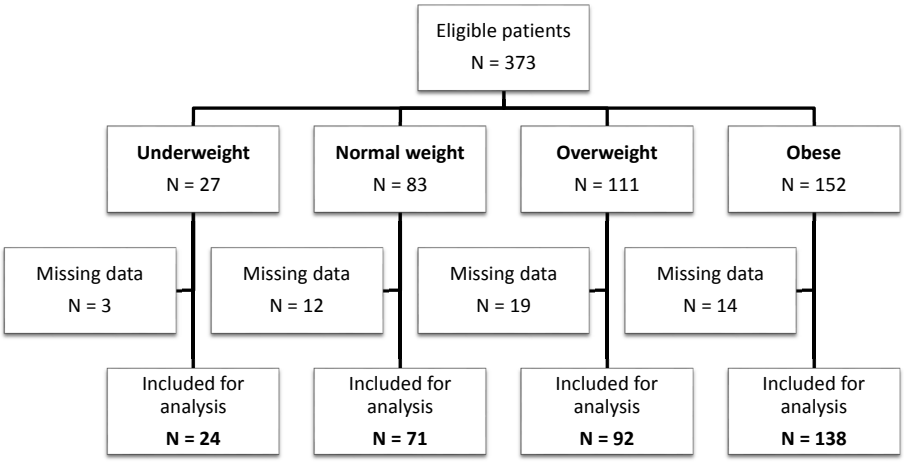

Figure 1. Inclusion flowchart based on preconception BMI groups. Missing longitudinal data 5maternal weight measurements or fetal biometry measurements) were excluded.

outs (lost to follow-up or migration) or miscarriage, termination of pregnancy, fetal death and twins, leaving a total of 325 viable, singleton pregnancies included in the final analysis (Figure 1).

All women underwent first trimester sonographic estimation of gestational age (GA) based on crown rump length (CRL) obtained between 7 and 14 weeks of gestation.

Maternal weight measurements were performed using a calibrated balance (Seca Alpha Model 770, Teleflex medical, Belgium), accurate to 0.1 kg in a standardized way (without shoes, with indoor clothing). Height was measured with a microtoise to the nearest 0.5 cm, all as described earlier in Guelinckx et al (1). Preconception BMI was calculated using height measurement at the first visit and the self-reported weight prior to conception (kg/m^2) (1,7). The total GWG was computed using the final weight measured on the day of delivery minus the self-reported weight prior to conception.

Each subject followed routine antenatal care including a minimum of three ultrasound examinations (first, second and third trimester) and additional examinations if necessary. All scans were performed by experienced ultrasonographers on a high-end ultrasound machine (Voluson 730-E or Voluson E-8, General Electric, USA) and they measured the bilateral parietal diameter (BPD), the abdominal circumference (AC) and the femur length (FL). The estimated fetal weight (EFW) was calculated using Hadlock IV curves [log(10) EFW = 1.335 - 0.0034(AC)(FL) + 0.0316(BPD) + 0.0457(AC) + 0.1623(FL)] (28). These curves are the same for both male and female fetuses.

Birth weight was measured one hour after delivery according to the recommendations of the WHO's rules on the Baby Friendly Hospital Initiative. The birth weight was measured on a standard calibrated neonatal scale (Seca, model 376, 0.1-7.5kg, Teleflex medical, Belgium).

Statistical analysis

Firstly, a cluster analysis was performed on the weight gain patterns of all women (Figure 2) (26). Subsequently the measurements were retrospectively assigned to four different clustered groups based on their GWG. Next, the maternal weight gain cluster assignment was used as an explanatory variable in a mixed effects longitudinal regression model to assess the evolution of the EFW (Hadlock IV curves) over time (29). In this analysis, the actual birth weight of the neonate was added to the fetal weight estimates as an extra observation per case (Figure 3). Other explanatory variables in the analysis were BMI (in kg/m^2), (G)DM (yes/no), GA (in days), parity (0-5), maternal age (in years), vitamin use (yes/no) and complications (yes/no). This model was further reduced by tests for the presence of random effects (GA, GA^2 and GA^3) and their structure and for the main and interaction effects of the explanatory variables. However maternal weight gain profile and BMI were forced in the model. Finally, F-tests were performed for comparing the evolution of the EFW over time for each pair of weight gain profile with correction for

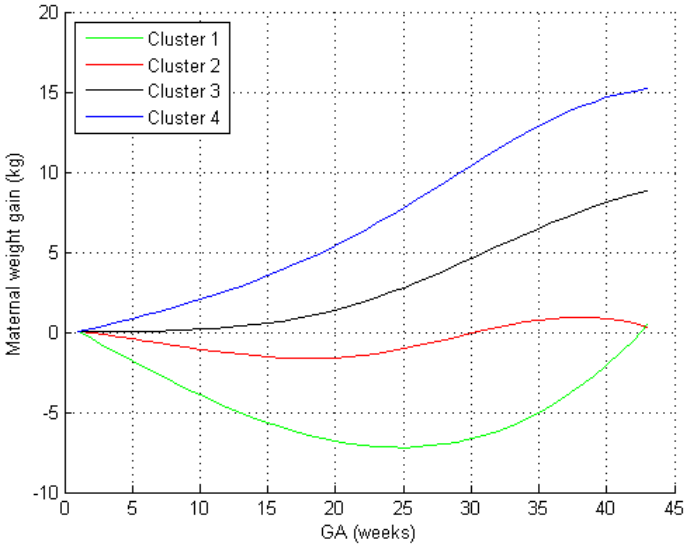

Figure 2. Maternal weight gain profile during gestational age (GA) reduced to four clusters.

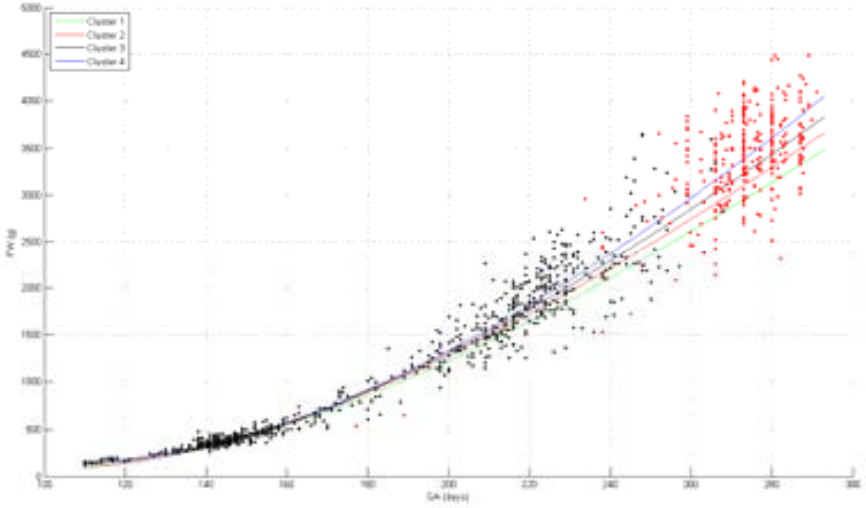

Figure 3. Evolution of estimated fetal weight (EFW) during pregnancy according to gestational weight gain (GWG) cluster. Birth weight measurements are presented by red dots.

multiple testing being applied using the approach by Hochberg (30). Statistical analyses were performed using SAS 9.2 (SAS Institute Inc., Cary, NC, USA) and Matlab 7.4 (The MathWorks Inc., Natick, Massachusetts, USA).

RESULTS

Study population:

A total of 325 out of 373 prospectively included pregnant women from different preconception BMI subgroups were included in the final analysis: 24 (7.4%) were underweight, 71 (21.8%) showed normal weight, 92 (28.3%) were overweight and 138 (42.5%) were obese (Figure 1). Baseline characteristics of the study population are summarized in Table 1.

Table 1. Subjects baseline characteristics after exclusion

	BMI category (kg/m^2) [a]			
	Underweight	**Normal weight**	**Overweight**	**Obese**
	< 18.5	18.5 – 24.9	25.0 – 29.9	≥ 30.0
	N = 24 (7.4%)	N = 71 (21.8%)	N =92 (28.3%)	N =138 (42.5%)
Nulliparous (%)	8 (33.3)	48 (67.6)	48 (52.2)	47 (34.1)
Mean age in years (range)	29.38 (21-35)	29.76 (19-41)	28.74 (17-41)	28.90 (20-41)
Smoking at inclusion (%)	1 (4.2)	4 (5.6)	4 (4.3)	20 (14.5)
Bariatric surgery (%)	0 (0)	0 (0)	3 (3.3)	2 (1.4)

(N = 325). body mass index (BMI) group (WHO criteria).

Cluster analysis:

Individual patients were divided into eight different groups according to their GWG. These were reduced to four clusters, as described above, by assigning the lowest GWG group as cluster 1, the second group as cluster 2, the following three groups taken together as cluster 3 and the subsequent three groups as cluster 4 (Figure 2). Therefore four different clusters with specific GWG patterns remained: 12 cases (3.7%) that ended up at their starting weight or decreased in weight (cluster 1), 16 cases (4.9%), that slightly increased in weight (maximum 4 kg) as compared to their initial weight, 114 cases (35.1%) that gained between 4 and 12 kg in weight (cluster 3) and 183 cases (56.3%) that showed the most weight gain, with more than 12 kg (cluster 4). In general the weight of women in cluster 1 decreased during the first trimester of pregnancy while for example the gestational weight for women in cluster 4 showed the largest increase. Table 2 shows the characteristics per cluster.

Table 2. Subjects (n=325) characteristics per cluster group.

	Cluster I	Cluster II	Clusters III	Cluster IV
	N =12	N =16	N =114	N =183
Age in years [a]	28.33 (2.35)	26.63 (5.51)	29.37 (3.62)	29.28 (4.28)
GA at birth in days [b]	273 (267, 275)	270 (266, 278)	273 (267, 280)	274 (269, 280)
Birth weight in gram [c]	3265 (3060, 3565)	3070 (2923, 3434)	3380 (3080, 3610)	3550 (3159, 3818)
Preconception BMI in kg/m² [d]	41.27 (36.82, 45.06)	33.68 (31.90, 36)	30.17 (25.31, 33.43)	27.16 (21.47, 30.61)

a mean (standard deviation); b Gestational age (GA) at birth: median (first- and third quartile; Q1and Q3); c Birth-weight: median (Q1, Q3); d Preconception body mass index (BMI): median (Q1, Q3).

Effect of BMI and Clusters on EFW and birth weight:

The longitudinal regression model showed that the weight gain profile has a statistically positive significant effect on fetal growth (p-value < 0.0001) (Figure 3). The following p-values were obtained for the pair-wise comparisons: 0.6234 (cluster 1 versus cluster

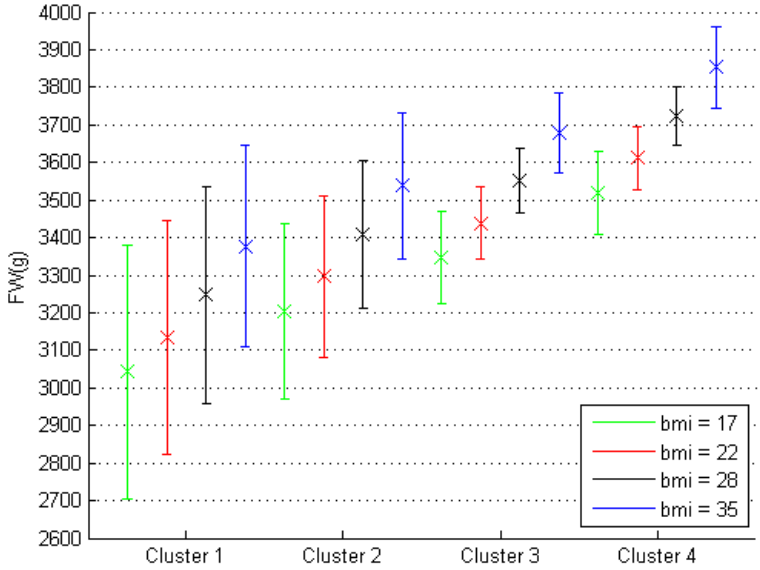

Figure 4. Mean (CI) estimated fetal weight (EFW) at 280 days (term) for the different gestational weight gain (GWG) clusters divided per BMI category (under-, normal-, over-weight and obese).

Table 3. Difference of fetal weight in gram at 280 days for different clusters and BMI's.

	Cluster I	Cluster II	Cluster III	Cluster IV		BMI 17	BMI 22	BMI 28	BMI 35
Cluster IV					BMI 35				
Cluster III				+173	BMI 28				+130
Cluster II			+142	+315	BMI 22			+111	+241
Cluster I		+162	+304	+477	BMI 17		+93	+204	+334

2), 0.0895 (cluster 1 versus cluster 3), 0.0007 (cluster 1 versus cluster 4), 0.0022 (cluster 2 versus cluster 3), 0.0004 (cluster 2 versus cluster 4) and < 0.0001 (cluster 3 versus cluster 4). Based on the multiple comparison method of Hochberg, we found statistically significant differences between the clusters' EFW growth curves, except for fetal growth in cluster 1 versus clusters 2 and 3. The relatively large p-value for comparing cluster 1 and cluster 2 is likely to be due to the relatively small number of observations in these clusters.

The model also shows that pre-conception BMI had an effect on EFW and birth weight, with a larger BMI resulting in a correspondingly larger EFW (p-value < 0.0001).

We subsequently compared BMI prior to conception with the different weight gain clusters to define their relative influence on birth weight at 280 days. Figure 4 and Table 3 show the estimated weight gain at 280 days with 95% confidence intervals for the four maternal weight gain profiles at BMI levels of 17, 22, 28 and 35 (under-, normal-, overweight and obese).

DISCUSSION

This article describes the first cluster analysis of GWG and demonstrates the positive influence of increased GWG on fetal growth and birth weight in a predominantly obese sample of pregnant women.

During recent decades the prevalence of obesity (>30kg/m^2) prior to conception has increased to as high as 25% in some developed countries (5,6). Both obesity and excessive GWG increase the risk for pregnancy-related maternal and fetal complications, like GDM, hypertension, pre-eclampsia, emergency caesarean section, macrosomia, intra uterine growth restriction (IUGR) and fetal demise (10,12,15,17). Additionally excessive GWG results in maternal weight retention after pregnancy with increasing risk for obesity and consequently induces a transgenerational effect influencing the metabolic and vascular traits in the offspring (8,14). Therefore, it seems essential to focus on maternal weight gain during pregnancy as an important modifiable factor to decrease these risks (30). In 2009 the Institute Of Medicine (IOM) produced recommendations for total weight gain in pregnancy for each BMI category: 12.5-18 kg (underweight women),

11.5-16 kg (normal weight women), 7.0-11.5 kg (overweight women) and 5.0-9.0 kg (obese women) (7). The aim of these recommendations is to avoid the additional burden of total excessive GWG, especially in the obese. Previous studies used the preconception BMI class and total GWG to examine their influence on pregnancy and pregnancy outcome, showing that particularly the pre-conception obese group and the excessive total GWG group have an increased risk for fetal overgrowth or macrosomia and early or late onset overweight in the offspring (32-35).

As fetal growth is not linear, but accelerates at mid trimester, it can be hypothesized that besides total GWG and preconception BMI, the pattern of weight gain during pregnancy also influences fetal growth (1,22-24,28). If so, this could lead to an instrument in pregnancy that is easy to monitor and is easier to influence than preconception BMI. Additionally, such a methodology could also be of importance for the non-obese group, as both normal weight and overweight women can gain significant weight during pregnancy and hereby increase the risks to both themselves and their fetus (1,4,6).

The strengths of the current study are its prospective design and the fact that detailed information from a single center on clinical parameters and ultrasound data were available for analysis in a large cohort of pregnancies including a significant number of well categorized obese patients (n=138, 42.5%). It furthermore represents the first study in which the pattern of GWG is taken into account by clustering rather than merely the resultant total GWG. This knowledge could add important information on understanding normal and abnormal weight gain, especially in the overweight and obese.

Our results show that patients, who start to gain weight in the first trimester and continue doing so during the entire pregnancy, show the largest positive effect on fetal (over-)growth (Figure 3). Around 180-200 days gestation (26-28 weeks), the clusters start to separate from each other in relation to their effect on EFW. These findings were independent of the BMI groups found in these clusters.

When comparing the different BMI groups within each cluster by taking representatives from each group (BMI 17, 22, 28 and 35), we found that preconception BMI remains an important determinant of fetal growth and birth weight. Therefore, it seems that besides the preconception BMI to select high-risk pregnancies, it is equally important to discuss the trajectory of the GWG. Because normal BMI, but excessive maternal weight gain and matching higher cluster will lead to fetal overgrowth and consequently have an effect on future generations through fetal programming by increasing the risk of child obesity. Two different tools to achieve a reduction of this phenomenon is to discuss the risks of overweight and obesity in the preconception period and to acquire customized maternal weight gain curves (Figure 2). The latter can be adjusted if an abnormal pattern is observed.

Ideally, this information could be useful in clinical management and urge the clinician to intervene when rapid first trimester weight gain is detected in this high-risk subset.

Naturally, pregnancy poses a specific problem as any treatment (surgical or pharmacological) might have an adverse effect on the fetus and is therefore ethically and medically not feasible. Much effort has been taken to study the effect on gestational weight gain by interventional programs such as diet and/or physical activity advice. They are sometimes hard to compare, because of their heterogeneity. The American College of Obstetrics and Gynecology (ACOG) guidelines recommend at least 30 minutes activity per day for five days per week (36). A recent meta-analysis on interventions appears to favor the diet-based advice on physical activity for the effect on maternal weight gain and obstetric outcome (37). New techniques to promote lifestyle alterations by motivational interviewing are currently under investigation for the obese and high-risk subset pregnancies.

Some limitations of our study also need to be discussed. Our cohort was part of a larger study group in which nutritional intake was monitored during pregnancy. Patients included could therefore be more aware of the problem of excessive GWG. This knowledge could potentially have influenced GWG in this group. Also, weight prior to conception used to calculate BMI was self-reported, which is difficult to avoid as recruitment took place when women were already pregnant. But self-reported weight has been studied in the context of pregnancy and does not lead to significant bias (38,39). Further studies on more heterogeneous groups will give more insight into the effect of GWG using clustering on fetal growth and birth weight. Also, it would be of interest to examine the effect on subsequent pregnancies of GWG control in the obese mothers.

We have shown using cluster analysis that GWG in pregnancy has an effect on fetal growth and actual birth weight partly irrespective of the women's preconception BMI. This effect has most impact from 180-200 days gestation when there is a separation between the different GWG clusters. By identifying this relationship between GWG, growth and birth weight we may now have the time potential to influence weight gain and so prevent both perinatal complications and possibly improve outcomes in future pregnancies and the metabolic and vascular traits in the offspring.

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ABDOMINAL AORTA FETAL ABDOMINAL
INTIMA MEDIA THICKNESS FETAL FAT
FUNCTIONAL LINEAR DISCRIMINANT
ADDITIVE MODEL LOCATION, SCALE AND
MELLITUS NON-EXCESSIVE GESTATIONAL
GROWTH FACTOR INTIMA-MEDIA THICKNESS
SOCIETY ON OBSTETRICS AND
GROWTH LARGE-FOR-GESTA
NUTRITION EPIGENOME
GROWTH STUDY GROUP
IMAGING MRI FATTY LIVER DISEASE
GLUCOSE TOLERANCE TEST OGTT ODDS RATIO
ARTERIAL STIFFNESS QUALITY
BLOOD PRESSURE SKINFOLD TRICEPS
FAT THICKNESS SMALL-FOR-GESTATIONAL AGE
VASCULAR AND BIOMETRY ULTRASOUND
STUDY VABULOS YOUNG ELASTIC MODULUS

Chapter 5

Fetal central adiposity *in utero* is affected by both excessive gestational weight gain and obesity: a prospective cohort study in normoglycemic women

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Submission in progress

ABSTRACT

Introduction

Neonatal adiposity is associated with high maternal pre-gestational body mass index (BMI), high gestational weight gain (GWG) and an abnormal maternal glucose metabolism. In this prospective cohort study we aimed to detect early fetal predictors of neonatal adiposity in normoglycemic mothers.

Methods

One hundred sixty-two women from different BMI classes were recruited during the first trimester of pregnancy and prospectively followed-up for maternal weight gain, fetal biometry and anthropometry. Exclusion criteria included: pre-existing diabetes mellitus (DM), development of gestational DM (GDM), premature delivery and missing birth outcome. Excessive GWG (e-GWG) was defined according to the revised criteria of the Institute of Medicine. Fetal anthropometry was assessed ultrasonographically for liver-length (LL), subscapular- and abdominal fat thickness (SFT and AFT) and lean- and fat mass calculations from the mid-portion of the fetal humerus and femur. Generalized Additive Model Location, Scale and Shape (GAMLSS) was used to align the timeframes of measurements.

Results

One hundred twenty-six pregnancies in normoglycemic women were included in the final analysis: 63/126 (50%) normal weight, 19/126 (15%) overweight and 44/126 (35%) obese. Sixty-one women (48%) showed an e-GWG. In the obese group the fetal LL and AFT growth trajectory increased significantly more than in the normal weight group ($p= 0.01$ and $p= 0.03$). E-GWG positively correlated with the growth in fetal abdominal circumference ($p= 0.03$), LL ($p= 0.002$), SFT ($p= 0.01$) and AFT ($p= 0.006$) when compared with the non-excessive GWG (ne-GWG) group. Neonatal weight, length and head circumference were significantly greater in the e-GWG group as compared to the ne-GWG.

Conclusion

Obesity and e-GWG both positively correlate to adiposity during fetal and neonatal life, with e-GWG clearly affecting fetal size in general as confirmed by birth anthropometry. In this normoglycemic population fetal trunk measures (LL, SFT, AFT and AC) are potentially useful in the prediction of neonatal adiposity.

INTRODUCTION

It has been demonstrated that environmental factors influence health and the development of diseases. Certainly the *in utero* and early neonatal life periods may induce responses, that lead to a higher susceptibility to develop diseases that occur in adult life. This is known as 'fetal programming'.[1,2] Abnormal environmental factors may lead to fetal overgrowth due to increased transplacental nutrients or to fetal growth restriction due to decreased transplacental transfer. Both effects can be transgenerational and are probably due to an epigenetic transmission mediated by the mother.[3,4] There is growing insight on the influence of maternal BMI, excessive Gestational Weight Gain (e-GWG) and abnormal glucose tolerance on fetal nourishment and development.[5] It has been demonstrated that maternal obesity is associated with fetal overgrowth and subsequent perinatal complications, such as fetal death, shoulder dystocia, operative delivery and neonatal metabolic complications.[6] Excessive maternal weight gain occurs in up to 50% of pregnancies and is on its turn associated with abnormal fetal growth, obstetric complications and post-partum weight retention.[7,8] Neonatal ('birth') data are available, but studies on fetal body composition and development in obesity, inappropriate GWG and (G)DM during pregnancy are scarce. Anthropometric ultrasound measurements, indicative of fetal body composition of normal fetuses, have shown a unique exponential pattern of the growth profile during the second half of gestation, both in lean mass and in fat mass.[9] This suggests that the measurement of fetal fat could provide a more sensitive and specific marker of abnormal fetal growth ('fetal adiposity').[10] In this prospective cohort study we aimed to detect early signs of fetal adiposity in normoglycemic mothers and study the relationships of maternal BMI and gestational weight gain category with fetal/neonatal anthropometric parameters.

DESIGN AND METHODOLOGY

This prospective cohort study was performed at the Department of Obstetrics and Gynecology of the University Hospitals Leuven (Belgium). Pregnant women of different pre-gestational BMI classes were included at their first trimester ante-natal visit between January 2012 and August 2014. This sub-study was part of three prospective studies for low-risk, obese and non-obese pregnancies monitored for GWG, development of GDM and nutrition.[11] All studies were approved by the Ethics Committee for Human Experimentation of the Faculty of Medicine, Katholieke Universiteit Leuven (S52172, S54026 and S55325). All women have signed written informed consent to take part in the study. Exclusion criteria were: pre-existing diabetes (DM) or development of GDM, multiple

pregnancies, premature delivery (<34 weeks), missing longitudinal data and adverse events (major fetal abnormality, fetal death and/or immature birth).

Maternal measurements

Pre-pregnancy weight was self-reported and if not available, patients were weighed at the first ante-natal visit in the first trimester on a standard weighing scale (SECA Alpha model 888 or 877, Teleflex, Belgium) with light, indoor clothes to the nearest 0.1kg. The height was measured at the first ante-natal visit with a microtoise to the nearest 0.5cm (SECA model 206, Leicester Height Measure, Burmingham, UK) without shoes. Height and weight was used to calculate the BMI (kg/m^2). The BMI was categorized as normal weight (≤24.9 kg/m2), overweight (25-29.9 kg/m2) and obese (>30kg/m2) as defined by the WHO guidelines. The total GWG was computed using the final weight measured at the last prenatal visit, minus the pre-gestational weight. GWG gain was categorized according to the Institute of Medicine (IOM) 2009 revised recommendations: 11.5-16kg (normal weight), 7.0-11.5kg (overweight) and 5.0-9.0kg (obese).[8] If women crossed the upper IOM threshold, they were categorized in the excessive-GWG (e-GWG) group. If they had either adequate or insufficient total GWG they were categorized in the non-excessive GWG group (ne-GWG). If women were considered high risk (history of GDM, BMI≥30kg/m^2), a fasting glucose measurement <92mg/dL (<5.1mmol/L) at baseline was used to exclude pre-existing DM. Another screening was performed at 24-28 weeks using a 75g oral glucose tolerance test (OGTT) based on the 2013 World Health Organization (WHO) criteria.[12] The non-obese population underwent an OGTT if the initial 50g glucose challenge test showed a glycemia ≥140mg/dL (≥7.8mmol/L) at 24-28weeks (local protocol).

Fetal measurements

Determination of gestational age (GA) was based on a crown rump length (CRL) measured between 7 and 14 weeks of gestation in all patients.[13] Ultrasound examinations were performed at three time-points in gestation: 12-18 weeks, 26-32 weeks and between 35-37 weeks of gestation. A proportion (51 participants) was also examined between 20-22 weeks of gestation. Fetal measurements included: Head Circumference (HC), Abdominal Circumference (AC) and Femur Length (FL). The measurements were obtained in accordance to the guidelines of the International Society for Ultrasound in Obstetrics and Gynecology (ISUOG, www.isuog.org).[14] Additional measurements of fetal body composition were obtained, as described earlier.[9-11,15-17] At 12-16 weeks the fetal liver length (LL) was measured and at all the subsequent visits also the subscapular fat thickness (SFT), abdominal fat thickness (AFT) and the lean- and fat mass at the mid-portion of the humerus and femur were measured (figure 1). All measurements were performed by a single operator (SG) on a high resolution real-time B-mode ultrasound

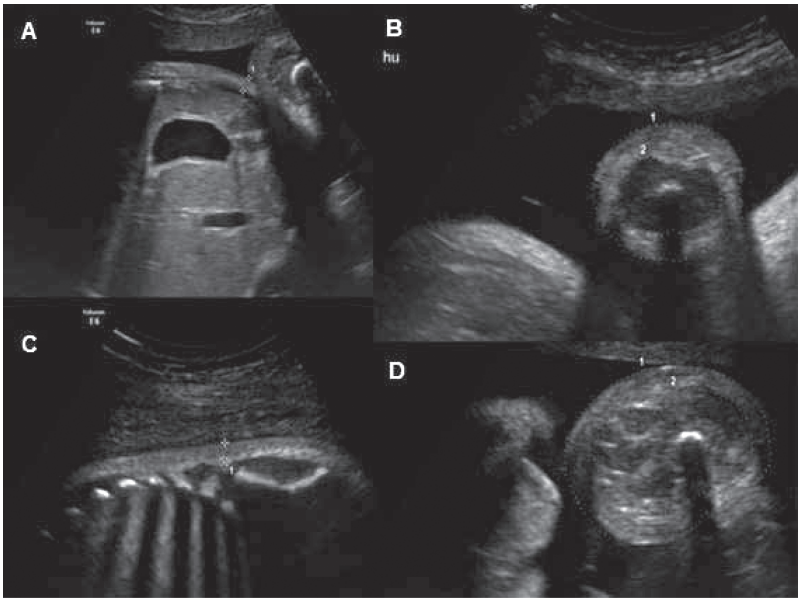

Figure 1. Figure 1. Fetal body composition (ultrasound images) at 33weeks gestation. A. Abdominal Fat Tissue (AFT); B. Humerus lean- (2) and fat mass ([1-2]) in mm^2; C. Subscapular Fat Thickness (SFT); D. Femur lean- (2) and fat mass ([1-2]) in mm^2.

system (E6, E8 or 730-Expert Voluson®, General Electric healthcare Medical systems, Kretztechnik, Zipf, Austria) with a convex, high frequency, 4-8 MHz volumetric 3D abdominal probe. Neonatal measurements (birth weight -BW-, birth length –BL- and head-circumference -BHC-) were obtained within 2 hours after birth. The ponderal index (PI) was calculated by the weight divided by the cubic root of the length (birthweight/ $length^3$) and can be regarded as a neonatal proportionality index in line with the adult BMI.[18] The PI was calculated for all BMI and GWG groups.

Statistical analysis

GAMLSS (Generalized Additive Models for Location, Scale and Shape, www.gamlss.org) R-package was applied to construct the growth curves for conventional fetal biometry measurements: HC, AC and FL.[19,20] We assessed several distributions: Box-Cox-t, Box-Cox Cole and Green, and Box-Cox Power Exponential. Goodness-of-fit of the models was assessed with QQ plots, AIC (Akaike Information Criterium) and worm plots. The goodness-of-fit was investigated covering the gestational period of 12-40 weeks, and for substrata of this period. GAMLSS smoothened the ante-natal growth curves for each prenatal measurement. Percentiles (P10, P50 and P90) for each parameter were constructed. Only complete fetal data-sets were analyzed. The effect of BMI category (normal weight, overweight and obese) and the effect of e-GWG versus ne-GWG were

added to the GAMLSS model. All prenatal and birth data were corrected for gestational age and birth measurements (BW, BL, BHC and PI) were correlated to the fetal parameters in the GAMLSS model.

RESULTS

From the 162 recruited pregnant women, 22 were excluded based on an abnormal fasting glucose measurement (GDM or pre-existent DM) at baseline or on the development of GDM at the 24-28 weeks screening. Seventy-seven percent (17/22) of these were obese. Another 14 pregnancies were excluded for various reasons: one early second trimester miscarriage, 3 premature births, 4 drop-outs and 6 missing longitudinal data (figure 2, flowchart). Therefore, 126 normoglycemic women with pregnancies resulting

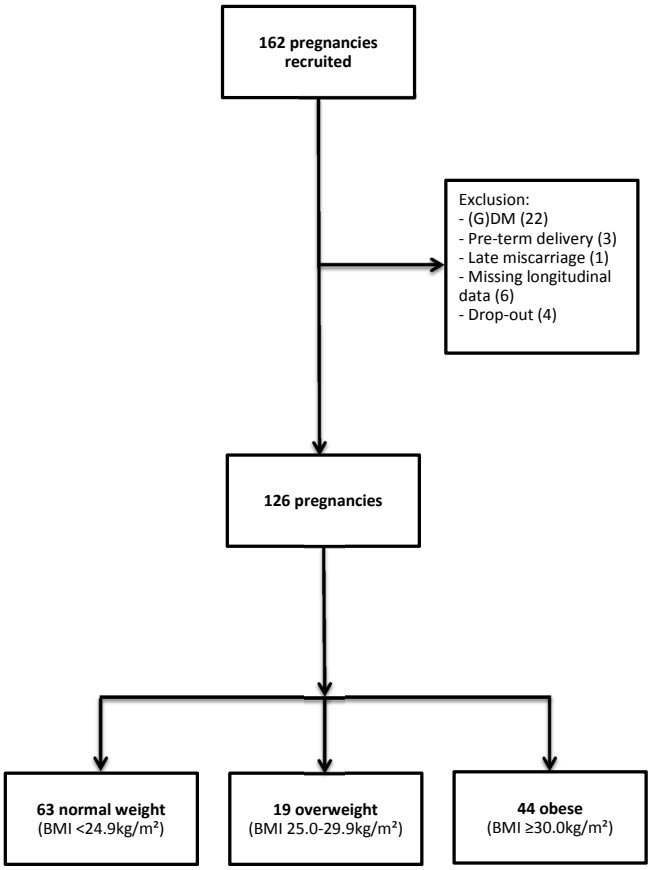

Figure 2. Flowchart of study population. (G)DM= Gestational Diabetes Mellitus; BMI= (pre-gestational) Body Mass Index.

Table 1. Maternal and feto-neonatal demographic data.

	Total (N= 126)	Normal BMI (N= 63, 50%)	Obese (N= 44, 35%)	P-value*	ne-GWG (N= 65, 52%)	e-GWG (N= 61, 48%)	P-value‡
Maternal age (years), mean ± std	29.7 ± 4.3	30.3 ± 3.8	29.0 ± 4.8	0.11	29.4 ± 3.9	30.0 ± 4.6	0.45
Nulliparity	56%	51%	64%	0.19	51%	61%	0.26
Obese	35%	-	100%	-	28%	43%	**0.08**
na-GWG	48%	32%	59%	**0.005**	-	100%	-
GA at birth (wks,0/7d), mean ± std	39w3d ± 1w1d	39w4d ± 1w1d	39w1d ± 1w1d	**0.03**	39w3d ± 1w2d	39w4d ± 1w0d	0.41
Birth weight (g), mean ± std	3404 ± 500	3425 ± 476	3350 ± 525	0.44	3281 ± 522	3534 ± 443	**0.004**
Birth Length (cm), mean ± std	50.2 ± 2.4	50.3 ± 2.1	49.8 ± 2.9	0.29	49.5 ± 2.5	50.9 ± 2.2	**0.002**
Birth HC (cm), mean ± std	34.4 ± 1.5	34.3 ± 1.4	34.4 ± 1.5	0.78	34.0 ± 1.5	34.7 ± 1.4	**0.01**
PI (g/cm^3), mean ± std	0.0269 ± 0.0026	0.0268 ± 0.0025	0.0270 ± 0.0023	0.64	0.0269 ± 0.0027	0.0268 ± 0.0025	0.90
Boys	48%	51%	43%	0.44	42%	56%	0.11

BMI = body mass index; (n)e-GWG = (non-)excessive gestational weight gain; (G)DM= (gestational) diabetes mellitus; GA = gestational age; wks = weeks; d = days; g = gram; cm = centimeter; HC = head circumference; PI = ponderal index (birthweight/birthlength^3); Std. = standard deviation. * P-value represents difference Obese vs Normal BMI; ‡ P-value represents difference e-GWG vs ne-GWG.

in a >34 weeks live birth were included in the final analysis. Of these 63/126 (50%) had a normal pre-pregnancy BMI, 19/126 (15%) were overweight and 44/126 (35%) obese. Sixty-one women (48%) had an e-GWG, according to the 2009-IOM revised criteria. The maternal, fetal and neonatal demographics for the total group are outlined in table 1.

Standard fetal biometry (HC, AC and FL) was measured in all three trimesters (e-figure 5.1a,b-5.2a). There was no difference in the evolution in these standard fetal biometry measurements throughout gestation between the overweight or obese versus the normal BMI group (table 2). There was a significant positive correlation (p= 0.03) between the e-GWG for fetal AC evolution as compared with the ne-GWG group (table 2; figure 3). The fetal liver-length (LL) and fetal body composition measurements are described throughout pregnancy from early in pregnancy and second half to late third trimester respectively (e-figure 5.2b-5.7a,b). The increase in fetal liver size was positively correlated with high maternal BMI (obesity) and e-GWG (respectively p= 0.01 and p= 0.002) when compared to the normal BMI and the ne-GWG group (table 2; figure 4a,b).

Table 2. Longitudinal Fetal Measurements related to maternal BMI and GWG.

Fetal Measurements	Overweight vs Normal BMI	Obese vs Normal BMI	Obese vs overweight&nl BMI	ne-GWG vs e-GWG
Head Circumference (HC)	0.68	0.94	0.85	0.08
Abdominal Circumference (AC)	0.82	0.97	0.89	**0.03**
Femur Length (FL)	0.29	0.13	0.20	0.13
Liver Length (LL)	0.35	**0.01**	**0.02**	**0.002**
Subscapular Fat Tissue (SFT)	0.22	0.08	0.14	**0.007**
Abdominal Fat Tissue (AFT)	0.06	**0.03**	0.07	**0.005**
H-FLMA	0.66	0.52	0.60	0.89
H-FLMA%	0.28	0.47	0.29	0.69
H-FFMA	0.06	0.61	0.39	0.91
H-FFMA%	0.26	0.48	0.29	0.67
F-FLMA	0.10	0.46	0.77	0.30
F-FLMA%	0.48	0.67	0.80	0.93
F-FFMA	0.32	0.27	0.39	0.89
F-FFMA%	0.46	0.68	0.80	0.86

BMI = body mass index; (n)e-GWG = (non-)excessive gestational weight gain; H-FLMA = humerus fetal lean mass area; H-FLMA% = % of total surface of mid-portion transvers section of humerus; H-FFMA = humerus fetal fat mass area; H-FFMA% = % of total surface of mid-portion transvers section of humerus; F-FLMA = femur fetal lean mass area; F-FLMA% = % of total surface of mid-portion transvers section of femur; F-FFMA = femur fetal fat mass area; F-FFMA% = % of total surface of mid-portion transvers section of femur; Significance is considered if $p < 0.05$

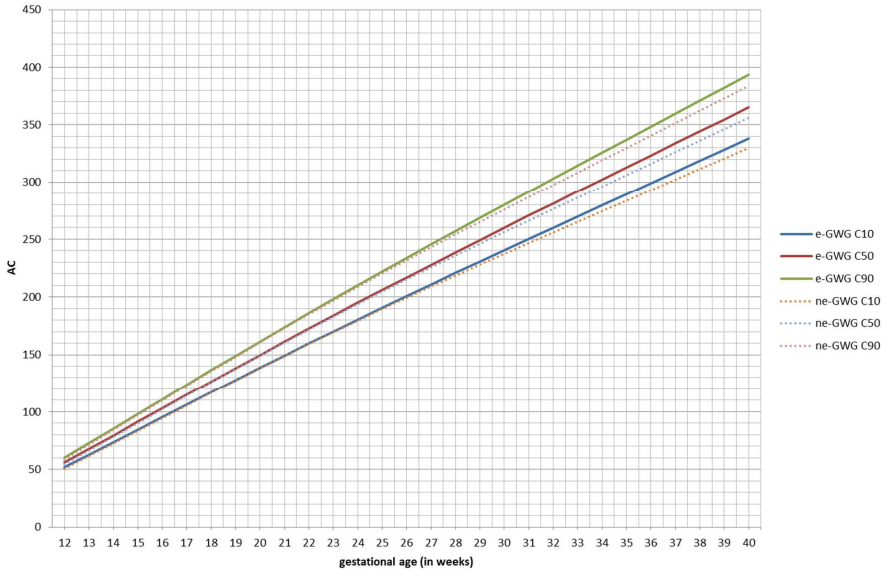

Figure 3. Fetal Abdominal Circumference (AC) in mm and gestational age (x-axis) in weeks. Percentiles 10, 50 and 90 for ne-GWG ('adequate/insufficient' GWG) and e-GWG ('excessive' GWG).

