

Antenatal characterisation and postnatal validation of fetal nutritional status using novel fetal imaging methods, neonatal body composition data and anthropometry

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Declaration

I, Caroline Knight, declare that this thesis and the work presented in it are my own. Work done in collaboration with others is indicated as such, and our respective contributions clearly stated. This thesis has not been submitted for any other degree in this or any other university or tertiary institution.

Dedication

This thesis is dedicated to:

- **The past:** my seventeen year-old self who dreamed of Oxbridge life. It was all you hoped it would be, and so very much more. The stress, tears, and white hairs have been more than outweighed by the phenomenal experiences, both academic and personal, amongst the Dreaming Spires.
- **The present:** my parents, Dave and Sandra Knight. Thank you again for being there through the highs and the lows, and for quietly encouraging me along every step of the journey, from the 11+, GCSEs and A-levels through to finals, MRCOG, and now an Oxford DPhil.
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Guide to symbols used in figures

Symbol or line and its representation	
GRAPH SYMBOLS	
	Fat – limb (arm or leg), volume or area
	Lean – limb (arm or leg), volume or area
	Limb (arm or leg), volume or area
	Fat volume / area expressed as a percentage of the limb (arm or leg) volume / area
GRAPH CENTILE LINES	
.....	3 rd / 97 th centile lines on graphs
-- --	10 th / 90 th centile lines on graphs (occasionally 5 th / 95 th centile line – clearly marked)
—	50 th centile line: fat [blue]
—	50 th centile line: lean [green]
—	50 th centile line: limb (arm or thigh) [red]
—	50 th centile line: fat as a percentage [purple]
BLAND-ALTMAN PLOT LINES	
—	Mean difference line on Bland-Altman plots [bright red]
-- --	Limits of agreement on Bland-Altman plots [bright red dashed]

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List of abbreviations

2D	two-dimensional ultrasound	ICC	intraclass correlation coefficient
3D	three-dimensional ultrasound	IGF-I	insulin-like growth factor I
AC	abdominal circumference	IGT	impaired glucose tolerance
ADP	air displacement plethysmography [for infants: PEA POD machine]	IQR	interquartile range
AFI	amniotic fluid index	ISUOG	International Society of Ultrasound in Obstetrics and Gynecology (conference)
AFV	amniotic fluid volume	LBW	low birthweight (below 2500g)
AGA	appropriate for gestational age (applied to size and/or weight)	LGA	large for gestational age (applied to size and/or weight)
AVol	fractional (50%) arm volume	LMP	last menstrual period
BMI	body mass index	MCA	middle cerebral artery (Doppler measurement)
BPD	biparietal diameter	MHRA	Medicines and Healthcare products Regulatory Agency (UK)
CPR	cerebroplacental ratio	MRI	magnetic resonance imaging
CRL	crown-rump length	MTC	mid-thigh circumference (usually refers to neonatal measurement)
CT	computerised tomography (imaging)	MUAC	mid-upper arm circumference
CTG	cardiotocograph(y)	MVP	maximum vertical pocket (amniotic fluid)
DICOM	digital imaging and communications in medicine (imaging file type)	NDOG	Nuffield Department of Obstetrics and Gynaecology, University of Oxford
DM	diabetes mellitus	NICU	Neonatal Intensive Care Unit
DXA	dual-energy X-ray absorptiometry	OUH	Oxford University Hospital (NHS Trust)
EFW	estimated fetal weight	PI	pulsatility index
EPSRC	Engineering and Physical Sciences Research Council	PMHx	past medical history
e-THRIVE	enhanced T1-weighted High Resolution Isotropic Volume Examination (MRI sequence)	POHx	past obstetric history
FDA	(U.S.) Food and Drug Administration	r	Pearson's correlation coefficient
FFI	fetal fat index	r^2	coefficient of determination
FGLS	Fetal Growth Longitudinal Study (part of INTERGROWTH-21 st project)	RCOG	Royal College of Obstetricians and Gynaecologists, UK
FGR	fetal growth restriction	SD	standard deviation
FL	femur length	SGA	small for gestational age (applied to size and/or weight)
GA	gestational age	ThC	thigh circumference (usually refers to antenatal ultrasound measurement)
GDM	gestational diabetes mellitus	UA	umbilical artery (Doppler measurement)
GTT	glucose tolerance test (diabetes screening)	UK	United Kingdom
HC	head circumference	UKCRN	United Kingdom Clinical Research Network
HL	humerus length	UtA	uterine artery (Doppler measurement)
HT	hypertension	VOCAL	Virtual Organ Computer-aided Analysis
IBME	Institute of Biomedical Engineering, University of Oxford	XI-VOCAL	eXtended Imaging Virtual Organ Computer-aided AnaLysis

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MAVIDOS study

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Validating fetal ultrasound imaging using MRI

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(See appendix C.)

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ABSTRACT

Background: Fetal growth restricted (FGR) infants have increased perinatal morbidity and mortality risks. Standard fetal biometry may identify some FGR babies; amniotic fluid measurement and Doppler assessment of blood vessels provide additional, functional assessments, but are often normal in babies with late-onset FGR who are difficult to diagnose. A marker reflecting nutritional status should help to identify FGR fetuses, enabling effective intervention: increased monitoring or delivery and neonatal management. Arm and/or thigh measurements have previously shown potential in 2D and 3D. Limb fat volume has never been measured and could provide an *in utero* marker of fetal nutritional status.

Aims: 1. To develop an ultrasound scanning protocol to acquire 2D images and 3D volumes of fetal arms and thighs. 2. To develop method(s), suitable for use in clinical practice, to measure fat in these images and volumes. 3. To assess the reproducibility of these methods. 4. To assess the validity of these methods by comparing them with MRI images of fetal limb fat. 5. To use these methods in a healthy population to develop reference ranges. 6. To correlate these methods with validated neonatal measurements, to assess whether the antenatal methods reflect neonatal body composition. 7. To apply these methods to longitudinal prospective ultrasound images from multiple countries to assess SGA fetuses.

Method: Ultrasound imaging protocols were developed to obtain accurate images and volumes of fetal arms and thighs. Segmentation tools were designed with biomedical engineers to measure fat, lean and limb compartments, and applied to 2D images and 3D volumes (n=500), with calculation of reference centiles in optimally healthy women (INTERGROWTH-21st study) and MRI validation of the ultrasound measurements. Additional methods were assessed: a two-ellipse method, and a three-thickness Fetal Fat Index (FFI). Reproducibility was assessed using Bland-Altman plots and ICCs. Fetal measurements were correlated with neonatal body composition data and anthropometry. Third trimester fetal thigh fat volumes were compared in sub-cohorts of AGA and SGA fetuses.

Results: Reference centiles were calculated for novel fractional arm and leg volumes (fat and lean), from 16 to 41 weeks. 2D reference ranges were also calculated. The FFI technique – quick, simple, 2D – correlated well with fat area and fat volume. DXA analyses showed a strong correlation between neonatal limb and whole body fat. Correlation analyses showed that infants with above- and below-average arm circumferences have significantly different amounts of arm fat as early as 30-35 weeks. The strength of correlation between antenatal limb fat and neonatal PEA POD whole body fat increased with increasing gestational age. Scans at 30-34 weeks showed a significant difference in fractional thigh fat between those who would be born SGA compared with AGA.

Conclusion: This thesis explores, in detail, the measurement of fetal arm and thigh fat using 2D and 3D ultrasound, and demonstrates that it is correlated to neonatal body composition thus allowing “fetal body composition” to be established as a research tool; the ultimate aim is to be able to distinguish growth-restricted fetuses from those of normal nutritional status. Novel measurements have been developed, acquisition protocols described, reproducibility assessed, and reference centiles calculated in an optimally healthy population: 2D Fetal Fat Index, 3D fractional limb fat and lean volumes, and limb fat (2D and 3D) as a percentage.

Preface. CONCEPTS: THE SEARCH FOR A NUTRITIONAL MARKER

This preface explores the “background to the background” of my work: areas I investigated whilst honing my thesis, including some which were initially considered but later rejected.

I came to this research as an O&G registrar, having acquired my Membership of the Royal College of Obstetricians and Gynaecologists (MRCOG), and keen to undertake a higher research degree. I wanted, if possible, to maintain a clinical aspect to my research. Whilst exploring options, I heard of several DPhil opportunities at the Nuffield Department of Obstetrics and Gynaecology (NDOG) at the University of Oxford. One was ultrasound-based, linked to the INTERGROWTH-21st project, and involved multidisciplinary collaboration with both biomedical engineers and neonatologists. Funding was already in place, through the Wellcome Trust and Engineering and Physical Sciences Research Council (EPSRC), for a DPhil student / clinical research fellow to work within a Personalised Perinatal Imaging project.

I met with the head of department, Stephen Kennedy, and the Senior Clinical Fellow, Aris Papageorghiou, who would become my supervisor. The brief was broad: to be part of the imaging project and to explore a functional marker, possibly nutritional. I would be involved with ultrasound scanning (2D and 3D) at the Oxford site for the INTERGROWTH-21st project, providing an optimally healthy local research population. After preliminary literature searches, I proposed to explore nutritional markers for my DPhil, particularly looking at limb fat and building on the work of Lee et al’s fractional limb volumes, as well as the liver. As part of the INTERGROWTH-21st project, NDOG had invested in a PEA POD machine to assess fetal body composition so I also planned to compare neonatal and fetal measurements. I was selected for the DPhil at competitive interview and began in Michaelmas term 2010.

Detailed literature searches expanded my list of possible functional and nutritional markers, particularly those related to growth – an ever-present issue in obstetrics. I considered fetal brain assessment and development but ultimately came back to the evaluation of fetal nutrition, particularly fat. As an O&G registrar, I had delivered many growth-restricted babies, both anticipated and unanticipated. Some showed the classic ‘brain-sparing’ pattern of having a relatively larger head to a starved body; many others looked undernourished rather than starved. This latter group had less central signs but noticeably scrawny limbs: slender, with loose skin and little fat. It seemed biologically plausible that babies in the early stage of growth restriction would lay down less peripheral (limb) fat, and that this might be a step before the onset of a reduction in abdominal circumference, with brain-sparing.

My initial plan was therefore to assess both central (abdominal and subscapular) and peripheral (limb) fat, together with measurements of the central metabolic organ, the liver. I also considered other measures in the literature, such as the fetal cheek-to-cheek diameter, a surrogate for buccal fat pads. I planned to correlate these fetal measurements with neonatal measurements: body composition, anthropometry, skinfold thickness, and cord blood biomarkers. The latter would be obtained as part of the INTERBIO-21st study, which was to follow the INTERGROWTH-21st project.

I balanced the literature searches with ultrasound sessions, learning the INTERGROWTH-21st scanning protocols and developing my own research protocols. I began with 2D and 3D scans of the more familiar thigh and liver, quickly expanding to include arm and subscapular scans. I finalised my research scanning protocols other than some minor amendments, such as changing the order of the acquisition of the 2D and 3D images to improve image quality.

A feasibility study confirmed my suspicions about the impracticality of cheek measurements, so these were abandoned. It also became evident that I was overly ambitious in aiming to measure liver volumes (a challenge in itself) as well as limb volumes. I continued to collect axial and sagittal abdominal volumes for possible future research, as well as fetal MRI liver sequences, but these did not form part of my thesis. Subscapular images were similarly collected but not measured: further literature review showed this to have been of limited previous use, and I also abandoned the neonatal skinfold thickness (including subscapular) as it was feared that parents would find the technique unacceptable.

Other neonatal aspects of my research also underwent review. The body composition analysis using the PEA POD machine was retained, and I began work with the anthropometry team shortly after my arrival to design a protocol, as well as writing the documents for the ethics application. Oxford's involvement with the MAVIDOS study offered another method of body composition analysis (DXA), and sub-analyses of limb composition. Neonatal anthropometry was retained, although protocol development and training of the team took longer than anticipated. This ensured reproducibility of the measurements and correct techniques, but resulted in a smaller number of cases than originally planned. Finally, the cord blood biomarkers were abandoned. I had considered various candidate biomarkers including leptin, ghrelin and adiponectin, but it became evident that the disparate timing of the INTERBIO-21st study and my DPhil would not allow sufficient samples to be collected.

This thesis thus presents my work assessing fetal arm and leg fat (and lean) areas and volumes, together with neonatal measurements of body composition and anthropometry.

Chapter 1. INTRODUCTION

This chapter outlines the background to my research, exploring developments to date and providing a context for this thesis.

Initially, **sections 1a, 1b, 1c and 1d** discuss ultrasound, both two-dimensional (2D) and three-dimensional (3D), including principles, safety, and their uses in pregnancy.

Sections 1e and 1f explore the use of ultrasound in assessment of fetal growth, both in terms of biometry and in the use of functional measurements: assessment of amniotic fluid volume, and the use of Doppler velocimetry.

Section 1g moves to fetal fat assessment, beginning with the development of fat and evidence for, or against, fetal biometry and fetal body composition. I then explore the work that has been done to date to measure fetal arm and leg, both in terms of simple biometry and then moving to assessment of limb fat, using both 2D and 3D ultrasound techniques.

Section 1h explores the use of magnetic resonance imaging (MRI) in pregnancy, including principles, uses and safety.

Finally, **sections 1i and 1j** consider neonatal assessment: anthropometry and the more complicated field of neonatal body composition analysis.

1a. 2D ultrasound: principles and use in pregnancy

Ultrasound imaging uses high frequency sound waves (3-15MHz) to assess internal structures. The waves, travelling at 1540m/s, reflect off tissue interface(s) where tissues with different acoustic impedances meet; these echoes are then used to generate the image.⁴ The advantages of ultrasound include that it is non-ionising (and is thus safe in pregnancy), provides real-time imaging, involves low costs, and is portable; the disadvantages include imaging artefacts and safety concerns.

Ultrasound has been used in clinical obstetrics since the 1960s, beginning with measurement of the biparietal diameter (BPD) to estimate gestational age.⁵ Abdominal circumference (AC) followed in the 1970s,⁶ and femur length (FL) in the 1980s.⁷ It has become a routine part of pregnancy assessment in most developed countries; two scans are routinely offered in the UK. The first, at 11-13 weeks, confirms fetal viability and number, and screens for structural anomalies suggestive of chromosomal abnormalities such as trisomy 21. The second, at 18-21 weeks, assesses fetal anatomy and placental location.⁸ Women may undergo additional scans for various indications, including threatened miscarriage, multiple pregnancy or suspected fetal growth restriction (FGR).

Most expectant parents find routine ultrasound scans a positive and reassuring experience.⁹ They provide early visual confirmation of pregnancy and contact with their baby, and reassurance about fetal well-being. There is also a trend towards increased attachment following ultrasound.¹⁰ Negative experiences include a lack of pre-scan information about the purpose of the scan, and resulting distress if an abnormality is diagnosed.¹¹ Safety concerns, raised by parents when ultrasound was unfamiliar, are now rarely expressed.^{10,11}

1b. Safety of ultrasound

The safety of ultrasound has been an ongoing concern since its first use as a medical imaging modality. No clinically evident, immediate adverse effects have been reported in humans in pregnancy,¹² but it is well recognised that high levels of ultrasound can produce biological thermal and mechanical effects.¹³ Ultrasound is a form of non-ionising imaging, so lacks the ionisation effects of X-rays and CT (computerised tomography) scans.¹⁴

Thermal effects occur due to tissues absorbing the ultrasound waves and converting the energy into heat; the resulting intracellular hyperthermia could cause teratogenic effects (as demonstrated in animal studies). Mechanical effects are due to cavitation: the interaction between ultrasound waves and small gas bubbles, which may expand, oscillate and/or collapse within a tissue and could theoretically result in cellular damage.⁴

Multiple clinical outcomes have been assessed with respect to ultrasound exposure. A 2009 WHO systematic review did not find any association with adverse maternal or perinatal outcome, including impaired physical or neurological development, increased risk for malignancy in childhood, subnormal intellectual performance, or mental diseases.¹² There appeared to be a weak association between exposure to ultrasound and non-right handedness in boys;¹² this association was evident in both boys and girls in a later meta-analysis.¹⁵

To reduce these risks, thermal and mechanical indices (TI and MI) were developed by the American Institute for Ultrasound Medicine in 1992.^{13,16} These are now standard on all machines and guide sonographers as to the risk of thermal or mechanical damage.⁴ The ALARA (as low as reasonably achievable) principle, used throughout imaging, is also applied to ultrasound. This balances obtaining necessary medical information with the minimal possible settings and examination time, so as to minimise risks to the mother and fetus.¹³ These principles were adhered to in all studies described in this thesis.