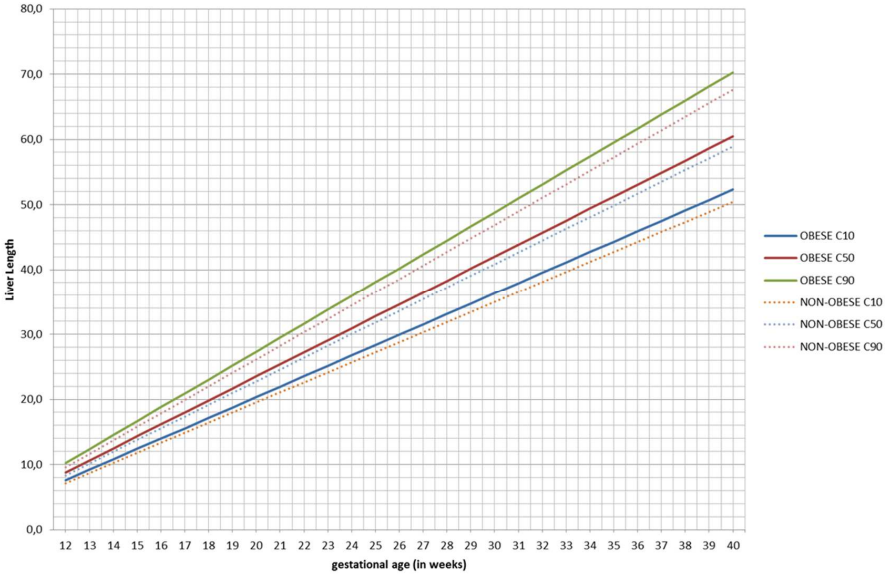

Figure 4.a. Fetal liver length in mm from 12weeks gestational age onwards (x-axis). Percentiles 10, 50 and 90 for obese and normal BMI ('non-obese').

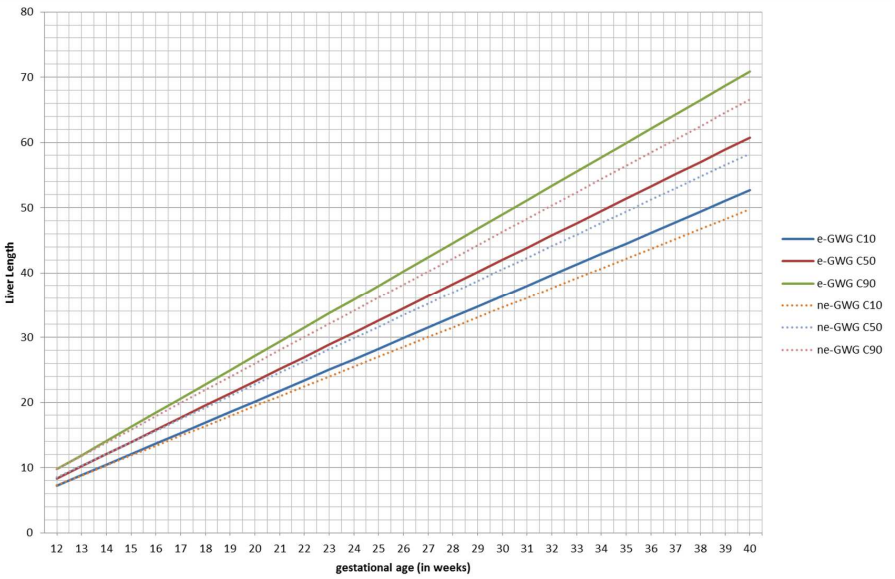

Figure 4.b. Fetal liver length in mm from 12weeks gestational age onwards (x-axis). Percentiles 10, 50 and 90 for ne-GWG ('adequate/insufficient' GWG) and e-GWG ('excessive' GWG).

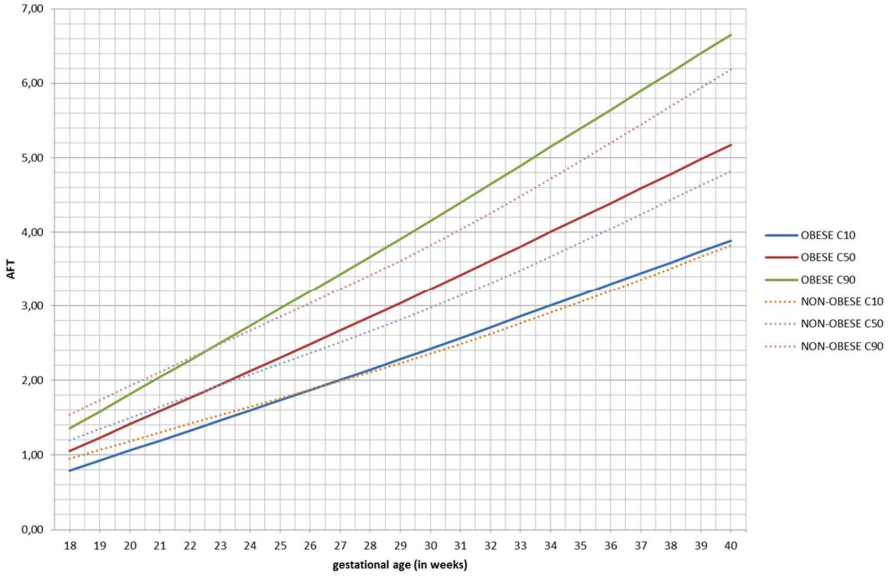

Figure 5.a. Fetal abdominal fat tissue (AFT) in mm from 18weeks gestational age onwards (x-axis). Percentiles 10, 50 and 90 for obese and normal BMI ('non-obese').

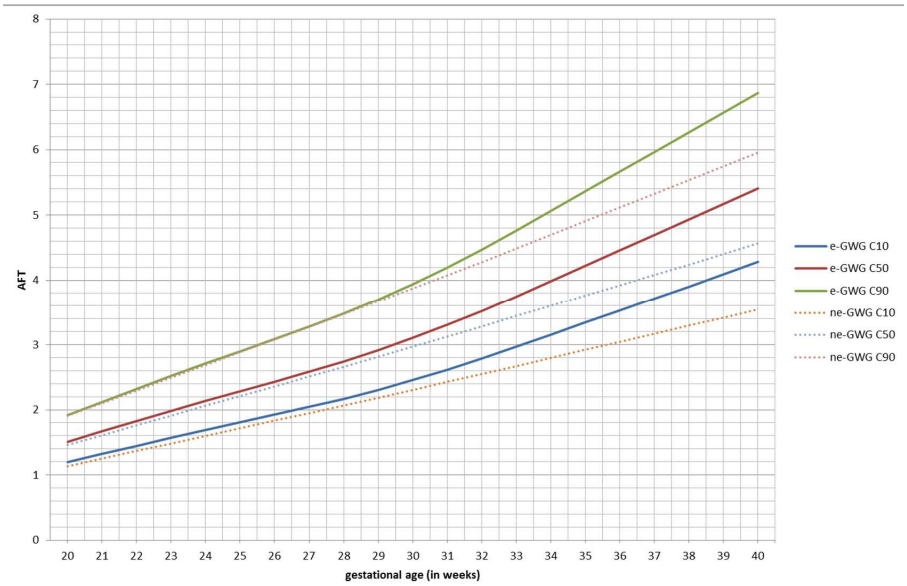

Figure 5.b. Fetal abdominal fat tissue (AFT) in mm from 18weeks gestational age onwards (x-axis). Percentiles 10, 50 and 90 for ne-GWG ('adequate/insufficient' GWG) and e-GWG ('excessive' GWG).

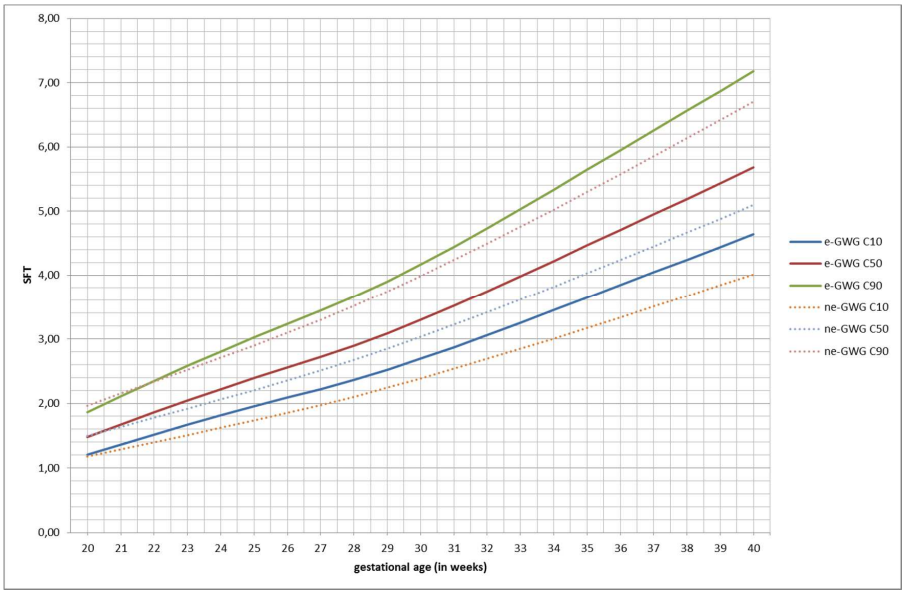

Figure 6. Fetal subscapular fat tissue (SFT) in mm from 18weeks gestational age onwards (x-axis). Percentiles 10, 50 and 90 for ne-GWG ('adequate/insufficient' GWG) and e-GWG ('excessive' GWG).

For the peripheral fetal fat- and lean mass measurements of the extremities (humerus and femur) there were no differences between the different pre-gestational BMI and weight gain groups (table 2). For abdominal fat deposition, AFT positively correlated with maternal BMI (table 2; figure 5a). In the e-GWG group the fetal central fat deposition increased significantly in pregnancy as shown by an increase for both AFT and SFT (table 2; figure 5b, 6).

Neonatal weight, -length and head-circumference did not differ significantly between the separate BMI groups, but were different between the two GWG groups (table 1). The PI on the other hand did not differ between BMI and GWG groups. The obese group delivered significant earlier than the normal BMI group (p= 0.03), but the difference can be considered clinically irrelevant (39w1d±1w1d versus 39w4d±1w1d respectively).

DISCUSSION

This longitudinal study in normoglycemic women offers interesting new insight in the differential effects of maternal obesity and e-GWG on fetal body composition during pregnancy. Additionally, we provide information of the evolution in fat content for specific central and peripheral fetal compartments during pregnancy, and how maternal obesity and e-GWG affect these. Fetal (central) adiposity emerged from 22 weeks gestation onwards.

While maternal obesity specifically promoted the fetal liver size and abdominal subcutaneous fat accumulation, the excessive GWG also affected the fetal abdominal circumference and subscapular fat thickness. Excessive GWG certainly correlated well with an increased fetal size at birth (higher birthweight, -length and headcircumference). The ponderal index, a neonatal body proportionality parameter, on the other hand was not influenced by the maternal pre-pregnancy weight or weight gain during pregnancy. Since both birth-weight and birth-length were increased, leaving the resultant of the PI equation balanced. In contrast, peripheral fat- and lean mass were not influenced by pre-gestational BMI or GWG.

Intrauterine growth is the result of genetic potential, substrate availability and endocrine regulation that will determine the growth trajectory during intrauterine life.[21] Most fetal weight is gained during the second half of gestation, when the pattern of fetal growth is accompanied by a large and exponential deposition of fat tissue. Fetal fat mass growth is previously reported to correlate with the intrauterine environment, whereas fat-free mass shows stronger relationships with genetic factors.[22,23] Evaluation of the nutritional state *in utero*, by estimating fetal body composition, might improve the prediction of short-term perinatal risks (related to fetal adiposity) in conditions of under- and overgrowth.[10] But the intra-uterine development of the fetus might also af-

fect long-term outcomes such as cardiovascular diseases.[24] Anthropometric ultrasound measurements, indicative of fetal body composition of normal fetuses, have shown an unique exponential pattern of the growth profile during the second half of gestation, both in lean mass and in fat mass.[15,16] This suggests that the measurement of fetal fat could provide a more sensitive and specific marker of abnormal fetal growth or development in different extra-uterine nutritional circumstances like in high maternal BMI, (n) e-GWG and (G)DM.[25,26]

In previous studies it has been demonstrated that obesity, e-GWG and (G)DM are associated with abnormal fetal growth, birth-weight and perinatal outcome.[6,27-30]

Studies on the influence of abnormal maternal glucose metabolism, obesity and GWG often report on neonatal outcomes, where birth-weight and neonatal body composition is examined as a resultant of fetal growth.[31,32] Not so many reports have focussed on the fetal period, while this represents the period of fastest growth in life.[32] Cross-sectional studies focusing at the late third trimester, exist, but longitudinal studies on fetal anthropometry (standard biometry and fat- and lean mass development) in a normal glucose tolerance population are scarce or non-existing.[10,34,35]

Rigano et al report on a measurement of fetal abdominal wall thickness in a small group with a normal (n=25) and abnormal (n=45) glucose challenge test, that was followed up prospectively.[36] The abnormal group received either treatment with diet only or in combination with insulin. The fetal abdominal wall thickness at recruitment differed between the GDM group and non-GDM group and they suggested that the treatment probably reduced the growth rate of the abdominal wall thickness later in pregnancy. While we excluded all glucose intolerant women, we still found a sustained difference of fetal abdominal fat thickness from recruitment throughout pregnancy in higher maternal BMI and GWG. In a cross-sectional study, Larciprete et al. reported on fetal subcutaneous fat thickness during pregnancy in a high risk population and a GDM group.[9] They confirmed the relatively small differences between GDM foetuses and controls, as reported by Rigano and colleagues. This could be explained by the fact that GDM women are being tightly controlled after the diagnosis of GDM. In an elegant study, Parretti et al. studied the role of abnormal glucose tolerance on fetal growth and body composition across gestation in a non)obese population.[36] Higher fetal fat measurements were found in the abnormal GCT group, while fetal lean mass did not differ. Also, they found a positive association between the 1hr postprandial glucose value and fetal fat development, suggesting that even in minor glucose alterations fetal development changes with higher deposition of fat tissue in the fetus. Unfortunately they did not examine an obese population. Although we excluded all glucose intolerant women, we included obese women in our study cohort with fetuses who showed increased fetal liver size and abdominal fat thickness. Hence we showed the increase of these insulin sensitive tissues in the fetuses of obese (and e-GWG) women. This could indicate that

even in a normoglycemic population obesity and e-GWG sensitize the fetal insulin sensitive tissues. Obesity and e-GWG are important reflections of maternal nutritious status and hence the nutritional supply for the unborn child.[38] They may alter the epigenome of the fetus and influence the phenotype at birth and subsequently the metabolic profile, predisposing them to diseases in later life. Central adiposity is one of the phenotypes associated with increased risk of insulin resistance, hypertension and cardio-vascular diseases.[39] In our study the e-GWG in particular, but also obesity affected central fetal adiposity (liver size, abdominal circumference and truncal fat measurements), potentially putting the fetus at risk for an aberrant metabolic phenotype. Although long term follow-up was not part of our study.

Particularly the fetal liver size, since it can be monitored from early gestation onwards (late first trimester), could potentially be a useful tool to detect fetal adiposity in an altered nutritional environment *in utero*. This increased fetal liver development could be the first *in utero* sign of a later pediatric non-alcoholic fatty liver disease (NAFLD).[40,41] Since we lack fetal liver enzymes from cord-serum and liver ultrasonography even in adults has poor agreement on NAFLD diagnosis, this remains merely speculative, but warrants further studies. The additional effect of e-GWG by enhancing the fetal AC development and SFT deposition, together with the distinct birth-outcome for weight and length stresses the importance of GWG on fetal growth and body proportions. One explanation for the different effect on fetal AC, which in its measurement contains not only liver and AFT, but also visceral fat tissue, could be the additional effect of GWG on fetal visceral fat deposition. Especially the relation of maternal nutritional intake, GWG and fetal metabolic (fat) tissue development needs further investigation.[42]

Strengths of our study include the relative large size sample, the longitudinal design throughout pregnancy and the fact that measurements were both performed in both the mother and the fetus. This allowed us to stratify for pre-gestational BMI and GWG. All pregnancies had a first trimester scan, that allowed us to standardize the gestational age determination.[13] Additionally, all patients had a normal glucose tolerance with a clearly defined protocol to exclude pre-gestational DM and the development of GDM. It has been demonstrated that fetal hyperinsulinemia results from transplacental passage of maternal hyperglycemia and therefore influencing fetal growth and fat accumulation.[3,5] Fetal lean- and fat mass measurements, were based on a previously described protocol and were validated in other studies.[10,37] All measurements were performed by a single operator, excluding inter-operator variability. Furthermore, the complexity of longitudinal fetal growth is tackled by the GAMLSS analysis, a statistical technique advised by the WHO Multicentre Growth Reference Study Group for the construction of growth standards.[19,20]

Some limitations have to be mentioned. The excessive GWG classification is based on the revised 2009-IOM criteria.[8] In the non-excessive group both adequate and insufficient

GWG are included. When insufficient weight gain is present, an increased incidence of SGA and decreased neonatal fat mass was reported in overweight and obese women.[43] It is possible that our non-excessive GWG group was influenced by the insufficient GWG group, although they only formed 22/126 (17.5%) of our cohort. Still, this may have yielded an increased difference of the fetal development in the e-GWG and ne-GWG mother. Furthermore, some of the obese participants were involved in a lifestyle intervention study to prevent the development of gestational diabetes.[11] This also explains the somewhat higher percentage of obese pregnancies, as compared to the general population. Consequently, the GWG in the obese pregnancies may be slightly lower than expected. The aim of our study was to examine early fetal discriminants related to maternal factors (obesity and GWG) that have the potential to influence fetal development and hence birth outcome. Although fetal liver and abdominal circumference can be investigated as early as in late first trimester, the other fetal parameters (AFT, SFT and humerus and femur fat- and lean mass), can only be reliably examined from 20 weeks onwards. This forms a general limitation not specific for our study, although our study is the first to our knowledge to examine these parameters from these early gestational periods. Further studies to examine the outstanding fetal discriminants (LL, SFT, AFT and AC) as predictors for neonatal adiposity are underway.

Our longitudinal study determined early signs of fetal adiposity associated with maternal obesity and particularly e-GWG in normoglycemic women. Maternal high BMI correlated with fetal liver size and abdominal fat, where e-GWG additionally correlated with increased subscapular fat and abdominal circumference development. Furthermore, fetal adiposity emerged from as early as 20 weeks as a reflection of a nutritious environment *in utero*. Especially the indicators of the fetal trunk (liver-length, AFT, SFT and AC) are discriminative and warrant further investigation, as potential early predictors of neonatal adiposity and long-term offspring metabolic risk.

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ABDOMINAL AORTA FETAL ABDOMINAL
INTIMA MEDIA THICKNESS FETAL FAT
FUNCTIONAL LINEAR DISCRIMINANT
ADDITIVE MODEL LOCATION, SCALE AND
MELLITUS NON-EXCESSIVE GESTATIONAL
GROWTH FACTOR INTIMA-MEDIA THICKNESS
SOCIETY ON OBSTETRICS AND
GROWTH LARGE-FOR-GESTA
NUTRITION EPIGENOME
GROWTH STUDY GROUP
IMAGING MRI FATTY LIVER DISEASE
GLUCOSE TOLERANCE TEST OGTT ODDS RATIO
ARTERIAL STIFFNESS QUALITY
BLOOD PRESSURE SKINFOLD TRICEPS
FAT THICKNESS SMALL-FOR-GESTATIONAL AGE
VASCULAR AND BIOMETRY ULTRASOUND
STUDY VABULOS YOUNG ELASTIC MODULUS

Chapter 6

Neonatal adiposity is predicted by fetal central fat accumulation *in utero*, a prospective cohort study in normoglycemic women

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Submission in progress

ABSTRACT

Introduction

Neonatal adiposity is the result of *in utero* development, significantly influenced by parental constitution, maternal pre-pregnancy weight, gestational weight gain (GWG) and glucose metabolism. Our aim was to detect early antenatal predictors for neonatal adiposity in normoglycemic women.

Methods

One hundred and sixty-two women were recruited in their first trimester and prospectively followed-up for maternal weight gain and fetal-neonatal biometry and anthropometry. Exclusion criteria included: pre-existing diabetes mellitus (DM), development of gestational DM, premature delivery and missing birth outcome. Total GWG was categorized according to the revised criteria of the Institute of Medicine. Birth outcome measurements, obtained within 48hrs, included neonatal anthropometry measurements (skinfolds and circumferences of abdomen, lower- and upper extremity), from which neonatal fat mass (% NFM) and the ponderal index (PI) were calculated. Generalized Additive Model Location, Scale and Shape (GAMLSS) was used to align the timeframes of the antenatal measurements and to correlate them with birth outcome parameters and NFM.

Results

One hundred and twenty-one pregnancies in normoglycemic women were included in the final analysis. The mean NFM at birth was 11.0% (SD±3.4%), with a cut-off of 15% for the 90th percentile (P90). No association was found between the maternal pre-pregnancy body mass index (BMI) and NFM. However, children born to mothers who had an excessive GWG (e-GWG, 59/121=49%) had a significantly higher NFM (11.6%) as compared to the non-excessive GWG mothers (10.4%, p=0.045). Fetal measurements were highly correlated with neonatal measurements. Fetal abdominal circumference (AC, p=0.04), liver length (LL, p=0.03), subscapular- and abdominal fat thickness (p<0.001), humerus (p<0.001) and femur fat mass (p<0.001) development correlated significantly with 90th NFM percentile. Fetal abdominal fat thickness (AFT) did not (p=0.25) correlate with the PI.

Conclusion

In the normoglycemic women, e-GWG was associated with an increased neonatal adiposity (>P90 NFM). The neonatal PI and high adiposity were preceded by an increased development of fetal central- and peripheral fat mass parameters. The fetal abdominal development (AC, LL), reflecting fetal central adiposity, correlated significantly to the

90th percentile of neonatal fat mass, showing their potential role as early predictors for neonatal fat deposition.

INTRODUCTION

In *utero* development is the fastest growth trajectory a human will ever experience in life. It has been demonstrated that the prenatal period is vulnerable to environmental factors and parental constitution, both leading to epigenetic effects responsible for diseases that occur in later life.[1] This is called the developmental origins of health and diseases or fetal/developmental programming.[2,3] The environmental factors can cause fetal overgrowth or growth restriction depending on increased or decreased transplacental nutrients transfer. Pregnancy related factors include maternal pre-gestational body mass index (BMI), excessive gestational weight gain (GWG) and abnormal glucose tolerance (gestational diabetes mellitus, GDM).[4-6] The influence of these three maternal determinants, are associated with an 25% increased incidence of higher birth-weight and increased perinatal complications like abnormal fetal growth, shoulder dystocia, caesarean section rates and fetal death.[7-10]

Studies of fat- and lean mass parameters at birth have mostly been performed on glucose intolerant populations to study their association with gestational glucose levels.[8] Antenatal ultrasound studies, focusing on fetal body composition ('fetal adiposity'), are often undertaken cross-sectionally in the third trimester and (again) in GDM populations.[11-14] Longitudinal prenatal adiposity measurements in relation to neonatal body composition ('birth adiposity') in normal glucose tolerant population sare therefore scarce, and if available, they concern mostly single sonographic markers of fetal fat deposition.[15-17] We suggested in chapter 5 of this thesis after investigating a wide range of antenatal fetal body characteristics, that fat features of the trunk and fetal liver size could serve as sensitive markers of abnormal fetal adiposity.[18] Our aim was to correlate these markers in a prospective study in normoglycemic women with neonatal outcome measurements in order to detect specific (early) antenatal predictors for neonatal adiposity. This could help to elucidate the mechanisms of an aberrant metabolic start in life.

DESIGN AND METHODOLOGY

This prospective study was performed at the Department of Obstetrics and Gynecology of the University Hospitals Leuven (Belgium). Pregnant women of different pregestational BMI classes were recruited at their first trimester ante-natal visit between January 2012 and August 2014. This sub-study was part of three prospective studies for

low-risk, obese and non-obese pregnancies monitored for GWG, development of GDM and nutrition.[19] All studies were approved by the Ethics Committee for Human Experimentation of the Faculty of Medicine, Katholieke Universiteit Leuven (S52172, S54026 and S55325). All women have signed written informed consent to take part in the study. Exclusion criteria for the sub-study were: pre-existing diabetes (DM) or development of GDM, multiple pregnancies, premature delivery (<34 weeks), missing longitudinal data and adverse events (major fetal abnormality, fetal death and/or immature birth).

Maternal measurements

At their first ante-natal visit, parameters for BMI (height and pre-pregnancy weight) were obtained as described elsewhere.[18] The BMI was categorized as normal weight (≤24.9 kg/m^2), overweight (25-29.9 kg/m^2) and obese (>30kg/m^2) as defined by the WHO guidelines. The total GWG gain was computed using the final weight measured at the last prenatal visit, minus the pre-gestational weight. GWG was categorized according to the Institute of Medicine (IOM) 2009 revised recommendations: 11.5-16kg (normal weight), 7.0-11.5kg (overweight) and 5.0-9.0kg (obese).[20] If women crossed the upper IOM threshold, they were considered excessive-GWG (e-GWG) or non-excessive (ne-GWG) if they had either adequate or insufficient total GWG. If women were considered high risk (history of GDM, BMI≥30kg/m^2), a fasting glucose measurement <92mg/dL (<5.1mmol/L) at baseline was used to exclude pre-existing DM. Another screening was performed at 24-28 weeks using a 75g oral glucose tolerance test (OGTT) based on the 2013 World Health Organization (WHO) criteria.[21] The non-obese population underwent an OGTT if the initial 50g glucose challenge test showed a glycemia of ≥140mg/dL (≥7.8mmol/L) at 24-28 weeks (local protocol).

Fetal measurements

Determination of gestational age (GA) was based on the crown rump length (CRL) measured between 7 and 14 weeks of gestation in all patients.[22] Ultrasound examinations were performed at three time-points in gestation: 12-18 weeks, 26-32 weeks and between 35-37 weeks of gestation. A proportion (48 participants) was also examined between 20-22 weeks of gestation. Fetal measurements (head circumference -HC-, abdominal circumference –AC-, and femur length –FL-) were performed in accordance to the International Society for Ultrasound in Obstetrics and Gynecology (ISUOG, www.isuog.org) guidelines as described elsewhere.[18,23] Additional measurements of fetal body composition (lean- and fat mass) and fetal liver length (LL) were obtained as previously described.[18,24] All fetal measurements were performed by a single operator (SG) on a high resolution real-time B-mode ultrasound system (E6, E8 or 730-Expert Voluson®, General Electric healthcare Medical systems, Kretztechnik, Zipf, Austria) with a convex, high frequency, 4-8 MHz volumetric 3D abdominal probe.

Neonatal measurements

Neonatal measurements were obtained within 48hrs after birth as described formerly.[19] In summary, the birth measurements consisted of birth-weight (BW), birth-length (BL), head circumference (BHC), abdominal circumference (BAC) and skinfold (SF) measurements of the flank (Fl-SF), triceps (Tr-SF), subscapular (S-SF) and thigh (Th-SF) with a Harpenden® skinfold calliper. The skinfolds were obtained using a strict protocol and measured at least two times and a third measurement was necessary, if there was a difference of >0.2mm between the first two performances. The mean of these measurements was recorded.[19] The circumferences of the upper arm and -leg and lower arm and -leg were also obtained.[19] Neonatal body composition measurements were performed by the same operator (SG). Different neonatal body composition algorithms were calculated as an end-point of the prenatal development measures: BW, BL, BHC, BW/BL, BW/BL^2 and the ponderal index (PI). The PI was calculated by the weight divided by the cubic root of the length (birthweight/$length^3$) and can be regarded as a neonatal proportionality index in line with the adult BMI.[25] The neonatal fat mass (NFM) was calculated using the following (validated) equation[26]: 0.39055 (BW, kg) + 0.0453 (Fl-SF, mm) – 0.03237 (BL, cm) + 0.54657. The percent of body fat is fat mass/BWx100. Total BW minus fat mass was calculated as lean-mass.

Statistical analysis

GAMLSS (Generalized Additive Models for Location, Scale and Shape, www.gamlss.org) R-package was applied to construct the growth curves for conventional fetal biometry measurements: HC, AC and FL.[27,28] We assessed several distributions: Box-Cox-t, Box-Cox Cole and Green, and Box-Cox Power Exponential. Goodness-of-fit of the models was assessed with QQ plots, AIC (Akaike Information Criterium) and worm plots. The goodness-of-fit was investigated covering the gestational period of 12-40 weeks, and for substrata of this period. GAMLSS smoothened the ante-natal growth curves for all prenatal measurements. Percentiles (P10, P50 and P90) for each parameter were constructed. Only complete consecutive fetal- and neonatal data-sets were analyzed. The effect of BMI (normal weight, overweight and obese) and the effect of e-GWG versus ne-GWG were added in the GAMLSS model. The NFM (%) at birth was correlated in the GAMLSS model as a continuous variable and at the cut-off points of 10, 12.5 and 15% for the development of all prenatal measurements. Also the birth proportionality measurements BW, BL, BHC, BW/BL, BW/BL^2 and PI, were correlated in the GAMLSS model for the development of all prenatal measurements. All prenatal and birth data were corrected for gestational age.

RESULTS

From the 162 recruited pregnant women, 22 were excluded based on an abnormal fasting glucose measurement (pre-existing DM/GDM) at baseline or the development of GDM at the 24-28 weeks screening. Seventy-seven percent (17/22) of the excluded women were obese. Another 19 pregnancies were excluded for various reasons: one early second trimester miscarriage, 3 premature births, 4 drop-outs and 11 missing longitudinal data (figure 1, flowchart). Therefore 121 normoglycemic women with pregnancies resulting in a >34 weeks live birth were included in the final analysis. Of these 59 (49%) had a normal pre-pregnancy BMI, 19 (16%) were overweight and 43 (35%) were obese. Sixty women (50%) had an e-GWG, according to the 2009-IOM revised criteria. The maternal, fetal and neonatal demographics are outlined in table 1. The fetal growth

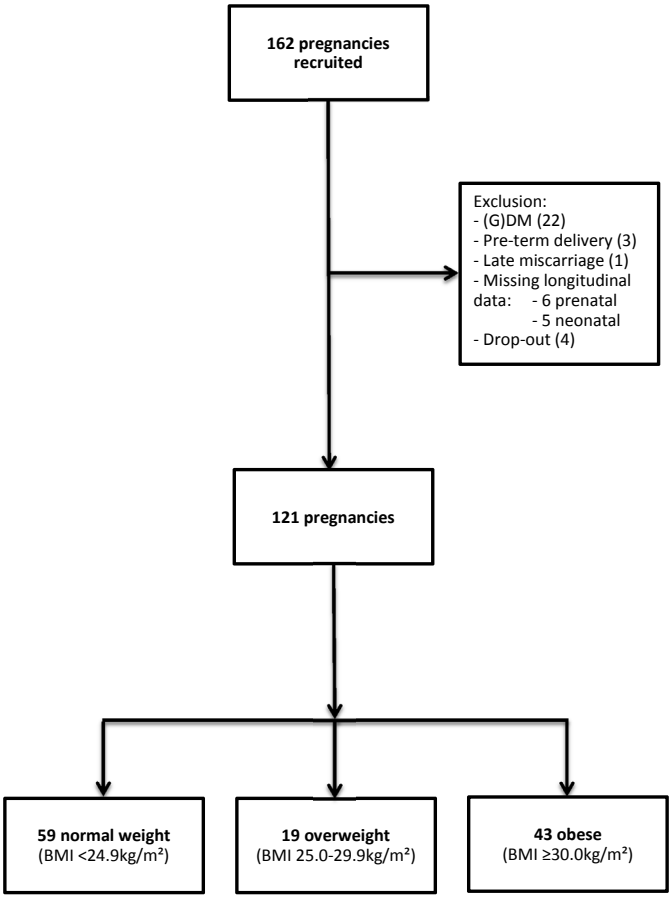

Figure 1. Flowchart of study population. (G)DM= Gestational Diabetes Mellitus; BMI= (pre-gestational) Body Mass Index.

Table 1. Maternal baseline characteristics.

	Total (N= 121)
Maternal age (years), mean ± std	29.7 ± 4.1
Nulliparity (%)	67 (55%)
Obese BMI (%)	43 (35%)
Overweight BMI (%)	19 (16%)
Normal weight BMI (%)	59 (49%)
Excessive GWG (%)	60 (50%)
GA at birth (wks,0/7d), mean ± std	39w3d ± 1w2d
Birth weight (g), mean ± std	3396 ± 499
Birth Length (cm), mean ± std	50.2 ± 2.4
Birth HC (cm), mean ± std	34.3 ± 1.5
Boys (%)	59 (49%)

BMI = body mass index; GWG = gestational weight gain; GA = gestational age; wks = weeks; d = days; g = gram; HC = head circumference; std. = standard deviation.

and body composition measurements throughout gestational age are shown in chapter 5 (e-figures 5.1-5.7, e-tables 5.1-5.6). The birth measurements are shown in e-figures 6.1-6.7. The mean NFM at birth was 11.0% (SD±3.4%) and mean neonatal lean mass was 90% (SD±5.4%). Of the neonates 81/121 (66.9%) had a NFM percentage of >10%, 41/121 of >12.5% and 14/121 of >15% (figure 2). The fifteen percent NFM represented the 90th percentile (P90). Maternal BMI did not correlate with the NFM (p=0.25). A NFM of 11.1%, 11.0% and 10.8% corresponded to the normal, overweight and obese BMI class, respectively. However, children born to mothers who showed an e-GWG (49%) had a significantly higher NFM (11.6%) compared to the ne-GWG mothers (10.4%, p=0.045).

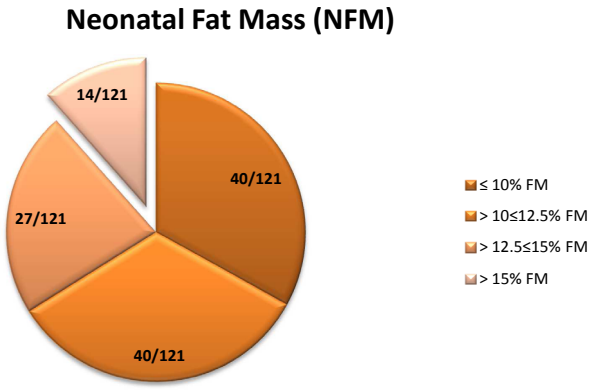

Figure 2. Neonatal fat mass (NFM) in % of total birth weight for 121 neonates. Fat mass (FM) ≤10% (40/121), >10≤12.5% (81/121), >12.5≤15% (41/121) and >15% (14/121=90th percentile) of total birth weight.

Vice versa, the lean-mass percentage was lower in e-GWG mothers (89%) versus the ne-GWG mothers (90%).

The development of all fetal parameters (biometry and body composition) correlated with the BW, BL, BHC and the proportionality indices BW/BL and BW/BL^2 (table 2). The development of fetal AC (p=0.04), LL (p=0.03), SFT (p=0.02), humerus fat mass (p=0.002) and femur lean- (p<0.001) and fat mass (p<0.001) correlated significantly with the PI (table 3). The AFT did not show a correlation with PI (p=0.25). The development of all fetal parameters (biometry and body composition) correlated highly with the NFM, as a continuous variable (table 3).

Table 2. Longitudinal Fetal Measurements related to neonatal outcome.

	Neonatal Measurements					
Fetal Measurements	**BW**	**BL**	**BHC**	**Weight/ length**	**Weight/ length^2**	**Weight/ length^3 (PI)**
Head Circumference	<0.001	<0.001	<0.001	<0.001	0.002	0.56
Abdominal Circumference	<0.001	<0.001	<0.001	<0.001	<0.001	**0.04**
Femur Length	<0.001	<0.001	0.005	<0.001	0.01	0.50
Liver Length	<0.001	<0.001	<0.001	<0.001	<0.001	**0.03**
Subscapular Fat Tissue	<0.001	<0.001	<0.001	<0.001	<0.001	**0.02**
Abdominal Fat Tissue	<0.001	<0.001	<0.001	<0.001	<0.001	0.25
H-FLMA	<0.001	<0.001	<0.001	<0.001	<0.001	0.06
H-FLMA%	0.008	0.15	0.10	0.001	0.001	0.09
H-FFMA	<0.001	<0.001	<0.001	<0.001	<0.001	**0.002**
H-FFMA%	0.008	0.15	0.09	0.001	0.001	0.08
F-FLMA	<0.001	0.02	<0.001	<0.001	<0.001	**<0.001**
F-FLMA%	0.34	0.89	0.47	0.26	0.22	0.25
F-FFMA	<0.001	<0.001	<0.001	<0.001	<0.001	**<0.001**
F-FFMA%	0.28	0.85	0.44	0.23	0.22	0.29

BW = birth weight; BL = birth length; BHC = birth head circumference; ponderal index = PI; H-FLMA = humerus fetal lean mass area; H-FLMA% = % of total surface of mid-portion transvers section of humerus; H-FFMA = humerus fetal fat mass area; H-FFMA% = % of total surface of mid-portion transvers section of humerus; F-FLMA = femur fetal lean mass area; F-FLMA% = % of total surface of mid-portion transvers section of femur; F-FFMA = femur fetal fat mass area; F-FFMA% = % of total surface of mid-portion transvers section of femur; Significance is considered if p <0.05

Table 3. Longitudinal Fetal Measurements related to birth measurements.

Fetal Measurements	NFM% at 40weeks (n= 121)				BAC	Uparm	Loarm	Upleg	Loleg	TrSF	SSSF	FlSF	ThSF
	Continuous variable	>10% (81/121)	>12.5% (41/121)	**>15%** (14/121)									
Head Circumference	0.01	0.18	0.10	0.09	0.003	0.37	0.02	0.03	0.006	0.64	0.05	0.41	0.14
Abd. Circumference	<0.001	<0.001	<0.001	**0.04**	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	0.002	<0.001
Femur Length	0.006	0.09	0.13	0.23	<0.001	<0.001	<0.001	<0.001	<0.001	0.02	0.003	0.03	<0.001
Liver Length	<0.001	0.001	0.005	**0.03**	<0.001	0.009	0.04	0.01	<0.001	0.35	0.002	0.005	0.007
Subscap. Fat Tissue	<0.001	<0.001	<0.001	**<0.001**	<0.001	<0.001	0.005	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Abd. Fat Tissue	<0.001	<0.001	<0.001	**<0.001**	<0.001	<0.001	0.06	<0.001	<0.001	0.08	<0.001	<0.001	<0.001
H-FLMA	<0.001	<0.001	0.001	0.17	<0.001	<0.001	<0.001	<0.001	<0.001	0.02	0.01	0.09	0.04
H-FLMA%	<0.001	0.03	0.04	<0.001	0.03	0.10	0.98	0.005	0.008	0.009	<0.001	<0.001	<0.001
H-FFMA	<0.001	<0.001	<0.001	**<0.001**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
H-FFMA%	<0.001	0.03	0.04	<0.001	0.03	0.09	0.96	0.005	0.008	0.008	<0.001	<0.001	<0.001
F-FLMA	<0.001	<0.001	<0.001	**<0.001**	<0.001	<0.001	0.01	<0.001	<0.001	0.06	<0.001	0.005	<0.001
F-FLMA%	0.11	0.27	0.97	0.57	0.25	0.19	0.42	0.14	0.40	0.12	0.07	0.78	0.008
F-FFMA	<0.001	<0.001	<0.001	**<0.001**	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.008	<0.001
F-FFMA%	0.08	0.23	0.95	0.56	0.26	0.17	0.42	0.14	0.38	0.11	0.07	0.84	0.007

NFM = neonatal fat mass; BAC = birth abdominal circumference; Uparm = Upper arm; Loarm = Lower arm; Upleg = Upper leg; Loleg = Lower leg; TrSF = Triceps skinfold; SSSF = Subscapular skinfold; FlSF = Flank skinfold; ThSF = Thigh skinfold; H-FLMA = humerus fetal lean mass area; H-FLMA% = % of total surface of mid-portion transvers section of humerus; H-FFMA = humerus fetal fat mass area; H-FFMA% = % of total surface of mid-portion transvers section of humerus; F-FLMA = femur fetal lean mass area; F-FLMA% = % of total surface of mid-portion transvers section of femur; F-FFMA = femur fetal fat mass area; F-FFMA% = % of total surface of mid-portion transvers section of femur; Significance is considered if $p < 0.05$

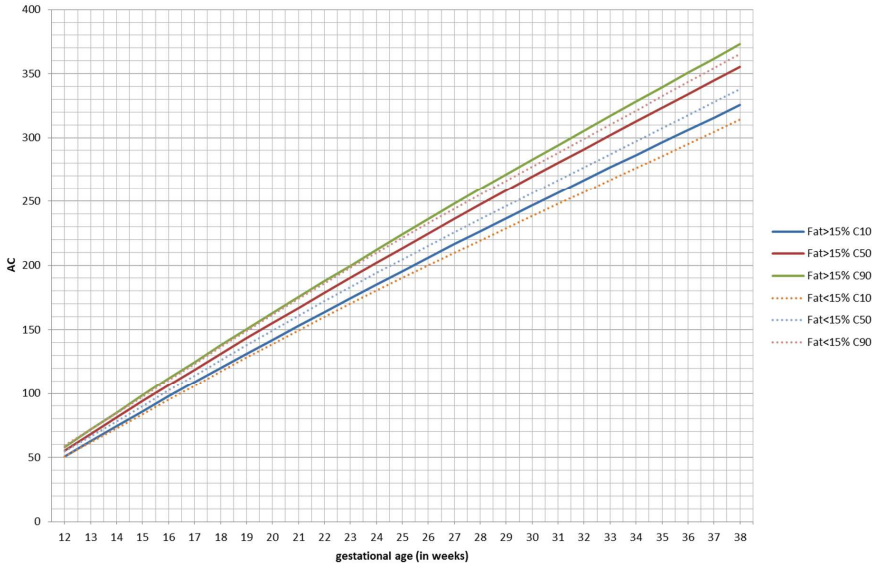

Figure 3.a. Fetal abdominal circumference (AC) in mm from 12 weeks gestational age onwards (x-axis). Percentiles 10, 50 and 90 for neonatal fat mass >15% (P90) and ≤15%.