International regulatory and advisory bodies have also produced guidelines on safe and appropriate use of ultrasound in pregnancy, including the International Society for Ultrasound in Obstetrics and Gynaecology (ISUOG);¹⁷ World Federation for Ultrasound in Medicine and Biology;¹⁸ European Federation of Societies for Ultrasound in Medicine and Biology;¹⁹ U.S. Food and Drug Administration (FDA);^{20,21} American College of Radiology, American College of Obstetricians and Gynecologists, American Institute for Ultrasound in Medicine, Society of Radiologists in Ultrasound;²¹ British Medical Ultrasound Society;²² and the Australian Society for Ultrasound in Medicine.^{23,24}

1c. 3D ultrasound: principles

Three-dimensional ultrasound was first developed in the 1980s,²⁵⁻²⁹ and entered clinical practice in the 1990s.^{28,30} It uses ultrasound technology to assess fetal structures in three-dimensional volumes. The three fundamental steps of 3D ultrasound are: image acquisition, image processing / reconstruction, and image display.³¹

There are three main volume acquisition approaches: freehand scanning, mechanical devices integrated into the transducer, and matrix array technology.^{32,33}

Freehand scanning involves moving the transducer across the area of interest in a steady and regular motion whilst multiple 2D images are acquired. This can be aided by position-sensing tracking with acoustic, magnetic field, or articulated arm technology. However instability may result in misregistration of 2D images and the generation of an inaccurate volume.³⁴

Mechanical devices integrated into the transducer (“volume probes”) have become the most commonly-used volume acquisition technology. Tilt-scanning is frequently used, whereby the probe is held still while the internal transducer is tilted about the probe’s face at predefined angular separations – this results in a fan-like volume acquisition. Linear and rotational scanning mechanisms are also available.^{33,34} In each case, multiple 2D images are processed to create a single 3D volume. [Figure 1]

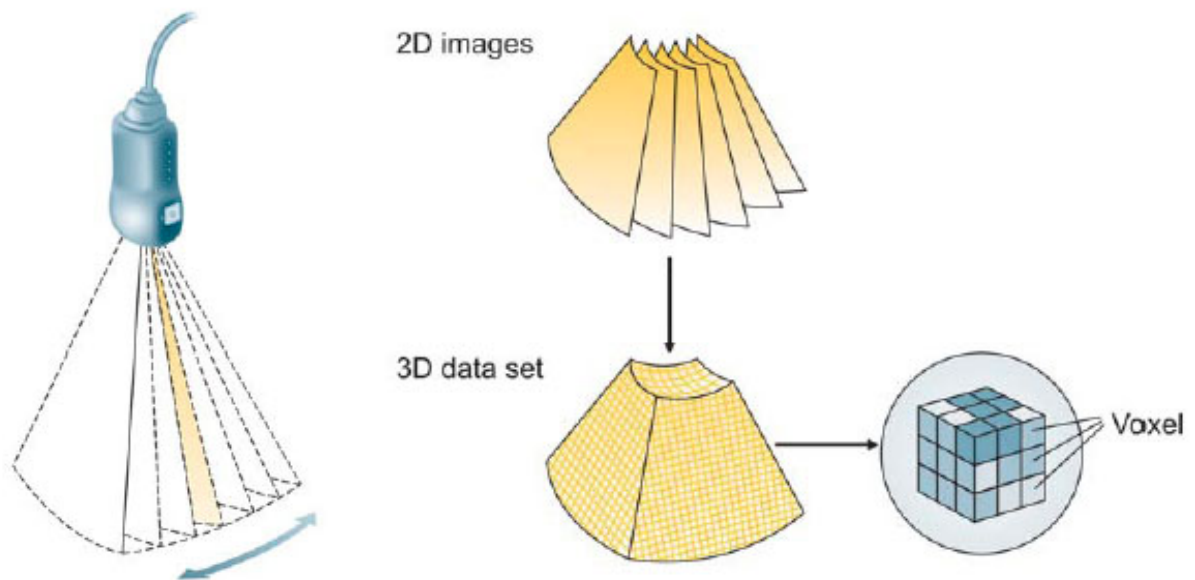


Figure 1: 3D volume probe showing tilt-scanning acquisition, and the combination of multiple 2D slices to form a 3D volume dataset (from Baba 2010³⁵).

Matrix array transducers contain multiple detectors: a square-faced transducer generates a pyramidal pulse of ultrasound and then processes the echoes to generate 3D information in real time. This also allows real-time assessment of two planes simultaneously.³⁴

The 3D ultrasound information is processed and stored as a 'block' known as a voxel ("volume pixel"; pixel, the 2D equivalent, is an abbreviation of "picture element"), which is the smallest definable unit of a 3D image. Each voxel has three planes: x, y, and z. [Figure 2]

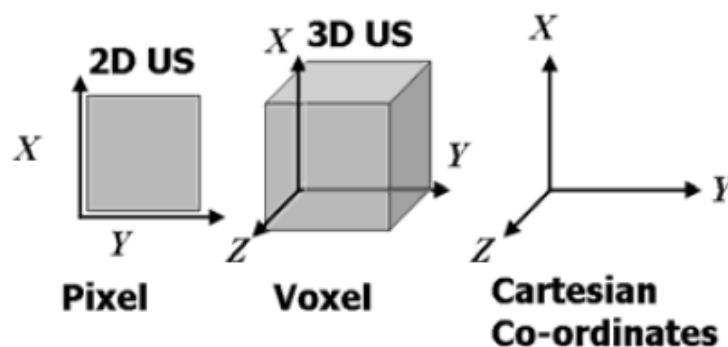


Figure 2: Image processing in three-dimensional ultrasound. A pixel is the smallest definable unit of the two-dimensional (2D) image and defines the location in a 2D plane. A voxel is the counterpart of a pixel in three-dimensional (3D) imaging and defines the location in 3D space. X, Y, and Z represent the orthogonal planes of Cartesian coordinates for spatial location of a voxel. (from Zalud 2006³¹).

The data also undergoes scan-conversion with interpolation to “fill in” the spaces within the scan volume. Speckle-reduction and/or smoothing algorithms may also be applied to the volume to improve clarity.²⁹

After computer processing, 3D volumes can then be displayed on 2D screens, either using multiplanar reconstruction / reformatting (MPR) or surface rendering.

Multiplanar reconstruction involves three orthogonal planes: axial, sagittal and coronal. Simultaneous display of the three planes provides better understanding of the position and orientation of each plane within the 3D volume. The volume can be explored by scrolling through parallel sections in any of the three views, and by rotating the volume to obtain an optimal view of the structures of interest.³⁶

The planes are typically called A, B, and C when displayed on-screen. The A plane is the image acquisition plane, and by convention is displayed in the upper left corner of a multiplanar reference image. The B plane is perpendicular to A, but still parallel to the ultrasound beam (reconstructed “vertical” plane), and is in the upper right corner of a multiplanar image. The C plane is perpendicular to both A and B (reconstructed “horizontal” plane), and is in the lower left corner of a multiplanar image³⁷ [Figure 3].

The A plane has the best resolution of the three planes, while the B plane has slightly degraded resolution (this is evident in image analysis although not necessarily obvious to the naked eye). The C plane is perpendicular to the original beam axis and is heavily interpolated from slice thickness information, so has noticeably degraded resolution compared to the other two planes³⁸ [Figure 3].

Surface (and similar skeletal) rendering uses algorithms to extract the boundaries of the desired structure / area of interest from the 2D images. Various techniques are then applied, including applying shadows and illumination, transparency, depth shading, texture mapping and image smoothing. The resulting 3D surface model of the anatomy – for example, the fetal face or vertebrae – is then displayed on the 2D screen.^{29,33,34,39}

The multiplanar display can also be shown simultaneously with a rendered image, allowing correlation of the planar image with the 3D image [Figure 3]

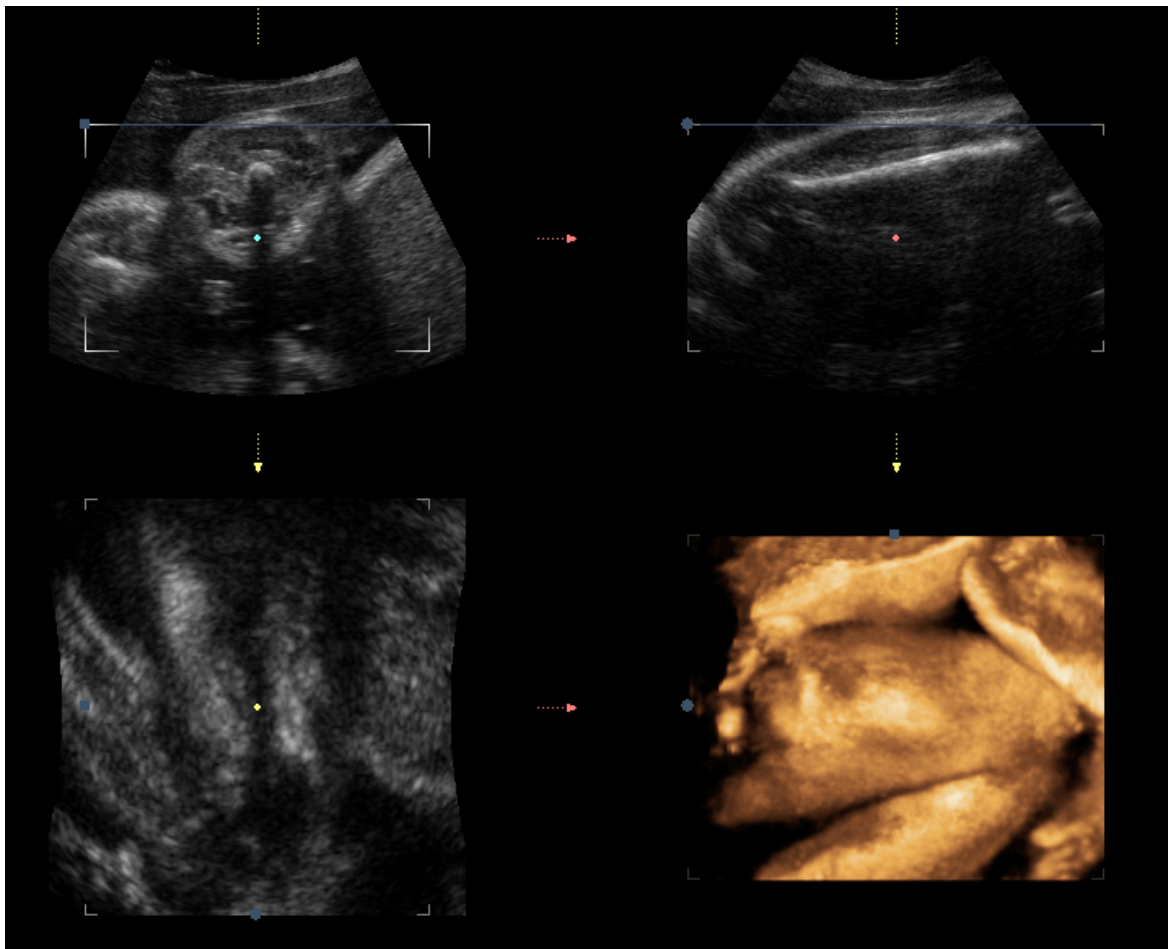


Figure 3: Multiplanar and surface rendered views of a 3D fetal thigh volume at 35 weeks' gestational age. In this volume, the A plane (top left) is axial, B plane (top right) is sagittal, C plane (bottom left) is coronal - note the decreased resolution of the latter. The surface rendered view, showing the flexed fetal leg, is bottom right.

1d. 3D ultrasound: use in pregnancy

Three-dimensional ultrasound has become popular in private practice settings; patients enjoy obtaining “photography-like” images of their fetus, particularly of the face, and to see live 3D moving images (“4D” scanning), including facial expressions⁴⁰ [Figure 4]. Studies suggest that the increased visibility and recognition of fetal structures by the parents at 3D scans result in increased bonding,⁴¹ with stronger and more positive maternal reactions in terms of excitement, amazement, satisfaction and feeling closer to the baby, when compared with 2D scans.⁴²

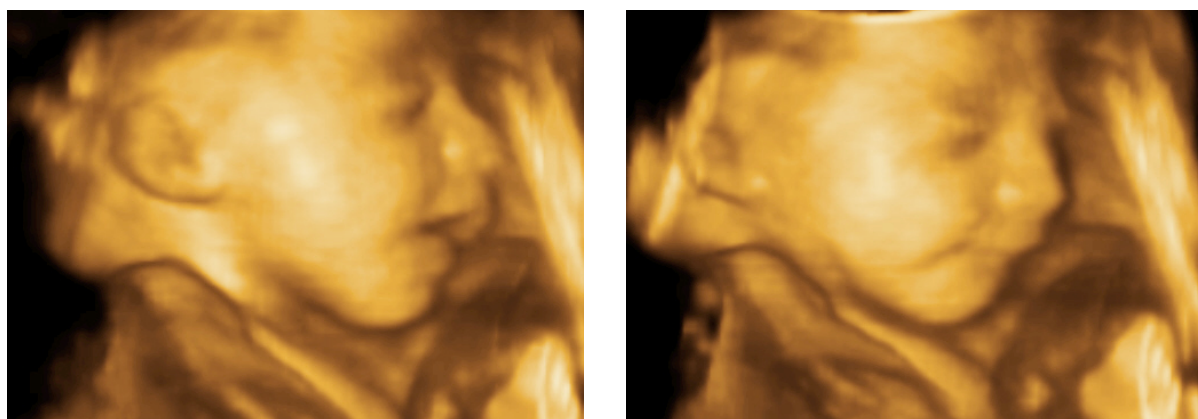


Figure 4: Facial expressions - 3D scan of an INTERGROWTH-21st fetus at 26 weeks of gestation.

In addition to these patient-centred benefits, there are also imaging/technological, clinical and research benefits to the use of 3D ultrasound.

Structures can be assessed in different planes to the acquisition / standard planes, and volumes can be rotated to assess structures from different angles.^{34,40,43,44} Volumes can also be manipulated to ensure ‘perfect’ 2D planes for optimally accurate measurements or visualisation of structures.

Different characteristics of structures can be assessed from a single volume – for example, surface rendering can demonstrate the surface of a meningocele (neural tube defect), whilst skeletal rendering can aid assessment of the underlying vertebral defect.⁴⁰ The use of 3D ultrasound can also provide additional diagnostic information for other abnormalities, such as limb extremity malformations or spinal abnormalities, with ‘transparent’ imaging allowing clear visualisation of the skeleton.^{40,43-45} Surface rendered views are particularly useful for facial clefts, as well as micrognathia, Trisomy 21 facies, and some cases of cleft palate.^{34,39,40,43,45} These three-dimensional views can also help in parents’ understanding of conditions, and preparation prior to the delivery of their baby.^{46,47}

Research using 3D ultrasound has produced volumetric measurements of many fetal organs and structures, including those of irregular shape: lungs, heart, liver, limb, placenta, and whole embryo or gestational sac volumes have been assessed.^{28,40,46,48} 3D ultrasound technology combined with power colour Doppler has allowed spatial investigation of vascular anatomy and pathology, for example, in fetal cardiac views, or pulmonary or placental vasculature.^{47,48} However, large studies comparing 2D and 3D ultrasound for the diagnosis of congenital anomalies assessed in routine anomaly scans have proved inconclusive in terms of the benefits of 3D ultrasound.^{40,43} As with 2D ultrasound, detection rates depend on the type of anomaly, gestational age, and the skill of the sonographer.^{39,43}

Volumes can be stored and assessed or revisited after the scan has been completed, whereas 2D ultrasound scans are stored as 2D images or brief cine video files. Offline assessment of volumes thus provides educational and training benefits as well as allowing for second opinions in challenging cases (whether in person, or via electronic transmission of volume data to distant sites).^{34,40,43,44,49}

1e. Assessment of fetal growth (including growth restriction) using ultrasound

The standard 2D ultrasound biometry measurements described in section 1a – BPD, head circumference (HC), AC and FL [Figure 5] – can be combined using various equations to estimate fetal weight (EFW).⁵⁰