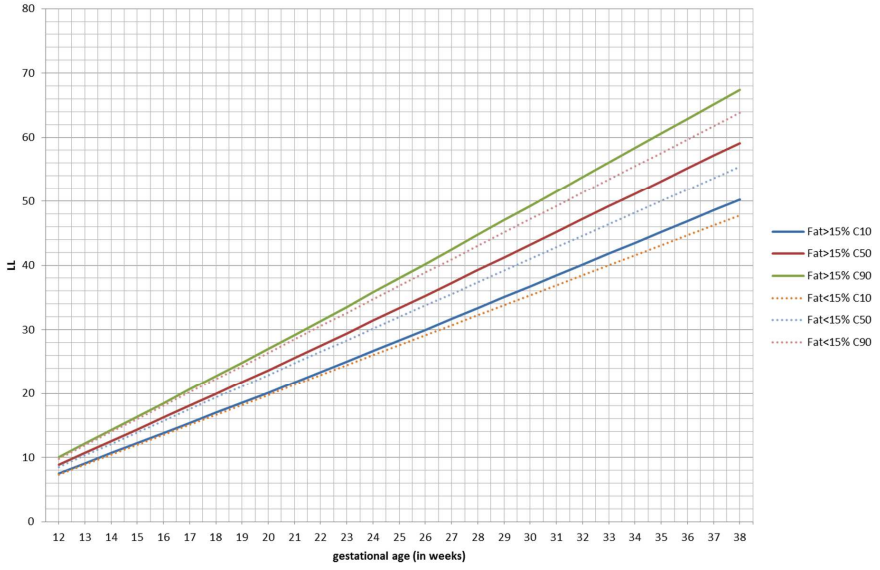

Figure 3.b. Fetal liver length in mm from 12 weeks gestational age onwards (x-axis). Percentiles 10, 50 and 90 for neonatal fat mass >15% (P90) and ≤15%.

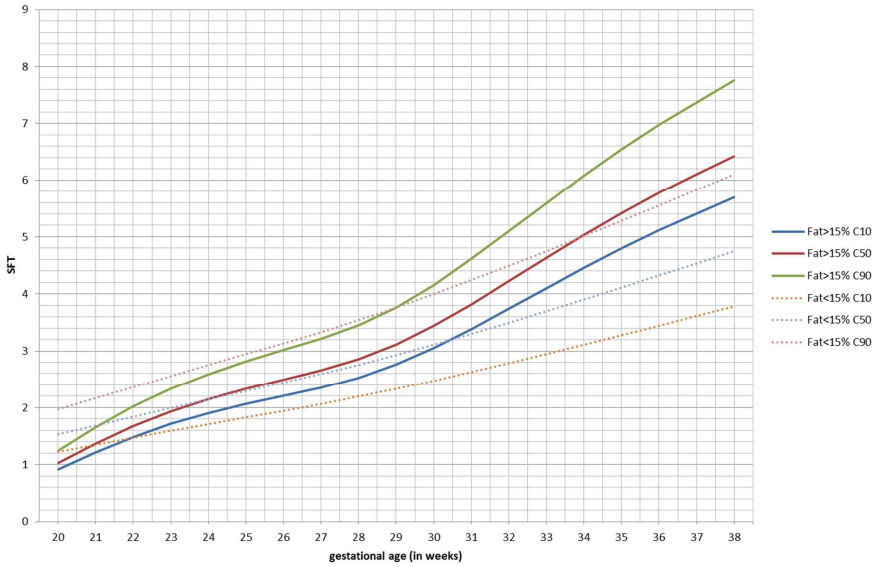

Figure 4.a. Fetal subscapular fat thickness (SFT) in mm from 20 weeks gestational age onwards (x-axis). Percentiles 10, 50 and 90 for neonatal fat mass >15% (P90) and fat mass ≤15%.

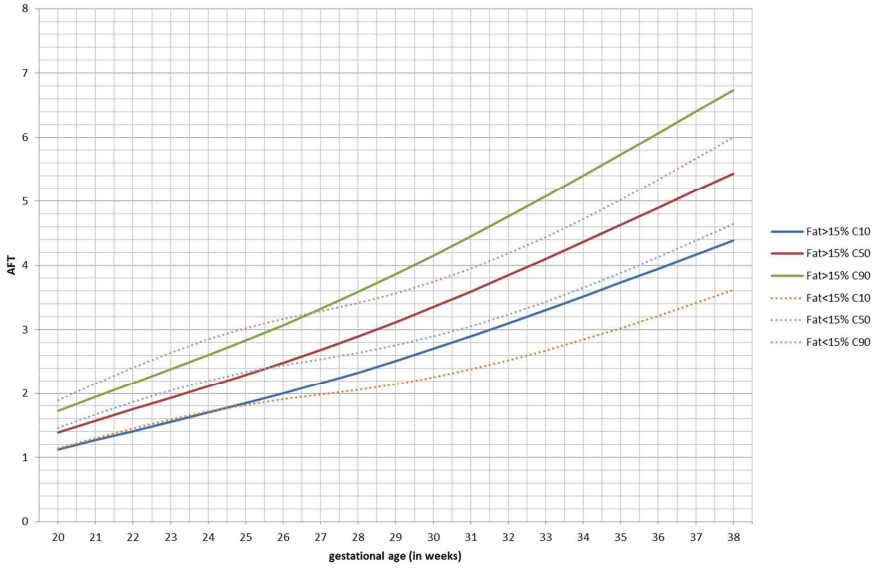

Figure 4.b. Fetal abdominal fat thickness (AFT) in mm from 20 weeks gestational age onwards (x-axis). Percentiles 10, 50 and 90 for neonatal fat mass >15% (P90) and fat mass ≤15%.

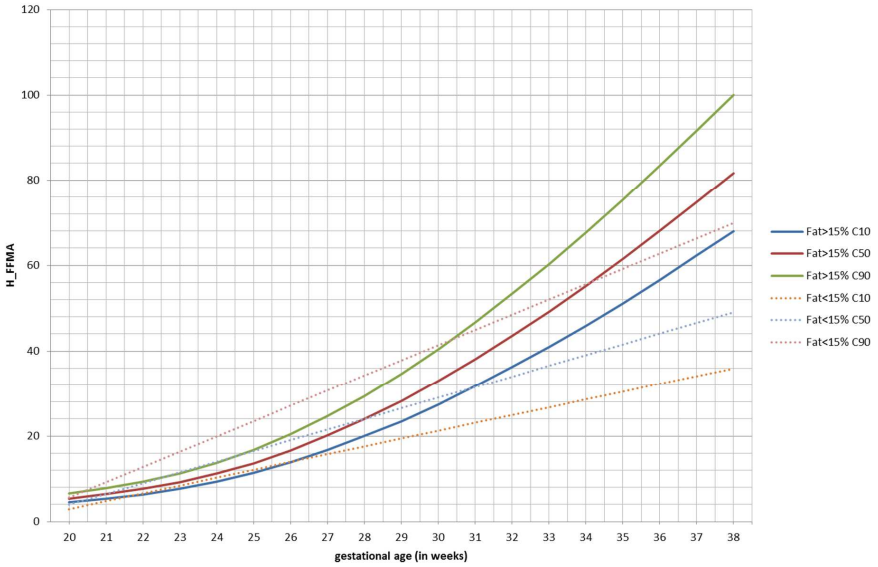

Figure 5.a. Humerus fetal fat mass area (H_FFMA) in mm^2 from 20 weeks gestational age onwards (x-axis). Percentiles 10, 50 and 90 for neonatal fat mass>15% (P90) and fat mass ≤15%.

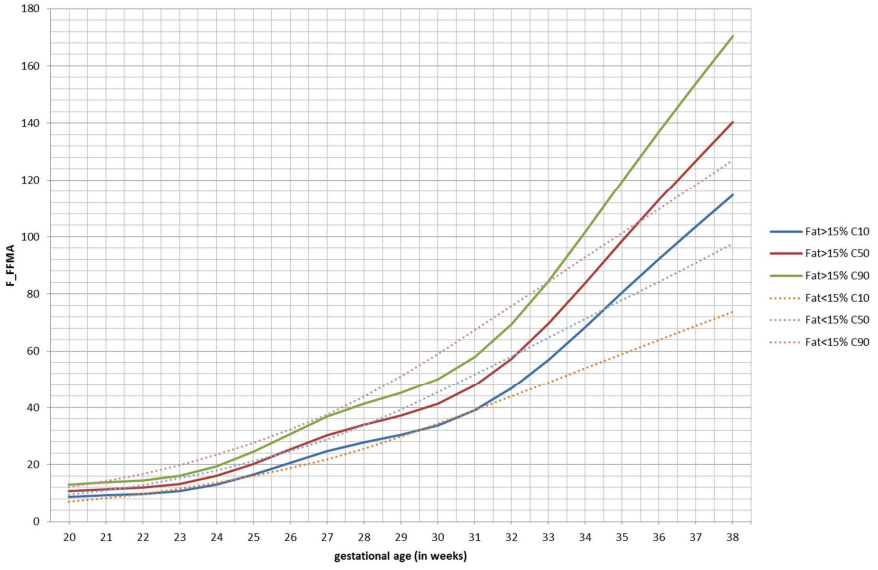

Figure 5.b. Femur fetal fat mass area (F_FFMA) in mm^2 from 20 weeks gestational age onwards (x-axis). Percentiles 10, 50 and 90 for neonatal fat mass>15% (P90) and fat mass ≤15%.

Globally, for the adipose babies (P90 NFM >15%) there were significant correlations with the development of the fetal AC (p=0.04), LL (p=0.03), SFT (p<0.001), AFT (p<0.001), the humerus fat mass (p<0.001) and both femur lean- and fat mass (P<0.001, table 3). More specifically, the fetal abdominal development (fetal AC and fetal LL), showed a more linear increase from the end of the first trimester towards the term period (figure 3a-b). Reference values are displayed in supplement 2. In the adipose babies (NFM>15%, P90), the fetal truncal fat measurements, SFT and AFT, showed an increase from 27-29 weeks gestation (figure 4a-b). Reference values are shown in e-tables 6.1-6.4. For the peripheral fetal fat measurements (humerus and femur) the increase started at 30-32 weeks gestation (figure 5a-b, e-tables 6.5-6.6).

DISCUSSION

This longitudinal study in normoglycemic women demonstrates interesting new insights in the correlation between maternal obesity, excessive GWG and neonatal adiposity. Excessive GWG and the subsequent development of abnormal fetal growth and adiposity, are associated with an increased risk for neonatal adiposity. While fetal fat measurements (SFT, AFT and peripheral fat depositions) show a high degree of correlation for neonatal fat mass and ponderal index, the fetal AFT correlates specifically to increased adiposity at birth (NFM >P90). The fetal AC and liver size are the first parameters to show a different growth trajectory in babies with a higher neonatal fat percentage, from first trimester onwards. Hereafter followed by the AFT and SFT in the late second and the peripheral (humerus and femur) fat measurements in the early third trimester. High maternal pre-pregnancy BMI was recognized by the IOM as a significant risk factor for adverse pregnancy outcome.[20] Out of that, excessive GWG also has an influence on the long-term cardio-metabolic health of the offspring.[29] Therefore the IOM advises a maximum GWG level per BMI class, negatively correlated to the prepregnancy BMI.[20] Obesity is seen as a pre-diabetic state, with increased insulin resistance, glucose levels and lipids in pregnancy. This potentially influences the developing fetus.[30,31] Even in the offspring of obese mothers, insulin resistance and increased neonatal body fat have been reported as short term outcomes.[9] Therefore we aimed to examine a normoglycemic population by applying a strict glucose screening and monitoring protocol, particularly in the high risk obese mother. Our results demonstrate a significantly higher neonatal fat deposition (NFM) in the mothers that have gained excessive weight irrespective of their BMI. Indeed, the BMI was negatively correlated, although not significantly, with neonatal fat mass in our selected population. In chapter 5 we demonstrate in a similar population that obesity promotes fetal liver size development and abdominal fat deposition, but does not affect ponderal index. While excessive GWG additionally, positively, influenced

fetal abdominal circumference and fetal subscapular fat thickness, it also affected birthweight, birth length and head-circumference, but not ponderal index.

Birth size is associated with childhood obesity and overweight.[32,33] BW, BL and PI have also been positively associated with higher BMI during childhood.[34] Neonatal body composition in the first days of life has been used as representative of *in utero* development by several studies.[26] While the importance of the early life period is widely acknowledged, the reports on the prenatal period are scarce. Most studies just summarize the *in utero* period by studying 'birthweight', which in itself is merely the outcome of fetal life. Buhling et al. performed a cross-sectional study in patients with and without GDM using late third trimester (>37 weeks) ultrasound. They correlated the fetal thigh and abdomen fat thickness measurement to postnatal skinfold measurements.[17] O'Connor et al. measured thigh and fetal abdominal fat thickness at 28 weeks and 37 weeks to improve the birthweight estimation in 328 pregnancies. The fetal thigh fat improved the prediction of birthweight.[15] They measured the fat component as follows: cross sectional plane of the mid-thigh with longitudinal tissue (subcutaneous fat and bone-muscle) distance. We decided to perform the more widely accepted surface area measurements of fat- and lean mass of both fetal humerus and femur.[13] A prospective longitudinal study was performed by the same group in 62 pregnancies using fetal abdominal fat thickness, thigh fat and thigh muscle at 28, 33 and 38 weeks and validated with air displacement plethysmography (ADP).[16] Our study confirms in a larger, normoglycemic cohort that fetal fat deposition increases from 26-28 weeks of gestation onwards. Additionally we showed that fetal measurements have a high degree of correlation with general birth outcome measurements, but are more specific to the profound neonatal adiposity indices (ponderal index and fat mass %). Several studies have addressed neonatal body composition by ADP, bio-electrical impedance analysis (BIA), anthropometry measurements or skinfold measurements.[16,35-37] Currently there are no parameters available in routine use, to indicate body proportionality in young children, under the age of two years. Body composition indexes as BW/BL, BW/BL^2 have been proposed by the WHO multicenter growth study group, but still have to prove their value as predictors of metabolic changes in later life.[35,38] Skinfolds measurements are practical, but require additional training, and there have been concerns on the reproducibility. The reported coefficients of variation are: 2.91% for suprailiac skinfolds, 2.57% for subscapular skinfolds and 2.73% for triceps skinfolds.[8] Furthermore, skinfolds inform on the distribution of neonatal fat (trunk or extremities), a property that is not present in the alternatives (ADP or BIA). ADP and BIA give a general, but objective, fat- and lean-mass outcome and seems to be the current normative for study purposes, but indeed no information is available on the location of the fat depositions.[37] We have BIA information on a limited group of our study (n=48) for 6 weeks, 6-12-18-24 months postpartum and this is currently under investigation.

The strengths of our study included the relative large sample size, longitudinal design and the fact that measurements were performed in both the mother and the fetus. All pregnancies had a first trimester scan, that allowed us to standardize the gestational age determination.[22] We did not focus on just one fetal fat index, but performed a whole sequence of central and peripheral fat measurements. A strict protocol to exclude (G) DM was implemented. All prenatal and postnatal measurements were performed by a single operator, overcoming the inter-observer variation, for both fetal and neonatal fat (skinfold) measurements. A validated algorithm was used to calculate neonatal fat mass percentage.[26] Furthermore, the complexity of longitudinal fast fetal growth was tackled by the GAMLSS analysis, a statistical technique advised by the WHO Multicentre Growth Reference Study Group for the construction of growth standards.[27,28]

Some limitations have to be mentioned. We categorized total GWG as excessive or non-excessive based on the 2009-revised IOM criteria.[20] In the non-excessive group both adequate and insufficient GWG are grouped. Recently our group showed a reduction in perinatal adverse outcome in obese women who lost weight in pregnancy, without an increased risk for low birth weight or small for gestational age (SGA) neonates.[39] On the other hand the group of Catalano et al. reported on a combined cohort of obese and overweight mothers, where they found an increased incidence of SGA and decreased fat mass at birth, when mother showed an insufficient weight gain.[40] Our insufficient GWG group formed 19/121 (15.7%) of our cohort. We can only estimate their influence on the ne-GWG group, since we did not select them separately, but it may have yielded an increased difference between the e-GWG and ne-GWG. Furthermore we did not correlate for the timing of the GWG, while first trimester weight gain profile may be most predisposing on the long-term to an aberrant cardio-metabolic outcome.[41] Finally, we did not collect information on nutritional intake during pregnancy from all included participants. A recent study, investigating a single fetal fat measurement (abdominal fat thickness) in an adolescent group, reported the AFT to be more affected by sugar intake than GWG.[42] Unfortunately only 24hrs recalled food-intake and basic birth measurements were collected (BW, BL, BHC and PI). We have 5-7days food-records for a certain group of our cohort (n=96), which will be used for future investigation.

In conclusion, this study demonstrated that in normoglycemic women, excessive GWG was associated with increased neonatal adiposity. In our cohort the P90 cut-off for neonatal fat mass could be set at 15% of the total body-weight. This increased neonatal adiposity (>P90) and the ponderal index were preceded by an increased development of fetal central- and peripheral fat mass parameters. The fetal fat measurements proved a high degree of correlation for neonatal adiposity, with a gestational age specific timing of first onset of aberrant fetal adiposity development: first (late first trimester) the abdominal circumference and fetal liver length, secondly (late second trimester) the truncal fat depositions of abdominal- and subscapular fat thickness and lastly (early

third trimester) the peripheral fat depositions on the extremities (humerus and femur). The fetal abdominal fat development, a marker of fetal central fat deposition, correlated specifically to the 90th percentile of neonatal fat mass, for which further studies are needed to show its potential role as a more specific predictor for neonatal adiposity.

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ABDOMINAL AORTA FETAL ABDOMINAL
INTIMA MEDIA THICKNESS FETAL FAT
FUNCTIONAL LINEAR DISCRIMINANT
ADDITIVE MODEL LOCATION, SCALE AND
MELLITUS NON-EXCESSIVE GESTATIONAL
GROWTH FACTOR INTIMA-MEDIA THICKNESS
SOCIETY ON OBSTETRICS AND
GROWTH LARGE-FOR-GESTA
NUTRITION EPIGENOME
GROWTH STUDY GROUP
IMAGING MRI FATTY LIVER DISEASE
GLUCOSE TOLERANCE TEST OGTT ODDS RATIO
ARTERIAL STIFFNESS QUALITY
BLOOD PRESSURE SKINFOLD TRICEPS
FAT THICKNESS SMALL-FOR-GESTATIONAL AGE
VASCULAR AND BIOMETRY ULTRASOUND
STUDY VABULOS YOUNG ELASTIC MODULUS

Chapter 7

Intima-Media Thickness (IMT) measurements in the fetus and the mother during pregnancy: *a feasibility study*

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ABSTRACT

Fetal intima-media thickness (IMT) has been suggested as a marker of pre-clinical atherosclerosis, and maternal IMT could be altered through dynamic circumstances related to pregnancy. We investigated the feasibility of measurement of IMT at four pre-defined fetal and four pre-defined maternal arterial locations in order to determine vascular changes that could be associated with impaired vascular function. IMT was measured from first to third trimester (12-34 weeks), in 38 low-risk pregnancies. We imaged a 10mm region of interest, using a Mindray (Shenzhen, China) high-resolution ultrasound machine with automated IMT measurement software.

Fetal abdominal aorta IMT was measurable during the second trimester in 71% and during the third trimester in 100% of the cases and the umbilical artery IMT was measurable in 50% and in 82% of the cases during the second and third trimester respectively. Fetal IMT measurements were not possible during the first trimester. The fetal common carotid artery, fetal renal artery and maternal external iliac artery IMT were not often feasible to measure (maximal 20% of cases). Maternal common carotid artery, abdominal aorta and uterine artery IMT were measurable throughout pregnancy. There was a significant relation between gestational age and IMT in the umbilical artery ($p = 0.03$) and a significant relation between body mass index and IMT in the maternal common carotid artery ($p = 0.01$). IMT measurements are feasible in some maternal and fetal vessels of interest. Further studies are underway to obtain more insight in the vascular development during normal and pathologic pregnancies.

INTRODUCTION

The fetal environment has been shown to be at the origin of conditions that present during adulthood. Low birth weight or intra-uterine growth restriction (IUGR) is associated with cardiovascular disease (CVD), type 2 diabetes mellitus and hypertension in adult life (Barker et al. 1989). This 'fetal origin of adult disease' theory was also proposed by Law et al. (1993). In these studies adults, who were small at birth, showed higher blood pressure and increased prevalence of CVD. It has been suggested that relative fetal undernourishment causes alterations that involve cardiovascular development, especially at the arterial level (Crispi et al. 2012).

Arteries are composed of three concentric layers. The intima is composed of endothelium; the media is a distinct layered structure of smooth muscle cells, collagen and elastic fibers; and the outer part of the vessel is the adventitia, which also comprises collagen and elastic fibers. In pathology studies, the intima-media thickness (IMT) in the abdominal aorta is reported to be the first site involved in the process of atherosclerosis in adults (McHill et al. 2000). Histology of the abdominal aorta wall in an intra-uterine growth restricted stillborn infant with a prenatally increased IMT revealed inflammation of the thickened intima layer. Lo Vasco et al. (2011) suggested that this is a very early sign of future adult lesions. The assessment of preclinical IMT changes may be an important predictor for future atherosclerosis and cardiovascular risk. Ultrasonography has the potential to detect these vascular changes.

Few studies have measured IMT in fetuses during pregnancy: Cosmi E et al measured IMT of the abdominal aorta in 38 fetuses with IUGR and 32 Appropriate-for-Gestational-Age (AGA) fetuses (Cosmi et al. 2009). The measurements were performed around 33 weeks of gestational age (range 30-34 weeks). They focused on a single fetal vessel, the fetal abdominal aorta, and found the IMT in the IUGR group was increased compared with that in the AGA group. Their consecutive studies represent the first on the sole use of a sonographic technique to investigate IMT in the fetus (Zanardo et al. 2011; Zanardo et al. 2013). IMT in the mother has also been poorly investigated so far. Existing studies have been performed on the common carotid artery (CCA) only. Furthermore studies were performed only in late pregnancy and compared these values with those of non-pregnant groups and hypertensive versus non-hypertensive groups. These reports are inconsistent on whether the CCA-IMT is increased in pregnancy (Sator et al. 1999). There are also some reports suggesting the Intima/Media (I/M) ratio is increased, as a result of inflammation and recruitment of inflammatory cells that occur in the atherosclerotic process, primarily in the intima layer (Akhter T et al. 2013). It has been hypothesized that the pregnancy-related increased hemodynamic load causes adaptations of elastic arteries, which could be gestational age dependent (Yuan LJ et al, 2013). Also, acute changes

in IMT in response to acute blood pressure and vascular tone modifications have been reported (Thijssen D et al. 2011).

No direct maternal influences on vascular wall property changes in the fetus have been reported yet. One possible factor could be the hyper-estrogenic state of the mother, which may affect the fetus through transplacental passage (Sator et al, 1999; Crispi et al 2012). Also, the pre-gestational maternal Body Mass Index (BMI) could be an identifiable metabolic risk factor (Raitakari OT et al. 2003).

There seems to be a lack of research on other vascular sites and vascular wall properties in both the fetus and the mother.

Therefore we aimed to investigate the feasibility of performing IMT measurements at predefined vessel locations using automated software in the fetus and the mother during pregnancy. Furthermore, the IMT measurements were investigated in relation to gestational age, BMI and distance from the ultrasound probe to the target vessel (depth).

METHODS

We performed a cross-sectional study between March and August 2011 in pregnant women with a low risk of CVD: no known maternal hypercholesterolemia, diabetes, pre-existing hypertension, pregnancy induced hypertension or pre-eclampsia. The study was performed in the context of the pilot phase of the 'DALI' (vitamin D And Lifestyle Intervention) study to prevent gestational diabetes, a 7th Framework Program of the European Community (FP7, Grant agreement No 242187). Patients, who came for their routine ultrasound scans at the prenatal care unit at the University Hospital Leuven, were recruited. The scans are planned at about 12, 20 and 30 weeks of gestation and gestational age was confirmed during the first trimester. Additional patients were included from admissions at the maternal intensive care unit with threatened premature birth but in the absence of cardiovascular compromise (hypertension, pre-eclampsia or hemolysis, elevated liver enzymes, low platelet count syndrome). Preconception BMI (in kg/m^2) was calculated using height measurements at the first antenatal visit and the self-reported weight before conception. Approval by the medical ethics committee was given and informed consent was obtained at inclusion.

Intima-media thickness was measured by a single experienced operator (S.G.) with the M7 high-resolution real-time B-mode ultrasound machine (Mindray, Shenzhen, China), equipped with both a 6.0-14.0 MHz, 4.0-7.0 MHz, and a 3.0-7.0 MHz linear array transducer and automated and validated IMT measurement software (figure 1 and 2). The probe was chosen on the basis of the depth of penetration required. In the majority of cases (n=35) we used the high-frequency (3.0-7.0 MHz) probe. In all measurements, both fetal and maternal, we used a minimum region of interest (ROI) window of 10mm,

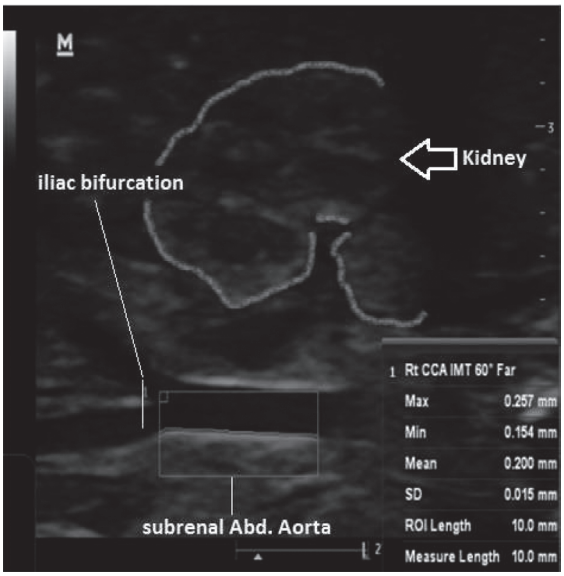

Figure 1. Ultrasound coronal view of the fetal lower abdominal region. Intima-media thickness was measured in the fetal abdominal aorta, below the renal artery and above the iliac bifurcation at gestational age of 20 weeks and 2 days with a 3.0-7.0 MHz linear transducer. CCA = Common carotid artery, IMT = Intima media thickness, ROI = Region of interest, SD = standard deviation.

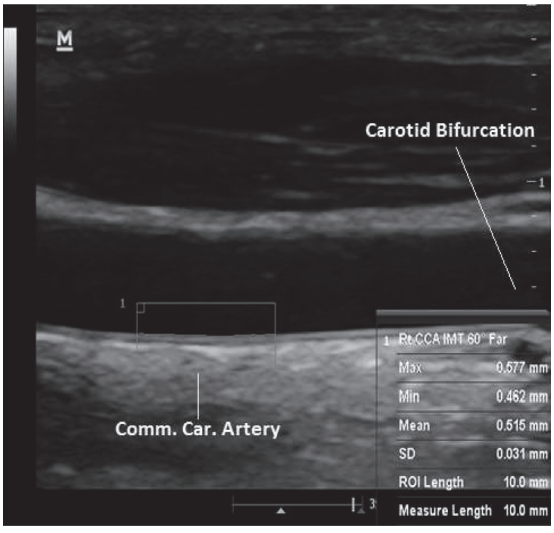

Figure 2. Ultrasound measurement of intima-media thickness in the maternal common carotid artery with ultrasound, proximal to the carotid bulb (bifurcation of the external-internal carotid artery), at GA of 20 weeks and 2 days using a 3.0-7.0 MHz linear transducer. CCA = Common carotid artery, IMT = Intima media thickness, ROI = Region of interest, SD = standard deviation.

an angle of insonation of 60º - 90º in a coronal or sagittal view and the vessel in end-diastolic cardiac phase, as determined on the B-mode image. The IMT was defined as the distance between the leading edge of the blood-intima interface, and the leading edge of the media-adventitia interface on the far wall of the vessel and was measured in micrometres (μm). The automated IMT wall tracking software allowed an online measurement of the target vessel: minimum, maximum and mean IMT values were outlined immediately. Hereby a feedback mechanism was available for the relationship of IMT vessels lumen properties. If the maximum IMT measurement crossed the anatomic delineation of the vessel and hence created a large discrepancy with the minimum IMT value, a large Standard Deviation (SD) resulted. For fetal vessels a SD of ≤100 μm and for maternal vessels a SD of ≤130 μm was considered as a valid measurement and otherwise rejected for recording. These cut-off points were chosen to obtain realistic values for IMT as a quality control for the innovative IMT measurements. Vessel depth was measured in a straight angle from the centre of the ultrasound probe surface to the far end of the target vessel wall.

We aimed to obtain one image per predefined location of the vessel at three consecutive time points and analyzed these immediately, bed-side. Maternal measurements were performed after completion of the fetal measurements, allowing the mothers to reach a state of relative rest.

Fetal and maternal arteries were measured at predefined locations:

- Fetal abdominal aorta: below the renal artery junction and above the iliac bifurcation (figure 1).
- Fetal umbilical artery: in a longitudinal plane, a horizontal trajectory part of the vessel.
- Fetal common carotid artery (fetal CCA): on the left or right side (depending on fetal position), proximal to the junction of the carotid bulb.
- Fetal renal artery: in the left or right renal artery (depending on position of the fetus).
- Maternal common carotid artery (maternal CCA): patient in supine position, head tilted slight left laterally (angle of 45°), probe approximately 6 cm above the clavicle; measurement 1-2 cm proximal to the junction of the internal-external carotid artery, the carotid artery bulb (figure 2) as described by Willekes et al (1999).
- Maternal abdominal aorta: right lateral tilted position, identification of left kidney and renal artery; measurement below the junction of the renal artery and above the iliac bifurcation.
- Maternal uterine artery: median of the cross-point of the uterine artery and external iliac artery; preferably on the ipsilateral side of the placental location.
- Maternal external iliac artery: probe positioning by movement laterally from the uterus and identification of the external iliac artery from the vein by Doppler signal.

Statistical analysis was performed with SPSS software version 20.1 (IBM, Armonk, NY, USA). Categorical values were compared using the Fisher exact test. The relationship between BMI and feasibility or non-feasibility of measurement of IMT was tested with independent samples t-test because BMI was distributed normally. Repeated measurements were compared with an intra-class correlation coefficient (two-way random effects). The relationship between IMT, gestational age and BMI, and also that between the coefficient of variation and depth, were tested with Spearman's bivariate correlation. A p-value of <0.05 was considered to indicate significance. All tests were two-tailed.

RESULTS

Study population

During the study period, 38 pregnant women were included; there were 36 Caucasians and two Africans. Median age was 31±2.3 years (range: 17-38 years). Two women were measured at a different gestational age, in one mother both twins were measured resulting in a total of 41 ultrasound examinations.

Gestational age (GA) ranged from 12 weeks to 33 weeks and 5 days, with a median of 29 weeks. Five ultrasound examinations were performed during the first trimester (GA <14 weeks), 14 ultrasound examinations during the second trimester (GA $\geq$14 and <28 weeks) and 22 ultrasound examinations during the third trimester (GA $\geq$28 weeks). Mean arterial pressure measured after their ultrasound scan was 103,8 ±8,2 mmHg (range 85,7-123mmHg).

Pre-pregnancy BMI of the women ranged from 18 to 30 kg/m^2 (median 23 kg/m^2). There were 2 ultrasound examinations in underweight women (BMI <18.5 kg/m^2), 27 ultrasound examinations in normal weight women (BMI $\geq$18.5 and <25 kg/m^2), 10 ultrasound examinations in overweight women (BMI $\geq$25 and <30 kg/m^2) and 2 ultrasound examinations in obese women (BMI ≥30 kg/m^2) (WHO classification of body mass index, 1995, kg/m^2).

Six twin pregnancies were included in the study: 2 ultrasound examinations were performed in dichorionic diamniotic twins, 4 ultrasound examinations in monochorionic diamniotic twins and 2 in monochorionic monoamniotic twins.

Seven women smoked during pregnancy and seven women received medication (1 tenovir/emtricitabine, lopinavir/ritonavir and levetiracetam; 3 atosiban in combination with betamethasone; 1 aspirin cardio; 1 levothyroxine; 1 calcitriol and 1 calcium carbonate).

The majority of the examinations (n=35), was performed with the high frequency 3.0-7.0 MHz linear ultrasound transducer.

Feasibility of measurement of IMT

IMT was measurable in the *fetal abdominal aorta* (figure 1) in the second (71%) and third (100%) trimester of pregnancy. IMT in *umbilical artery* was measurable in the second (50%) and third (82%) trimester of pregnancy, as outlined in table 1. However these measurements were not feasible during the first trimester.

It was not feasible to measure the *fetal common carotid artery* and *fetal renal artery* in any trimester in our study. The *maternal common carotid artery* (figure 2) was measurable in all examinations (100%) all trimesters (table 1). Throughout pregnancy the *maternal abdominal aorta* and the *uterine artery* were measurable in half of the cases (40%-60%). For the *maternal external iliac artery*, IMT measurements were not feasible at any gestational age in our study.

Table 1. Feasibility of at least one good quality IMT measurement in pre-defined regions of fetal and maternal arteries in first, second and third trimester of pregnancy.

Assessed vessels	**1st trim. (n=5)**	**2nd trim. (n=14)**	**3rd trim. (n=22)**	***Total (n=41)***
Fetal				
Abdominal Aorta	0	10 (**71%**)	22 (**100%**)	*32 (**76%**)*
Umbilical Artery	0	7 (**50%**)	18 (**82%**)	*25 (**61%**)*
Common Carotid Artery	0	0	4 (**18%**)	*4 (**10%**)*
Renal Artery	0	0	0	*0*
Maternal				
Common Carotid Artery (CCA)	4 (**100%**)	11 (**100%**)	16 (**100%**)	*31 (**100%**)**
Abdominal Aorta	2 (**40%**)	7 (**50%**)	9 (**41%**)	*18 (**44%**)*
Uterine Artery	2 (**40%**)	7 (**50%**)	13 (**59%**)	*22 (**54%**)*
External Iliac Artery	1 (**20%**)	1 (**7%**)	0	*2 (**5%**)*

* CCA-IMT measurement was examined in respectively 4/5, 11/14 and 16/22 of the women. Trim. = trimester

Effect of gestational age and BMI on feasibility

The proportion of patients in whom it was feasible to measure IMT at the level of the fetal abdominal aorta, increased significantly with increasing gestational age. It increased from 0% in the first trimester, to 71% in the second trimester (p=0.01). It further increased from 71% to 100% between the second and third trimester (p=0.02). The feasibility of measurement of IMT at the level of the fetal umbilical artery was also significantly related to gestational age, with a significant increase between first and third trimester (0% to 82%, p=0.002), although the difference between second and third trimester was not significant (50% to 82%, p=0.067). The feasibility of measurement of IMT in the fetal CCA was not related to gestational age, with an insignificant difference between second

and third trimester (18% versus 10% p=0.14). The IMT in the fetal renal artery was never measurable (table 1).

The feasibility of measurement of IMT in the maternal arteries studied was independent of gestational age. The measurement of IMT in the maternal external iliac artery was difficult to measure in all trimesters of pregnancy (table 1).

No relationship was found between maternal BMI and the feasibility of measurement of IMT for any fetal or maternal blood vessel studied: fetal abdominal aorta (p=0.18), umbilical artery (p=0.19), fetal CCA (p=0.19), maternal CCA (p=0.50), maternal abdominal aorta (p=0.69), maternal uterine artery (p=0.64) and maternal external iliac artery (p=0.51).

Intra-observer agreement

For each blood vessel measured, there was no significant difference between the first and second measurement, or between second and third and first and third repeated measurement. This was determined with the inter-class correlation coefficient (ICC): fetal abdominal aorta (ICC=0.965), fetal umbilical artery (ICC=0.975), maternal CCA (ICC=0.946), maternal abdominal aorta (ICC=0.999) and maternal uterine artery (ICC=0.747).

Range of IMT values throughout pregnancy and effect of BMI and depth

The IMT range and the median are outlined per separate vessel per trimester in table 2. In our small population, no relationship was evident between the IMT and gestational age in the fetal abdominal aorta (p=0.13), fetal CCA (p=0.20), maternal CCA (p=0.92), maternal abdominal aorta (p=0.54) and maternal uterine artery (p=0.77). However, we did find a significant relationship between IMT and gestational age in the fetal umbilical artery (p=0.03), with a median IMT of 289 µm in second trimester and 301 µm in third trimester.

We did not find a significant relationship between the IMT and maternal BMI in the fetal abdominal aorta (p=0.18), fetal umbilical artery (p=0.78), fetal CCA (p=0.20), maternal abdominal aorta (p=0.24) and maternal uterine artery (p=0.86). However, there was a significant relationship between the IMT and BMI in the maternal CCA (p=0.01), with an IMT of 445 µm in one underweight woman, a median of 510 µm in normal weight women, 540 µm in overweight women and 507 µm in one obese woman.

The mean coefficient of variation (SD/mean; SD and mean as reported by the Mindray software per measurement) and the mean with 95% confidence interval (CI)of the IMT values are reported in µm (table 3). For the dispersion of the range of IMT values, the minimum, maximum and median IMT values are also listen in Table 3, as are the mean depth and range of depths (in cm) of the vessels (table 3). The coefficient of variation

Table 2. Median and range of IMT measurement (in µm) in pre-defined regions of fetal and maternal arteries in first, second and third trimester of pregnancy.

Assessed vessels	1st trimester		2nd trimester		3rd trimester	
	Median	**Range IMT**	**Median**	**Range IMT**	**Median**	**Range IMT**
Fetal						
Abdominal Aorta	-	-	**330**	181-431	**342**	201-586
Umbilical Artery	-	-	**289**	225-572	**301**	212-417
Common Carotid Artery	-	-	-	-	**295**	185-367
Renal Artery	-	-	-	-	-	-
Maternal						
Common Carotid Artery	**452**	400-539	**545**	440-712	**504**	378-740
Abdominal Aorta	**580**	523-616	**561**	367-680	**652**	495-735
Uterine Artery	**229**	163-415	**246**	195-351	**238**	160-535
External Iliac Artery	-	582	-	601	-	-

Table 3. Mean with 95% CI, minimum, median and maximum IMT per measured fetal and maternal artery throughout pregnancy in µm. Vessels' depth (mean and range) to the far end of the vessels' wall is defined in cm. * Data on fetal renal artery too scarce.