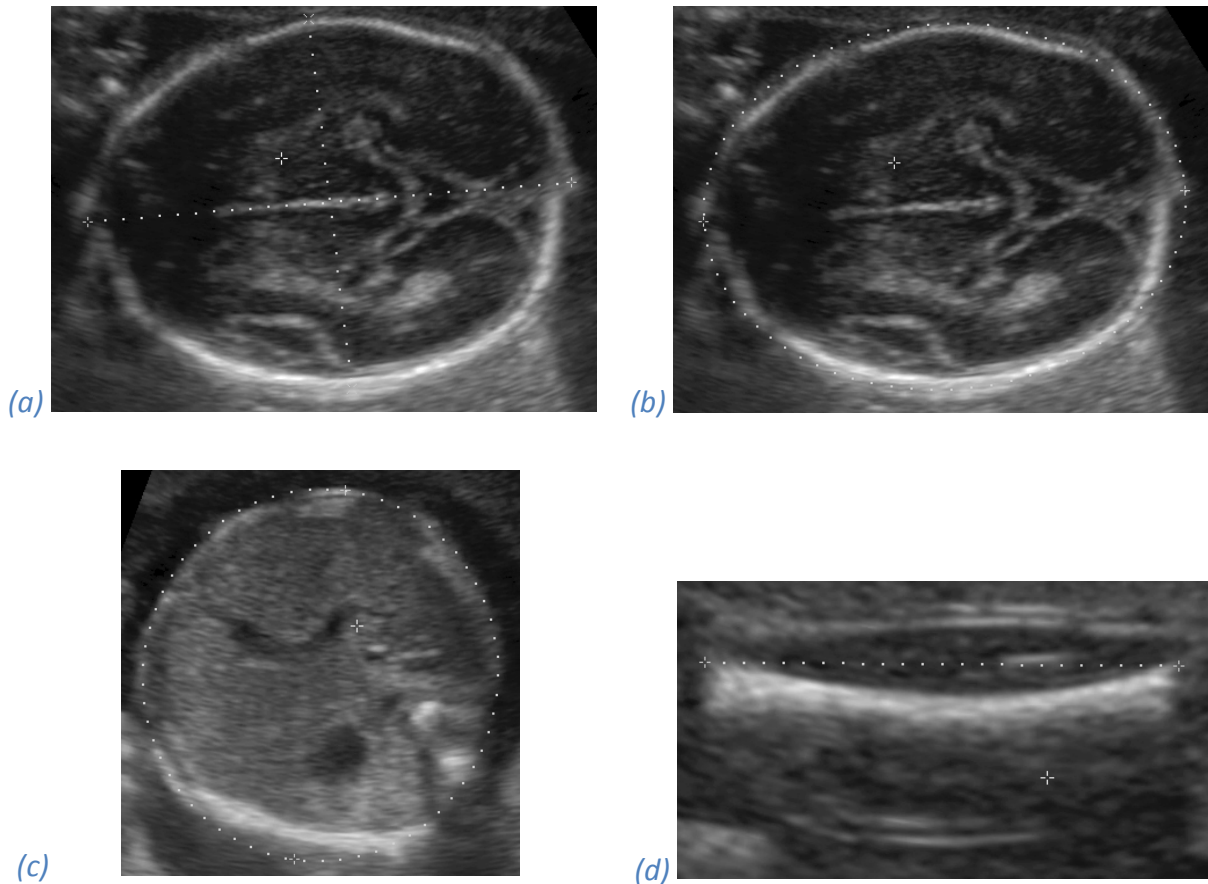


Figure 5: 2D ultrasound biometry from INTERGROWTH-21st scans, showing (a) BPD (vertical line) at 22⁺4 weeks of gestation, (b) HC on the same image, (c) AC at 20⁺3 weeks and (d) FL at 22⁺1 weeks.

EFW and/or AC centiles enable identification of small (<10th) or large (>90th) for gestational age (SGA, LGA) fetuses [Figure 6].⁵¹ The AC is often used as a surrogate marker for nutritional status as it is a soft tissue, rather than skeletal, measurement. The liver – the major metabolic organ of the body – occupies most of the AC plane, and the subcutaneous fat layer is also included in the measurement.

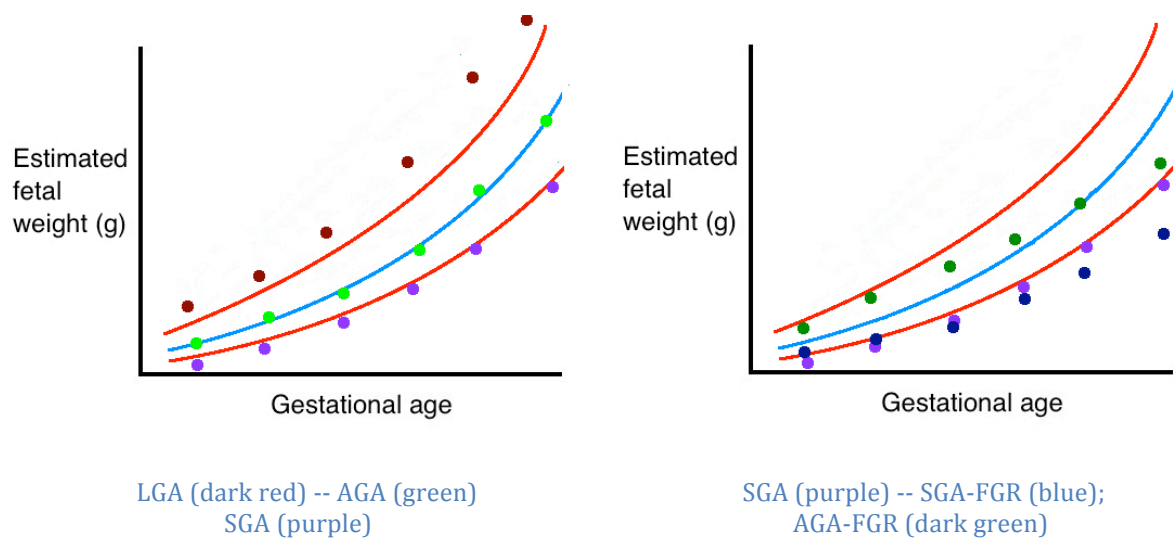


Figure 6: Illustrative examples of EFW from serial 2D ultrasound scans, showing different growth patterns.

Assuming correct gestational age assessment, SGA babies may be small due to constitutional factors, growth restriction (many with placental insufficiency) or congenital abnormalities.⁵² Antenatal screening programmes detect many major congenital anomalies, leaving two hard-to-distinguish subgroups: 50-70% are constitutionally small⁵³ and the remainder pathologically growth-restricted.

Compared with appropriate size for gestational age (AGA) babies, SGA babies have higher rates of stillbirth, seizures, Apgar scores <4, cord pH ≤ 7.0 , admission to neonatal intensive care unit (NICU), hypothermia, and hypoglycaemia.⁵⁴⁻⁵⁷ On closer assessment, however, it is the FGR babies within this group that seem to have the highest risks of complications,^{58,59} whilst labour and delivery outcomes in constitutionally small babies appear similar to AGA babies.^{52,60}

Longer-term risks for low birthweight babies may include increased hypertension, stroke, coronary heart disease and/or diabetes in adult life (effects described in the Developmental Origins of Health and Disease (DOHaD) or Barker Hypothesis).⁶¹



Figure 7: Term baby with undiagnosed FGR, delivered by the author. Photograph at 2 days of age showing little adipose tissue (birthweight 2520g at 41 weeks). [Written parental consent obtained for clinical photograph as per NHS policy.]

These increased risks explain the importance of distinguishing FGR [Figure 7] from constitutionally SGA fetuses: the former may need increased monitoring, early delivery and increased neonatal support; the latter, whilst usually undergoing more monitoring than AGA fetuses, can usually be allowed to await spontaneous labour at term.^{60,62}

Late-onset FGR is a further diagnostic challenge. Whilst early-onset FGR is more severe, more commonly associated with pre-eclampsia, demonstrates abnormal fetoplacental Doppler indices, and has worse perinatal morbidity and mortality, it may only account for as few as 7% of FGR cases. Of these, >70% may be detected. Meanwhile, late-onset FGR (up to 93% of cases) may only be detected in 32%.⁶³ Even when diagnosed, monitoring late-onset FGR is challenging, as functional assessment tools – such as amniotic fluid assessment and Doppler velocimetry – are often normal and thus less useful.^{64,65} It also appears that late-onset FGR, being far more common, accounts for greater numbers of stillbirths overall;⁶³ FGR (all types) is found in >50% of stillbirths.⁶⁶

The differences between the two sub-types of FGR are summarised below [Table 1].

Table 1: Early and late onset FGR (adapted from Figueras 2014⁶⁰).

	Early onset FGR	Late onset FGR
Prevalence in pregnancy	1-2%	3-5%
Percentage of all FGR cases	20-30%	70-80%
Clinical problem	Management	Diagnosis
Placental disease	Severe placental insufficiency	Mild placental disease
Umbilical artery Doppler PI	Usually abnormal	Usually normal
Association with pre-eclampsia	High association (up to 50% cases)	Low association (approx. 10% cases)
Hypoxia and cardiovascular (CVS) adaptation	Hypoxia ++ (chronic) Systemic CVS adaptation	Hypoxia +/- Central CVS adaptation
Fetal effects	Immature fetus with higher tolerance to hypoxia → natural history seen eg. worsening UA Dopplers, followed by increasing ductus venosus PI	Mature fetus with lower tolerance to hypoxia → no / very short natural history many have abnormal cerebroplacental ratio (CPR); 25% low MCA PI
Morbidity and mortality	High morbidity High mortality	Lower mortality (but common cause of late stillbirth); poor long-term outcome

It is even more difficult to diagnose AGA babies who remain within 10th-90th centiles for EFW and AC, but have failed to reach their growth potential. At present there are no reliable *in utero* markers to distinguish these babies from those of normal size that have grown appropriately.⁶⁷

1f. Importance of functional assessment of SGA/FGR fetuses

Whilst fetal biometry provides an indication of fetal size, it has long been recognised that other measures, reflecting fetal function and/or physiological adaptation, are needed to help with the identification of FGR babies.

i. Amniotic fluid assessment

The earliest such measurement was the introduction of amniotic fluid volume assessment; this was first introduced in 1980 as part of the biophysical profile, which involves ultrasound-based assessment of fetal movements, breathing, tone and amniotic fluid; and assessment of the fetal heart rate via cardiotocography (CTG) if any of the ultrasound parameters are abnormal. Assessment of amniotic fluid was by measurement of the maximal vertical pocket (MVP), with normal MVP being one pocket of at least 2cm.⁶⁸

In 1981 amniotic fluid volume (AFV) was used to stratify pregnancies referred with SGA: 93% of those with normal AFV (defined as at least one pool of >1cm depth) delivered normal babies, whereas 90% of those with abnormal AFV delivered FGR babies. They also suggested a ten-fold increase in perinatal morbidity in patients with decreased AFV.⁶⁹ Normal MVP is now classified as 2-8cm; oligohydramnios <2cm; and severe oligohydramnios <1cm.⁷⁰

The amniotic fluid index (AFI) was introduced in 1987, and involves measuring the maximum vertical liquor depth in each of four quadrants of the uterus, then adding the four measurements to obtain the AFI⁷¹ [Figure 8]. As a general rule, a normal AFI is 5-25cm; oligohydramnios is <5cm, although there are specific gestational age AFI ranges.⁷²



Figure 8: Amniotic fluid index (AFI) demonstrating measurement of vertical liquor depth in four quadrants of the uterus.

A recent Cochrane review comparing the two methods favoured the single deepest vertical pocket (SDVP) measurement since the use of AFI increased the rates of diagnosis of oligohydramnios, induction of labour, and Caesarean section for fetal distress, without improvement in peripartum outcomes (NICU admission, umbilical artery pH <7.1, presence of meconium, 5 minute Apgar <7, overall Caesarean section rate);⁷³ however, both methods are still in use.

ii. Doppler velocimetry

FGR, particularly early-onset FGR, is associated with Doppler changes suggesting haemodynamic redistribution as a reflection of fetal adaptation to under nutrition / hypoxia; there may be histological and/or biochemical signs of placental disease, and such pregnancies carry an increased risk of developing pre-eclampsia.⁶⁰

Umbilical artery (UA) Doppler assessment [Figure 9], and the differences in waveforms in AGA and SGA fetuses, were first described in 1984.⁷⁴ A 2010 Cochrane review assessing the use of fetal and umbilical Doppler ultrasound in unselected and low-risk pregnancies (>14,000 women) found no benefit to either mother or baby.⁷⁵ However, a similar review in high-risk pregnancies (>10,000 women) in 2013 suggested that the use of Doppler ultrasound reduced the risk of perinatal deaths and resulted in fewer obstetric interventions (induction of labour and caesarean sections).⁷⁶ Unfortunately, whilst umbilical artery Doppler measurements are helpful in monitoring early-onset FGR fetuses, they tend to be normal in late-onset FGR fetuses⁶⁴ and not helpful in identifying those who will have suboptimal perinatal outcomes.⁶⁵

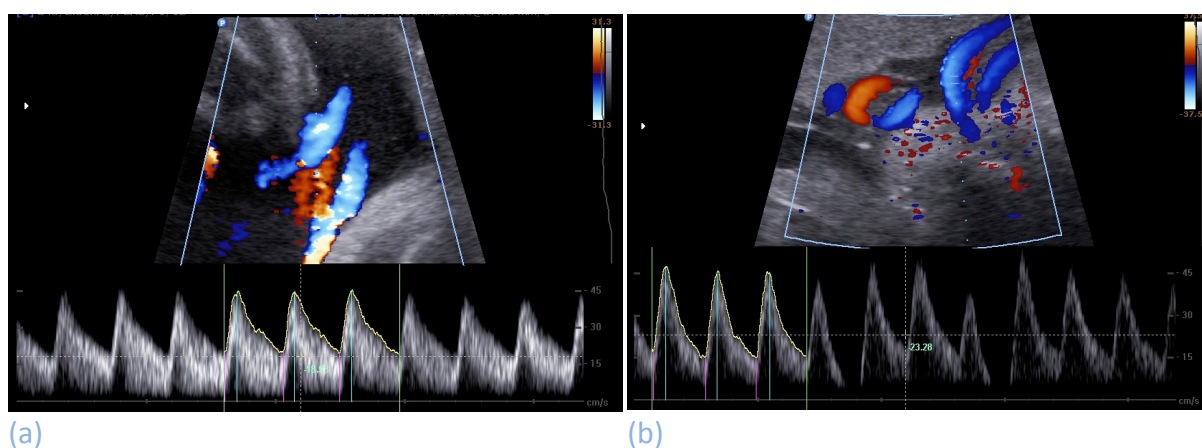


Figure 9: Umbilical artery Doppler assessment in two INTERBIO-21st fetuses at 34 weeks of gestation showing (a) normal waveform and (b) high resistance to flow.

Many other vessels have been assessed with Doppler ultrasound in SGA/FGR babies, including uterine artery (UtA),⁷⁷ thoracic aorta, common carotid artery, middle cerebral artery (MCA), renal artery,⁷⁸ superior and transverse cerebellar arteries,⁷⁹ ductus venosus,⁸⁰ descending aorta, and inferior vena cava.⁸¹ These have been assessed individually and as ratios comparing one vessel with another.

Although Doppler waveforms of many vessels do show changes in SGA/FGR babies compared with AGA babies, the cerebroplacental ratio (CPR) appears to have the best performance in identifying FGR babies, particularly late-onset; and the best correlation with adverse outcomes. The CPR was first proposed by Gramellini et al in 1992,⁸² and is calculated by dividing the MCA pulsatility index (PI) by the UA PI. It thus reflects, in a combined fashion, mild increases in placental resistance with mild reductions in brain vascular resistance.⁶⁰

A group from Barcelona have recently revisited the challenges of diagnosing, classifying and managing FGR. They propose a modified definition, whereby the presence of any one of the following in an SGA fetus (<10th centile for EFW) should result in a diagnosis of FGR:

- (1) CPR below 5th centile
- (2) UtA PI above 95th centile
- (3) EFW below 3rd centile

These criteria are based upon the evidence that supports CPR (above); that UtA >95th centile predicts a poorer outcome in smaller fetuses (and can be abnormal whilst the UA PI is normal); and that SGA fetuses <3rd centile for EFW have a much higher risk of adverse perinatal outcome irrespective of the CPR or UtA PI.⁶⁰

1g. Fetal limb / fat measurement

i. Fetal fat

Assessment of *in utero* nutritional state, rather than EFW, might improve identification of FGR fetuses (whether SGA or AGA) and add to the currently available functional markers.