	Fetal*			**Maternal**			
Vessels	**AbdAo**	**UmbArt**	**CCA**	**CCA**	**AbdAo**	**UtArt**	**ExtIlArt**
mean	334	314	286	517	575	264	592
95%CI mean	313-354	294-334	165-406	498-537	534-616	231-296	471-712
Minimum	181	212	185	378	367	160	582
Median	335	301	295	511	578	236	592
Maximum	586	572	367	740	735	535	601
CoeffVar.	.12	.09	.17	.13	.11	.15	.18
Depth mean	5.8	5.0	5.2	2.2	10.4	3.1	5.9
Depth range	3.9-9.2	3.1-8.4	4.4-6.0	1.5-2.7	7.5-14.2	1.6-4.9	4.7-7.0

AbdAo = Abdominal Aorta; UmbArt = Umbilical Artery; CCA = Common Carotid Artery; UtArt = Uterine Artery; ExtIArt = External Iliac Artery. CoeffVar = Coefficient of Variation

was found to be the lowest for IMT in the fetal umbilical artery and was also low in the fetal abdominal aorta, the maternal CCA and the maternal abdominal aorta.

There was a significant positive relationship between vessel depth and coefficient of variation for the IMT value in the maternal CCA (rho=0.27; p=0.019) and maternal uterine artery (rho=0.45; p=0.014). However, there was no significant relationship in the

fetal abdominal aorta (p=0.67), fetal umbilical artery (p=0.33), fetal CCA (p=0.60) and maternal abdominal aorta (p=0.40).

DISCUSSION

This is the first study to investigate the feasibility of arterial IMT measurement in mother and fetus throughout pregnancy using ultrasound. We were able to measure IMT in the fetal abdominal aorta and the umbilical artery during the second and third trimester. Furthermore, we found a positive relationship between values of IMT and gestational age in the umbilical artery. No relationship between IMT value and gestational age was evident in any of the other fetal and maternal blood vessels. The maternal CCA IMT could be measured throughout pregnancy and exhibited a positive association with pre-gestational BMI. The maternal abdominal aorta and uterine artery IMT measurements were feasible, although more difficult than measurement of maternal CCA IMT.

Cardiovascular disease generally becomes apparent in older patients. However, the intra-uterine environment has been postulated as having a crucial role in programming the fetus for short term survival in the early postnatal period, but with impaired consequences for cardiovascular function and metabolic changes in latter adult life, when intra-uterine life is unfavorable (Barker et al. 1989; Law et al. 1993; Galjaard et al. 2013). Pathology and histology studies indicate that IMT thickening is caused by inflammation and that the abdominal aorta artery is the first location of increasing IMT as a sign of atherosclerosis (McGill et al. 2000; Lo Vasco et al. 2011; Jarvisalo et al. 2001). Therefore assessment of pre-clinical IMT changes may be used to predict future atherosclerosis and cardiovascular risk.

In newborns, the association between birthweight, abnormal growth and increased IMT in the abdominal aortic artery and development of atherosclerosis later in life is supported by several studies (Jarvisalo et al. 2001; Skilton et al. 2005; Koklu et al. 2007; Satoru et al. 2012). In early childhood, Crispi et al. reported early signs of cardiovascular dysfunction at 3-6 years of age in children with IUGR and Small for Gestational Age (SGA) children as compared to Appropriate for Gestational Age (AGA) children. Not only was their cardiac function affected, but the CCA IMT was also increased in both children with IUGR and SGA childresn (Crispi et al. 2012). In a recent study, Dratva et al. (2013) reported an increased CCA IMT at the age of 11 years to be associated with increased birth weight.

Very few studies however, have measured IMT in fetuses and mothers during pregnancy. Cosmi et al. (2009) measured IMT in the abdominal aorta in 38 fetuses with IUGR and 32 AGA children. They found the IMT to be higher in IUGR fetuses than in AGA fetuses (1.9mm vs 1.15mm, P<.001). The measurements were performed around 33 weeks of gestational age (range 30-34 weeks) and this study represents the first sonographic

investigation of IMT in fetuses. Sarikabadayi et al. (2012) measured the umbilical *wall* thickness in the third trimester with routine obstetrical ultrasound equipment, but did not measure umbilical IMT. IMT in carotid arteries in pregnant women was found to differ from IMT in the non-pregnant fertile female population. This was largely related to the increase in carotid media and decrease in carotid intima layer, that supposedly occurs because of the different oestrogen levels in the two groups (Sator et al. 1999). There is lack of research on other vascular sites in pregnant women. Furthermore, relationships between IMT development and pre-gestational weight (under-, normal-, overweight and obese mothers), excessive gestational weight gain or gestational and type I/II diabetes mellitus have not yet been reported during pregnancy.

Our study employed a clear IMT measurement protocol to study the feasibility in relevant maternal and fetal arterial vessels at different gestational ages, using a cross-sectional design. The automated IMT wall tracking software allowed an instant measurement of the target vessel and therefore immediate feedback on the relationship of IMT to vessel lumen properties was available. If the maximum IMT measurement crossed the anatomical delineation of the vessel and hence created a large discrepancy with the minimum IMT value, a large standard deviation resulted. These cut-off values were used to define a successful measurement as a quality control for the innovative IMT measurements. Furthermore, through bedside analysis of three different consecutive images of the identical predefined vessel location instead of simply three off-line analyzes of the same image, we could take the image acquisition process into account, being a large source of intra-observer variability.

The present study revealed several impediments to measurement of IMT in fetal arteries. In the first trimester it was not possible to obtain a ROI window of 10mm for all fetal vessels because of their small size. In second and third trimester, because of fetal movements, the position and distance of the target vessel from the ultrasound probe ('depth') could impair a successful IMT measurement. In the umbilical artery, the coiling of the cord could prevent acquisition of a straight ROI window of at least 10mm. The fetal CCA was often not visualized because of fetal position (anterior flexing of the fetal neck) and hence a ROI window of 10mm could not be defined. The main obstacle to obtaining reliable IMT measurements in the fetal renal artery was the limited angle of insonation ($<60^{o}$ or $>90^{o}$) at which the vessel could be visualized.

The impediments to measuring the IMT in maternal arteries generally were limited examination time and maternal discomfort, making it sometimes impossible to complete all measurements. Because its trajectory is superficial, the maternal CCA is at each attempt reachable for ultrasound examination. During pregnancy it was more difficult to reach the abdominal aorta because of the interposition of the fetus and uterus and the 'depth' sometimes prevented successful measurement. The uterine artery is characterized by a very curly trajectory, making it hard to obtain a ROI window of 10mm in the

correct angle of insonation (<60° or >90°). The correct angle was also the main obstacle for successful measurement of the maternal external iliac artery IMT in pregnancy.

It is not easy to compare our quantitative IMT data with those of other studies as the available information relies mostly on histopathology or postnatal IMT studies, therefore including the fetal-to-infancy transition period in IMT development. The median IMT's in the fetal CCA and abdominal aorta in our study were 295 and 335 µm respectively, which are comparable to the results obtained by Jarvisalo et al. (2001). They performed an ultrasound study in 11 year old children with a high risk (hypercholesterolemia/ diabetes) and a low risk for atherosclerosis. In the control group they obtained an IMT value in the CCA and abdominal aorta of 420 and 440 µm, respectively. In the high-risk group both carotid IMT and abdominal aorta IMT were increased compared with values for the low-risk group. The abdominal aorta IMT was higher than the CCA IMT, indicative of the predilection of the abdominal aorta as the first location of IMT changes related to atherosclerosis. The small variation with our study could be related to the different time frame (fetuses vs. children) in which the IMT was measured by Jarvisalo et al. (2001). But generally, the studies support the robustness of our findings (Dratva et al. 2013).

In preterm neonates the abdominal aorta IMT was found to increase from 316 µm to 348 µm for children born at respectively 25 and 37 weeks of gestation (Koklu et al. 2007). This is very similar to our results (335 µm), although we could not report a significant positive relationship between IMT and gestational age in our study, probably because of our small study population. Furthermore it is unknown whether birth has an effect on vessel properties, for example, as a result of a change in blood pressure and hence a different distention of arteries after birth. In their ultrasound study, Cosmi et al. (2009) reported an IMT of 1150 µm in third trimester fetuses was found, which can probably partly be explained by the fact that the authors studied a growth-restricted population. Also, they used different equipment and ultrasound resolution in their elegant studies (Zanardo et al. 2011; Zanardo et al. 2012).

The median IMT of the umbilical artery in our study was 301 µm in the third trimester. In their histopathology study, Junek et al. (2000) reported an IMT of about 30 µm in umbilical cords, measured after birth. They compared the evolution of IMT in umbilical arteries and veins after birth from IUGR fetuses (controls) and fetuses born of mothers with pre-eclampsia (PE). They found that the total IMT increased with advancing gestational age at birth, because the increase in the intima was larger than the observed decrease in the media. Between the two groups the IMT was 15% thicker in the umbilical arteries from foetuses of mothers with PE than in the controls. Of course, the umbilical cord changes significantly after birth with contraction of Wharton's jelly, the umbilical vein and arteries. We can only speculate if this may explain the difference in IMTs between our study and that of Junek et al. (2000). Sarikabadayi et al (2012) measured the umbilical wall thickness in the third trimester with standard obstetric ultrasound and

measured umbilical IMT and wall thickness in postnatal pathology specimens. Although there was a positive correlation between prenatal and postnatal findings, the absolute values cannot be compared between these two time frames because of the different structures examined.

Maternal CCA IMT was, on average, 517 µm in our study. This is comparable to the results obtained by Sator et al. (1999) who reported 560 µm in pregnant women. We did not find a correlation with gestational age, but our study was designed to study feasibility at different time points in gestation and not gestational age-related effects.

Interestingly, we found a positive relationship between maternal CCA IMT and maternal preconception BMI. This is in accordance with the study of Dick et al. (2013) who found a positive relationship between IMT and obesity, although their study was conducted in male and non-pregnant female subjects. Yuan et al. (2013) found very recently, in late third trimester pregnancy, a difference in maternal CCA IMT between normotensive and pre-eclamptic pregnancies (351±85 µm vs 459±95 µm).

Several limitations to our present study have to be addressed. The number of patients studied was relatively small and comprised a heterogeneous, although cardiovascular low-risk, obstetric population. Our study also compiled some longitudinal data with mostly cross-sectional measurements. Although significant relations were observed, the study may have been underpowered to show other correlations such as for gestational age or smoking. Furthermore, by defining a limitation in restricting the SD range for successful fetal and maternal IMT measurements, there is the possibility of underestimating the IMT measurements and hence probable first signs of inflammation or atherosclerosis. From a methodological point of view, we chose to focus on the arterial vessel wall and a ROI of 10mm. Another possibility is to include the lumen diameter of the vessel of interest in the measurements. Satoru et al. (2012) combined the lumen diameter of the abdominal aorta and IMT to an adjusted IMT (aIMT in mm/mm) measurement in newborns. For first trimester measurements, it could be considered to choose a smaller ROI window then 10mm to be able to measure fetal IMT or measure IMT in such tortuous vessels as the umbilical artery and the uterine artery. Finally, more information on the plasticity of the vessel can be acquired when automated software would take into account the influences of blood pressure, blood velocity and wall velocity from the vessels. The shear stress or arterial stiffness could thereby be defined through different cardiac cycles (Rossi et al. 2009; Yuan et al. 2013). A longitudinal study would be required to observe intra-individual changes in IMT in selected blood vessels during gestation.

CONCLUSION

This is the first study to investigate the possibility of measuring IMT during pregnancy in both fetal and maternal blood vessels. The study proves the feasibility of reliably measuring IMT with automated software in the fetal abdominal aorta and the umbilical artery in second and third trimester of gestation. Strong correlations between our findings on fetal abdominal aorta IMT and those from the study by Koklu et al. on newborns of different gestational ages, support the robustness of our findings. Obesity of the mother did not hamper measurement of IMT in fetal arteries. The positive relationship between maternal CCA IMT and BMI invites further investigation into abnormal pregnancies. Furthermore, fetal IMT development in abnormal pregnancy is of interest, as it could contribute to elucidating the mechanisms of intra-uterine fetal programming and the prediction of the origin of adult metabolic diseases.

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ABDOMINAL AORTA FETAL ABDOMINAL
INTIMA MEDIA THICKNESS FETAL FAT
FUNCTIONAL LINEAR DISCRIMINANT
ADDITIVE MODEL LOCATION, SCALE AND
MELLITUS NON-EXCESSIVE GESTATIONAL
GROWTH FACTOR INTIMA-MEDIA THICKNESS
SOCIETY ON OBSTETRICS AND
GROWTH LARGE-FOR-GESTA
NUTRITION EPIGENOME
GROWTH STUDY GROUP
IMAGING MRI FATTY LIVER DISEASE
GLUCOSE TOLERANCE TEST OGTT ODDS RATIO
ARTERIAL STIFFNESS QUALITY
BLOOD PRESSURE SKINFOLD TRICEPS
FAT THICKNESS SMALL-FOR-GESTATIONAL AGE
VASCULAR AND BIOMETRY ULTRASOUND
STUDY VABULOS YOUNG ELASTIC MODULUS

Chapter 8

Excessive gestational weight gain increases maternal common carotid artery stiffness in pregnancy

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Submission in progress

ABSTRACT

Introduction

Pregnancy causes hemodynamic changes in the mother. The aim of our study was to detect modifications of the vascular properties in the maternal common carotid artery (CCA) with radio-frequency ultrasound in relation to maternal characteristics.

Methods

Women with low-risk pregnancies were recruited during the first trimester and prospectively followed-up for intima media thickness (IMT) and elastic properties (arterial stiffness) measurements of the right maternal CCA. High resolution radio-frequency (RF) ultrasound was performed in early, mid- and late pregnancy. Blood pressure and gestational weight gain (GWG) were obtained at every visit. Total GWG was categorized according to the 2009-revised Institute of Medicine criteria as excessive- or non-excessive (e-GWG and ne-GWG). CCA elastic indices were calculated: carotid artery compliance (CAC), young elastic modulus (YEM) and stiffness index (SI). Generalized Additive Model Location, Scale and Shape (GAMLSS) was used to align the timeframes of the measurements.

Results

Eighty women (51% nulliparous, 50% with e-GWG), were included in the final analysis. All CCA properties and indices were significantly associated with gestational age in terms of decreasing compliance ($p<0.001$) and increasing arterial stiffness (YEM and SI, $p<0.001$). All indices (CAC, YEM and SI) were also significantly correlated with both maternal age and e-GWG, but when corrected for maternal age, the YEM remained positively correlated with GWG ($p= 0.004$). No correlation was found with respect to pre-gestational BMI.

Conclusion

Maternal CCA stiffness increased throughout pregnancy age and was positively correlated with higher maternal age, e-GWG, but not with pre-gestational BMI category, based on RF ultrasound data in pregnancy. When e-GWG was corrected for maternal age, the IMT-independent stiffness index (YEM) remained positively associated with e-GWG. Further studies are warranted on the long-term vascular effects of maternal weight gain during pregnancy as a risk factor for future cardiovascular disease.

INTRODUCTION

Hemodynamic changes in normal pregnancy have been well described.[1,2] At the vascular level, the changes of elastic properties are regionally distributed. The systemic vasculature (aorta and direct aortic branches) shows an increased compliance, while the common carotid artery (CCA) compliance decreases in normal pregnancy.[3,4] In studies with non-pregnant individuals, increased arterial stiffness is used as a first sign of remodelling and inflammation of the arterial wall and therefore as a risk factor for the development of atherosclerosis.[5] In pregnancy the research focus is mainly on hyperdynamic circumstances, pregnancy complications and the risk for later disease. Preeclampsia for example can alter arterial wall properties with long term effects on vascular stiffening.[6-8] Different tools have been used to investigate the vascular structure in pregnancy, all based on non-invasive imaging methods. The most commonly used technique is ultrasound.[9-11] Radio-frequency (RF) ultrasound is capable to detect small vessel wall properties and functionality.[12,13] Recently CCA behaviour in pregnancy has been elucidated using radio-frequency ultrasound.[14,15] These small studies have focussed mainly on the effects of hypertension and pre-eclampsia, although it has been demonstrated that other maternal determinants, like (excessive) GWG, maternal BMI and an increased insulin resistant state (gestational diabetes mellitus) influence pregnancy outcome.[16-17] It is therefore possible that these maternal characteristics also influence arterial stiffness in pregnancy.[5] The aim of our study was to describe vascular properties of the CCA in normal pregnancy and to detect modifications of the vascular properties (IMT and arterial stiffness) with RF ultrasound in relation to maternal characteristics.

DESIGN AND METHODS

This prospective cohort study was performed at the Department of Obstetrics and Gynecology of the University Hospitals Leuven (Belgium). Pregnant women of different pre-gestational BMI classes were included at their first trimester ante-natal visit between August 2012 and December 2013. This vascular sub-study was part of three prospective studies for low-risk, obese and non-obese pregnancies monitored for GWG, development of GDM and nutrition.[18] All studies were approved by the Ethics Committee for Human Experimentation of the Faculty of Medicine, Katholieke Universiteit Leuven (S52172, S54026 and S55325). All women have signed written informed consent to take part in the study. Exclusion criteria for the sub-study were: pre-existing diabetes (DM), pre-existing hypertension, development of pre-eclampsia, multiple pregnancies, missing longitudinal data and adverse events (major fetal abnormality, fetal death and/or immature birth).

Maternal determinants

At their first ante-natal visit, parameters for BMI (height and pre-pregnancy weight) were obtained as described elsewhere.[19] The BMI was categorized as obese (≥30kg/m^2), overweight (25-29.9kg/m^2) or normal weight (≤24.9kg/m^2) as defined by the WHO guidelines. The total GWG was computed using the final weight measured at the last prenatal visit, minus the pre-gestational weight. GWG gain was categorized according to the Institute of Medicine (IOM) 2009 revised criteria: 11.5-16kg (normal weight), 7.0-11.5kg (overweight) and 5.0-9.0kg (obese).[20] If women crossed the upper threshold, they were considered excessive-GWG (e-GWG) or non-excessive (ne-GWG) if they had either adequate or insufficient total GWG.[21] If women were considered a high risk (history of GDM, BMI≥30kg/m^2), a fasting glucose measurement <92mg/dL (<5.1mmol/L) at baseline was used to exclude pre-existing DM. Another screening was performed at 24-28 weeks using a 75g oral glucose tolerance test (OGTT) based on the 2013 World Health Organization (WHO) criteria.[22] The non-obese population underwent an OGTT if the initial 50g glucose challenge test showed a glycaemia of ≥140mg/dL (≥7.8mmol/L) at 24-28weeks (local protocol).

Vascular measurements and blood pressure

Determination of gestational age (GA) was based on the fetal crown rump length (CRL) measured between 7 and 14 weeks of gestation in all patients.[23] Ultrasound examinations were performed at three time points in gestation: 12-18 weeks, 26-32 weeks and between 35-37 weeks of gestation. A proportion (29 participants) was also examined between 18-22 weeks of gestation. First a trans-abdominal obstetrical ultrasound was performed, which took at least 25-30 minutes and gave the women the opportunity to acquire a resting state. Right maternal CCA was examined as follows: patient lying in supine position, head tilted slight left lateral (angle of 45°), probe approximately 6 cm above the clavicle; measurement 1-2 cm proximal to the junction of the internal-external carotid artery, the carotid artery bulb over a region of interest (ROI) of the vessel of 15mm.[12,24] High resolution RF, real-time B-mode ultrasound (ESAOTE, Genua, Italy) was used, with integrated applications (QIMT -Quality IMT- and QAS -Quality arterial stiffness-) as developed in ART-LAB systems (figure 1). The technique is based on an automated arterial wall tracking system, which preserves the authenticity of the acoustic wave and therefor the examined tissue.[13] For IMT the far-wall measurement was taken. The QAS provided the vascular diameter (DIA) between diastolic and systolic phases and the arterial distension (DIS). A 3-11 MHz, linear-array ultrasound probe was used. All measurements for both QIMT and QAS were performed three times over 6 consecutive cardiac cycles at every time point by the same operator (SG). An average of the three mean values was taken, based on 18 cardiac cycles, compensating for the hemodynamic fluctuations characteristic in vascular measurements. Blood pressure was

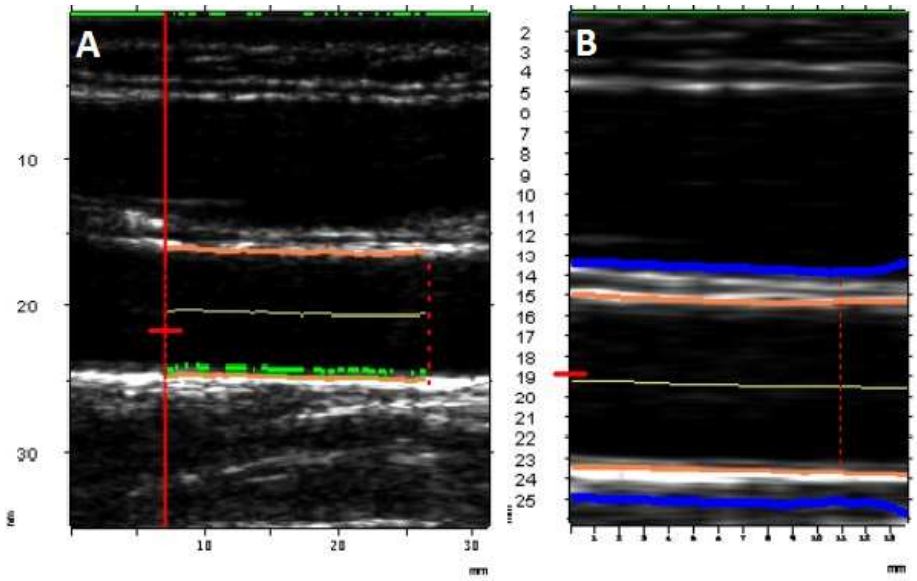

Figure 1. a,b. Maternal right common carotid artery measurement with radio-frequency ultrasound A. Quality intima media thickness (QIMT), far-wall measurement; green line = IMT, orange line = adventitia B. Quality arterial stiffness (QAS) measurement; blue line = arterial distension (DIS), orange line = arterial diameter (DIA). X-axis, region of interest in mm; Y-axis distance in mm.

measured concomitantly using an automated blood-pressure machine (OMRON M6 Intellisense, Healthcare Co.Ltd, Japan) and a mean arterial pressure (MAP) was calculated from systolic blood pressure (SBP) and diastolic blood pressure (DBP) as: (2xDBP)+SBP/3. After analysing the RF-data via ART-LAB software, the following CCA elastic indices were calculated[25,26]:

- Carotid artery compliance (CAC), as the ability of the vessel to expand in response to the cardiac pulse pressure: ([systolic diameter-diastolic diameter]/diastolic diameter)/(SBP-DBP) in %/10mmHg.
- Young elastic modulus (YEM), provides an estimate of the arterial stiffness, but is less dependent on IMT (arterial wall characteristic)[27]: ([SBP-DBP]x diastolic diameter)/([systolic diameter-diastolic diameter]/IMT in mmHg/mm.
- Stiffness index (SI), an elastic property relatively independent of blood pressure[28]: ln(SBP/DBP)/([systolic diameter-diastolic diameter]/diastolic diameter).

Variability

Since 3 consecutive measurements of at least 6 cardiac cycles at each time point were performed, the intra-observer variability could be calculated for IMT, DIA and DIS. The three measurements cycles are regarded as separate observations (figure 1, 2 and e-

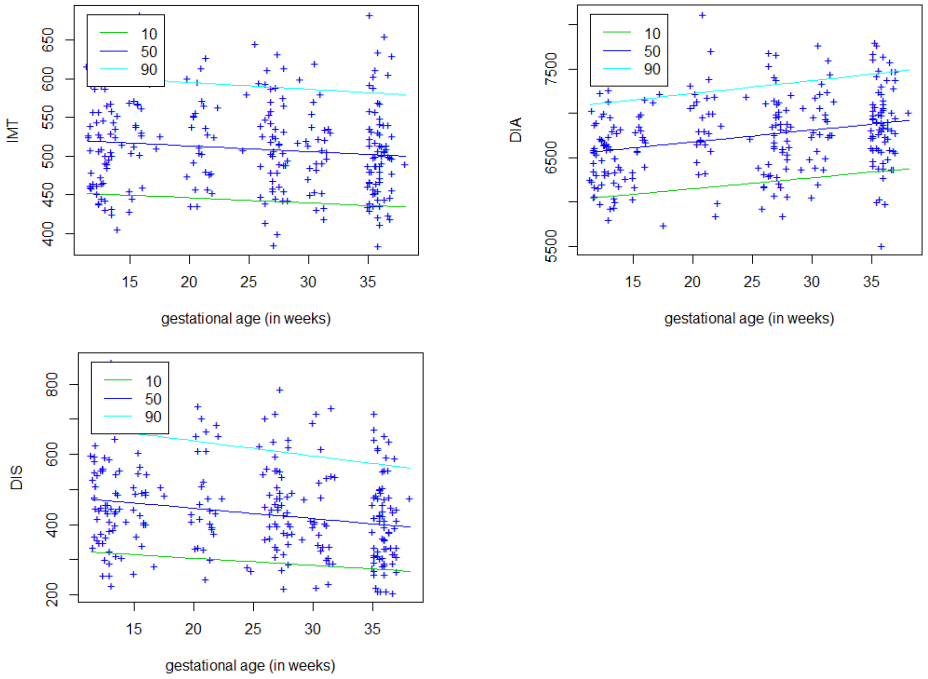

Figure 2. Correlation (scatterplots) of vascular properties in the right common carotid artery with the gestational age (x-axis in weeks). Y-axis: Intima media thickness (IMT) in µm, diameter (DIA) in µm and distension (DIS) in µm. Percentiles, P10, P50 and P90.

figures 8.1-8.3). The intra-class correlation coefficients for IMT, DIA and DIS were: 0.80-0.95 and 0.87, respectively.

Statistical analysis

GAMLSS (Generalized Additive Models for Location, Scale and Shape, www.gamlss.org) R-package was applied to construct the growth-evolution curves for the vascular properties IMT, DIA, DIS, CAC, YEM and SI.[29,30] We assessed several distributions: Box-Cox-t, Box-Cox Cole and Green, and Box-Cox Power Exponential. Goodness-of-fit of the models was assessed with QQ plots, AIC (Akaike Information Criterium) and worm plots. The goodness-of-fit was investigated covering the gestational period of 12-40 weeks. GAMLSS smoothed the ante-natal growth curves for IMT, DIA, DIS, CAC, YEM and SI. Percentiles (P10, P50 and P90) for each investigated parameter were constructed.

Only complete vascular data-sets including a minimum of two consecutive time points were analyzed. The effect of BMI (normal weight, overweight and obese) and the effect of excessive GWG versus non-excessive GWG was assessed by adding these variables into the GAMLSS model. The same analysis was performed for maternal age (continuous) and parity (nulli-parous versus parous). All vascular data and maternal characteristics were

corrected for gestational age if applicable and introduced as consecutive measurements within the GAMLSS model.

RESULTS

From the 101 recruited pregnant women, 5 were excluded based on an abnormal fasting glucose measurement (pre-existent DM) at baseline. There were 4 drop-outs, one late first trimester miscarriage, 4 pre-eclamptic participants and 8 participants were excluded because of only a single time-point vascular measurement. This resulted in 80 participants in the final analysis: 35 (44%) had a normal pre-gestational weight, 15 (19%) were overweight and 30 (38%) obese. Forty women (50%) showed e-GWG and 14/80 (17.5%) had an insufficient GWG. The maternal baseline characteristics are outlined in table 1. All CCA properties (IMT, DIA and DIS) were highly dependent on gestational age (table 2). The IMT decreased, the DIA increased and the DIS decreased during pregnancy (figure 2). An overview of the measurements at different time-points is given in table 3. Detailed reference values per gestational week are provided in e-table 8.1. Also all CCA elasticity indices were significantly dependent on gestational age (table 2). The CAC decreased ($p<0.001$), while the YEM and SI both increased significantly ($p<0.001$), with advancing gestational age (figure 3). The elasticity indices at different time points are detailed in table 3. Reference values per gestational week are provided in e-table 8.2.

Table 1. Maternal baseline characteristics.

	Total (N= 80)
Maternal age (years), mean ± std	29.7 ± 4.0
Nulliparity (%)	41 (51%)
Obese (%)	30 (38%)
Overweight (%)	15 (19%)
Normal weight	35 (44%)
Excessive GWG (e-GWG)	40 (50%)
GA at birth (wks,0/7d), mean ± std	39w3d ± 1w2d
Birth weight (g), mean ± std	3420 ± 510
Smoking	5 (6.3%)
GDM	6 (7.5%)
PIH	10 (12.5%)

GWG = gestational weight gain; GA = gestational age; wks = weeks; d = days; g = gram; GDM= gestational diabetes mellitus; PIH = pregnancy induced hypertension; std. = standard deviation.

Table 2. Correlations of maternal characteristics in the development of vascular properties and elasticity indexes of the right maternal common carotid artery (CCA); Characteristics were introduced as explanatory variables in the GAMLSS model; p values are given.

Properties & Elasticity	GA#	Nulliparous vs Parous	Obese vs Non-obese	Obese vs Normal weight	Obese vs Overweight	Maternal age#	e-GWG vs ne-GWG	*e-GWG vs ne-GWG**
IMT	**0.03** (↓)	0.53	0.44	0.59	0.34	**0.006** (↑)	*0.12 (↑)*	0.33
DIA	**<0.001** (↑)	0.82	0.13	0.13	0.31	0.24	0.34	0.48
DIS	**<0.001** (↓)	0.52	0.74	0.90	0.56	**<0.001** (↓)	**0.05** (↓)	0.27
CAC	**<0.001** (↓)	0.63	0.50	0.61	0.46	**<0.001** (↓)	**0.03** (↓)	0.16
YEM	**<0.001** (↑)	0.46	0.86	0.94	0.61	**<0.001** (↑)	**<0.001** (↑)	**0.004** (↑)
SI	**<0.001** (↑)	0.33	0.18	0.28	0.22	**<0.001** (↑)	**0.01** (↑)	0.06

GA = gestational age; e-GWG = excessive gestational weight gain; ne-GWG = non-excessive GWG; IMT = intima media thickness; DIA = diameter; DIS = distension; CAC = carotid artery compliance; YEM = young elastic modulus; SI = stiffness index; * = corrected for maternal age; # = continuous variable; ↑ = promotes development vascular unit; ↓ = inhibits development vascular unit; Significance is considered if p <0.05.

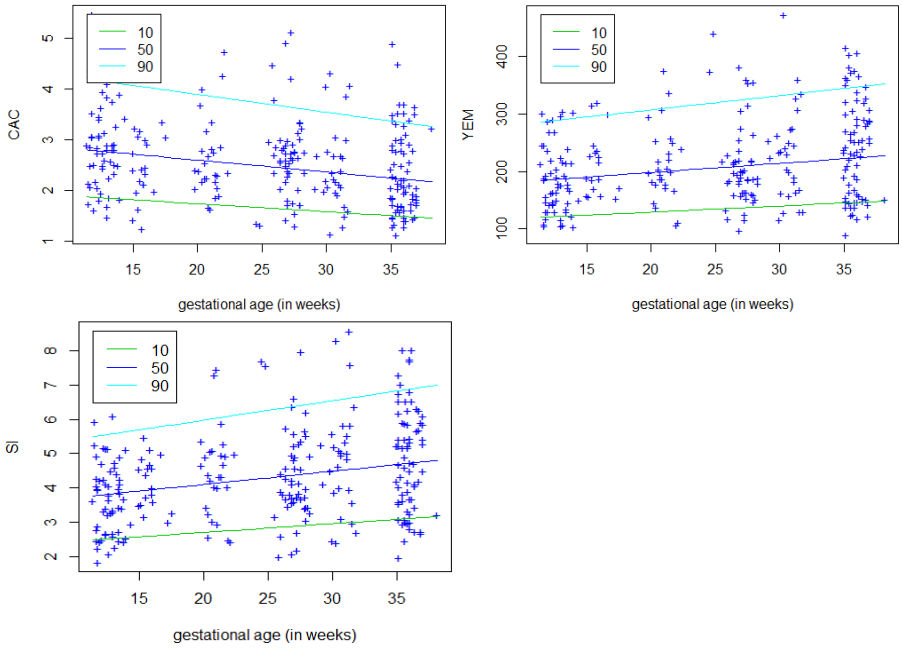

Figure 3. Correlation (scatterplots) of elasticity indexes for the right common carotid artery and the gestational age (x-axis in weeks). Y-axis: carotid artery compliance (CAC) in %/10mmHg; young elastic modulus (YEM) in mmHg/mm; stiffness index (SI); Percentiles, P10, P50 and P90.

Table 3. Blood pressure, vascular properties and elasticity indexes of the right maternal common carotid artery per gestational age.

Gestational age	<18w	18-34w	>34w
Number	78	105	73
DBP (in mmHg), mean ± std	125 ± 11	123 ± 10	126 ± 11
SBP (in mmHg), mean ± std	73 ± 9	70 ± 8	74 ± 8
MAP (in mmHg), mean ± std	90 ± 8	88 ± 8	92 ± 8
IMT (in µm), mean ± std	521 ± 60	515 ± 56	505 ± 60
DIA (in µm), mean ± std	6565 ± 381	6804 ± 449	6880 ± 439
DIS (in µm), mean ± std	469 ± 125	456 ± 132	406 ± 124
CAC (in %/10mmHg), mean ± std	2.86 ± 0.86	2.60 ± 0.82	2.35 ± 0.82
YEM (in mmHg/mm), mean ± std	200 ± 72	218 ± 74	241 ± 84
SI, mean ± std	3.97 ± 1.22	4.53 ± 1.40	4.86 ± 1.48

DBP = diastolic blood pressure; SBP = systolic blood pressure; MAP = mean arterial pressure; IMT = intima media thickness; DIA = diameter; DIS = distension; CAC = carotid artery compliance; YEM = young elastic modulus; SI = stiffness index.

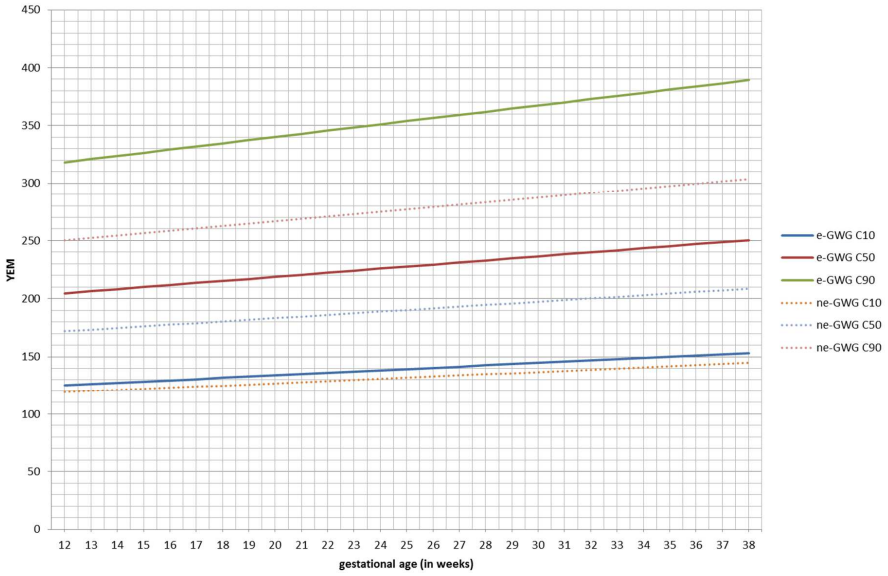

Figure 4. Young elastic modulus (YEM) in the maternal right common carotid artery from 12-38 weeks of gestational age for excessive and non-excessive gestational weight gain (GWG). Na-GWG = non appropriate or excessive gestational weight gain (solid lines); a-GWG = appropriate or non-excessive gestational weight gain (dotted lines). C10, C50 and C90 = Percentile 10, 50 and 90. X-axis, gestational age in weeks; Y-axis, YEM in mmHg/mm.

Increasing maternal age, as a continuous variable, was associated with decreasing distension ($p<0.001$), higher IMT values ($p=0.006$) and lower compliance ($p<0.001$) and therefore higher arterial stiffness indices (YEM/SI, both $p<0.001$) in the CCA throughout gestation. In non-corrected analysis, the GWG, excessive versus non-excessive, provided an effect on CCA elasticity parameters by lower distension ($p= 0.05$) and compliance ($p = 0.03$) and higher stiffening for YEM ($p<0.001$) and SI ($p=0.01$). When corrected for maternal age, a significant effect on the IMT-independent stiffness index, the YEM ($p=0.004$; figure 4) remained. In the e-GWG group the median age was 31 years, versus 29 years in the ne-GWG group ($p<0.001$). No effect of obesity as compared to being non-obese (normal- or overweight), or overweight in all CCA properties and elasticity indices was demonstrated (table 2). Also being overweight did not result in any effect on the CCA measurements as compared to normal pre-gestational BMI (data not shown). Lastly, parity (being nulliparous or parous) also did not show any effect on the CCA wall properties during pregnancy.

DISCUSSION

To our knowledge we present the first large longitudinal radio-frequency ultrasound-study on the vascular remodeling of the maternal carotid artery during pregnancy in low-risk women. Our study offers important new information on the natural development of vascular properties and elasticity in the CCA during pregnancy in relation to maternal age, weight gain and pre-gestational BMI. We demonstrate that, when corrected for maternal age, increased arterial stiffening during gestation correlates with gestational weight gain, as a short term and modifiable variable.

Pregnancy induces hemodynamic changes in women: predominantly a decreased peripheral resistance, increased plasma volume and increases cardiac output are observed. These adaptations are required for a successful pregnancy outcome and hypertensive disorders may occur during pregnancy. These disorders have recently been identified as being a risk factor for cardiovascular diseases later in life by decreasing arterial elasticity.[31] Pregnancy is therefore considered a 'hypertensive stress-test' in women.[32] Many different methodologies have been used to obtain information on vascular function. Therefore, in normal pregnancy various results concerning arterial compliance are reported. Applanation tonometry (pulse wave velocity analysis) was one of the first reported methods used in pregnant women and is therefore commonly used as the standard for arterial elasticity research.[7,9,11] This method measures the pulse wave velocity, produced by the central aorta at the level of the peripheral brachial artery, which serves as a reflection of the aortic compliance. In normal pregnancies it reveals a nadir of arterial stiffness in the second trimester, and a similar level of arterial stiffness in the first and third trimester.[11] There is some debate over whether these peripheral measurements reflect real elastic artery remodeling.[11,14,15] Therefore other studies, like ours, investigated local vascular elastic alterations in peripheral vessels that are easily accessible like the CCA. The Cardiovascular Risk in Young Finns study used high frequency B-mode ultrasound scanning combined with biomarkers to investigate atherosclerotic risks factors in early life in elegant long-term studies. In a cross-sectional study of approximately 30 pregnant women per different trimester and matched controls, they reported a decrease in compliance and an increase in stiffness during pregnancy for the CCA.[25] In the current study, we confirmed this finding. In contrast to the Finnish study however, we found a negative correlation between gestational age and IMT evolution and the mean values per trimester we obtained for CAC, YEM and SI were all lower with smaller SD's. Besides our larger sample size and longitudinal design, the differences can probably be also explained by the highly sensitive automatic arterial wall tracking system we used and the fact that all measurements were performed by a single operator. Intima media thickness measurement is traditionally considered the gold standard in detecting atherosclerosis. In pregnancy it appears that the intima/media ratio (I/M ratio) seems to

better reflect the wall remodeling than the IMT complex.[10] Our methodology did not enable us to differentiate the separate IMT-layers, but we were capable of identifying a significant decrease in IMT during gestation, reflecting the expected adaptation of the vascular wall to an increased CCA diameter due the higher plasma volume throughout pregnancy. Hypothetically, the modified estradiol levels in our 'older' mothers could have contributed to an altered I/M ratio (through an increased intima and decreased media) and therefore to a higher IMT complex in our study. Recently the first reports of radio-frequency (RF) ultrasound findings in late third trimester pregnancies were published.[14,15,33] These small studies focused mainly on the hyperdynamic conditions (like pre-eclampsia), but included long term follow up. They revealed with this sensitive technique persistent vascular wall modifications at eighteen months postpartum in the preeclamptic group. The technique used in our study, RF ultrasound, is a highly sensitive detection technique for small structures (up to 17µm), respecting the tissue characteristics by preserving the acoustic wave for amplitude and phase. Based on M-mode, even small differences in vascular wall remodeling can be detected in variable hemodynamic circumstances.[13] Also the linked automated wall tracking technique, allows the measurement to include at least six cardiac cycles, allows to compensate for time-frame vascular fluctuations and provides a high quality mean result for both vascular morphology and functionality. Using this highly precise methodology, we were able to detect a decrease in IMT as pregnancy progresses, that, as far as we know, was never described before. Based on the 50th percentile at 12 weeks of gestation the IMT decreased at 38 weeks from 519µm to 500µm (table 1, supplement 2). This even outbalanced the six months increase of IMT of 7.4µm (annually 14.7µm), which is the natural IMT increase ('aging'), as previously reported in healthy men.[34] Furthermore, when maternal age was added as an explanatory variable in the GAMLSS model, there was a positive correlation with IMT development. This suggests that older mothers could be more vulnerable to vascular wall remodeling during pregnancy. Whether this effect is transient and reversible, can only be elucidated in long term follow up studies. Possibilities for funding of these studies are currently being investigated. The negative effects of excessive GWG on the CCA elasticity indices are striking. When corrected for maternal age (the e-GWG group had a slight but significant higher maternal age), the YEM remained significant, although the CAC and SI still demonstrated a trend. The YEM is a stiffness index unrelated to IMT development and therefore less dependent on maternal age. This has not been described before and the etiology is also unknown. Pregnancy is a period where there is a general insulin insensitivity and hence a greater degree of glucose intolerance. The glucose intolerance could lead to increased insulin concentrations, purported to be associated with arterial stiffening.[5] Furthermore, maternal GWG reflects a placental transfer of nutrients, glucose and free fatty acids and if there is excessive GWG this accompanies a larger fat deposition.[35] Together with the higher insulin levels characteristic

in pregnancy, this might explain the negative effects on vascular wall remodeling of the maternal CCA in women with e-GWG, even in the absence of IMT increase. We can only speculate on this issue, and since we only measured insulin in the obese pregnancies, further investigations needed to address this issue are currently underway.[18] In contrast to e-GWG a higher maternal BMI did not affect CCA elasticity and wall property development in pregnancy. Previous studies have reported a decreased arterial elasticity possibly mediated through an obesity mediated hyperinsulinemia, although this did not concern a pregnant study group.[36] We did not observe this BMI effect while our cohort contained a large (38%) number of obese patients. This, and the fact that we also closely monitored and excluded patients showing an abnormal glucose tolerance, enable us to distinguish the effects of maternal adiposity (BMI), nutrient intake and storage (GWG) and abnormal glucose tolerance. These three separate contributors play an important role in the development of complications in the mother and the fetus, both short and long term.[37] It is important to distinguish the different contributors and their specific effects, especially as GWG is potentially modifiable. Interventions to reduce GWG in pregnancy can have benefits for both the mother and the fetus. These interventions often reflect lifestyle, physical activity or a combination of both. But, although there has been a reduction in GWG, the effect on the prevention of the adverse outcomes are mixed.[38] Strengths of this study include the longitudinal design and the application of well-defined inclusion/exclusion protocols, while not customizing the cohort too tightly. This prevented the data from skewing the results and provided real reference charts, other than standard charts. Also, the use of RF-ultrasound technique, all performed by a single operator, therefore overcoming the inter-observer variability. Finally the statistical GAMLSS method, as recommended by the WHO Multicentre Growth Reference Study Group especially for development and regeneration data.[30] Some limitations also need to be mentioned: Obese women were slightly overrepresented in our cohort, but this did not lead to increasing rates of e-GWG or GDM. Possibly because the obese women were enrolled in a lifestyle intervention program and women with pre-existing GDM were excluded.[16] There was no data available on the nutrition in the full cohort to explain the positive correlation between excessive GWG and decreased vascular elasticity. We have 5-7days food records in 61/80 of the cohort and further analysis is currently in progress. Our results could not be correlated with vascular biomarkers and no long-term follow up was performed. Therefore leaving the question unanswered whether the observed vascular changes are transient and could be reversible or not.

In summary, our study provides to our knowledge the first longitudinal radio-frequency vascular data during pregnancy for the common carotid artery in low risk pregnant women. It offers interesting new insights on the general behavior of the arterial elasticity in relation to gestational age, maternal age, gestational weight gain and pre-pregnancy BMI class. We demonstrate a positive correlation of both maternal age and excessive

GWG with increased arterial stiffness during gestation in CCA. Whether this concerns a transient, reversible phenomenon requires further investigation.

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ABDOMINAL AORTA FETAL ABDOMINAL
INTIMA MEDIA THICKNESS FETAL FAT
FUNCTIONAL LINEAR DISCRIMINANT
ADDITIVE MODEL LOCATION, SCALE AND
MELLITUS NON-EXCESSIVE GESTATIONAL
GROWTH FACTOR INTIMA-MEDIA THICKNESS
SOCIETY ON OBSTETRICS AND
GROWTH LARGE-FOR-GESTA
NUTRITION EPIGENOME
GROWTH STUDY GROUP
IMAGING MRI FATTY LIVER DISEASE
GLUCOSE TOLERANCE TEST OGTT ODDS RATIO
ARTERIAL STIFFNESS QUALITY
BLOOD PRESSURE SKINFOLD TRICEPS
FAT THICKNESS SMALL-FOR-GESTATIONAL AGE
VASCULAR AND BIOMETRY ULTRASOUND
STUDY VABULOS YOUNG ELASTIC MODULUS

Chapter 9

Fetal abdominal aorta remodelling correlates with maternal obesity independently of fetal size : a longitudinal study *in utero*

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Submission in progress

ABSTRACT

Introduction

Maternal obesity may influence cardiovascular development in the offspring. The aim of our study was to detect modifications in the fetal abdominal aorta (fAA) with radio-frequency ultrasound in relation to maternal characteristics and fetal growth and development.

Methods

Mothers with low-risk pregnancies were recruited during the first trimester and prospectively followed-up, gestational weight gain (GWG) was categorized according to the 2009-revised Institute of Medicine guidelines. The fetus was monitored for growth and vascular development of the fAA intima media thickness (fAA-IMT) and fAA elastic properties (arterial stiffness) with high resolution radio-frequency (RF) ultrasound. FAA elastic indices were calculated: fetal abdominal aortic compliance (fAAC), young elastic modulus (YEM) and stiffness index (SI). Generalized Additive Model Location, Scale and Shape (GAMLSS) was used to align the timeframes of the measurements.

Results

Seventy-four mothers were included in the final analysis of whom 50% were nulliparous and 49% showed excessive GWG (e-GWG). fAA measurements could reliably be obtained from 25 weeks onwards. All fAA properties (IMT, diameter (DIA) and distension (DIS)) showed a positive correlation ($p<0.001$) with gestational age. FAA elasticity indices decreased during pregnancy with decreasing fAAC ($p<0.001$) and increasing arterial stiffness (YEM, $p<0.001$ and SI $p = 0.04$). Elasticity indices (fAAC, YEM and SI) were also significantly influenced by fetal size. The fAA-IMT and DIS showed a significant smaller increase in obese mothers ($p = 0.03$ and $p = 0.009$), compared to normal weight ($\leq$24.9kg/m^2 body mass index (BMI)) mothers, even after correction for fetal size.

Conclusion

Arterial stiffness in the fAA increases towards the end of gestation, and is more pronounced if fetal growth is fast. FAA-IMT development is negatively associated with higher maternal BMI, suggesting a modulation of the fetal vessel-wall in obese and overweight mothers.

INTRODUCTION

In Western populations cardiovascular disease (CVD) is the most important cause of mortality.[1] While clinical manifestations appear more during adulthood, the process of atherosclerosis, as a first sign of CVD, already starts early in life.[2,3] This very early exposure to an adverse environment during pregnancy, and the subsequent predisposition to disease later in life is referred to as the 'fetal origin of adult disease', as proposed by Law et al. and is one of the features of the Barker's hypothesis 'Developmental origins of health and disease (DOHaD)', or 'fetal programming'.[2,4]

Environmental factors can exert their influence on the metabolic health of the offspring during pregnancy by abnormal gestational weight gain (GWG), (gestational) diabetes mellitus ((G)DM) and maternal obesity.[5,6] These maternal metabolic determinants are associated with vascular remodelling in the offspring.[7] It has been reported that relative fetal undernourishment causes alterations on the arterial level, influencing cardiovascular development,thereby confirming a possible mechanism for the correlation of low birth-weight and CVD in adulthood.[8] At the other end of the spectrum fetal overnutrition and high birth weight predisposes for childhood obesity and increased blood pressure in adulthood.[9-11] And although studies are still limited, there are data showing that being born large for gestational age (LGA) is associated with an increased aortic intima media thickness in childhood.[12-14] Many studies on cardiovascular risk predictors have been performed with imaging of arterial wall layers and properties, mainly focussing on the common carotid artery (CCA), as this vessel is easily accessible in children and adults. In foetuses and newborns, the AA is a better target to study vascular wall properties. Indeed histopathology studies reveal that this is the first region of vascular inflammation and remodelling preceding future adult lesions.[15,16] Furthermore, in a previous feasibility study we have found the fetal abdominal aorta (fAA) to be the most accessible fetal vessel during the second and third trimester.[17] So far, studies examining the fetal vascular development are limited and the two reports availablefocus on cross-sectional third trimester measurements.[18,19] With current high standard vessel-wall tracking techniques using radio-frequency (RF) ultrasound, it is possible to detect arterial properties and elasticity.[20,21] The aim of our study was to prospectively detect modifications of the fAA during pregnancy using RF ultrasound and to relate these to maternal characteristics and fetal growth.

DESIGN AND METHODOLOGY

This prospective cohort study was performed at the Department of Obstetrics and Gynecology of the University Hospitals Leuven (Belgium). Pregnant women of different

pre-gestational BMI classes were included at their first trimester ante-natal visit between August 2012 and December 2013. This vascular sub-study was part of three prospective studies for low-risk, obese and non-obese pregnancies monitored for GWG, development of GDM and nutrition.[22] All studies were approved by the Ethics Committee for Human Experimentation of the Faculty of Medicine, Katholieke Universiteit Leuven (S52172, S54026 and S55325). All women have signed written informed consent to take part in the study. Exclusion criteria for the sub-study were: pre-existing DM, pre-existing hypertension, development of pre-eclampsia, multiple pregnancies, missing longitudinal data and adverse events (major fetal abnormality, fetal death and/or immature birth).

Maternal determinants

At the first ante-natal visit, parameters for BMI (height and pre-pregnancy weight) were obtained as described elsewhere.[23] The total GWG was computed using the final weight measured at the last prenatal visit, minus the pre-gestational weight. GWG gain was categorized according to the Institute of Medicine (IOM) 2009 revised criteria: 11.5-16kg (normal weight), 7.0-11.5kg (overweight) and 5.0-9.0kg (obese).[24] If women crossed the upper threshold, they were considered excessive-GWG (e-GWG) or non-excessive (ne-GWG) if they had either adequate or insufficient GWG.[25] If women were considered high risk (history of GDM, BMI≥30kg/m^2), a fasting glucose measurement <92mg/dL (<5.1mmol/L) at baseline was performed to exclude pre-existing DM. Another GDM screening was performed at 24-28 weeks using a 75g oral glucose tolerance test (OGTT) based on the 2013 World Health Organization (WHO) criteria.[26] The non-obese population underwent an OGTT if an initial 50g glucose challenge test showed a glycaemia of ≥140mg/dL (≥7.8mmol/L) at 24-28weeks (local protocol).

Vascular and fetal measurements

Determination of gestational age (GA) was based on the fetal crown rump length (CRL) measured between 7 and 14 weeks of gestation in all patients.[27] Ultrasound examinations were performed at three time points in gestation: 12-18 weeks, 26-32 weeks and between 35-37 weeks of gestation. A proportion (29 participants) was also examined between 18-22 weeks of gestation. Fetal measurements included: Biparietal Diameter (BPD), Head Circumference (HC), Abdominal Circumference (AC) and Femur Length (FL). The measurements were obtained in accordance to the guidelines of the International Society for Ultrasound in Obstetrics and Gynecology (ISUOG, www.isuog.org).[28] Estimated fetal weight (EFW) was calculated based on the BPD, HC, AC and FL.[29] The trans-abdominal obstetrical ultrasound was performed first, and took at least 25-30 minutes and gave the women the opportunity to acquire a resting state. FAA measurements were obtained with the mothers in a supine position (or in slight

lateral tilted position, if necessary when facing aorto-caval compression at advanced gestation), trans-abdominal, below the fetal renal artery junction and above the iliac bifurcation in a coronal or saggital plane with an angle of insonation of 60°–90° over a region of interest (ROI) of the vessel of 15mm (e-figure 9.1). High resolution RF, real-time B-mode ultrasound (ESAOTE, Genua, Italy) was used, with integrated applications (QIMT -Quality IMT- and QAS -Quality arterial stiffness-) as developed in ART-LAB systems. The technique is based on an automated arterial wall tracking system, which preserves the authenticity of the acoustic wave and therefore the examined tissue.[20] For IMT the far-wall measurement was taken. The QAS provided the vascular diameter (DIA) between diastolic and systolic phases and the arterial distension (DIS). A 3-11 MHz, linear-array ultrasound probe was used. All measurements for both QIMT and QAS were performed three times over 6 consecutive cardiac cycles at every time point by the same operator (SG). This resulted in three mean values for every parameter. An average of the three mean (3x6) values was taken, based on 18 cardiac cycles, compensating for the hemodynamic fluctuations characteristic in vascular measurements. Fetal blood pressure was calculated using the following algorithm: systolic blood-pressure= 1.06*GA +15.91 and diastolic blood-pressure= 0.67*GA+2.47, as previously reported.[30] After analysing the RF-data via ART-LAB software, the following CCA elastic indices were calculated.[31,32]

- Fetal abdominal artery compliandce (fAAC), as the ability of the vessel to expand in response to the cardiac pulse pressure: ([systolic diameter-diastolic diameter]/ diastolic diameter)/(SBP-DBP) in %/10mmHg.
- Young elastic modulus (YEM), provides an estimate of the arterial stiffness, but is less dependent on IMT (arterial wall characteristic)[33]: ([SBP-DBP]x diastolic diameter)/ ([systolic diameter-diastolic diameter]/IMT in mmHg/mm.
- Stiffness index (SI), an elastic property relatively independent of blood pressure[34]: ln(SBP/DBP)/([systolic diameter-diastolic diameter]/diastolic diameter).

Birth outcomes (weight and gestational age at birth) were obtained from the electronic obstetrical records of the individual patients.

Variability

Since multiple measurements were obtained based on at least 6 cardiac cycles at each time point, the intra-observer variability could be calculated for IMT, DIA and DIS. The three measurements cycles were regarded as separate observations (e-figures 9.2-9.4). The intra-class correlation coefficients for IMT, DIA and DIS were: 0.63-0.59 and 0.65, respectively.

Statistical analysis

GAMLSS (Generalized Additive Models for Location, Scale and Shape, www.gamlss.org) R-package was applied to construct the growth-evolution curves for the vascular

properties IMT, DIA, DIS, fAAC, YEM, SI and for the fetal growth EFW.[35,36] We assessed several distributions: Box-Cox-t, Box-Cox Cole and Green, and Box-Cox Power Exponential. Goodness-of-fit of the models was assessed with QQ plots, AIC (Akaike Information Criterium) and worm plots. The goodness-of-fit was investigated covering the gestational period from 25 to 40 weeks. GAMLSS smoothed the ante-natal growth curves for IMT, DIA, DIS, fAAC, YEM, SI and EFW. Percentiles (P10, P50 and P90) for each investigated parameter were calculated.

Only the vascular data-sets including a minimum of two consecutive time points were analyzed. The effect of maternal BMI (obese if ≥30kg/m^2; overweight if 25-29.9kg/m^2; normal weight if ≤24.9kg/m^2) and the effect of excessive GWG versus non-excessive (adequate or insufficient) GWG was assessed by adding these variables to the GAMLSS model. The same analysis was performed for maternal age (continuous), parity (nulliparous versus parous), birthweight (continuous) and fetal gender. The effect of BMI (obese versus normal BMI) was added for EFW, fAA-IMT, fAAC. The effect of exceptionally large for gestational age fetuses (EFW>P90) and normal BMI was added to the model for DIA. Also the effect of EFW>P90 for all BMI classes, e-GWG and birth-weight was added. All fetal growth and vascular data and maternal characteristics were corrected for gestational age if applicable and introduced as consecutive measurements in the GAMLSS model.

RESULTS

From the 101 recruited pregnant women, 5 were excluded based on an abnormal fasting glucose measurement (pre-existent DM) at baseline. There were 4 drop-outs, one late first trimester miscarriage, 4 pre-eclamptic participants and 14 participants were excluded because fewer than two time-points of vascular measurement were available for analysis. This resulted in 74 mother-fetus pairs in the final analysis: 34 (46%) had a normal pre-gestational weight, 13 (18%) were overweight and 27 (36%) obese. Thirty-six women (49%) showed e-GWG and 14/74 (18.9%) had an insufficient GWG. The maternal baseline characteristics are outlined in table 1. All fAA properties (IMT, DIA and DIS) were highly dependent on gestational age (table 2). The IMT, DIA and the DIS all increased during pregnancy (figure 1). An overview of the measurements at each gestational week is given in table 3. Detailed reference values per gestational week are provided in e-table 9.2. Also all CCA elasticity indices changed during gestational age (table 2). The fAAC decreased ($p<0.001$), while the YEM and SI both increased significantly ($p<0.001$), with advancing gestational age (figure 2). The elasticity indices at different time-points are detailed in table 3. Reference values per gestational week are provided in e-table 9.3.

Table 1. Study group characteristics.

	Total (N= 74)
Maternal age (years), mean ± std	29.8 ± 4.1
Nulliparity (%)	37 (50%)
Obese (%)	27 (36%)
Overweight (%)	13 (18%)
Normal weight (%)	34 (46%)
Excessive GWG (%)	36 (49%)
GA at birth (wks, 0/7d), mean ± std	39w3d ± 1w1d
Birth weight (g), mean ± std	3425 ± 516
Smoking (%)	5 (6.8%)
GDM (%)	6 (8.1%)
PIH (%)	4 (5.4%)

GWG = gestational weight gain; GA = gestational age; wks = weeks; d = days; g = gram; GDM= gestational diabetes mellitus; PIH = pregnancy induced hypertension; std. = standard deviation.

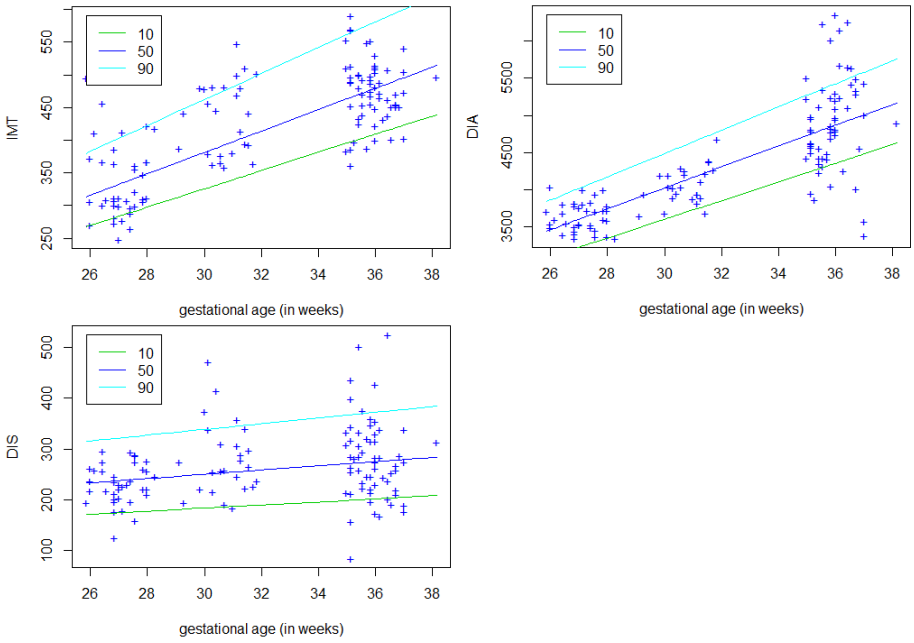

Figure 1. Correlation (scatterplots) of vascular properties in the fetal abdominal aorta artery with the gestational age (x-axis in weeks). Y-axis: Intima media thickness (IMT) in µm, diameter (DIA) in µm and distension (DIS) in µm. Percentiles, P10, P50 and P90.

Table 2. Correlations of maternal & fetal characteristics in the development of vascular properties and elasticity indexes for the fetal abdominal aorta (fAA); Maternal & fetal characteristics were introduced as explanatory variables in the GAMLSS model; p values are given. Italic means without the EFW>P90 (LGA fetus).

Properties & Elasticity	GA#	Obese vs Non-obese	*Obese vs Non-obese**	Obese vs Normal	*Obese vs Normal**	Overweight vs Normal	*Overweight vs Normal**	Birth-weight#	*Birth-weight**	Males vs Females
IMT	**<0.001** (↑)	0.16	0.17	**0.03** (↓)	**0.03** (↓)	**0.02** (↓)	**0.02** (↓)	0.26	0.21	0.84
DIA	**<0.001** (↑)	0.54	0.30	0.29	0.14	0.09	0.07	**0.02** (↑)	0.13	0.11
DIS	**0.009** (↑)	**0.01** (↓)	**0.01** (↓)	**0.009** (↓)	**0.008** (↓)	0.25	0.26	0.08	**0.04** (↓)	0.54
fAAC	**<0.001** (↓)	0.10	0.11	0.09	0.10	0.68	0.68	**0.009** (↓)	**0.004** (↓)	0.82
YEM	**<0.001** (↑)	0.20	0.23	0.34	0.40	0.40	0.40	**<0.001** (↑)	**<0.001** (↑)	0.56
SI	**0.04** (↑)	0.18	0.19	0.19	0.22	0.19	0.92	**0.01** (↑)	**0.01** (↑)	0.39

GA = gestational age; IMT = intima media thickness; DIA = diameter; DIS = distension; fAAC = fetal abdominal aorta artery compliance; YEM = young elastic modulus; SI = stiffness index; * = corrected for estimated fetal weight (EFW) >P90 from normal BMI group; # = continuous variable. ↑ = promotes development vascular unit; ↓ = inhibits development vascular unit; Significance is considered if p <0.05.

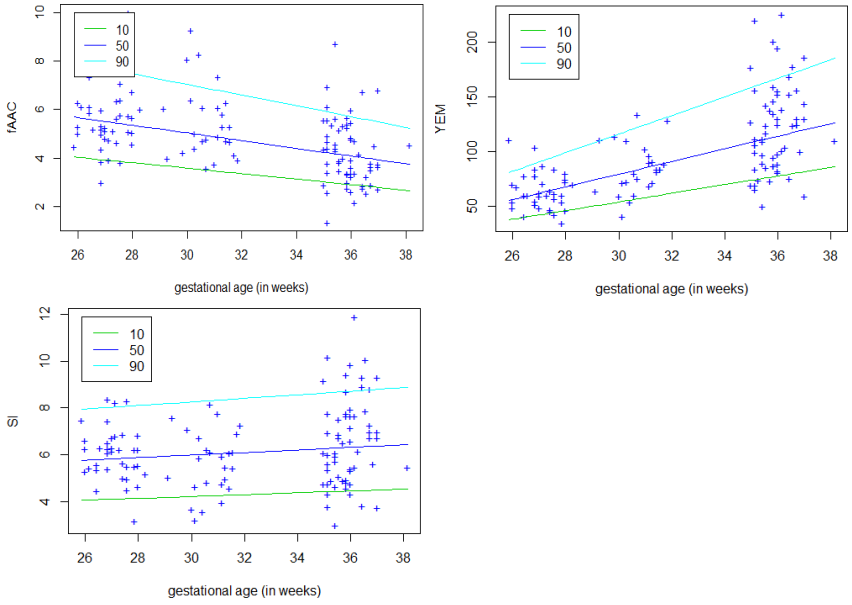

Figure 2. Correlation (scatterplots) of elasticity indexes for the fetal abdominal aorta artery and the gestational age (x-axis in weeks). Y-axis: fetal abdominal aorta artery compliance (fAAC) in %/10mmHg; young elastic modulus (YEM) in mmHg/mm; stiffness index (SI); Percentiles, P10, P50 and P90.

Table 3. Vascular properties en elasticity measurements of the fetal abdominal aorta artery for each gestational week. Values are displayed for 50th percentile as calculated by the GAMLSS model.

GA	IMT (in µm)	DIA (in µm)	DIS (in µm)	fAAC (in %/10mmHg)	YEM (in mmHg/mm)	SI
26	316	3467	234	5.67	56.0	5.77
27	332	3607	238	5.51	61.8	5.82
28	349	3747	242	5.35	67.6	5.88
29	365	3887	246	5.19	73.4	5.93
30	381	4028	251	5.04	79.2	5.99
31	398	4168	255	4.88	85.0	6.04
32	414	4308	259	4.72	90.8	6.10
33	430	4448	263	4.56	96.6	6.15
34	447	4588	267	4.40	102.4	6.21
35	463	4729	271	4.24	108.2	6.26
36	479	4869	276	4.09	114.0	6.32
37	496	5009	280	3.93	119.8	6.37
38	512	5149	284	3.77	125.6	6.43

GA = gestational age; IMT = intima media thickness; DIA = diameter; DIS = distension; fAAC = fetal abdominal aorta artery compliance; YEM = young elastic modulus; SI = stiffness index.

Fetal characteristics

Birth weight on a continuous scale positively correlated with the increase in diameter, and showed a trend for lower distension increase for the fAA. Birth weight and IMT were not significantly correlated, but the elasticity parameters were significantly enhanced for the compliance (fAAC) decrease and for the YEM/SI increase (table 2). Since birth weight was associated, we corrected for the LGA fetus: EFW>P90 as obtained at every time point in the normal BMI group (table 2 and e-table 9.1). If corrected for the LGA fetus (EFW>P90), birthweight is significantly associated with a reduced increase in distension (p= 0.04) and greater decrease in compliance (p= 0.004, table 2). The corrected birth weight is significantly correlated with increasing YEM and SI (table 2). Gender did not show any association with vascular development of the fAA (table 2).

Maternal characteristics

A higher pre-gestational BMI was associated with a lower increase in fAA-IMT and a lower increase in DIS during pregnancy, especially when comparing the obese group versus the normal BMI group (p = 0.03 and p = 0.009, respectively). Furthermore, when correcting for the LGA fetuses, there still remained a significant correlation of higher BMI as compared to normal BMI with the IMT (p = 0.03) and DIS (p = 0.008) evolution *in utero* (table 2). Similarly being overweight had a negative association with the IMT increase (p = 0.02) compared to normal weight women, even when corrected for LGA fetuses (p = 0.02, table 2). When the obese group was compared to the other BMI categories (normal and overweight) together, the association with obesity remained significant for the fetal aortic distension (p= 0.01), which persisted after correction for LGA fetuses (p = 0.01, table 2).

Parity, maternal age and GWG did not show any significant effect on the fAA development *in utero* (e-table 9.1).

DISCUSSION

To our knowledge this is the first longitudinal radio-frequency ultrasound study of vascular remodeling in the fetal abdominal aorta. Our study offers important new information and insight inro the natural development of vascular properties and elasticity in the fAA in relation to gestational age, fetal weight and maternal pre-gestational BMI category. We demonstrate that, even when corrected for the LGA fetus (EFW>P90), increased arterial stiffening during gestation in the fAA positively correlates with fetal growth. Furthermore, maternal pre-gestational BMI category isnegatively associated with vessel-wall remodeling of the fAA.

Pregnancy induces hemodynamic changes , and is therefore considered a 'hypertensive stress-test' in women.[37,38] When this adaptive mechanism fails, this may predispose to pathologic situations like gestational hypertension, preeclampsia and fetal growth restriction. This is most frequent in women with an altered metabolic state.[39] Women with diabetes, obesity and excessive weight gainare at higher risk to fail the stress test. For the offspring of these women, it is likely that this maladaptation has repercussions on fetal growth and long term vascular development. This is suggested by the many studies that focus on the relationship of maternal characteristics and birth-outcome on long-term cardiovascular effects in the mother and offspring. In contrast, studies including the *in utero* environment and prenatal changes are scarce.[40,41] The intima-media vessel wall layer has been reported as the first site of atherosclerosis and thickening is regarded as an early pre-clinical sign of future lesions.[15,16] Cosmi et al and Crispi et al have confirmed increased IMT of the intra uterine growth restricted fetuses and the small for gestational age babies.[8,18] IMT measurements in school aged children were higher in association with higher birthweight.[13,14] Most postnatal reports concern the common carotid artery, but Koklu et al, reported on the IMT of the abdominal aorta in neonates, born at different gestational ages.[42] Their IMT results correspond well with the ones from our *in utero* study. One of the reasons forthe limited availability of data, regarding prenatal information on vascular development, is that the parameters (e.g. blood pressure) necessary to gain information on the fetal cardiovascular system are difficult to obtain. Furthermore, the available research methods require a subject in a resting state and require close contact with the subject (applanation tonometry), neither of which are feasible in a moving fetus. RF ultrasound offers a non-invasive possibility to overcome these limitations.

Recently the first reports of radio-frequency (RF) ultrasound findings in late third trimester in the maternal common carotid artery were published.[43,44] The technique used in our study, RF ultrasound, is a highly sensitive detection technique for small structures (up to 17µm), respecting the tissue characteristics by preserving the acoustic wave for amplitude and phase. Based on M-mode, even small differences in vascular wall remodeling can be detected in variable hemodynamic circumstances.[21] Also the linked automated wall tracking technique, allows to include at least six cardiac cycles and to compensate for time-frame vascular fluctuations and to provide a high quality mean result for both vascular morphology and functionality. Using this highly precise methodology, we were able to describe vessel structures and their longitudinal development during pregnancy for the first time.

Not surprisingly we found gestational age to have a significant effect on all fetal vessel properties (IMT, DIA and DIS). But the progressive arterial stiffening in the fetal aorta (decrease of fAAC, increase of YEM and SI) during pregnancy is a novel finding. In addition the association between birth weight and fetal growth with the elasticity properties of

the fAA in low-risk pregnancy fetus has never been reported before. Most interestingly, and also novel, is the finding that maternal obesity was significantly associated with altered vascular development in the fetus, shown by the correlation between maternal BMI and decreased IMT and distension.

We can offer some potential mechanisms for our findings. It has been demonstrated that the extracellular matrix (ECM) of the vessel wall develops rapidly during late gestation and in the newborn. Elastin which provides elasticity, and collagen, are both components of the ECM and contribute to vascular stiffness. It is elastin that accumulates rapidly in late gestation and in the early neonatal period, slowly degrades as the individual ages. Collagen on the other hand accommodates increased blood pressure and vascular shear stress, and is positively correlated with aging.[45] Therefore the ECM organization and development strongly relies on intravascular pressure and flow. *In vitro* studies in humans have been performed with umbilical arteries, with an increased umbilical IMT in pre-eclamptic versus non-hypertensive subjects.[46] An IUGR-induced sheep model was used to study the effect of IUGR on carotid vessel compliance with pressure-diameter measurements and histo-morphology. They reported a changed collagen-to-elastin ratio in the carotid arteries of the IUGR-sheep fetus. They conclude that a decreased elastin, due to higher pressure, and increased collagen stiffen the walls of the aorta and great arteria, especially in the growth inflicted individual.[47] One could therefore hypothesize that our observations of increased arterial stiffness in fetal aorta are probably due to the rapid vessel development in late gestation, with increasing fetal blood flow and hence change in architecture and function of the vessel wall. Based on our observations, the DIA increase was much higher than the increase of DIS, which might explain the relative stiffening of the vessel-wall . Whether this observation reflects a natural vessel-wall remodeling, as found in the histo-morphology study referred to above, is unknown. The finding that a higher maternal BMI is associated with a lesser IMT increase, could also reflect a remodeling effect of the arterial vessel layers. This can lead to a thinner (and less compliant) fAA. In a previous study we have reported a positive association between higher BMI and fetal weight, therefore we corrected for the LGA fetus (EFW>P90) in the GAMLSS analysis for the BMI-effect.[48] The effect of obesity and overweight on the fAA remained even after this correction.

Strengths of this study include the longitudinal design and the application of well-defined inclusion/exclusion protocol, while not customizing the cohort too tightly. This prevented the data from skewing the results and provided real reference charts, rather than standard charts. Also, the use of RF-ultrasound technique, all performed by a single operator, helped overcoming the inter-observer variability. Finally the statistical GAMLSS method used is recommended for development and regeneration data by the WHO Multicentre Growth Reference Study Group.[36] Some limitations to this study include that obese women were slightly overrepresented in our cohort, but this did not lead to

increasing rates of e-GWG or GDM. This may have been because the obese women were being part of a lifestyle intervention program and therefore tightly controlled for pre-existing glucose intolerance.[22] There was no data available on nutrition in the full cohort to explain the positive correlation of BMI and the decreased fAA-IMT development. We have 5-7days food records in 57/74 of the cohort and further analysis is currently in progress. We had to calculate blood pressure for the fetus, since this was not feasible to measure in utero.[30] We cannot rule out that individual blood-pressure fluctuations from the fetus influenced our results on the elasticity indices. Furthermore, our results could not be correlated with vascular biomarkers and no long-term follow up was performed.

In summary, our study provides the first longitudinal radio-frequency vascular data during pregnancy for the fetal abdominal aorta in low risk pregnant women. It offers interesting new insights on the general behavior during early life of the fetal arterial elasticity in relation to gestational age, fetal growth and pre-pregnancy BMI class. We demonstrate a positive correlation of both fetal growth and maternal BMI with increased arterial remodeling during gestation in the fetal abdominal aorta. Whether this concerns a transient or persistent phenomenon requires further investigation.

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ABDOMINAL AORTA FETAL ABDOMINAL
INTIMA MEDIA THICKNESS FETAL FAT
FUNCTIONAL LINEAR DISCRIMINANT
ADDITIVE MODEL LOCATION, SCALE AND
MELLITUS NON-EXCESSIVE GESTATIONAL
GROWTH FACTOR INTIMA-MEDIA THICKNESS
SOCIETY ON OBSTETRICS AND
GROWTH LARGE-FOR-GESTA
NUTRITION EPIGENOME
GROWTH STUDY GROUP
IMAGING MRI FATTY LIVER DISEASE
GLUCOSE TOLERANCE TEST OGTT ODDS RATIO
ARTERIAL STIFFNESS QUALITY
BLOOD PRESSURE SKINFOLD TRICEPS
FAT THICKNESS SMALL-FOR-GESTATIONAL AGE
VASCULAR AND BIOMETRY ULTRASOUND
STUDY VABULOS YOUNG ELASTIC MODULUS

Chapter 10

Summary and future perspectives

SUMMARY

The effects of maternal adiposity (high body mass index - high BMI -), nutrient intake and storage (gestational weight gain - GWG -) and (abnormal) glucose tolerance (gestational diabetes - GDM -) are regarded important cornerstones in metabolic research in pregnancy. In **Chapter 1**, I explained, that they play an important role in the development of complications in the mother and the fetus, both short- and long-term. I also reported on how the current state of research in pregnancy has evolved and how it can be used for detailed studies of the unborn child. In this field of research, the fetal period is often conveniently summarized by its resultant birthweight. This outcome parameter is used to compare intra-uterine effects on long- and short term complications in epidemiologic studies. High and low birth weight have been linked to future increased cardiovascular risk profiles, fitting the 'fetal origin of diseases' theory. Beside birth weight, more detailed information on the neonates' body composition (fat distribution), in both lean- and fat mass might reflect the *in utero* development better, although fetal predictors for neonatal adiposity still have to be developed. Furthermore, there is no generally accepted definition of 'neonatal BMI' or neonatal adiposity. At present ponderal index or fat-mass percentage fill this lacuna. Finally, ultrasound in pregnancy is capable of obtaining information on the unborn child and uses an *estimation* for fetal weight. Accuracy for this estimate fluctuates around 6-12% of the actual fetal weight. In the obese mother, on the other hand, visualization of the fetus is even more limited.

As stated in **Chapter 2**, the environment in utero and in early neonatal life may induce a permanent response in the fetus and the newborn, leading to enhanced susceptibility to later diseases. Fetal overgrowth is related to a diabetic intra-uterine environment. The increased prevalence of diabetes mellitus (DM) in children of diabetic mothers is consistent with an epigenetic effect of hyperglycemia in pregnancy acting in addition to genetic factors that induce diabetes in next generations (human and animal data). Fetal growth restriction due to malnutrition on the other hand, leads to a higher incidence of CVD and type-2 DM in later life. Particularly when catch-up growth is present by high caloric postpartum intake. The third trimester seems to be a vulnerable period for the influence of maternal malnutrition, and hence fetal growth restriction (human and animal data). The role of environmental influences, such as excessive nutrition, nutritional restriction or low folic acid and vitamin B12 levels, on DNA methylation in early life development is called epigenetics. The effects of maternal and fetal nutrition stresses the importance of the embryonic environment on epigenetic imprinting for metabolic mechanisms on the future generations. Not every overweight newborn and not every newborn with intra-uterine growth restriction will develop problems in later life. Other factors of course influence final outcomes: in general it depends on the extent of adaptation and on its plasticity later on.

In view from the fetus, the intra-uterine period has been associated with gender specific physical adaptations to a changed nutrient supply from the mother. The male infant body composition has been shown to be more sensitive to maternal influences such as higher pre-gestational BMI and excessive gestational weight gain. And although gender is recognized as a determining factor for differences in outcome in adult medicine, little information is available on fetal gender influence in the prenatal period. Given these complexities the World Health Organization (WHO) Multicentre Growth Reference Study Group (MGRS) recommended GAMLSS (Generalized Additive Model Location, Scale and Shape) for the construction of (WHO) Growth Standards. In **Chapter 3**, we constructed fetal growth curves in a Caucasian population (12368 pregnancies) and further customized for fetal gender. The fetal head measurements were significantly larger in boys compared to girls from 20 weeks' gestation onwards, equating to a 3 day difference at 20-24 weeks. Boys were significantly heavier, longer and had greater head circumference than girls at birth. The Apgar score at one minute and arterial cord pH were lower in boys. These longitudinal fetal growth curves for the first time allow integration with neonatal and pediatric WHO gender specific growth curves. Immediate birth outcomes are worse in boys than girls, and gender differences in intra uterine growth are sufficiently distinct to have a clinically important effect on fetal weight estimation and second trimester dating. Gender differences might play a role in obstetric or immediate neonatal viability management. The implication of these findings is that a boy and a girl at exactly 24weeks gestation might, based on the current late second trimester dating protocols with head measurements, be assigned a gestation as much as 3 days different and an estimated weight difference of 21gram at 24weeks favoring the boys; The term difference adds to 121gram, favoring the boys. Also the boys' head circumference is larger (+0.6cm) at birth. In conclusion, the boys seem to be more vulnerable around viability age (22-24weeks), since they are estimated older than they are in reality. The girls are more vulnerable for this erroneous estimate at the later post-term period in pregnancy. Because at this stage, decisions on ending the pregnancy are often taken and they are older than estimated. But around term, boys do worse for the immediate start (lower first minute Apgar Score and cord-pH) compared to girls.

In **Chapter 4**, a prospective cohort analysis was performed for the influence of maternal GWG and BMI on fetal growth. These factors influence independently fetal growth development during pregnancy and birth weight. Cluster analysis discerned four total GWG clusters: I: ≤0kg, II: 0-4kg, III: 4-12kg and IV: >12kg. For each cluster there was a different fetal weight evolution and also finally, different birth size. When cluster IV was compared to cluster I, the additive effect of the cluster difference on birth weight was +477g. The maternal BMI class acted independently on the fetal weight evolution. A higher BMI class increased fetal growth and hence birth weight. When BMI of 35 was compared to a BMI of 17, the additive effect on birth weight was +334g. The total GWG

effect had most impact from 180 to 200 days gestation when there was a separation between the different GWG clusters. By identifying this relationship between GWG, growth and birth weight, we may now have time potential to influence weight gain during pregnancy and so possibly prevent perinatal complications, improve the metabolic and vascular traits of the infant and possibly improve outcomes in future pregnancies.