Fetal fat begins to appear, at a microscopic level, from the beginning of the second trimester (14 weeks); the adipose cells continue to differentiate and proliferate until 24 weeks.⁸³ Fat lobules then develop and increase in size until 29 weeks,⁸⁴ with ongoing transport of maternal fatty acids and increasing fetal lipogenesis and triglyceride storage thereafter (fetal lipogenesis increases and accounts for 80% of fat deposition at term) in both subcutaneous (white) and deep (brown) fat.⁸⁵ Subcutaneous fat represents an energy store that can be mobilised when food availability is limited, and is laid down earlier in fetal life. Brown fat has a highly specialised role in heat generation, ensuring that the newborn can effectively adapt to the cold challenge of the extra-uterine environment.⁸⁶ Overall, however, post-mortem and surgical studies suggest that term infants only have relatively small amounts of brown fat, in abdominal and perirenal sites, with 70-80% of fat being subcutaneous.⁸⁷

Reflective of this development, initially fat accounts for only 0.5% of the body composition, but increases to 3.5% at 28 weeks and reaches 16% or more at full term,^{85,88} In the third trimester, intrauterine growth is accompanied by a large deposition of fat tissue;⁸⁹ this is reflected in the caloric requirements of the fetus, with 50% of caloric accretion being used for the deposition of fat at 27 weeks, and 90% at term (when the total daily caloric requirement is 90-100 kcal/kg).⁹⁰

Maternal glucose is the major fetal substrate for energy production. It is actively transported across the placenta, with increasing transport protein (GLUT) activity in the second half of pregnancy. The glucose stimulates fetal insulin, which then stimulates lipogenesis. In addition, insulin-like growth factor-I (IGF-I) is stimulated, which is the dominant endocrine regulator of fetal growth, particularly in the second half of pregnancy.⁸⁵

A compromised nutritional supply to the fetus will result in multiple adaptations, including metabolic and endocrine systems. A fall of oxygen and glucose delivery below a certain threshold causes gluconeogenesis from hepatic glycogen stores. Further hypoglycaemia leads to corresponding lower levels of insulin and IGF-I with a reduction in fetal growth. Corresponding increasing glucagon, adrenaline and noradrenaline levels result in further, peripheral, gluconeogenesis and inhibition of lipogenesis. There is also down-regulation of leptin-coordinated deposition of fat stores.⁹¹

In addition to the assessment of fetal growth, there have been various attempts to assess fetal fat and nutritional status. Fetal biometry and EFW – the most common surrogates for nutrition – have been compared with neonatal measurements including fat percentage (body composition analysis) and limb circumferences.

Comparing ultrasound EFW with postnatal dual-energy X-ray absorptiometry (DXA) calculated percentage body fat, Schmelzle et al found that 10% of AGA babies had <10% body fat, and that there was overlap of body composition values between SGA and AGA babies, and between AGA and LGA babies [Figure 10]. They concluded that the SGA-AGA-LGA classification did not reflect body composition.⁹²

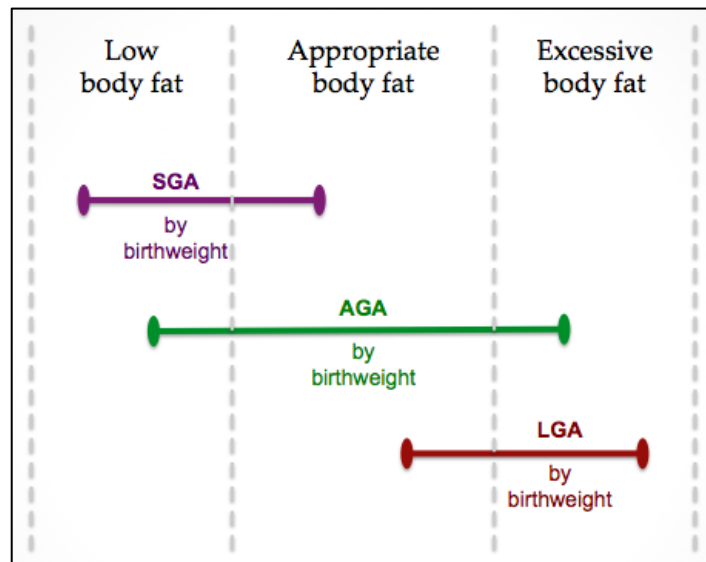


Figure 10: Schematic diagram (not to scale) illustrating the relationship between neonatal body composition and birthweight classification.

Moyeur-Mileur et al compared fetal ultrasound biometry with neonatal anthropometry and air displacement plethysmography (ADP) body composition. Standard fetal biometry was only weakly associated with neonatal body fat. There was, however, a stronger association between neonatal thigh circumference and body fat, suggesting that limb measurements may correlate with body composition (fetal thigh circumference was not measured).⁹³

Deter et al assessed fetuses at risk of FGR and followed them up as neonates. They found that thigh circumference and weight were the two most important predictors of FGR, followed by AC, whereas crown-heel length (CHL) and HC were far less helpful. These findings support the concepts of a decrease in soft tissue mass as the initial step in the development of FGR and the protection of head growth (“brain sparing”).⁹⁴

Ultrasound assessment has shown variation between fetuses in limb fat content with relatively constant lean content;^{95,96} it therefore seems logical to investigate the former as a marker of nutritional status.

Research using ultrasound to assess fetal fat (arm, leg, abdominal, subscapular) began in the 1980s,⁹⁷ as summarised below [Table 2] and outlined in the following sub-sections.

Table 2: Summary of ultrasound assessment of fetal limbs and fetal limb fat.

	ARM: assessment aim / population(s) studied	THIGH: assessment aim / population(s) studied
2D		
Circumferences	EFW calculation ^{98,99}	EFW calculation ⁹⁸⁻¹⁰⁰ Reference ranges ¹⁰¹ Plane identification ¹⁰²
Subcutaneous thickness (fat + muscle)	Correlation with gest'l age ⁹⁷ Diabetes (DM) ^{103,104} Macrosomia ^{105,106} EFW calculation ^{107,108}	Correlation with gest'l age ⁹⁷ FGR ¹⁰⁹ LGA/macrosomia ^{104-106,109} Gestational DM (GDM)/DM ^{110,111}
Fat area	Reference ranges ¹¹² Varying altitudes ^{95,113} FGR ^{114,115} GDM / impaired glucose tolerance (IGT) ^{96,116}	Reference ranges ¹¹² Varying altitudes ^{95,113} FGR ^{114,117} GDM / IGT ^{96,116} EFW calculations ^{107,108}
3D		
Mid-limb plane / circumference	EFW calculation ¹⁰⁷⁻¹⁰⁹	EFW calculation ¹⁰⁷⁻¹⁰⁹ Comparison with neonatal thigh circumference (ThC) ¹¹⁸
Limb volume	Reference ranges ¹¹⁹ EFW calculation ¹²⁰⁻¹²²	Reference ranges ^{123,124} EFW calculation ^{112,121}
Soft tissue volume (limb – bone)	SGA ¹²⁵	-----
Fractional limb volume	Reference ranges ^{3,119} EFW calculation ^{126,127} EFW + body composition ¹²⁸⁻¹³⁰	Reference ranges ³ EFW calculation ^{126,127,131-134} EFW + body composition ^{128-130,135} FGR ¹³⁷ LGA ¹³⁶
VOCAL limb volume	Reference ranges ^{1,2} Accuracy ^{1,2}	Reference ranges ^{137,138} Accuracy ^{1,2}

The arm would seem to be the easier limb to measure as, in axial section, there are only two major muscle groups, and less prominent intra- and intermuscular septae than the thigh [Figure 11]. Fetal atlas images confirmed these findings [Figure 12]. Fetal fat also occupies a larger percentage of limb cross-sectional area in the arm than the thigh [Figure 13].

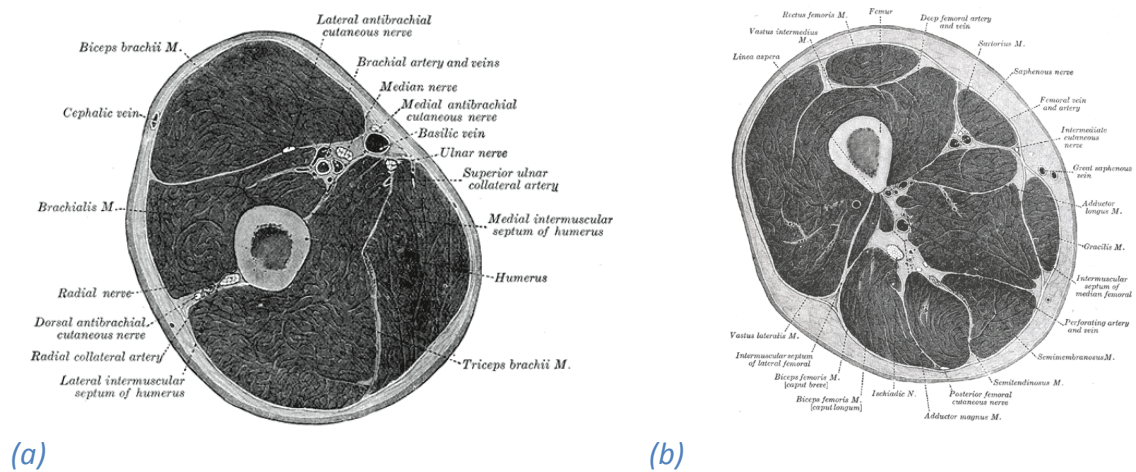


Figure 11: Cross-sections showing adult anatomy of (a) the upper arm and (b) the thigh (from Gray's Anatomy¹³⁹).