Since birthweight is the resultant of the fetal period, the aim of **Chapter 5**, was to increase knowledge on the intra-uterine development with regard to fetal body composition. This was prospectively monitored in a highly controlled glucose tolerant cohort of 126 mothers. GWG was regarded 'excessive' (e-GWG) if the revised-2009 IOM upper thresholds were crossed. If not, the GWG was considered non excessive GWG (ne-GWG). In the prepregnant obese group, the fetal liver size (LL) and the abdominal fat thickness (AFT) growth trajectory increased significantly compared to normal weight women. E-GWG positively correlated with the growth in fetal abdominal circumference , LL, subscapular fat thickness (SFT) and AFT as compared to the ne-GWG group. Birth outcomes were significantly different for weight, length and head circumference in the e-GWG group as compared to the ne-GWG. Obesity and e-GWG both positively affect adiposity during fetal and neonatal life, with e-GWG clearly affecting fetal size in general, which was confirmed by birth anthropometry. In the normoglycemic pregnant women, determinants of the fetal trunk (LL, SFT, AFT and AC) are potentially useful in the prediction of neonatal adiposity. Our longitudinal study determined early signs of fetal adiposity associated with maternal obesity and particularly e-GWG in normoglycemic women. Maternal high BMI correlated with fetal liver size and abdominal fat, where e-GWG additionally correlated with increased subscapular fat and abdominal circumference development. Furthermore, fetal adiposity emerged as a reflection of a nutritious environment in utero with effects detectable as early as 20 weeks. Especially the indicators of the fetal trunk (liver-length, AFT, SFT and AC) are discriminative of fetal adiposity and warrant further investigation as potential early predictors of neonatal adiposity.

Given these results we correlated in **Chapter 6** these measurements in 121, highly controlled glucose tolerant mothers, prospectively, with neonatal body composition indices: neonatal fat mass (NFM), ponderal index (PI) and birthweight. The mean NFM at birth was 11.0% (SD±3.4%), with a cut-off of 15% for the 90th percentile (P90). No association was found between the maternal body mass index (BMI) and NFM. However, children born to mothers who had an e-GWG had a significantly higher NFM (11.6%) as compared to the ne-GWG mothers (10.4%). Fetal measures were highly correlated with neonatal measurements. Fetal abdominal circumference (AC), liver length (LL), subscapular- and abdominal fat thickness, humerus and femur fat mass development correlated significantly with 90th NFM percentile. Fetal AFT did not correlate with the PI. In the normoglycemic woman, e-GWG was associated with an increased neonatal adiposity. The neonatal PI and high adiposity (>P90 NFM) were preceded by an increased

development of fetal central- and peripheral fat mass parameters. The fetal abdominal tissue development (AC, LL), reflecting fetal central adiposity, correlated highly to the 90th percentile of neonatal fat mass, showing their potential role as early predictors for neonatal fat deposition. In conclusion, the fetal fat measurements showed a high degree of correlation for neonatal adiposity, with a gestational age specific timing of first onset of aberrant fetal adiposity development: first (late first trimester) the abdominal circumference and fetal liver length, secondly (late second trimester) the truncal fat depositions of abdominal- and subscapular fat thickness and lastly (early third trimester) the peripheral fat depositions on the extremities (humerus and femur). The fetal abdominal fat development, a marker of fetal central fat deposition, correlated specifically to the 90th percentile of neonatal fat mass, for which further studies are needed to show its potential role as a more specific predictor for neonatal adiposity.

Fetal developmental programming is influenced by maternal BMI, GWG and glucose intolerance. These factors affect the fetal growth and development, often conveniently summarized by its resultant 'birthweight'. Birth weight is used in epidemiological studies as a proxy for long- and short term complications. Cardiovascular risks have been linked to birth weight, fitting the 'fetal origin of diseases' theory. Intima-media thickness (IMT) has been suggested as a marker of pre-clinical atherosclerosis. In the mother, IMT can be altered through dynamic circumstances related to pregnancy. In 38 low-risk pregnancies we investigated in **Chapter 7**, the feasibility of measurement of IMT at four pre-defined fetal and four pre-defined maternal arterial locations to determine vascular changes that could be associated with impaired vascular function throughout pregnancy. Fetal abdominal aorta IMT was measurable during the second trimester in 71% and during the third trimester in 100% of the cases, and umbilical artery IMT was measurable in 50% and 82% of cases during the second and third trimesters, respectively. Fetal IMT measurements were not possible during the first trimester. It was not often feasible to measure the IMT of the fetal common carotid artery, fetal renal artery and maternal iliac artery (maximal 20% of cases). Maternal common carotid artery, abdominal aorta and uterine artery IMTs were measurable throughout pregnancy. There was a significant relation between gestational age and IMT in the umbilical artery and a significant relation between body mass index and IMT in the maternal common carotid artery. IMT measurements are feasible in some maternal and fetal vessels of interest. Furthermore, fetal IMT development in abnormal pregnancy is of interest, as it could contribute to elucidating the mechanisms of intra-uterine fetal programming and the prediction of the origin of adult metabolic diseases.

Pregnancy induces hemodynamic changes in women. The common carotid artery (CCA) is an easily accessible artery and is therefore often used in research (but also clinically) as representative for great artery health. Vascular changes in the CCA throughout normal pregnancy have not been elucidated before. In **Chapter 8**, we performed high

resolution, radio-frequency (RF) ultrasound measurements of the CCA in 80 low risk mothers, prospectively, from first trimester onwards. All CCA properties and indices were significantly associated with gestational age in terms of decreasing compliance (Carotid Artery Compliance -CAC-) and increasing arterial stiffness (Young Elastic Modulus -YEM- and Stiffness Index -SI-). All indices (CAC, YEM and SI) were also significantly influenced by both maternal age and e-GWG, but when corrected for maternal age, the YEM remained positively correlated with GWG. Interestingly, no correlation was found with respect to pre-gestational BMI. In summary, our study provides to our knowledge the first longitudinal radio-frequency vascular data during pregnancy for the common carotid artery in low risk pregnant women. Whether the observed changes concern a transient, reversible phenomenon or represent a more durable modification with correlates later in life requires further investigation. Particularly the long-term vascular effects of maternal weight gain during pregnancy as a risk factor for future cardiovascular disease.

More challenging - when we look at our feasibility study reported in chapter 7 - is the research on the fetal abdominal aorta (fAA) development throughout pregnancy. In **Chapter 9**, we included seventy-four mothers in the final analysis. fAA measurements could reliably be obtained prospectively from 25 weeks onwards. All fAA properties (IMT, diameter (DIA) and distension (DIS)) showed a positive correlation with gestational age. FAA elasticity indices decreased during pregnancy with decreasing fAA Compliance (fAAC) and increasing arterial stiffness (YEM and SI). Elasticity indices (fAAC, YEM and SI) were also significantly influenced by fetal size. Interestingly, the fAA-IMT and DIS showed a significantly smaller increase in obese mother when compared to normal weight (≤24.9kg/m2 BMI) mothers, even after correction for fetal size. In conclusion, arterial stiffness in the fAA increases towards the end of gestation, and is more pronounced if fetal growth is fast. FAA-IMT development is negatively affected by high maternal BMI, suggesting a modulation of the fetal vessel-wall in the obese and overweight mothers. In summary, our study provides the first longitudinal radio-frequency vascular data during pregnancy for the fetal abdominal aorta in low risk pregnant women. We demonstrate a positive correlation of both fetal growth and maternal BMI with increased arterial remodeling during gestation in the fetal abdominal aorta. As with the results achieved with measures of the maternal CCA, whether this represents a transient or persistent phenomenon requires further investigation.

FUTURE PERSPECTIVES

The objective of this thesis was to investigate the effects of pregnancy dynamics (GWG and glucose intolerance) on the short term outcomes of infant body and cardiovascular

development from the perspective of different pregestational BMI categories. These early life developments impact the infants' start in life and it is thought that this start may potentially induce long-term metabolic effects.

The research conducted for this thesis is unique as it combines: very high quality ultrasonic sensing with prospective and longitudinal measurements performed with a substantial number of patients under highly controlled conditions. Also, the longitudinal measurements for chapters 5 to 9 were obtained by one single operator, the doctoral candidate (SG), hereby overcoming the inter-observer variation bias. This allowed for a series of primary studies which advance the current state of knowledge on the dynamics of pregnancy and infant development. The thesis consist of the following chapters. Chapter one provides an overall overview and introduction of key history and concepts. Chapters two and three reveal general risk factors for adverse fetal growth and the role of fetal gender on separate intra-uterine development and (adverse) perinatal outcome. Chapter 4 highlights maternal influences on the timing of adverse fetal growth. Chapters 5 and 6 perform a fetal sequencing in order to find early predictors for neonatal adiposity. And chapters 7 to 9, discern with novel techniques interesting new information on the modulation of maternal and fetal great arteries during pregnancy. This thesis certainly opens up new perspectives. And besides the strengths, stated above, also shortcomings have to be highlighted, most of them will additionally open up new research perspectives, of which most can be managed with the available data enclosed in the cohorts from this thesis.

Gestational Weight Gain

In chapters 5,6,8 and 9 the GWG has been categorized as 'excessive' and 'non-excessive' according to the 2009-revised IOM criteria. In the non-excessive group, both adequate and insufficient GWG has been enclosed. And although the group of insufficient GWG was small, there has been some concern of the effect on fetal size ('birthweight'). Therefore it is interesting in further studies, to categorize the revealed effects of chapters 5,6,8 and 9 for this insufficient GWG group. Furthermore, we looked at final GWG (total-GWG). There have been reports that 'timing' of GWG in pregnancy differentiate the fetal adaptation towards either higher or lower susceptibility for later adverse outcomes. Particularly the first trimester weight gain profile seems an important period. As we have followed these cohorts prospectively, we measured their weight gain every visit, so we should be able to discern GWG profiles per trimester easily.

Nutrition

In chapters 5,6,8 and 9 an effect of maternal GWG and BMI has been demonstrated. More in detail it is exciting to look at the different lifestyle factors (nutritional intake and physical activity) of these mothers and to correlate them with GWG, BMI and fetal de-

velopment. For more than two-third of the participants (from 'DALI' and 'MANOE' study) we have 5-7 days food questionnaires and -diaries, providing information on maternal nutrition during pregnancy. For the same sub-study groups we will have information on physical activity. Analysis is currently being performed. Also, from a small subgroup (approx. 50 participants from 'MANOE' study) from which we have both maternal and fetal-neonatal data, epigenetics analysis is currently being performed in maternal- and cord blood.

(G)DM - (Gestational) Diabetes Mellitus -

In chapters 5,6,8 and 9, we performed a close control for glucose intolerance and excluded to our best, the highly glucose intolerant women. Since some of them have been excluded at recruitment, we do not have longitudinal information on most of the overt diabetic women. For this we have to rely on the multicentre 'DALI' study, in which we hope to have enclosed a nice cohort of overt DM and GDM. Analyses are currently being performed and outcomes (key findings and deliverables) have to be reported at the end of 2015.

Biomarkers

In chapters 5,6,8 and 9 we did not correlate with maternal or fetal/neonatal biomarkers. This is a limitation for both the vascular and the fetal-neonatal body composition correlates. We will have the bio-markers for the obese group of women and their off-spring ('DALI' participants and cord blood), and analysis is currently being performed.

Fetal predictors

In chapters 5 and 6 we aimed to define fetal distinctive body composition measurements in order to predict neonatal adiposity. Although there is no clear definition for neonatal adiposity, it seemed that for early antenatal predictors, the fetal abdominal circumference and fetal liver size could provide a good estimate for future neonatal adiposity. As a predictive model, further investigations are being performed to obtain relative risks (RR), odds ratio's (OR) and area under the curves (AUC's). Future research in large study samples is needed to obtain a practical and widely accepted definition or index for neonatal adiposity, which correlates with future metabolic risk profile.

Neonatal adiposity

Continuing this subject, in chapter 5 and 6, we defined neonatal adiposity with an algorithm, including skinfolds measured after birth, within the 48hrs. In many research settings, a more objective bio-impedance analysis (BIA) or air-displacement plethysmography (ADP) is being performed. Although there has been a good correlate between skinfold measurements and ADP/BIA, the skinfold measurement technique needs

proper training and exercise to overcome inter- and intra-observer variability. Benefit of the skinfolds is that they provide more information on fat distribution of the infant. We have for as subgroup ('MANOE' participants) additional BIA information on mothers, and also for infants at 6 weeks, 6, 12, 18 and 24 months post-partum. These measurements are still being performed and analyses follow.

Vascular properties

In chapters 7, 8 and 9 we performed high-technological measurements concerning the vascular wall properties of the great arteries of mother (CCA) and fetus (fAA). Since these radio-frequency data encloses raw imaging information on tissue characteristics, we will be able in the near future to extract even more information on these vessels of interest, by outcome parameters for augmentation index and pulse-wave velocities. The last parameter being regarded as the golden standard for vascular shear-stress properties. In highlighting the high-standard technique, we at the same time acknowledge the difficulty of repeating these studies in clinical settings. It requires not only skills and patience from the investigator (and participant!), but the equipment itself is currently only being used in research settings and not in clinical practice. On the other hand, long-term follow-up is needed to address the vascular changes observed in both mother and fetus (e.g. in -preferably the same- children at school age). Funding to embed this high technology for long-term follow-up is currently being investigated.

Long term outcomes

In chapters 5,6,8 and 9 only short term outcomes were monitored. Indeed for both fetal and neonatal adiposity measurements and vascular measurements long-term follow-up is warranted, to estimate the consequences of the findings of the studies. This will hopefully provide new insights in the origin of child-hood obesity (a current hot topic) and metabolic (aberrant) changes in later life. Together with the DALI consortium currently further alternatives to finance these long-term follow studies are being looked at, via European, but also via more international resources.

CONCLUSION

In conclusion, this thesis adds:

- Gender-tailored insights in fetal development and outcome consequences, generally favoring girls above boys, except for the post-term period.
- New sensing techniques to allow for a much more accurate view on fetal body- and vascular development *in utero*.
- Correlates of fetal development and neonatal adiposity (neonatal fat measurement).

- Measurement approaches in high standard ultrasound technique (RF ultrasound) , whichhave been refined for vascular wall property measurements (functional and morphology) on the maternal CCA and fAA showing the most promise for reliable execution and meaningful insight.
- Measures that show an overall tendency for increased arterial stiffness during pregnancy with some interesting interactions and exceptions.

As these results represent the first studies of this kind to date considerable care needs to be taken with the overall interpretation of results.

It is clear that high GWG, high BMI and increased glucose intolerance are important risk factors for neonatal adiposity. High quality ultrasound measures of vascular development could potentially provide clinicians a sensitive tool to detect early the development of adiposity. This could allow for targeted interventions to influence GWG and thus potentially influence infant adiposity. Whether such an intervention strategy could be designed and will prove to be successful is a prime option for future research.


ABDOMINAL AORTA FETAL ABDOMINAL
INTIMA MEDIA THICKNESS FETAL FAT
FUNCTIONAL LINEAR DISCRIMINANT
ADDITIVE MODEL LOCATION, SCALE AND
MELLITUS NON-EXCESSIVE GESTATIONAL
GROWTH FACTOR INTIMA-MEDIA THICKNESS
SOCIETY ON OBSTETRICS AND
GROWTH LARGE-FOR-GESTA
NUTRITION EPIGENOME
GROWTH STUDY GROUP
IMAGING MRI FATTY LIVER DISEASE
GLUCOSE TOLERANCE TEST OGTT ODDS RATIO
ARTERIAL STIFFNESS QUALITY
BLOOD PRESSURE SKINFOLD TRICEPS
FAT THICKNESS SMALL-FOR-GESTATIONAL AGE
VASCULAR AND BIOMETRY ULTRASOUND
STUDY VABULOS YOUNG ELASTIC MODULUS

Appendices

List of abbreviations

Abstract of Research

Nederlandse Samenvatting

Supplement

Collaborators' list

Dankwoord

CV & Publications

ABBREVIATIONS

aIMT	aortic Intima Media Thickness
AC	Abdominal Circumference
ADP	Air Displacement Plethysmography
AFT	Abdominal Fat Thickness
AGA	appropriate-for-gestational age
AS	Apgar Score
BE	Base Excess
BHC	Birth Head Circumference
BIA	bio-electrical impedance analysis
BL	Birth Length
BMI	Body Mass Index
BPD	BiParietal Diameter
BW	Birth Weight
CAC	Carotid Artery Compliance
CCA	Common Carotid Artery
CRL	Crown Rump Length
CVD	CardioVascular Disease
DALI	Vit D And Lifestyle Intervention on the prevention of GDM
DBP	Diastolic Blood Pressure
DIA	Diameter
DIS	Distension
EFW	Estimated Fetal Weight
fAA	Fetal Abdominal Aorta
fAAC	Fetal Abdominal Artery Compliance
faIMT	fetal abdominal aortic Intima Media Thickness
FFMA	Fetal Fat Mass Area
FL	Femur Length
FLMA	Fetal Lean Mass Area
FLDA	Functional Linear Discriminant Analysis
GA	Gestational Age
GAMLSS	Generalized Additive Model Location, Scale and Shape
(G)DM	(Gestational) Diabetes Mellitus
(n-e)-GWG	(non-excessive) Gestational Weight Gain
HC	Head Circumference
IGF	Insulin Growth Factor
IMT	Intima-Media Thickness
IOM	Institute of Medicine
ISUOG	International Society on Ultrasound in Obstetrics and Gynaecology
IUGR	Intra-Uterine Growth Restriction

LGA	large-for-gestational age
LL	Liver Length
MANOE	MAternal Nutrition and Offspring's Epigenome
MAP	Mean Arterial Pressure
MGRS	Multicentre Growth Reference Study Group
MHz	Mega Hertz
MRI	Magnetic Resonance Imaging
NAFLD	Non-Alcoholic Fatty Liver Disease
NFM	Neonatal Fat Mass
OGTT	Oral Glucose Tolerance Test
OR	Odds Ratio
PE	Pre-Eclampsia
PI	Ponderal Index
QAS	Quality Arterial Stiffness
QIMT	Quality Intima-Media THickness
RF	Radio-Frequency
SBP	Systolic Blood Pressure
SF (FI-SF, Tr-SF, S-SF, Th-SF)	SkinFold (Flank-, Triceps-, Subscapular-, Thigh-)
SFT	Subscapular Fat Thickness
SGA	Small-for-Gestational Age
SI	Stiffness Index
US	UltraSound
Vabulos	VAscular and Biometry Ultrasound measurements in LOw risk pregnancy Study
YEM	Young Elastic Modulus
WHO	World Health Organization

ABSTRACT OF THE RESEARCH

Three important cornerstones for metabolic research in pregnancy are maternal adiposity (body mass index -BMI-), nutrient intake and storage (gestational weight gain -GWG-) and abnormal glucose tolerance (GDM). These contributors play an important role in the intra-uterine development. Beside birth weight, detailed information on the neonates' body composition (fat distribution) in both lean- and fat mass, reflect the *in utero* development better. Accuracy for the estimated fetal growth fluctuates and in the obese mother visualization of the fetus is more limited (**Chapter 1**).

The environment *in utero* and in early neonatal life may induce a permanent response in the fetus and the newborn, leading to enhanced susceptibility to later diseases. Human and animal data confirm this important early life period. The effects of maternal and fetal nutrition stress the importance of the embryonic environment on epigenetic imprinting for metabolic mechanisms, but in general it depends on the extent of embryonic adaptation and on its plasticity later on (**Chapter 2**).

The male fetus body composition has been shown to be more sensitive to maternal influences and gender is recognized as a determining factor for differences in outcome in adult medicine. GAMLSS (WHO) based growth charts for the male and female fetus were constructed to align with WHO neonatal and pediatric curves. At birth boys were heavier, longer, had greater head circumference with lower first minute Apgar score and arterial pH than girls. Second trimester dating estimated boys 3 days ahead to girls. The implication is that boys seem to be more vulnerable around viability age (overestimated age) and at term (worse birth outcome), although girls are more vulnerable for the latter, post-term period in pregnancy, since they are underestimated for their real age (**Chapter 3**).

Maternal GWG and BMI both effect fetal growth and birth weight, independently. Cluster analysis discerned four total GWG clusters: I: ≤0kg, II: 0-4kg, III: 4-12kg and IV: >12kg. Cluster IV added effect of +477g, when compared to cluster I. A high BMI class (35) added +344g, when compared to a low BMI (17). The total GWG effect had most impact from 180 to 200 days gestation when there was a separation between the different GWG clusters, offering us a time estimate to influence weight gain during pregnancy and so possibly prevent perinatal complications, improve the metabolic and vascular traits of the infant and possibly improve outcomes in future pregnancies (**Chapter 4**)

Fetal body composition can be monitored, additional to fetal weight for fetal adiposity estimation. The fetal liver size (LL) and the abdominal fat thickness (AFT) growth trajectory increased significantly in the high BMI group. Excessive GWG (above IOM guideline, e-GWG) positively correlated with the growth in fetal abdominal circumference , LL, subscapular fat thickness (SFT) and AFT as compared to the non-excessive GWG (ne-GWG) group. Birth outcomes were significantly different for weight, length and head circum-

ference in the e-GWG group as compared to the ne-GWG. Interestingly, fetal adiposity emerged as a reflection of a nutritious environment in utero with effects detectable as early as 20 weeks. Especially the indicators of the fetal trunk (LL, AFT, SFT and AC) are discriminative of fetal adiposity and warrant further investigation as potential early predictors of neonatal adiposity (**Chapter 5**), represented by neonatal fat mass (NFM), ponderal index (PI) and birthweight. The mean NFM at birth was 11.0% (SD±3.4%), with a cut-off of 15% for the 90th percentile (P90). No association was found between the maternal BMI and NFM. However, children born to mothers who had an e-GWG had a significantly higher NFM (11.6%) as compared to the ne-GWG mothers (10.4%). Fetal AC, LL, SFT, humerus and femur fat mass development correlated significantly with 90th NFM percentile. Interestingly, fetal fat measurements showed a gestational age specific timing of first onset of aberrant fetal adiposity development: first (late first trimester) the AC and fetal LL, secondly (late second trimester) the truncal fat depositions of AFT and SFT and lastly (early third trimester) the peripheral fat depositions on the extremities (humerus and femur). The fetal abdominal fat development (LL, AC and AFT), a marker of fetal central fat deposition, correlated to NFM P90 warranting further studies as a neonatal adiposity predictor (**Chapter 6**).

Cardiovascular risks have been linked to birth weight, fitting the 'fetal origin of diseases' theory. A cross-sectional feasibility study in pregnancy revealed from 4 fetal and 4 maternal vessels, that the maternal common carotid artery (CCA) and the fetal abdominal artery (fAA) were most feasible to measure during pregnancy (**Chapter 7**).

Pregnancy induces hemodynamic changes in women. Vascular changes in the CCA throughout normal pregnancy have not been elucidated before. This is the first Radio-frequency (RF) study providing longitudinal vascular data during pregnancy for the CCA with novel insights on gestational age and e-GWG correlates. Whether the observed changes concern a transient, reversible phenomenon requires further investigation (**Chapter 8**).

More challenging, since less feasible, is fetal abdominal aorta (fAA) development throughout pregnancy with RF ultrasound from 25 weeks onwards. All fAA properties (IMT, diameter and distension) showed a positive correlation with gestational age. In conclusion, our study provides the first longitudinal radio-frequency vascular data during pregnancy for the fetal abdominal aorta in low risk pregnant women. We demonstrate a positive correlation of both fetal growth and maternal BMI with increased arterial remodeling during gestation in the fetal abdominal aorta. As with the results achieved with measures of the maternal CCA, whether this represents a transient or persistent phenomenon requires further investigation (**Chapter 9**).

Long-term follow up studies are needed to correlate some of the results in this thesis, particularly on the transience of the vascular changes and neonatal adiposity definition.

Correlates with weight gain patterns, lifestyle patterns, biomarkers and pediatric fat deposition are under way.

NEDERLANDSE SAMENVATTING

Gewichtstoename in de zwangerschap is voor veel aanstaande mamma's een belangrijk onderwerp. Vooral een teveel, maar soms ook een te weinig toename (of zelfs een afname) wordt gevreesd. Die ongerustheid hierover komt voornamelijk voort door de zorg over het ongeboren kind en dat is niet geheel onterecht. Het Amerikaanse Institute of Medicine (IOM), heeft in 2009 haar richtlijnen voor een gezonde gewichtstoename in de zwangerschap (her-)uitgegeven. Zij koppelt de bandbreedte van de totale gewichtstoename in de zwangerschap aan de categorie van de body mass index (BMI), zoals die voor de zwangerschap bepaald is. Er geldt een negatieve correlatie: hoe hoger de BMI, hoe lager de maximale en minimale gewichtstoename mag zijn. De IOM adviezen komen voort uit gegevens die o.a. laten zien dat een excessieve gewichtstoename niet alleen leidt tot negatieve effecten voor de mamma, maar ook voor de baby. Een van die negatieve effecten is een foetale overgroei, die kan ontstaan wanneer de mamma een heel voedingsrijke omgeving aanbiedt aan haar baby tijdens de zwangerschap. Dat vervolgens deze baby ook zelf weer een hogere kans heeft om later een hogere 'adipositeit' te ontwikkelen of zelfs obesitas noemen we het 'transgenerationele' effect. In dit proces worden de moederlijke zwaarlijvigheid, een afwijkende gewichtstoename en een afwijkende suikerstofwisseling als drie belangrijke factoren, ook wel 'de drie hoekstenen' genoemd. Behalve de lichamelijke veranderingen die bij de baby plaatsvinden, zijn er ook epigenetische veranderingen beschreven. Dat zijn veranderingen die het genoom niet veranderen, maar wel een aansturings-effect teweeg brengen, waardoor sommige genen kunnen worden 'aan-' of 'uitgezet'. Het lijkt er dus op dat er een modulerend effect uitgaat van de invloeden in de baarmoeder voor het ongeboren kind. Deze 'voorprogrammering' bepaalt de mogelijkheden van de boreling om zich aan het buiten-baarmoederlijke leven aan te kunnen passen. Dit wordt ook wel zijn of haar plasticiteit genoemd.

In dit doctoraatsonderzoek hebben we de invloeden van de drie hoekstenen op de intra-uteriene ontwikkeling van de foetus onderzocht, zowel met betrekking tot de lichaamssamenstelling, als ook tot de vaatontwikkeling. Verder hebben we de geslacht specifieke ontwikkeling van de foetus voor de geboorte gekarakteriseerd en gekoppeld aan de uitkomsten bij de geboorte. Via nieuwe hoog technologische methoden hebben we prospectief zeer gedetailleerd kleine structurele vaatveranderingen, zowel bij de moeder als bij de foetus kunnen vastleggen. Ook hebben we de zwangerschapsinvloeden (BMI, gewichtsverandering) op zowel vaatfunctie als -morfologie kunnen documenteren. Met de informatie over de lichaamssamenstelling en vaatontwikkeling krijgen we een goed idee van de foetale metabole voortgang. De informatie kan ons mogelijk helpen in de zoektocht naar interventiemogelijkheden in de zwangerschap, opdat de

transgenerationele overdracht van aberrante (moederlijke) omgevingsinvloeden naar baby tijdig kan worden onderbroken.

Om de achtergrond te begrijpen van de onderzoeksmethoden die sterk op beeldvorming zijn toegespitst, is een voorkennis van de evolutie van de niet invasieve technieken (echogeluidsonderzoek) van belang. Dit leg ik uit in **hoofdstuk 1**. Met dit echogeluid onderzoek ('echo') kan een zeer gedetailleerd beeld worden verkregen van het ongeboren kind. In epidemiologische studies wordt vaak teruggegrepen op het geboortegewicht als een afspiegeling van het intra-uteriene traject. Deze uitkomstmaat wordt gebruikt om korte en lange termijn effecten te verduidelijken. Zowel hoog als laag geboortegewicht wordt in verband gebracht met toekomstig verhoogd cardiovasculair risico, wat past in de 'foetale oorzaak voor latere ziektes' theorie. Naast het geboortegewicht, zou meer gedetailleerde informatie over de *lichaamssamenstelling* van de pasgeborene, zowel in vetmassa als in vetvrije massa, meer kunnen vertellen over de ontwikkeling in de baarmoeder. Er is helaas nog geen algemeen geaccepteerde definitie van 'neonatale BMI' of van neonatale zwaarlijvigheid voorhanden. Ook moeten foetale voorspellers voor neonatale zwaarlijvigheid nog ontwikkeld worden. Momenteel wordt de zogenaamde 'ponderal index', een soort baby-BMI en 'percentage vethoeveelheid' gebruikt om deze leemte op te vullen. Prenataal kunnen we een schatting doen van het foetale gewicht met een marge van ongeveer tussen 6 en 12% van het werkelijke gewicht. Ook is bekend dat bij een obese moeder de beeldvorming van de foetus meer beperkt is.

Zoals hierboven gemeld, zou het milieu binnen de baarmoeder en in het vroege neonatale leven, een blijvende reactie kunnen veroorzaken in de foetus en de pasgeborene, wat tot een verhoogde vatbaarheid voor latere ziekten kan leiden. In **hoofdstuk 2**, meld ik dat foetale overgroei is gerelateerd aan een diabetisch milieu in de baarmoeder. De verhoogde kans op diabetes mellitus (DM) voor kinderen van moeders met diabetes, komt overeen met een epigenetisch effect van hyperglycemie in de zwangerschap, naast genetische factoren waaronder diabetes in volgende generaties. Aan de andere kant zorgt een foetale groeibeperking veroorzaakt door ondervoeding, juist tot het vaker voorkomen van cardio-vasculaire ziekten en type-2 diabetes mellitus het latere leven. Vooral, als er sprake is van een inhaalslag voor de beperkt gegroeide foetus, via hoogcalorische voedselinname na de geboorte. Het derde trimester lijkt een kwetsbare periode te zijn voor de invloed van moederlijke ondervoeding, en daaruit voortkomende foetale groeiachterstand. Excessieve voeding, een beperkte voedingswaarde of een laag foliumzuur en vitamine B12 niveau, zijn omgevingsfactoren die een invloed hebben op DNA-methylatie in de ontwikkeling van het jonge leven, dit wordt 'Epigenetica' genoemd.

De effecten van moederlijke en foetale voeding benadrukken het belang van de embryonale omgeving op de epigenetische inprenting voor metabole mechanismen

op de toekomstige generaties. Niet iedere pasgeborene met overgewicht en niet elke pasgeborene met een intra-uteriene groeiachterstand zal later in het leven problemen ontwikkelen. Er zijn uiteraard andere factoren die invloed hebben op de uitkomst daarvan: over het algemeen hangt het af van de mate van aanpassing en de plasticiteit van de baby.

Vanuit de foetus gezien, wordt de periode in de baarmoeder geassocieerd met sexe-afhankelijke fysieke aanpassingen vanwege een veranderde voedingsvoorraad van de moeder. De lichaamssamenstelling van een jongen is meer gevoelig gebleken voor moederlijke invloeden, zoals een hogere BMI voor de zwangerschap en een excessieve gewichtstoename in de zwangerschap. En alhoewel geslacht wordt erkend als een bepalende factor voor uitkomstverschillen binnen de geneeskunde voor volwassenen, is er nog maar weinig informatie beschikbaar over de invloed van het foetale geslacht in de prenatale periode. Vanwege de complexiteit die gepaard gaat met onderzoek naar 'groei', heeft de Multicentre Growth Reference Study Group (MGRS) van de Wereld Gezondheids Organisatie (WHO) het GAMLSS model (Generalized Additive Model Location, Scale and Shape) aangeraden voor het ontwikkelen van (WHO) groei standaarden.

In **hoofdstuk 3** hebben we groeicurven gemaakt voor de foetus in een Kaukasische populatie (12368 zwangerschappen) en deze verder aangepast voor het foetal geslacht. Dit leverde interessante bevindingen op. De metingen van het foetale hoofd waren significant hoger voor jongens vergeleken met meisjes vanaf 20 weken zwangerschap, wat overeen komt met een verschil van 3 dagen op een leeftijd van 20 – 24 weken. Jongens waren significant zwaarder, langer en hadden een grotere hoofdomtrek dan meisjes bij de geboorte. De Apgar score op 1 minuut en de zuurgraad van het arterieel navelstrengbloed waren lager bij jongens.

Deze longitudinale foetale groeicurves, laten voor de eerste keer een integratie toe tussen neonatale en door de WHO gebruikte pediatrische, sekse-specifieke groeicurves. De uitkomst direct na de geboorte was voor jongens slechter dan voor meisjes, en het verschil tussen de intra-uteriene groei tussen de seksen was voldoende groot om een klinisch belangrijk effect te hebben op de schatting van foetaal gewicht (waarbij de grootte van het hoofd betrokken is) en tweede trimester zwangerschapsduur bepaling, die in veel landen in het tweede trimester op basis van het foetale hoofd wordt bepaalt. Daardoor is de implicatie van deze bevindingen dat voor een jongen en een meisje, op precies 24 weken zwangerschapsduur, een verschil kan worden toegewezen van wel 3 dagen en een geschat verschil in gewicht van 21 gram op 24 weken. Dit verschil is in het voordeel van de jongens. Het verschil op het eind van de zwangerschap loopt op tot 121 gram, in het voordeel van de jongens. Daarnaast vonden we de hoofdomtrek van de jongens groter (+0.6cm) is bij de geboorte. Samengevat, lijken jongens kwetsbaarder te zijn rond de levensvatbare leeftijd (22 – 24 weken), omdat ze ouder worden geschat dan ze in werkelijkheid zijn. Meisjes zijn kwetsbaarder voor deze onjuiste schatting tijdens

de latere, post-terme periode in de zwangerschap. In dit stadium worden immers vaak beslissingen genomen over het al dan niet beëindigen van een zwangerschap en ze zijn al ouder dan geschat. Rond de uitgerekende datum hebben jongens een slechtere start (lagere 1 minuut Apgar Score en navelstreng-PH) vergeleken met meisjes, maar of dit van voorbijgaande aard is, zonder neveneffecten hebben we door een gebrek aan verdere gegevens niet kunnen concluderen.

In **hoofdstuk 4** is een prospectieve cohort analyse uitgevoerd voor de invloed van moederlijke gewichtstoename in de zwangerschap en BMI op de foetale groei. Deze factoren bleken de foetale groei *onafhankelijk* van elkaar te beïnvloeden. Er konden vier verschillende clusters onderscheiden worden voor gewichtstoename in de zwangerschap: I: ≤0kg, II: 0-4kg, III: 4-12kg and IV: >12kg. In elk cluster was er een andere evolutie van het foetale gewicht en ook, uiteindelijk, een verschillend geboortegewicht. Bij het vergelijken van cluster IV met cluster I was het toevoegend effect van het cluster verschil op het geboortegewicht +477g. De moederlijke BMI had een onafhankelijke invloed op de evolutie van foetaal gewicht. Een hogere BMI klasse zorgde voor een toename van foetale groei en daardoor een hoger geboortegewicht. Wanneer een BMI van 35 werd vergeleken met een BMI van 17, was het toegevoegde effect op het geboortegewicht +344g.

Het totale effect van gewichtstoename in de zwangerschap had de grootste impact op 180 tot 200 dagen zwangerschapduur, toen er een scheiding was tussen de verschillende GWG clusters. Door de relatie tussen gewichtstoename in de zwangerschap (GWG), foetale groei en geboortegewicht te identificeren, kunnen we nu mogelijk de gewichtstoename beinvloeden tijdens de zwangerschap en mogelijk op die manier perinatale complicaties voorkomen en op de langere termijn zelfs cardiovasculaire risico's en metabole aandoeningen.

Aangezien het geboortegewicht de uitkomst is van de foetale periode, was het doel van **hoofdstuk 5** om de kennis te vergroten over de ontwikkeling van de foetale lichaamssamenstelling in de baarmoeder.

Dit werd prospectief in een zeer goed gedocumenteerde populatie onderzocht via een gecontrolleerde glucose-tolerante groep van 126 moeders. De GWG werd als 'excessief' beschouwd (e-GWG) als de herschreven-2009 IOM bovenste limieten werden overstegen. Als deze drempel niet werd gepasseerd, dan werd de gewichtstoename als niet-excessief gedocumenteerd (ne-GWG). In de groep obese vrouwen, steeg het groei traject van de foetale lever grootte (LL) en de dikte van het buikhuidvet (ATF) significant vergeleken met vrouwen van een normaal gewicht. Excessieve-GWG correleert positief met de groei van foetale buikomtrek, LL, sub-scapulaire huidplooidikte (SFT) en AFT, vergeleken met de ne-GWG groep. Vergeleken met de ne-GWG waren de uitkomsten bij de geboorte in de e-GWG groep significant anders voor gewicht, lengte en hoofdomtrek. Obesitas en e-GWG hadden beiden een positieve invloed op zwaarlijvigheid

tijdens de foetale en neonatale periode, waarbij e-GWG duidelijk de foetale grootte in het algemeen beïnvloedde, wat werd bevestigd door metingen na de geboorte.

Ons longitudinaal onderzoek heeft vroege tekenen van foetale zwaarlijvigheid kunnen vaststellen die geassocieerd worden met moederlijke obesitas en vooral bij e-GWG in vrouwen met een normale glucose tolerantie. Een hoge BMI bij de moeder correleerde met de foetale levergrootte en buikvet, waar e-GWG daarbij ook correleerde met toegenomen buik-huidplooidikte en de ontwikkeling van de buikomtrek.

Foetale zwaarlijvigheid komt naar voren als een afspiegeling van een voedzame omgeving in de baarmoeder waarvan de effecten al op 20 weken gemeten kunnen worden. Vooral de indicatoren van de foetale romp (lever-lengte, AFT, SFT en AC) zijn discriminatief voor foetale zwaarlijvigheid en vragen verder onderzoek als potentieel vroege voorspellers van neonatale zwaarlijvigheid.

Naar aanleiding van deze resultaten correleerden we in **hoofdstuk 6** deze metingen in 121, streng opgevolgde, glucosetolerante moeders, prospectief, met een neonatale lichaamssamenstelling ('adipositas'-) index: neonatale vetmassa (NFM), ponderal index (PI) en geboortegewicht. De gemiddelde NFM bij geboorte was 11.0% (SD±3.4%), met een cut-off van 15% voor het 90ste percentiel (P90). Er werd geen associatie gevonden tussen maternale BMI en NFM. Echter, kinderen die geboren waren bij moeders met een e-GWG hadden een significant hoger NFM (11.6%) vergeleken met groep kinderen van de ne-GWG moeders (10.4%).

Foetale metingen in het algemeen vertoonden een hoge correlatie met de neonatale metingen. Foetale buikomtrek (AC), lever lengte (LL), subscapulaire huidplooi (SFT) en buikhuidvet dikte (AFT), humerus en femur vet massa ontwikkeling correleerden significant met het 90ste NFM percentiel. Foetale AFT correleerde niet met PI. Bij de normaal glucose tolerante vrouw, werd e-GWG geassocieerd met een verhoogde neonatale zwaarlijvigheid. De neonatale PI en hoge adipositeit (>P90 NFM) werden vooraf gegaan door een verhoogde ontwikkeling van foetale parameters voor centrale- en perifere vet massa.

De ontwikkeling van het foetale buikweefsel (AC, LL), een afspiegeling van foetale centrale adipositeit, correleerde sterk met het 90ste percentiel van neonatale vetmassa, waarmee hun potentiele rol als vroegtijdige voorspellers voor neonatale vet afzetting wordt aangetoond.

Samenvattend, de foetale vetmetingen bewezen een hoge graad van correlatie met neonatale adipositas. Verder was er een zwangerschapsduur gerelateerde timing voor een eerste aanzet (de eerste tekenen) van een afwijkende foetale vetopstapeling: als eerste, aan het eind van het eerste trimester, de buikomtrek en foetale lever grootte; ten tweede, aan het eind van het tweede trimester, de aanzet van lichaamsromp-vet 5AFT en SFT) *en* tenslotte, in het begin van het derde trimester, de perifere vetverdeling van de extremiteiten (humerus en femur). De ontwikkeling van het het foetale

buikhuidvet, wat een markeerpunt is voor de centrale vetverdeling, correleerde vooral met het 90ste percentiel van neonatale vetmassa, waarvoor verder onderzoek nodig is om de potentiele rol ervan als meer specifieke voorspeller voor neonatale adipositeit te kunnen aantonen.

Zoals hierboven reeds gemeld, wordt de programmering van foetale ontwikkeling beïnvloed door de BMI van de moeder, gewichtsveranderingen glucose-(in-)tolerantie. Deze factoren beinvloeden de foetale groei en onwikkeling, vaak gemakshalve samengevat door de parameter 'geboortegewicht'.

Cardiovasculaire risico's worden in verband gebracht met het geboortegewicht, wat past binnen de theorie van 'foetale oorzaak voor ziektes'. De dikte van de Intima-media (IMT) wordt gezien als een markering voor pre-klinische atherosclerose (aderverkalking). Verder is gekend dat IMT van de moeder kan worden veranderd door dynamische omstandigheden gerelateerd aan de zwangerschap. In **Hoofdstuk 7** beschrijf ik het onderzoek dat we hebben gedaan bij 38 laag-risico zwangerschappen, naar de haalbaarheid van het meten van de IMT op vier vooraf bepaald foetale en vier vooraf bepaalde maternale vaatpunten. Het doel is uiteindelijk om de vasculaire veranderingen te kunnen vaststellen die geassocieerd kunnen worden met veranderde vasculaire functies gedurende de zwangerschap.

De IMT van de foetale abdominale aorta (fAA) was meetbaar tijdens het tweede trimester in 71% en tijdens het derde trimester in 100% van de gevallen. De IMT van de navelstreng arterie was meetbaar in 50% en respectievelijk 82% van de gevallen tijdens het tweede en derde trimester. Foetale metingen van IMT waren niet mogelijk tijdens het eerste trimester. Het was vaak niet haalbaar om de IMT te meten van de foetale a. carotis communis, de foetale a. renalis en de maternale a iliaca (max. 20% van de gevallen). De IMT van de maternale a. carotis communis (CCA), de abdominale aorta en de a. uterina waren meetbaar gedurende de hele zwangerschap. Er was een significante relatie tussen de zwangerschapsduur en de IMT in de navelstreng arterie en een significante relatie tussen de moederlijke BMI en IMT in de maternale CCA. Kortom, IMT metingen zijn haalbaar in sommige maternale en foetale bloedvaten. Verder is de foetale ontwikkeling van IMT in abnormale zwangerschappen van belang, omdat dit zou kunnen bijdragen aan het verhelderen van de mechanismes van intra-uteriene programmering en het voorspellen van de oorsprong van metabole ziekten bij volwassenen.

Een zwangerschap veroorzaakt hemodynamische veranderingen bij vrouwen. Vanwege de makkelijke toegankelijkheid van de a. carotis communis (CCA) wordt deze vaak gebruikt in het onderzoek naar de conditie het vaatstelsel als zijnde representatief voor de (goede) gezondheid van de vaten. Vasculaire veranderingen in de CCA gedurende een normale zwangerschap zijn nog niet eerder opgehelderd. In **hoofdstuk 8** heb ik hoge-resolutie, radio-frequency (RF) echografische metingen gedaan van de CCA in

80 laag-risico moeders, prospectief, vanaf het eerste trimester. Alle CCA eigenschappen en indices waren significant geassocieerd met zwangerschapsduur wat betreft een afnemende compliantie (Carotid Artery Compliance -CAC-) en een toenemende 'vaatstijfheid' (Young Elastic Modulus -YEM- and Stiffness Index -SI-). Alle indices (CAC, YEM and SI) werden ook significant beïnvloed door zowel leeftijd van de moeder als e-GWG, maar na corrective voor de maternale leeftijd, bleef de YEM positief gecorreleerd met e-GWG. Interessant was dat er geen correlatie werd gevonden wat betreft de BMI voorafgaand aan de zwangerschap. Onze studie levert, voor zover wij weten, eerste longitudinale radio-frequency vasculaire gegevens tijdens zwangerschap voor de CCA binnen een laag-risico populatie. Vooral de vasculaire lange termijn effecten van maternale gewichtstoename tijdens de zwangerschap is interessant als een risicofactor voor toekomstige cardiovasculaire ziekten. Of de waargenomen veranderingen een tijdelijk, omkeerbaar fenomeen betreffen, of een meer langdurige aanpassing met gevolgen in het toekomstig leven, vraagt om verder onderzoek.

Een veel grotere uitdaging, wanneer we naar onze haalbaarheidsstudie kijken (hoofdstuk 7), is het onderzoek naar de ontwikkeling van de foetale abdominale aorta (fAA) tijdens de zwangerschap. In **hoofdstuk 9** includeerde ik vierenzeventig moeders in de uiteindelijke analyse. Metingen van de fAA konden prospectief betrouwbaar worden verkregen vanaf 25 weken zwangerschap. Alle kenmerken van fAA (IMT, diameter (DIA) en uitzetting (DIS)) lieten een positieve correlatie zien met zwangerschapsduur. FAA elasticiteits-indices namen af tijdens de zwangerschap. Er was een afnemende fAA compliantie (fAAC) en een toenemende vaatstijfheid (YEM and SI). Elasticiteits-indices (fAAC, YEM and SI) werden ook significant beïnvloed door grootte van de foetus. Interessant is dat de fAA-IMT en de DIS een significant kleinere toename lieten zien in obese moeders als deze worden vergeleken met de moeders met normaal gewicht (≤24.9kg/m2 BMI), zelfs na correctie voor foetale grootte.

Samenvattend, de vaatstijfheid in de fAA nam toe naar het einde van de zwangerschap en was meer uitgesproken wanneer de foetale groei ook snel is (derde trimester). De fAA-IMT ontwikkeling wordt negatief beinvloed door een hoge BMI bij de moeder, wat een aanpassing suggereert van de foetale aderwand in de obese moeder en moeder met overgewicht. Onze studie levert de eeste longitudinale radio-frequency vasculaire gegevens tijdens de zwangerschap over de foetale abdominale aorta in laag-risico zwangere vrouwen. We laten een positieve correlatie zien van zowel foetale groei en maternale BMI met een toegenomen arteriële remodellering tijdens de zwangerschap in de foetale abdominale aorta. Evenzo als bij de resultaten van de maternale CCA, geldt ook hier dat er verder onderzoek nodig is om te zien of dit een tijdelijk of een langdurig fenomeen betreft.

Toekomst Perspectieven

Het doel van dit doctoraat was om de dynamische effecten van de zwangerschap (gewichtsverandering en glucose intolerantie) te onderzoeken op de korte termijn uitkomsten van foetale en neonatale lichaams-samenstelling en de cardio-vasculaire ontwikkeling, binnen het perspectief van de verschillende BMI categorieën. Deze ontwikkelingen van het vroege bestaan, hebben impact op de begin van het leven van een kind met de gedachte dat dit ook lange termijn metabole effecten kan hebben. De uitkomsten zoals boven beschreven openen uiteraard nieuwe perspectieven voor verder onderzoek. Kort samengevat is er binnen de gewichtstoename per trimester nog verder onderzoek te verrichten, aangezien er tekenen zijn dat met name de gewichtsverandering tijdens het eerste trimester cardio-vasculaire effecten heeft op de lange termijn. Verder hebben we in de longitudinale studies nog veel (verborgen) informatie met betrekking tot de lifestyle van de deelnemers. Er zijn fysieke activiteits-rapporten en voedingsdagboeken beschikbaar. Verder is het gerapporteerde werk in deze thesis onderdeel van een grote multicentrische studie, waaruit nog meer gegevens zullen volgen. In deze zelfde studie werden ook veel 'biomarkers' bepaald, waaruit niet alleen de Insuline indices kunnen worden berekend, maar ook bijvoorbeeld nuchtere lipiden (triglyceriden, LDL, HDL, en vrije vetzuren). In hoofdstuk 6 wordt er gesproken over foetale voorspellers voor neonatale adipositeit. Het zou een volgende stap zijn om de foetale parameters die opvallend geassocieerd zijn, te combineren in een model, waarbij je ook relative risks (RR), odds ratio's (OR) and area under the curves (AUC's) zou kunnen geven voor de neonatale 'hoge' vetstapeling. Met betrekking tot die vetstapeling, is een vergelijking met de gouden standaard (bio-impedance analysis (BIA) of air-displacement plethysmography (ADP)) een volgende uitdaging. Voor een bepaalde groep kinderen hebben we de BIA gegevens van op 6weken, 6, 12, 18 en 24 maanden. Voor de vaatmetingen zou een lange-termijnstudie een logisch vervolg zijn op onze studie resultaten. Het zou daarmee mogelijk zijn om te zien of er permanente aanpassingen zijn gebeurd onder invloed van zwangerschap gerelateerde factoren, voor zowel de moeder als de baby.
Concluderend brengt deze thesis ons verder in:

- Geslacht specifieke nieuwe inzichten in de foetale ontwikkeling en uitkomsten, waarbij in het algemeen de meisjes een voordeel hebben ten opzichte van de jongens, behalve in de post-terme tijdsperiode.
- Nieuwe gevoelige technieken voor een gedetailleerder inzicht in lichaams-samenstelling en vasculaire ontwikelling in de baarmoeder.
- Correlaties van foetale ontwikkeling en neonatale vetstapeling.
- Metingen verricht met radio-frequency ultrasound voor arteriële vaat-karakteristieken (functionaliet en morfologie) van de maternale CCA en de foetale fAA, met vooruitzichten op verder onderzoek.

- Vaatmetingen: er wordt een algemene vaatstijfheid reactie gezien tijdens de zwangerschap, zowel voor de moeder als voor de foetus met interessante links naar zwangerschap gerelateerde kenmerken.

Opgemerkt dient te worden dat, aangezien veel uitkomsten in dit doctoraat de eerste zijn in hun soort, er nog bevestigende studie moeten volgen, vooraleer er definitieve conclusies kunnen worden getrokken.

Het is in ieder geval duidelijk dat hoge gewichtstoename, hoge BMI and een togenomen glucose intolerantie belangrijke risico factoren zijn voor neonatale adipositas. Echo-onderzoek van hoge kwaliteit, kunnen clinici een gevoelig instrument geven om vroege verandering en ontwikkeling te detecteren voor vaatfunctie metingen en voor vetstapeling. Hiermee kunnen vervolgens doelgerichte interventies worden voorzien om de gewichtsveranderingen aan te passen met als doel de vetstapeling te beïnvloeden. Of dergelijke interventie strategieën ontwikkeld en ook succesvol toegepast kunnen worden, moet bevestigd worden in verder onderzoek.

e-Table 3.2. Fetal charts characteristics.

Study	Year	Country	N° women	N° scans	Weeks	Measurement	Design	Data Collection	Research?	Quality score (%)*
Chitty et al[13-15]	1994	UK	594-649	425-649	12-42	BPD,HC,AC,FL	C1	Prospective	Yes	78
Johnsen et al[20]	2006	NO	650	2489-2589	10-42	BPD,HC,AC,FL	LL	Prospective	Yes	67
Kurmanavicius et al[16,17]	1999	CH	6557	5462-6217	12-42	BPD,HC,AC,FL	C1	Retrospective	No	65
Leung et al[19]	2008	CN	709	679-708	12-40	BPD,HC,AC,FL	C1	Prospective	Yes	70
Paladini et al[18]	2005	IT	626	623-625	16-40	BPD,HC,AC,FL	C1	Prospective	Yes	65
Snijders & Nicolaides[12]	1994	UK	1040	1040	14-40	BPD,HC,AC,FL	C1	Retrospective	No	61
Verburg et al[21]	2008	NL	3760‡	20.277-22.271	10-40	BPD,HC,AC,FL	LL	Prospective	Yes	75
Galjaard et al	*2015*	*BE*	*12.368*	*27.680*	*12-40*	*BPD,HC,AC,FL*	*LL*	*Retrospective*	*No*	*75**

Characteristics of the highest ranked fetal growth studies (*as categorized by Ioannou C et al. BJOG 2012; 119:1425-1439)[37]. ‡= actual number of women analyzed after exclusion. C1 = cross-sectional -each fetus measured ones-. L = Longitudinal data -each fetus measured longitudinal-. UK = United Kingdom; NO = Norwegian; CH = Suisse; CN = China; IT = Italy; NL = the Netherlands; BE = Belgium.

e-Table 3.10. Reference values for AbdominalCircumference (AC) in mm, *Boys & Girls*

GA	Boys (AC)					Girls (AC)				
	C5	**C10**	**C50**	**C90**	**C95**	**C5**	**C10**	**C50**	**C90**	**C95**
12	50,1	51,6	57,0	62,9	64,7	49,4	50,7	55,5	61,0	62,7
13	60,7	62,4	68,6	75,2	77,2	59,8	61,2	66,7	72,9	74,8
14	72,1	73,9	80,8	88,1	90,3	71,1	72,7	78,9	85,7	87,8
15	83,9	86,0	93,4	101,4	103,7	82,9	84,7	91,5	99,0	101,3
16	95,7	97,9	105,9	114,4	116,9	94,5	96,5	103,9	112,0	114,4
17	107,4	109,7	118,1	127,0	129,6	105,9	108,1	116,0	124,6	127,2
18	118,8	121,2	130,0	139,4	142,2	117,1	119,4	127,9	137,0	139,7
19	130,0	132,5	141,8	151,7	154,6	127,9	130,4	139,5	149,1	152,0
20	140,6	143,3	153,1	163,4	166,5	138,1	140,8	150,5	160,7	163,7
21	150,6	153,6	163,9	174,7	178,0	147,7	150,6	160,8	171,6	174,8
22	160,3	163,5	174,5	186,0	189,7	157,3	160,3	171,2	182,6	186,0
23	169,7	173,4	185,2	197,7	202,0	167,2	170,5	182,1	194,1	197,7
24	179,0	183,4	196,3	209,9	214,9	177,3	180,8	193,2	206,0	209,8
25	188,7	193,6	207,4	221,9	227,5	187,5	191,3	204,4	218,0	222,0
26	199,5	204,4	218,6	233,5	239,1	197,8	201,9	215,7	229,9	234,2
27	211,0	215,7	229,8	244,8	250,0	208,5	212,7	227,1	241,8	246,3
28	222,5	226,9	241,2	256,2	261,1	219,3	223,6	238,5	253,6	258,2
29	233,6	237,9	252,5	267,8	272,6	230,1	234,6	249,7	265,2	269,9
30	244,1	248,5	263,5	279,3	284,2	240,7	245,3	260,7	276,7	281,6
31	253,7	258,2	274,0	290,5	295,5	250,6	255,3	271,3	287,8	293,0
32	262,3	267,0	283,7	301,2	306,5	259,4	264,3	281,0	298,5	304,0
33	269,9	274,9	292,8	311,7	317,3	267,1	272,3	290,0	308,7	314,7
34	276,8	282,2	301,7	322,1	328,2	273,8	279,4	298,2	318,5	325,0
35	283,2	289,1	310,2	332,5	339,2	279,9	285,9	306,1	328,0	335,3
36	289,3	295,6	318,5	342,8	350,0	285,9	292,2	313,8	337,7	345,7
37	295,2	302,0	326,7	353,0	360,8	292,0	298,7	321,7	347,7	356,5
38	301,0	308,2	334,8	363,2	371,6	298,4	305,6	330,0	358,0	367,8
39	306,7	314,4	342,9	373,4	382,5	305,1	312,6	338,5	368,8	379,6
40	312,3	320,5	351,0	383,8	393,5	311,9	319,8	347,1	379,8	391,7

Reference values in mm for fetal abdominal circumference (AC) for boys and girls for each gestational week for the median and 5th, 10th, 90th and 95th centiles. GA = gestational weeks.

e-Table 3.11. Reference values for FemurLength (FL) in mm, *Boys & Girls*

GA	Boys (FL)					Girls (FL)				
	C5	**C10**	**C50**	**C90**	**C95**	**C5**	**C10**	**C50**	**C90**	**C95**
12	4,7	5,0	6,5	8,5	9,3	5,0	5,3	6,6	8,1	8,6
13	7,3	7,7	9,5	11,7	12,5	7,5	8,0	9,5	11,3	11,9
14	10,3	10,8	12,8	15,0	15,8	10,5	11,0	12,7	14,7	15,3
15	13,6	14,1	16,2	18,4	19,2	13,6	14,2	16,1	18,2	18,9
16	17,0	17,6	19,6	21,8	22,5	16,9	17,5	19,5	21,6	22,3
17	20,3	20,9	22,9	25,1	25,8	20,2	20,8	22,8	25,0	25,7
18	23,5	24,1	26,1	28,3	29,0	23,4	24,0	26,1	28,3	29,0
19	26,5	27,1	29,1	31,4	32,1	26,5	27,1	29,2	31,4	32,1
20	29,3	29,9	32,0	34,3	35,0	29,3	29,9	32,1	34,3	35,0
21	31,7	32,4	34,7	37,0	37,7	31,9	32,5	34,7	37,0	37,7
22	34,1	34,8	37,3	39,8	40,5	34,4	35,1	37,3	39,6	40,3
23	36,5	37,4	40,1	42,6	43,3	37,0	37,7	40,1	42,5	43,2
24	38,8	39,9	42,8	45,4	46,2	39,7	40,4	42,9	45,4	46,2
25	41,2	42,3	45,4	48,1	48,9	42,2	43,0	45,6	48,2	49,0
26	43,6	44,7	47,9	50,7	51,5	44,7	45,5	48,1	50,8	51,6
27	46,1	47,1	50,3	53,2	54,1	46,9	47,7	50,4	53,2	54,0
28	48,6	49,6	52,6	55,6	56,5	49,1	50,0	52,7	55,5	56,4
29	51,0	51,9	54,8	57,8	58,8	51,2	52,1	55,0	57,8	58,7
30	53,1	54,0	57,0	60,0	61,0	53,3	54,2	57,1	60,1	60,9
31	55,1	56,0	59,1	62,3	63,3	55,3	56,3	59,3	62,3	63,2
32	57,0	57,9	61,1	64,4	65,5	57,3	58,3	61,4	64,5	65,4
33	58,6	59,6	63,0	66,4	67,6	59,0	60,0	63,3	66,5	67,4
34	60,2	61,2	64,6	68,3	69,5	60,4	61,5	64,9	68,3	69,3
35	61,5	62,6	66,2	70,1	71,4	61,8	62,9	66,6	70,2	71,3
36	62,9	64,0	67,7	71,8	73,2	63,2	64,4	68,4	72,2	73,4
37	64,5	65,6	69,4	73,7	75,1	64,6	65,9	70,0	73,9	75,1
38	66,2	67,4	71,3	75,7	77,1	66,0	67,3	71,4	75,3	76,5
39	68,0	69,2	73,2	77,7	79,2	67,5	68,7	72,7	76,5	77,6
40	69,9	71,1	75,2	79,7	81,3	68,8	70,0	73,8	77,4	78,5

Reference values in mm for fetal femur length (FL) for boys and girls for each gestational week for the median and 5th, 10th, 90th and 95th centiles. GA = gestational weeks.

e-Table 3.13. BiParietalDiamater (BPD) for Boys and Girls in mm

Centiles	GA	Boys	Girls	SE	p-value
C5	20	44,0	43,1	0,059	<0,001
	25	58,6	57,3	0,4	<0,001
	30	72,9	71,6	0,1	<0,001
	35	82,5	81,3	0,3	<0,001
	40	88,4	88,2	1,1	*0,39*
C10	20	44,9	43,9	0,059	<0,001
	25	59,8	58,4	0,4	<0,001
	30	74,1	72,7	0,1	<0,001
	35	84,0	82,7	0,3	<0,001
	40	90,3	89,8	1,1	*0,36*
C50	20	47,8	46,7	0,059	<0,001
	25	63,8	62,3	0,4	<0,001
	30	78,4	76,9	0,1	<0,001
	35	88,9	87,3	0,3	<0,001
	40	96,6	95,3	1,1	*0,21*
C90	20	50,8	49,6	0,059	<0,001
	25	67,9	66,2	0,4	<0,001
	30	82,8	81,1	0,1	<0,001
	35	93,9	92,1	0,3	<0,001
	40	103,2	101,1	1,1	*0,07*
C95	20	51,7	50,5	0,059	<0,001
	25	69,1	67,3	0,4	<0,001
	30	84,2	82,4	0,1	<0,001
	35	95,5	93,5	0,3	<0,001
	40	105,3	102,8	1,1	0,03

Centiles = C (C5 =P5; C10=P10; C50=P50; C90=P90; C95=P95). GA = Gestational Age in weeks. SE = Standard Error

e-Table 3.14. HeadCircumference (HC) for Boys and Girls in mm

Centiles	GA	Boys	Girls	SE	p-value
C5	20	163,4	160,7	0,177	<0,001
	25	216,2	212,5	1,336	0,008
	30	265,3	261,6	0,327	<0,001
	35	297,7	292,6	1,038	<0,001
	40	323,5	319,4	2,696	*0,13*
C10	20	165,8	163,0	0,177	<0,001
	25	219,7	215,5	1,336	0,003
	30	269,0	265,0	0,327	<0,001
	35	301,6	296,7	1,038	<0,001
	40	327,6	323,7	2,696	*0,15*
C50	20	174,3	171,0	0,177	<0,001
	25	231,7	226,2	1,336	<0,001
	30	282,0	277,0	0,327	<0,001
	35	315,9	311,3	1,038	<0,001
	40	342,9	339,2	2,696	*0,16*
C90	20	182,9	179,2	0,177	<0,001
	25	243,6	237,3	1,336	<0,001
	30	295,1	289,5	0,327	<0,001
	35	332,2	326,5	1,038	<0,001
	40	364,2	355,4	2,696	0,002
C95	20	185,4	181,6	0,177	<0,001
	25	247,0	240,6	1,336	<0,001
	30	299,0	293,2	0,327	<0,001
	35	337,5	331,0	1,038	<0,001
	40	373,1	360,2	2,696	<0,001

Centiles = C (C5 =P5; C10=P10; C50=P50; C90=P90; C95=P95). GA = Gestational Age in weeks. SE = Standard Error

e-Table 3.15 AbdominalCircumference (AC) for Boys and Girls in mm

Centiles	GA	Boys	Girls	SE	p-value
C5	20	140,6	138,1	0,202	<0,001
	25	188,7	187,5	1,702	*0,32*
	30	244,1	240,7	0,373	<0,001
	35	283,2	279,9	1,538	0,04
	40	312,3	311,9	6,380	*0,40*
C10	20	143,3	140,8	0,202	<0,001
	25	193,6	191,3	1,702	*0,16*
	30	248,5	245,3	0,373	<0,001
	35	289,1	285,9	1,538	0,05
	40	320,5	319,8	6,380	*0,40*
C50	20	153,1	150,5	0,202	<0,001
	25	207,4	204,4	1,702	*0,09*
	30	263,5	260,7	0,373	<0,001
	35	310,2	306,1	1,538	0,01
	40	351,0	347,1	6,380	*0,33*
C90	20	163,4	160,7	0,202	<0,001
	25	221,9	218,0	1,702	*0,03*
	30	279,3	276,7	0,373	<0,001
	35	332,5	328,0	1,538	0,006
	40	383,8	379,8	6,380	*0,33*
C95	20	166,5	163,7	0,202	<0,001
	25	227,5	222,0	1,702	0,002
	30	284,2	281,6	0,373	<0,001
	35	339,2	335,3	1,538	0,02
	40	393,5	391,7	6,380	*0,38*

Centiles = C (C5 =P5; C10=P10; C50=P50; C90=P90; C95=P95). GA = Gestational Age in weeks. SE = Standard Error

e-Table 3.16. Femurlength (FL) for Boys and Girls in mm

Centiles	GA	Boys	Girls	SE	p-value
C5	20	29,3	29,3	0,046	*0,36*
	25	41,2	42,2	0,378	0,007
	30	53,1	53,3	0,073	0,03
	35	61,5	61,8	0,262	*0,27*
	40	69,9	68,8	1,167	*0,27*
C10	20	29,9	29,9	0,046	*0,20*
	25	42,3	43,0	0,378	0,05
	30	54,0	54,2	0,073	0,02
	35	62,6	62,9	0,262	*0,17*
	40	71,1	70,0	1,167	*0,27*
C50	20	32,0	32,1	0,046	*0,08*
	25	45,4	45,6	0,378	*0,36*
	30	57,0	57,1	0,073	0,02
	35	66,2	66,6	0,262	*0,08*
	40	75,2	73,8	1,167	*0,20*
C90	20	34,3	34,3	0,046	*0,40*
	25	48,1	48,2	0,378	*0,40*
	30	60,0	60,1	0,073	*0,38*
	35	70,1	70,2	0,262	*0,34*
	40	79,7	77,4	1,167	*0,06*
C95	20	35,0	35,0	0,046	*0,26*
	25	48,9	49,0	0,378	*0,39*
	30	61,0	60,9	0,073	*0,31*
	35	71,4	71,3	0,262	*0,39*
	40	81,3	78,5	1,167	0,02

Centiles = C (C5 =P5; C10=P10; C50=P50; C90=P90; C95=P95). GA = Gestational Age in weeks. SE = Standard Error

e-Table 3.17. Estimated Fetal Weight (EFW) for Boys and Girls in g

Centiles	GA	Boys	Girls	SE	p-value
C5	20	298,0	289,7	1,201	<0,001
	25	670,8	658,3	10,869	0,21
	30	1347,7	1314,3	5,163	<0,001
	35	2108,1	2059,9	30,165	0,11
	40	2776,4	2780,4	140,429	0,40
C10	20	308,1	299,5	1,201	<0,001
	25	697,6	683,6	10,869	0,17
	30	1400,3	1365,8	5,163	<0,001
	35	2208,8	2156,3	30,165	0,09
	40	2946,3	2943,0	140,429	0,40
C50	20	346,4	336,7	1,201	<0,001
	25	798,6	776,9	10,869	0,06
	30	1593,4	1550,2	5,163	<0,001
	35	2577,1	2499,6	30,165	0,01
	40	3569,6	3531,0	140,429	0,38
C90	20	391,3	379,8	1,201	<0,001
	25	916,5	884,1	10,869	0,005
	30	1814,1	1761,1	5,163	<0,001
	35	3007,5	2911,8	30,165	0,003
	40	4325,5	4300,3	140,429	0,39
C95	20	405,9	393,6	1,201	<0,001
	25	954,8	918,8	10,869	0,002
	30	1885,8	1831,4	5,163	<0,001
	35	3151,6	3058,9	30,165	0,004
	40	4590,7	4604,5	140,429	0,40

Centiles = C (C5 =P5; C10=P10; C50=P50; C90=P90; C95=P95). GA = Gestational Age in weeks. SE = Standard Error

SUPPLEMENT

The figures and some e-tables are not available in the printed version of this thesis, but can be downloaded from the electronic version of this thesis on www.e-pubs.nl

Chapter 3

e-Text 3.1. Ultrasound protocol guidelines

All operators performed the measurements as single observations in accordance to the following guidelines, subsequently defined by the International Society for Ultrasound in Obstetrics and Gynecology (ISUOG, www.isuog.org).[26] The **BPD** measurement was performed in the transverse plane of the skull at the level where the continuous midline echo is interrupted anteriorly by the *septum cavum pellucidum* and centrally by the thalami. Ideally, an angle of insonation of 90° on the midline echo is used. Measurements were performed from the proximal edge of the skull closest to the transducer to the proximal edge of the deep border (i.e. outer-inner edge of skull) with the calipers. The **HC** measurement was performed in the same plane. If the ultrasound equipment had an ellipse measurement capacity, then the HC could be measured directly by placing the ellipse around the outer edge of the skull. Alternatively, the HC could be calculated from the BPD and the Occipito-Frontal Diameter (OFD) by using the equation HC=1.62x(BPD+OFD). This formula required the placement of the calipers for BPD at leading edges and for OFD middle of the bone echo at forehead and occiput. The **AC** measurement was performed in the transverse plane of the fetal abdomen at the level of the stomach, as circular as possible. The umbilical vein should be visible at the level of the portal sinus. The kidneys should not be visible. The AC was measured at the outer surface of the skin line using ellipse calipers or it could be calculated from linear measurements made perpendicular to each other, usually the Anterior-Posterior Abdominal Diameter (APAD) and the Transverse Abdominal Diameter (TAD). To measure the APAD the calipers were placed at the outer borders of the body outline from the posterior aspect (skin covering the spine) to the anterior abdominal wall. To measure the TAD the calipers were placed on the outer borders of the body outline, across the abdomen at the widest point. The AC was calculated by a direct ellipse measurement or using the formula AC=1.57(APAD+TAD). The **FL** was optimally imaged with both ends of the ossified metaphyses clearly visualized. The longest axis of the ossified diaphysis was measured. An angle of 45-90° between the insonating ultrasound beam and the femur were aimed for. The calipers were placed at the ends of the ossified diaphysis if

it is visible avoiding the artificial triangle spurs that appear to extend the length of the femoral ends.

References

26. ISUOG Prenatal Ultrasound Screening Taskforce. Practice guidelines for performance of the routine mid-trimester fetal ultrasound scan. http://www.isuog.org/NR/rdonlyres/EA865840-6CA3-45AC-9E99-FBAF775119A9/0/PrenatalUS_GL_Final.pdf

REFERENCES

e-table 5.1. Fetal abdominal circumference in e-GWG and ne-GWG women

GA (in weeks)	e-GWG			ne-GWG		
	C10	C50	C90	C10	C50	C90
12	51,8	55,9	60,3	50,5	54,6	58,8
13	62,6	67,6	72,9	61,5	66,4	71,5
14	73,4	79,4	85,5	72,4	78,2	84,3
15	84,4	91,2	98,3	83,4	90,1	97,1
16	95,4	103,1	111,1	94,5	102,0	109,9
17	106,3	114,9	123,8	105,5	113,9	122,7
18	117,2	126,6	136,4	116,5	125,8	135,5
19	127,9	138,2	149,0	127,3	137,5	148,2
20	138,6	149,8	161,4	138,1	149,1	160,6
21	149,2	161,2	173,7	148,6	160,5	172,9
22	159,7	172,6	186,0	159,0	171,8	185,1
23	170,1	183,9	198,1	169,3	182,9	197,0
24	180,5	195,0	210,2	179,4	193,8	208,8
25	190,7	206,1	222,1	189,4	204,5	220,3
26	200,8	217,0	233,8	199,2	215,1	231,8
27	210,8	227,8	245,5	208,9	225,6	243,0
28	220,8	238,6	257,1	218,4	235,9	254,2
29	230,6	249,3	268,6	227,9	246,1	265,2
30	240,5	259,9	280,1	237,3	256,2	276,1
31	250,3	270,5	291,5	246,5	266,3	286,9
32	260,1	281,1	302,9	255,8	276,3	297,6
33	269,9	291,7	314,3	265,0	286,2	308,4
34	279,6	302,2	325,7	274,2	296,2	319,1
35	289,4	312,7	337,0	283,4	306,1	329,8
36	299,1	323,2	348,3	292,7	316,1	340,5
37	308,8	333,7	359,6	301,9	326,1	351,3
38	318,5	344,2	370,9	311,2	336,1	362,1
39	328,2	354,7	382,2	320,4	346,1	372,8
40	337,9	365,2	393,6	329,7	356,1	383,6

Fetal Abdominal Circumference (AC) reference values in mm and gestational age (GA) in weeks. Percentiles 10, 50 and 90 for ne-GWG (non-excessive GWG) and e-GWG (excessive GWG).

e-Table 5.2. Fetal liver length in obese and non-obese women

GA (in weeks)	OBESE			Normal BMI		
	C10	C50	C90	C10	C50	C90
12	7,6	8,8	10,2	7,1	8,3	9,6
13	9,2	10,6	12,3	8,7	10,1	11,6
14	10,8	12,5	14,5	10,2	11,9	13,7
15	12,4	14,3	16,6	11,8	13,7	15,8
16	14,0	16,2	18,8	13,3	15,6	17,9
17	15,6	18,0	20,9	14,9	17,4	19,9
18	17,2	19,8	23,1	16,4	19,2	22,0
19	18,8	21,7	25,2	18,0	21,0	24,1
20	20,4	23,5	27,4	19,5	22,8	26,1
21	22,0	25,4	29,5	21,1	24,6	28,2
22	23,6	27,2	31,6	22,6	26,4	30,3
23	25,2	29,1	33,8	24,1	28,2	32,4
24	26,7	30,9	35,9	25,7	30,0	34,4
25	28,3	32,8	38,1	27,2	31,8	36,5
26	29,9	34,6	40,2	28,8	33,6	38,6
27	31,5	36,5	42,4	30,3	35,4	40,7
28	33,1	38,3	44,5	31,9	37,2	42,7
29	34,7	40,1	46,7	33,4	39,1	44,8
30	36,3	42,0	48,8	35,0	40,9	46,9
31	37,9	43,8	50,9	36,5	42,7	49,0
32	39,5	45,7	53,1	38,1	44,5	51,0
33	41,1	47,5	55,2	39,6	46,3	53,1
34	42,7	49,4	57,4	41,2	48,1	55,2
35	44,3	51,2	59,5	42,7	49,9	57,3
36	45,9	53,1	61,7	44,3	51,7	59,3
37	47,5	54,9	63,8	45,8	53,5	61,4
38	49,1	56,8	66,0	47,4	55,3	63,5
39	50,7	58,6	68,1	48,9	57,1	65,6
40	52,3	60,4	70,2	50,5	58,9	67,6

Fetal Liver length reference values in mm from 12 weeks gestational age (GA) onwards. Percentiles 10, 50 and 90 for obese and normal BMI ('non-obese').

e-Table 5.3. Fetal liver length in e-GWG and ne-GWG women

GA (in weeks)	e-GWG			ne-GWG		
	C10	C50	C90	C10	C50	C90
12	7,2	8,4	9,7	7,3	8,5	9,7
13	8,8	10,2	11,9	8,8	10,3	11,8
14	10,5	12,1	14,1	10,3	12,1	13,8
15	12,1	13,9	16,2	11,8	13,9	15,8
16	13,7	15,8	18,4	13,3	15,6	17,9
17	15,3	17,6	20,6	14,9	17,4	19,9
18	16,9	19,5	22,8	16,4	19,2	21,9
19	18,5	21,4	24,9	17,9	21,0	23,9
20	20,1	23,2	27,1	19,4	22,8	26,0
21	21,8	25,1	29,3	20,9	24,5	28,0
22	23,4	27,0	31,5	22,5	26,3	30,0
23	25,0	28,9	33,7	24,0	28,1	32,1
24	26,6	30,7	35,8	25,5	29,9	34,1
25	28,2	32,6	38,0	27,0	31,6	36,1
26	29,9	34,5	40,2	28,5	33,4	38,2
27	31,5	36,4	42,4	30,0	35,2	40,2
28	33,1	38,2	44,6	31,6	37,0	42,2
29	34,8	40,1	46,8	33,1	38,8	44,2
30	36,4	42,0	49,0	34,6	40,5	46,3
31	38,0	43,9	51,2	36,1	42,3	48,3
32	39,6	45,7	53,4	37,6	44,1	50,3
33	41,3	47,6	55,6	39,1	45,9	52,4
34	42,9	49,5	57,8	40,7	47,6	54,4
35	44,5	51,4	60,0	42,2	49,4	56,4
36	46,1	53,3	62,1	43,7	51,2	58,5
37	47,8	55,1	64,3	45,2	53,0	60,5
38	49,4	57,0	66,5	46,7	54,8	62,5
39	51,0	58,9	68,7	48,2	56,5	64,5
40	52,7	60,8	70,9	49,8	58,3	66,6

Fetal Liver length reference values in mm from 12 weeks gestational age (GA) onwards. Percentiles 10, 50 and 90 for ne-GWG (non-excessive GWG) and e-GWG (excessive GWG).

e-Table 5.4. Fetal abdominal fat tissue in obese and normal BMI women

GA (in weeks)	OBESE			Normal BMI		
	C10	C50	C90	C10	C50	C90
18	0,79	1,06	1,36	0,95	1,20	1,54
19	0,93	1,23	1,59	1,07	1,35	1,73
20	1,06	1,41	1,81	1,19	1,50	1,92
21	1,19	1,59	2,04	1,30	1,64	2,11
22	1,33	1,77	2,27	1,42	1,79	2,30
23	1,46	1,95	2,50	1,53	1,94	2,49
24	1,60	2,13	2,73	1,65	2,08	2,67
25	1,73	2,31	2,96	1,76	2,22	2,85
26	1,87	2,49	3,20	1,88	2,37	3,04
27	2,00	2,67	3,43	1,99	2,51	3,23
28	2,14	2,85	3,67	2,11	2,66	3,42
29	2,28	3,04	3,91	2,23	2,82	3,62
30	2,43	3,23	4,15	2,36	2,97	3,82
31	2,57	3,42	4,40	2,49	3,14	4,03
32	2,71	3,61	4,64	2,62	3,31	4,25
33	2,86	3,81	4,89	2,76	3,49	4,48
34	3,00	4,00	5,14	2,91	3,67	4,72
35	3,15	4,20	5,39	3,06	3,86	4,96
36	3,30	4,39	5,65	3,21	4,05	5,20
37	3,45	4,59	5,90	3,36	4,24	5,45
38	3,59	4,78	6,15	3,51	4,43	5,69
39	3,74	4,98	6,40	3,66	4,62	5,94
40	3,89	5,17	6,65	3,82	4,82	6,19

Fetal abdominal fat tissue (AFT) reference values in mm from 18 weeks gestational age (GA) onwards. Percentiles 10, 50 and 90 for obese and normal BMI ('non-obese').

e-Table 5.5. Fetal abdominal fat tissue in e-GWG and ne-GWG women

GA (in weeks)	e-GWG			ne-GWG		
	C10	C50	C90	C10	C50	C90
18	0,90	1,14	1,45	0,93	1,20	1,55
19	1,03	1,30	1,66	1,04	1,34	1,74
20	1,16	1,47	1,87	1,16	1,49	1,93
21	1,29	1,63	2,07	1,27	1,63	2,12
22	1,42	1,79	2,28	1,38	1,78	2,31
23	1,55	1,95	2,49	1,49	1,92	2,49
24	1,68	2,11	2,69	1,61	2,07	2,68
25	1,80	2,27	2,89	1,72	2,21	2,87
26	1,93	2,43	3,09	1,83	2,36	3,06
27	2,06	2,60	3,30	1,95	2,50	3,25
28	2,19	2,77	3,52	2,06	2,65	3,44
29	2,34	2,95	3,75	2,18	2,80	3,64
30	2,49	3,14	3,99	2,30	2,96	3,84
31	2,65	3,34	4,25	2,42	3,11	4,04
32	2,81	3,54	4,51	2,55	3,27	4,25
33	2,98	3,76	4,78	2,67	3,43	4,46
34	3,15	3,98	5,06	2,80	3,60	4,67
35	3,33	4,20	5,34	2,93	3,76	4,89
36	3,50	4,42	5,62	3,06	3,93	5,11
37	3,68	4,64	5,91	3,19	4,10	5,33
38	3,86	4,87	6,19	3,32	4,27	5,55
39	4,03	5,09	6,47	3,45	4,44	5,77
40	4,21	5,31	6,76	3,59	4,61	5,99

Fetal abdominal fat tissue (AFT) reference values in mm from 18 weeks gestational age (GA) onwards. Percentiles 10, 50 and 90 for ne-GWG (non-excessive GWG) and e-GWG (excessive GWG).

e-Table 5.6. Fetal subscapular fat tissue in the e-GWG and ne-GWG women

GA (in weeks)	e-GWG			ne-GWG		
	C10	C50	C90	C10	C50	C90
18	0,88	1,07	1,36	0,96	1,22	1,60
19	1,03	1,26	1,60	1,07	1,36	1,78
20	1,19	1,45	1,84	1,18	1,50	1,97
21	1,34	1,64	2,08	1,29	1,64	2,15
22	1,50	1,83	2,32	1,40	1,78	2,33
23	1,65	2,01	2,55	1,51	1,92	2,52
24	1,80	2,20	2,78	1,62	2,06	2,70
25	1,94	2,38	3,01	1,74	2,20	2,89
26	2,09	2,56	3,24	1,85	2,35	3,08
27	2,24	2,74	3,47	1,97	2,51	3,28
28	2,40	2,93	3,71	2,10	2,67	3,50
29	2,56	3,13	3,97	2,24	2,85	3,73
30	2,73	3,34	4,23	2,39	3,03	3,97
31	2,90	3,55	4,50	2,54	3,22	4,22
32	3,08	3,77	4,77	2,69	3,42	4,48
33	3,26	3,99	5,05	2,85	3,62	4,74
34	3,44	4,21	5,33	3,02	3,83	5,01
35	3,63	4,44	5,62	3,18	4,04	5,29
36	3,81	4,66	5,90	3,35	4,25	5,57
37	4,00	4,89	6,19	3,52	4,47	5,85
38	4,18	5,12	6,48	3,69	4,69	6,14
39	4,37	5,34	6,76	3,86	4,90	6,42
40	4,55	5,57	7,05	4,04	5,12	6,71

Fetal subscapular fat tissue (SFT) reference values in mm from 18 weeks gestational age (GA) onwards. Percentiles 10, 50 and 90 for ne-GWG (non-excessive GWG) and e-GWG (excessive GWG).

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Gridcurves & Scatterplots for Total group and Boys & Girls

Reference values (gridlines) for outstanding fetal anthropometry measurements for neonatal fat mass (NFM) >15% (P90) and fat mass ≤15%.

e-Table 6.1. Fetal abdominal circumference for neonatal fat mass (NFM) >15% (P90) and NFM ≤15%

GA (in weeks)	Fat>15%			Fat≤15%		
	C10	C50	C90	C10	C50	C90
12	50,68	55,33	58,09	50,88	54,73	59,19
13	62,49	68,22	71,63	61,62	66,29	71,69
14	74,28	81,09	85,14	72,55	78,05	84,40
15	85,98	93,87	98,56	83,68	90,02	97,35
16	97,56	106,51	111,83	94,91	102,11	110,42
17	108,96	118,95	124,90	106,12	114,16	123,45
18	120,15	131,17	137,73	117,19	126,07	136,34
19	131,17	143,19	150,35	128,11	137,82	149,04
20	142,04	155,07	162,82	138,87	149,39	161,55
21	152,84	166,85	175,19	149,46	160,79	173,88
22	163,58	178,58	187,50	159,91	172,03	186,03
23	174,27	190,26	199,76	170,22	183,11	198,02
24	184,92	201,87	211,96	180,36	194,02	209,82
25	195,48	213,41	224,07	190,33	204,75	221,42
26	205,96	224,85	236,09	200,12	215,28	232,81
27	216,33	236,17	247,97	209,78	225,67	244,05
28	226,57	247,35	259,71	219,34	235,96	255,17
29	236,70	258,41	271,32	228,85	246,19	266,23
30	246,74	269,37	282,83	238,32	256,38	277,25
31	256,72	280,25	294,26	247,77	266,55	288,25
32	266,64	291,09	305,64	257,23	276,72	299,24
33	276,53	301,89	316,98	266,69	286,90	310,25
34	286,38	312,65	328,27	276,16	297,08	321,27
35	296,20	323,36	339,52	285,65	307,29	332,30
36	305,97	334,03	350,72	295,14	317,51	343,36
37	315,72	344,67	361,90	304,66	327,74	354,42
38	325,46	355,31	373,06	314,18	337,98	365,50

Fetal Abdominal Circumference (AC) reference values in mm and gestational age (GA) in weeks. Percentiles 10, 50 and 90 for neonatal fat mass >15% (P90) and fat mass ≤15%.

e-Figure 3.1. Grid reference curve and scatterplot for BiParietalDiameter (BPD) in mm for total group from 12-40 weeks of gestation. Percentiles 5, 10, 50, 90 and 95.

e-Table 6.2. Fetal liver length for neonatal fat mass (NFM) >15% (P90) and NFM ≤15%

GA (in weeks)	Fat>15%			Fat≤15%		
	C10	C50	C90	C10	C50	C90
12	7,50	8,83	10,07	7,32	8,49	9,77
13	9,07	10,68	12,17	8,87	10,29	11,85
14	10,64	12,52	14,27	10,43	12,10	13,93
15	12,21	14,37	16,38	11,99	13,90	16,00
16	13,78	16,22	18,49	13,54	15,71	18,08
17	15,36	18,07	20,60	15,10	17,51	20,16
18	16,94	19,94	22,73	16,66	19,32	22,24
19	18,53	21,81	24,86	18,21	21,12	24,32
20	20,13	23,70	27,01	19,77	22,93	26,40
21	21,75	25,59	29,17	21,33	24,73	28,47
22	23,37	27,51	31,35	22,88	26,54	30,55
23	25,01	29,43	33,55	24,44	28,35	32,63
24	26,66	31,37	35,76	26,00	30,15	34,71
25	28,32	33,33	37,99	27,55	31,96	36,79
26	29,98	35,29	40,22	29,11	33,76	38,87
27	31,66	37,26	42,47	30,67	35,57	40,94
28	33,34	39,24	44,73	32,22	37,37	43,02
29	35,03	41,23	46,99	33,78	39,18	45,10
30	36,72	43,22	49,26	35,33	40,98	47,18
31	38,41	45,21	51,53	36,89	42,79	49,26
32	40,10	47,20	53,80	38,45	44,59	51,34
33	41,79	49,19	56,07	40,00	46,40	53,41
34	43,49	51,18	58,34	41,56	48,20	55,49
35	45,18	53,17	60,61	43,12	50,01	57,57
36	46,87	55,16	62,87	44,67	51,82	59,65
37	48,56	57,15	65,14	46,23	53,62	61,73
38	50,25	59,14	67,41	47,79	55,43	63,81

Fetal Liver length reference values in mm from 12weeks gestational age (GA) onwards. Percentiles 10, 50 and 90 for neonatal fat mass >15% (P90) and fat mass ≤15%.

e-Table 6.3. Fetal subscapular fat thickness for neonatal fat mass (NFM) >15% (P90) and NFM ≤15%

GA (in weeks)	Fat>15%			Fat≤15%		
	C10	C50	C90	C10	C50	C90
20	0,91	1,02	1,24	1,22	1,53	1,97
21	1,21	1,36	1,65	1,34	1,69	2,17
22	1,48	1,67	2,02	1,46	1,84	2,36
23	1,71	1,93	2,33	1,58	1,99	2,56
24	1,90	2,14	2,59	1,70	2,14	2,75
25	2,06	2,33	2,81	1,82	2,29	2,94
26	2,21	2,49	3,01	1,94	2,44	3,13
27	2,35	2,66	3,21	2,06	2,59	3,33
28	2,53	2,85	3,45	2,19	2,75	3,54
29	2,75	3,11	3,76	2,33	2,92	3,76
30	3,04	3,44	4,15	2,47	3,11	3,99
31	3,37	3,81	4,60	2,62	3,30	4,23
32	3,73	4,21	5,09	2,78	3,49	4,49
33	4,10	4,63	5,59	2,94	3,69	4,75
34	4,46	5,03	6,08	3,10	3,90	5,01
35	4,80	5,42	6,54	3,27	4,11	5,28
36	5,11	5,77	6,97	3,44	4,32	5,55
37	5,40	6,10	7,37	3,61	4,53	5,82
38	5,69	6,42	7,76	3,78	4,74	6,09

Fetal subscapular fat thickness (SFT) reference values in mm from 20 weeks gestational age (GA) onwards. Percentiles 10, 50 and 90 for neonatal fat mass >15% (P90) and fat mass ≤15%.

e-Table 6.4. Fetal abdominal fat thickness for neonatal fat mass (NFM) >15% (P90) and NFM ≤15%

GA (in weeks)	Fat>15%			Fat≤15%		
	C10	C50	C90	C10	C50	C90
20	1,12	1,39	1,72	1,13	1,46	1,89
21	1,26	1,57	1,94	1,29	1,66	2,15
22	1,41	1,74	2,16	1,45	1,86	2,41
23	1,55	1,92	2,38	1,59	2,04	2,64
24	1,69	2,10	2,60	1,71	2,20	2,84
25	1,84	2,28	2,83	1,82	2,33	3,02
26	1,99	2,48	3,07	1,90	2,44	3,16
27	2,16	2,68	3,32	1,98	2,54	3,28
28	2,33	2,89	3,58	2,05	2,63	3,41
29	2,51	3,11	3,86	2,14	2,75	3,56
30	2,70	3,35	4,14	2,25	2,89	3,74
31	2,89	3,59	4,44	2,37	3,05	3,94
32	3,09	3,84	4,75	2,52	3,23	4,18
33	3,30	4,09	5,07	2,67	3,43	4,44
34	3,51	4,36	5,40	2,84	3,65	4,72
35	3,73	4,62	5,73	3,02	3,88	5,02
36	3,94	4,89	6,06	3,21	4,13	5,34
37	4,16	5,16	6,40	3,41	4,38	5,66
38	4,38	5,43	6,73	3,61	4,63	5,99

Fetal abdominal fat thickness (AFT) reference values in mm from 20 weeks gestational age (GA) onwards. Percentiles 10, 50 and 90 for neonatal fat mass >15% (P90) and fat mass ≤15%.

e-Figure 3.2. Grid reference curve and scatterplot for HeadCircumference (HC) in mm for total group from 12-40 weeks of gestation. Percentiles 5, 10, 50, 90 and 95.

e-Figure 3.3. Grid reference curve and scatterplot for Abdominal Circumference (AC) in mm for total group from 12-40 weeks of gestation. Percentiles 5, 10, 50, 90 and 95.

e-Figure 3.4. Grid reference curve and scatterplot for Femur Length (FL) in mm for total group from 12-40weeks of gestation. Percentiles 5, 10, 50, 90 and 95.

e-Figure 3.5. Grid reference curve and scatterplot for Estimated Fetal Weight (EFW) in g for total group from 12-40 weeks of gestation. Percentiles 5, 10, 50, 90 and 95.

e-Figure 3.6. Grid reference curves and scatterplots for BiParietalDiameter (BPD) in mm for Boys (upper right) and Girls (lower right) from 12-40 weeks of gestation. Percentiles 5, 10, 50, 90 and 95.

e-Figure 3.7.a,b. Grid reference curves for BiParietalDiameter (BPD) in mm for Boys and Girls: a) from 20-30 weeks of gestation (upper); b) from 30-40 weeks of gestation (down). Percentiles 5, 10, 50, 90 and 95.

e-Figure 3.8. Grid reference curves and scatterplot for HeadCircumference (HC) in mm for Boys (upper right) and Girls (lower right) from 12-40 weeks of gestation. Percentiles 5, 10, 50, 90 and 95.

e-Figure 3.9.a,b. Grid reference curves for HeadCircumference (HC) in mm for Boys and Girls: a) from 20-30 weeks of gestation (upper); b) from 30-40 weeks of gestation (down). Percentiles 5, 10, 50, 90 and 95.

e-Figure 3.10. Grid reference curves and scatterplot for Abdominal Circumference (AC) in mm for Boys (upper right) and Girls (lower right) from 12-40 weeks of gestation. Percentiles 5, 10, 50, 90 and 95.

e-Figure 3.11.a,b. Grid reference curves for Abdominal Circumference (AC) in mm for Boys and Girls: a) from 20-30 weeks (upper); b) from 30-40 weeks of gestation (down). Percentiles 5, 10, 50, 90 and 95.

e-Figure 3.12. Grid reference curves and scatterplot for Femur Length (FL) in mm for Boys (upper right) and Girls (lower right) from 12-40 weeks of gestation. Percentiles 5, 10, 50, 90 and 95.

e-Figure 3.13.a,b. Grid reference curves for Femur Length (FL) in mm for Boys and Girls: a) from 20-30 weeks of gestation (upper); b) from 30-40 weeks of gestation (down). Percentiles 5, 10, 50, 90 and 95.

e-Figure 3.14. Grid reference curves and scatterplot for Estimated Fetal Weight (EFW) in g for Boys (upper right) and Girls (lower right) from 12-40 weeks of gestation. Percentiles 5, 10, 50, 90 and 95.

e-Figure 3.15.a,b. Grid reference curves for Estimated Fetal Weight (EFW) in g for Boys and Girls: a) from 20-30 weeks of gestation (upper); b) from 30-40 weeks of gestation (down). Percentiles 5, 10, 50, 90 and 95.

Chapter 5

Scatterplots fetal measurements

e-Table 8.1. Percentile grids of IMT, DIA and DIS per gestational week

GA	IMT			DIA			DIS		
	P10	P50	P90	P10	P50	P90	P10	P50	P90
12	451,392	519,3619	602,1823	6050,135	6569,734	7106,3	320,6322	470,3141	672,2
13	450,7456	518,6181	601,3198	6062,539	6583,203	7120,869	318,6048	467,3401	667,9495
14	450,0991	517,8742	600,4574	6074,943	6596,673	7135,439	316,5773	464,3662	663,699
15	449,4526	517,1304	599,5949	6087,347	6610,142	7150,008	314,5499	461,3923	659,4485
16	448,8061	516,3866	598,7325	6099,751	6623,611	7164,578	312,5224	458,4183	655,1979
17	448,1596	515,6427	597,87	6112,155	6637,081	7179,147	310,495	455,4444	650,9474
18	447,5131	514,8989	597,0076	6124,559	6650,55	7193,716	308,4675	452,4705	646,6969
19	446,8666	514,1551	596,1451	6136,963	6664,019	7208,285	306,4401	449,4965	642,4464
20	446,2201	513,4112	595,2827	6149,367	6677,488	7222,854	304,4126	446,5226	638,1959
21	445,5737	512,6674	594,4202	6161,77	6690,956	7237,423	302,3851	443,5487	633,9454
22	444,9272	511,9236	593,5578	6174,173	6704,425	7251,992	300,3577	440,5747	629,6948
23	444,2807	511,1797	592,6953	6186,577	6717,894	7266,56	298,3302	437,6008	625,4443
24	443,6342	510,4359	591,8329	6198,98	6731,362	7281,128	296,3028	434,6269	621,1938
25	442,9877	509,6921	590,9704	6211,383	6744,83	7295,697	294,2753	431,6529	616,9433
26	442,3412	508,9482	590,108	6223,786	6758,298	7310,265	292,2479	428,679	612,6928
27	441,6947	508,2044	589,2455	6236,189	6771,766	7324,833	290,2204	425,7051	608,4422
28	441,0483	507,4606	588,3831	6248,592	6785,234	7339,401	288,193	422,7311	604,1917
29	440,4018	506,7167	587,5206	6260,994	6798,702	7353,969	286,1655	419,7572	599,9412
30	439,7553	505,9729	586,6582	6273,397	6812,17	7368,537	284,1381	416,7833	595,6907
31	439,1088	505,2291	585,7957	6285,8	6825,638	7383,105	282,1106	413,8093	591,4402
32	438,4623	504,4852	584,9333	6298,203	6839,106	7397,673	280,0832	410,8354	587,1897
33	437,8158	503,7414	584,0709	6310,605	6852,574	7412,24	278,0557	407,8615	582,9391
34	437,1693	502,9976	583,2084	6323,008	6866,042	7426,808	276,0283	404,8875	578,6886
35	436,5228	502,2537	582,346	6335,411	6879,51	7441,376	274,0008	401,9136	574,4381
36	435,8764	501,5099	581,4835	6347,814	6892,978	7455,944	271,9734	398,9397	570,1876
37	435,2299	500,7661	580,6211	6360,216	6906,446	7470,512	269,9459	395,9657	565,9371
38	434,5834	500,0222	579,7586	6372,619	6919,913	7485,079	267,9185	392,9918	561,6865

Percentile grids for IMT = intima media thickness in µm; DIA = diameter in µm; DIS = distension in µm; GA = gestational age. P10 = 10th percentile; P50 = 50th percentile; P90 = 90th percentile.

e-Figure 5.1.a,b. Scatterplots for Fetal Head Circumference (HC) in mm (upper) and for Fetal Abdominal Circumference (AC) in mm (down) during gestation (x-axis) in weeks. Percentiles 10, 50 and 90 using Box Cox Transformation (BCT).

e-Table 8.2. Percentile grids of CAC, YEM and SI per gestational week

GA	CAC			YEM			SI		
	P10	P50	P90	P10	P50	P90	P10	P50	P90
12	1,868591	2,79078	4,179133	120,6843	185,1391	287,2175	2,49731	3,789062	5,520322
13	1,852908	2,767357	4,144057	121,7242	186,7343	289,6922	2,522861	3,82783	5,576804
14	1,837225	2,743934	4,108981	122,764	188,3295	292,167	2,548412	3,866598	5,633285
15	1,821541	2,720511	4,073906	123,8039	189,9247	294,6417	2,573964	3,905366	5,689766
16	1,805858	2,697088	4,03883	124,8437	191,5199	297,1165	2,599515	3,944133	5,746247
17	1,790175	2,673664	4,003754	125,8836	193,1151	299,5912	2,625065	3,9829	5,802727
18	1,774492	2,650241	3,968678	126,9234	194,7103	302,0659	2,650616	4,021667	5,859206
19	1,758808	2,626818	3,933603	127,9633	196,3055	304,5407	2,676166	4,060432	5,915684
20	1,743125	2,603395	3,898527	129,0031	197,9007	307,0154	2,701715	4,099198	5,972162
21	1,727442	2,579971	3,863451	130,043	199,4959	309,4902	2,727264	4,137963	6,028639
22	1,711759	2,556548	3,828375	131,0828	201,0911	311,9649	2,752813	4,176727	6,085115
23	1,696076	2,533125	3,7933	132,1227	202,6863	314,4396	2,778362	4,215491	6,14159
24	1,680392	2,509702	3,758224	133,1625	204,2815	316,9144	2,80391	4,254254	6,198065
25	1,664709	2,486279	3,723148	134,2024	205,8768	319,3891	2,829458	4,293017	6,254539
26	1,649026	2,462855	3,688072	135,2422	207,472	321,8639	2,855006	4,33178	6,311014
27	1,633343	2,439432	3,652997	136,2821	209,0672	324,3386	2,880554	4,370543	6,367488
28	1,61766	2,416009	3,617921	137,3219	210,6624	326,8134	2,906103	4,409306	6,423962
29	1,601976	2,392586	3,582845	138,3618	212,2576	329,2881	2,931651	4,448069	6,480436
30	1,586293	2,369163	3,547769	139,4016	213,8528	331,7628	2,957199	4,486832	6,53691
31	1,57061	2,345739	3,512694	140,4414	215,448	334,2376	2,982747	4,525595	6,593384
32	1,554927	2,322316	3,477618	141,4813	217,0432	336,7123	3,008295	4,564358	6,649858
33	1,539243	2,298893	3,442542	142,5211	218,6384	339,1871	3,033842	4,60312	6,706332
34	1,52356	2,27547	3,407466	143,561	220,2336	341,6618	3,05939	4,641883	6,762805
35	1,507877	2,252046	3,372391	144,6008	221,8288	344,1366	3,084938	4,680645	6,819278
36	1,492194	2,228623	3,337315	145,6407	223,424	346,6113	3,110485	4,719407	6,875751
37	1,476511	2,2052	3,302239	146,6805	225,0192	349,086	3,136033	4,75817	6,932224
38	1,460827	2,181777	3,267163	147,7204	226,6144	351,5608	3,16158	4,796932	6,988697

Percentile grids for CAC = carotid artery compliance in %/10mmHg; YEM = young elastic modulus in mmHg/mm; SI = stiffness index. GA = gestational age. P10 = 10th percentile; P50 = 50th percentile; P90 = 90th percentile.

e-Figure 5.2.a,b. Scatterplots for Fetal Femur Length (FL) in mm (upper) and Fetal Liver Length (LL) in mm (down) during gestation (x-axis) in weeks. Percentiles 10, 50 and 90 using Box Cox Transformation (BCT).

e-Figure 5.3.a,b. Scatterplots for Fetal Subscapular Fat Tissue (SFT) in mm (upper) and Fetal Abdominal Fat Tissue (AFT) in mm (down) during gestation (x-axis) in weeks. Percentiles 10, 50 and 90 using Box Cox Transformation (BCT).

e-Figure 5.4.a,b. Scatterplots for Humerus Fetal *Lean* Mass Area (H-FLMA) in mm^2 (upper) and in % (down) of total surface of mid-portion transvers section of humerus during gestation (x-axis) in weeks. Percentiles 10, 50 and 90 using Box Cox Transformation (BCT).

e-Figure 5.5.a,b. Scatterplots for Humerus Fetal *Fat* Mass Area (H-FFMA) in mm^2 (upper) and in % (down) of total surface of mid-portion transvers section of humerus during gestation (x-axis) in weeks. Percentiles 10, 50 and 90 using Box Cox Transformation (BCT).

e-Figure 5.6.a,b. Scatterplots for Femur Fetal *Lean* Mass Area (F-FLMA) in mm^2 (upper) and in % (down) of total surface of mid-portion transvers section of femur during gestation (x-axis) in weeks. Percentiles 10, 50 and 90 using Box Cox Transformation (BCT).

e-Figure 5.7.a,b. Scatterplots for Femur Fetal *Fat* Mass Area (F-FFMA) in mm^2 (upper) and in % (down) of total surface of mid-portion transvers section of femur during gestation (x-axis) in weeks. Percentiles 10, 50 and 90 using Box Cox Transformation (BCT).

Chapter 6

e-Table 9.2. Percentile grids of IMT, DIA and DIS per gestational week

GA	IMT			DIA			DIS		
	P10	P50	P90	P10	P50	P90	P10	P50	P90
26	269,6516	315,8827	382,863	3102,313	3466,738	3862,983	171,6476	233,8431	316,1935
27	283,6074	332,2311	402,6779	3227,78	3606,943	4019,214	174,7107	238,0161	321,836
28	297,5631	348,5795	422,4929	3353,247	3747,149	4175,444	177,7738	242,189	327,4786
29	311,5189	364,9279	442,3078	3478,714	3887,354	4331,675	180,8369	246,362	333,1211
30	325,4746	381,2763	462,1228	3604,181	4027,56	4487,906	183,9	250,535	338,7636
31	339,4303	397,6247	481,9378	3729,648	4167,765	4644,137	186,9631	254,708	344,4062
32	353,3861	413,9731	501,7527	3855,115	4307,971	4800,368	190,0262	258,881	350,0487
33	367,3418	430,3216	521,5677	3980,582	4448,176	4956,599	193,0893	263,0539	355,6913
34	381,2976	446,67	541,3826	4106,049	4588,381	5112,829	196,1523	267,2269	361,3338
35	395,2533	463,0184	561,1976	4231,516	4728,587	5269,06	199,2154	271,3999	366,9763
36	409,209	479,3668	581,0125	4356,983	4868,792	5425,291	202,2785	275,5729	372,6189
37	423,1648	495,7152	600,8275	4482,45	5008,998	5581,522	205,3416	279,7459	378,2614
38	437,1205	512,0636	620,6425	4607,917	5149,203	5737,753	208,4047	283,9188	383,904

Percentile grids for IMT = intima media thickness in µm; DIA = diameter in µm; DIS = distension in µm; GA = gestational age. P10 = 10th percentile; P50 = 50th percentile; P90 = 90th percentile.

e-Table 9.3. Percentile grids of fAAC, YEM and SI per gestational week

GA	fAAC			YEM			SI		
	P10	P50	P90	P10	P50	P90	P10	P50	P90
26	4,033462	5,669164	7,92108	38,08719	56,00549	82,01378	4,062464	5,769922	7,955559
27	3,920861	5,510901	7,69995	42,03019	61,8035	90,5043	4,101144	5,824859	8,031307
28	3,808261	5,352637	7,478821	45,97319	67,6015	98,99483	4,139824	5,879796	8,107054
29	3,69566	5,194373	7,257691	49,91619	73,3995	107,4854	4,178504	5,934733	8,182802
30	3,58306	5,036109	7,036561	53,85918	79,1975	115,9759	4,217184	5,989671	8,258549
31	3,470459	4,877845	6,815431	57,80218	84,9955	124,4664	4,255864	6,044608	8,334297
32	3,357858	4,719581	6,594302	61,74518	90,7935	132,9569	4,294544	6,099545	8,410044
33	3,245258	4,561317	6,373172	65,68818	96,59151	141,4475	4,333224	6,154483	8,485791
34	3,132657	4,403053	6,152042	69,63118	102,3895	149,938	4,371904	6,20942	8,561539
35	3,020057	4,24479	5,930913	73,57418	108,1875	158,4285	4,410584	6,264357	8,637286
36	2,907456	4,086526	5,709783	77,51718	113,9855	166,919	4,449264	6,319295	8,713034
37	2,794855	3,928262	5,488653	81,46018	119,7835	175,4096	4,487944	6,374232	8,788781
38	2,682255	3,769998	5,267523	85,40317	125,5815	183,9001	4,526624	6,429169	8,864529

Percentile grids for fAAC = fetal abdominal aorta artery compliance in %/10mmHg; YEM = young elastic modulus in mmHg/mm; SI = stiffness index. GA = gestational age. P10 = 10th percentile; P50 = 50th percentile; P90 = 90th percentile.

Scatterplots neonatal / birth measurements

e-Figure 6.1.a,b. Scatterplots for Birth Head Circumference (BHC) in mm (upper) and Birth Abdominal Circumference (BAC) in mm (down) at birth in gestational weeks (x-axis). Percentiles 10, 50 and 90 using Box Cox Transformation (BCT).

e-Figure 6.2.a,b. Scatterplots for Birth Weight (BW) in g (upper) and Birth Length (BL) in cm (down) at birth in gestational weeks (x-axis). Percentiles 10, 50 and 90 using Box Cox Transformation (BCT).

e-Figure 6.3.a,b. Scatterplots for Birth upper arm circumference (Uparm) in mm (upper) and Birth lower arm circumference (Loarm) in mm (down) at birth in gestational weeks (x-axis). Percentiles 10, 50 and 90 using Box Cox Transformation (BCT).

e-Figure 6.4.a,b. Scatterplots for Birth upper leg (Upleg) in mm (upper) and Birth lower leg (Loleg) in mm (down) at birth in gestational weeks (x-axis). Percentiles 10, 50 and 90 using Box Cox Transformation (BCT).

e-Figure 6.5.a,b. Scatterplots for Birth Triceps Skinfold (Tr-SF) in mm (upper) and Birth Subscapular Skinfold (S-SF) in mm (down) at birth in gestational weeks (x-axis). Percentiles 10, 50 and 90 using Box Cox Transformation (BCT).

e-Figure 6.6.a,b. Scatterplots for Birth Flank Skinfold (Fl-SF) in mm (upper) and Birth Thigh Skinfold (Th-SF) in mm (down) at birth in gestational weeks (x-axis). Percentiles 10, 50 and 90 using Box Cox Transformation (BCT).

e-Figure 6.7.a,b. Scatterplots for Birth fat percentage ('neonatal adiposity') in % of total BW at birth in gestational weeks (x-axis). Percentiles 10, 50 and 90 using Box Cox Transformation (BCT).

Chapter 8

Intra-class correlation coefficient (ICC) of IMT, DIA and DIS

e-Figure 8.1. Maternal right common carotid artery measurement with radio-frequency US. Measurements of IMT. First, second and third mean values for each time-point. IMT1 = measurement 1; IMT2 = measurement 2; IMT3 = measurement 3; X/Y-axis, IMT values in µm.

e-Figure 8.2. Maternal right common carotid artery measurement with radio-frequency US. Measurements of DIA. First, second and third mean values for each time-point. DIA1 = measurement 1; DIA2 = measurement 2; DIA3 = measurement 3; X/Y-axis, DIA values in µm.

e-Figure 8.3. Maternal right common carotid artery measurement with radio-frequency US. Measurements of DIS. First, second and third mean values for each time-point. DIS1 = measurement 1; DIS2 = measurement 2; DIS3 = measurement 3; X/Y-axis, DIS values in µm.

Chapter 9

e-Figure 9.1. Fetal abdominal aorta artery measurement with radio-frequency US. Quality arterial stiffness (QAS) measurement; fast-b-mode image; blue line = arterial distension (DIS), orange line = arterial diameter (DIA). X-axis, region of interest in mm; Y-axis distance in mm.

e-Figure 9.2. Fetal abdominal aorta artery measurement with radio-frequency US. Measurements of IMT. First, second and third mean values for each time-point. IMT1 = measurement 1; IMT2 = measurement 2; IMT3 = measurement 3; X/Y-axis, IMT values in µm.

e-Figure 9.3. Fetal abdominal aorta artery measurement artery measurement with radio-frequency US. Measurements of DIA. First, second and third mean values for each time-point. DIA1 = measurement 1; DIA2 = measurement 2; DIA3 = measurement 3; X/Y-axis, DIA values in µm.

e-Figure 9.4. Fetal abdominal aorta artery measurement artery measurement with radio-frequency US. Measurements of DIS. First, second and third mean values for each time-point. DIS1 = measurement 1; DIS2 = measurement 2; DIS3 = measurement 3; X/Y-axis, DIS values in µm.

e-Figure 9.5. Correlation estimated fetal weight (EFW) >P90 in normal BMI group women versus EFW<P90 in the same normal BMI group for fetal abdominal aorta diameter (fAA DIA) from 26 weeks onwards. EFW>P90 and EFW<P90 were introduced as explanatory variables in the GAMLSS model; $p < 0.07$. X-axis: gestational age in weeks; Y-axis: abdominal aortic diameter in µm.

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Jakko! Ik heb je de laatste maanden weer eens anders leren kennen. Vond ik je al een hele fijne jongen, dan blijk je ook nog eens breed georiënteerd en zeer intelligent te zijn. Heel, heel erg veel dank voor de tekstuele en inhoudelijke reflecties van de afgelopen maanden. Je hebt een constructieve bijdrage gehad in dit werk en dank aan je gezin (**Judith**, **Isis** en **Abel**) dat ik je aandacht mocht lenen. Lieve ouders, lieve **pappa** en **mamma**. Het zijn julllie geweest die mij hebben geleerd hard te kunnen werken en ergens voor te gaan in het leven. Die basis is zo belangrijk. En ook om de *mens* in ieder persoon te waarderen, niet door zijn of haar afkomst. En hoewel ik nooit het gevoel gehad heb jullie 'gouden ei' te zijn, waren jullie natuurlijk ook bezorgd wanneer ik niet vreemd was van on-orthodoxe keuzes in het leven. Jullie zagen dat al van jongs af aan aankomen toen ik met de driewieler (en een pak melk achterop –blijf natuurlijk een Hollander-) de wijde wereld introk. Heel erg dank dat jullie die veilige en ondersteundende 'extra-uteriene' omgeving hebben gegeven! Last, maar eigenlijk most of all, lieve **Alies**, mijn grootste steun en toeverlaat! Jouw positieve insteek in het leven geeft mijn pieker-neigingen de beste aanvulling die ik mij kan wensen. Vrijwel nooit een onvertogen woord, de afgelopen maanden (en jaren), hoewel wel eens een traan. Ik hoop dat ik dat niet meer hoef te veroorzaken! Groot respect heb ik voor jou, om alles draaiende te houden met werk, twee kleine kinderen en vrienden. Ik ben echt een zondagskind, denk ik wel eens. Maar dat zeg jij van jezelf ook. Ik begrijp goed dat je mij nu weer voor jezelf terug wilt hebben. En voor onze twee prachtige jongens, **Lennard** en **Yannick**.

Voor de deelnemende patiënten.

CV

Sander Galjaard, born at 22 August 1975 in Leiden, the Netherlands, finished his secondary school at Stedelijk Gymnasium in Leiden in 1994 with one extra major. In 1995 he started medical school at the Leiden University (at that time Rijks Universiteit Leiden) and in 2002 he obtained his medical degree, after working as a 'daktari' for six months in the Misikhu Mission Hospital, Webuye, Kenia. For this he was nominated for the Leiden International Student Funds (LISF) award.

From 2003 to 2004 he worked as a clinical resident in the Obstetrics and Gynaecology department at the Groene Hart Ziekenhuis, Gouda, The Netherlands, under the supervision of dr. Hans van Huisseling. From 2004 to 2006 he worked in the Leiden University Medical Centre (LUMC), The Netherlands, at the centre for prenatal diagnosis and fetal therapy under the guidance of professor Humphrey Kanhai and professor Dick Oepkes. From 2006 to 2008 he worked at the Maastricht University Medical Centre (MUMC), The Netherlands, as a prenatal specialist, performing invasive prenatal diagnostic tests under supervision of professor Jan Nijhuis. Here he also became a tutor.

In August 2008 he started his specialist training in Obstetrics and Gynaecology at the University Hospitals KU Leuven, Belgium, under the supervision of professor Ignace Vergote, with the first two years of training in the Ziekenhuis Oost Limburg (ZOL), Genk, Belgium (head professor Willem Ombelet). In 2011, during his specialist training, he started his doctoral thesis: 'The Cradle of Metabolic Disease, vascular development and fetal body composition during pregnancy', at the Departments of Regeneration and Growth, KU Leuven under supervision of his promotor, professor Roland Devlieger and the co-promotors professor Dirk Timmerman and professor Jan Deprest. Sander was the coordinator in the DALI (vitamin D And Lifestyle Intervention study) consortium, a European FP7 program, together with his promotor, the Work Package 4 'core fetal measurements' and he organized training for fetal and neonatal protocols, which were also used in this doctoral thesis. During his doctoral period, he was also clinically involved as a fellow at the fetal therapy department of the University Hospitals KU Leuven, under the supervision of professor Jan Deprest.

Sander lives together with Alies van Kampen, who he loves intensely. And at the mountain, cordially called 'Gasthuisberg', where he spent so much time, their two beautiful sons, Lennard and Yannick, were born.

Curriculum Vitae

Sander Galjaard werd geboren op 22 augustus, 1975 te Leiden, Nederland. Van 1988 tot 1994 doorliep hij het Stedelijk Gymnasium te Leiden met een extra hoofdvak. In 1995 werd hij direct ingeloot voor de studie Geneeskunde aan de Universiteit Leiden (Rijks Universiteit Leiden, indertijd) en in 2002 behaalde hij zijn artsexamen. Hiervoor heeft hij eerst nog 6 maanden als een exceptioneel co-schap in het Misikhu Mission Hospital, Webuye, Kenia, gewerkt als een 'dakatari', alwaar hij de eerste kennismaking had met de zorg verantwoordelijkheid voor moeder en kind. Voor het rapport over deze periode werd hij genomineerd voor de Leiden International Student Funds (LISF) award. Van 2003 tot en met 2004 werkte hij als arts-assistent niet in opleiding (anios) op de afdeling Gynaecologie-Verloskunde in het Groene Hart Ziekenhuis te Gouda, Nederland (opleider dr. Hans van Huisseling). Van 2004 tot en met 2006 werkte hij in het Leiden University Medical Centre (LUMC), Nederland, op de afdeling prenatale disgnostiek en foetale therapie, onder supervisie van professor Humphrey Kanhai en professor Dick Oepkes. Van 2006 tot en met 2008 werkte hij in het Maastricht University Medical Centre (MUMC), Nederland, als prenatale arts, met de speciale bevoegdheid om zelfstandig invasieve prenatale diagnostiek te mogen uitvoeren, onder de supervisie van professor Jan Nijhuis. Sander volgde hier ook een opleiding tot tutor 'probleem gestuurd onderwijs' van de Universiteit Maastricht. In augustus 2008, begon hij zijn opleiding tot gynaecoloog in het Universitair Ziekenhuis ('Gasthuisberg') te Leuven, België (opleider professor Ignace Vergote). De eerste twee jaar van zijn opleiding ronde hij af in het Ziekenhuis Oost Limburg (ZOL), Genk, België (opleider professor Willem Ombelet). In 2011 werd hij door professor Roland Devlieger (promotor), professor Jan Deprest en professor Dirk Timmerman (co-promotores) aangesteld als promovendus, dat leidde tot de huidige thesis: 'The Cradle of Metabolic Disease -vascular development and fetal body composition during pregnancy-'. Tijdens dit promotietraject was Sander, samen met zijn promotor, intensief betrokken bij de coördinatie van de DALI (vitamin D And Lifestyle Intervention study) studie, een Europees FP7 programma, en verzorgde hij hiervoor trainingen voor de foetale en neonatale metingen als onderdeel van de Work Package 4, 'core foetal measurements'. Deze technieken werden ook gebruikt voor zijn doctoraat.

Tijdens zijn doctoraatsperiode, was hij ook klinisch betrokken als fellow aan de subafdeling 'foetale therapie' van de Universitaire Ziekenhuizen KU Leuven, onder supervisie van professor Jan Deprest. Sander leeft samen met Alies van Kampen, van wie hij erg veel houdt. En op 'de berg', warmhartig vernoemd tot 'Gasthuisberg', waar hij zoveel tijd doorbracht, werden in respectievelijk 2011 en 2014 hun twee prachtige zonen, Lennard en Yannick geboren.

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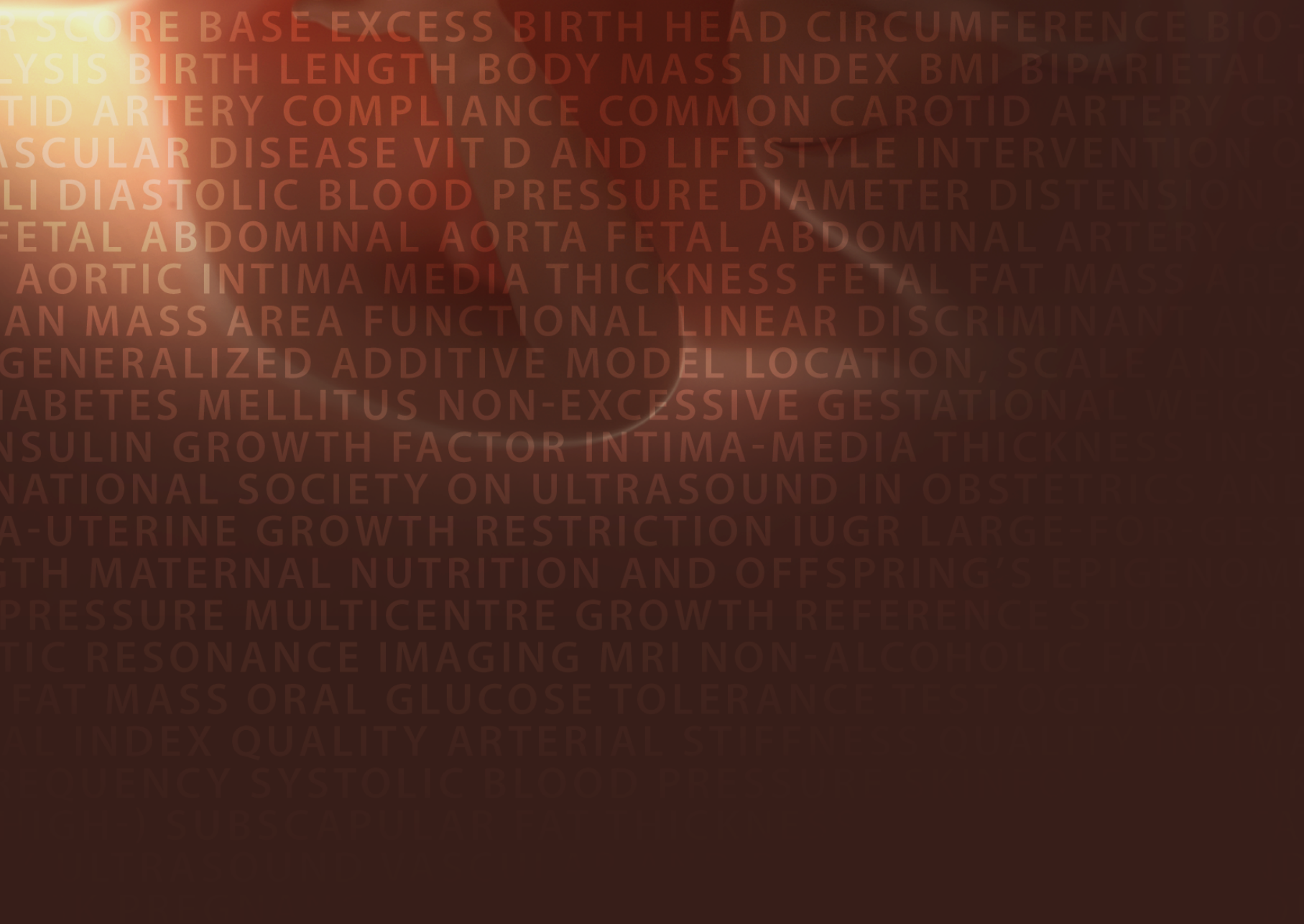
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PRESSURE MULTICENTRE GROWTH REFERENCE
TIC RESONANCE IMAGING MRI NON-ALCOHOLIC
FAT MASS ORAL GLUCOSE TOLERANCE
INDEX QUALITY ARTERIAL STIFFNESS
QUENCY SYSTOLIC BLOOD PRESSURE
SUBSCAPULAR