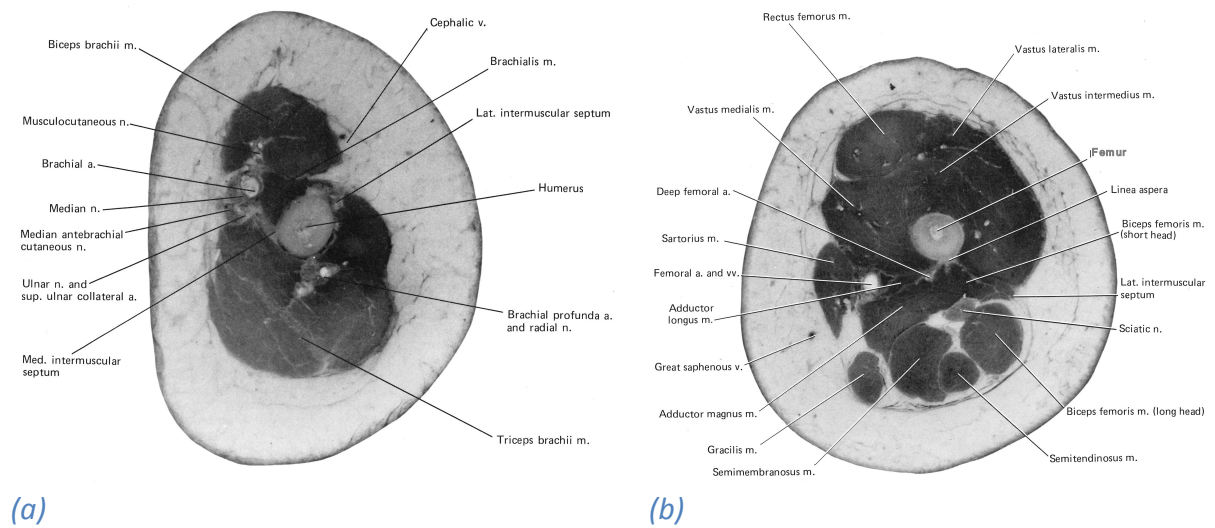


Figure 12: Cross sections showing fetal anatomy at 26-30 weeks (a) arm (middle third) and (b) thigh (mid region): "the disproportionate amount of subcutaneous fat is characteristic of the term fetus" (from Parke 1975¹⁴⁰).

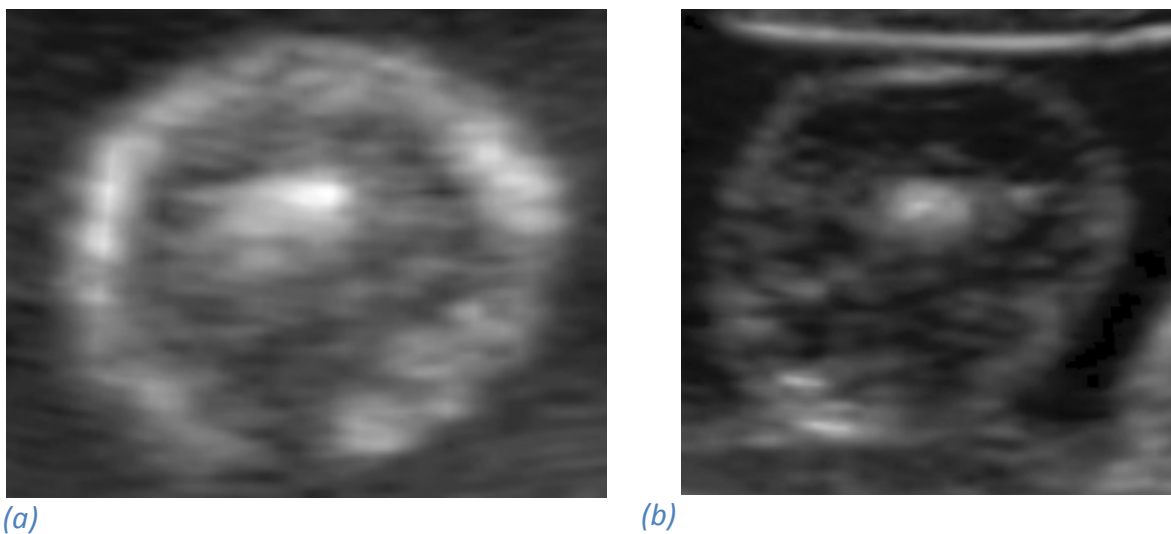


Figure 13: Axial images of (a) arm and (b) thigh from the same INTERGROWTH-21st fetus at 21 weeks, demonstrating a larger percentage of fat in the arm cross-sectional area than in the thigh.

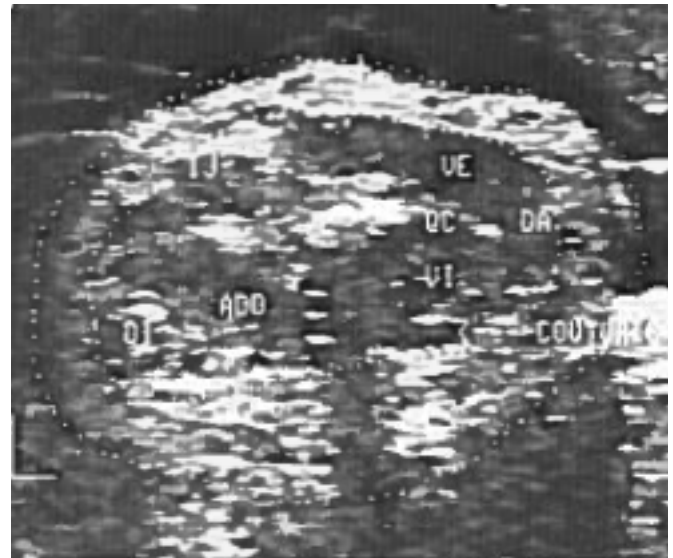
ii. Fetal arm assessment

Initial work used 2D ultrasound to measure arm circumferences, and to measure the entire soft tissue thickness (fat *and* muscle);⁹⁷ these were applied to diabetic women^{103,104} and those at risk of macrosomic babies,^{105,106} and incorporated into EFW calculations.^{107,108}



(a)

Sonographic image of humeral soft tissue measurement at term in pregnancies complicated by gestational diabetes (GDM); LGA fetus.



(b)

Coupe-repère: description anatomique.
The landmark cut, an anatomical description.

Figure 14: Initial assessment of fetal fat in term fetuses (a) fetal humeral soft tissue thickness (from Landon 1991¹⁰³) and (b) the tracing of fetal fat circumferences, as demonstrated on a fetal thigh (arm image not available) (from Balouet 1992⁹⁸). These images also demonstrate ultrasound image quality >20 years ago.

Bernstein et al were the first to measure the 2D areas of fetal arm fat and lean tissue in 1997 to assess fetal tissue growth.¹¹² Balouet et al were actually the first group to free-hand trace the outer skin and inner fat-muscle borders in 1992, using the resulting 'circumference' measurements to estimate fetal weight but not to assess fat or lean areas.^{98,99}

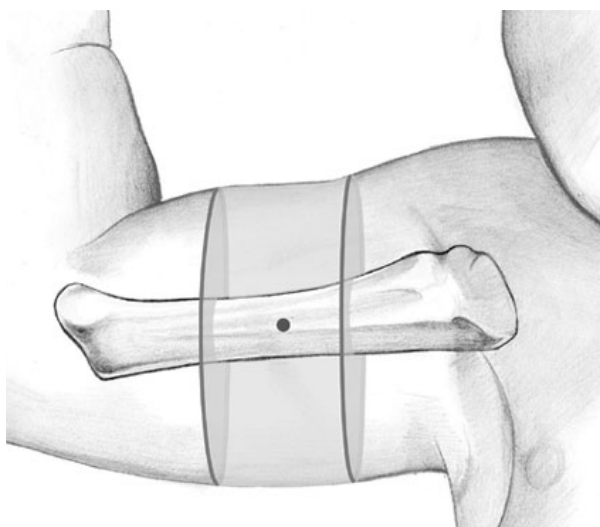
Galan et al compared fetal arm fat and lean areas in populations at sea level and altitude (1600m), finding that babies carried and delivered at higher altitude were of significantly lower birthweight (255g smaller on average) and had a smaller antenatal arm fat area compared with those born at sea level.⁹⁵ However, a later study by Schwartz et al comparing fetuses at 1600m and 3100m showed that, whilst the babies similarly had a lower birthweight at the greater altitude, there was only a non-statistically significant lowering of arm fat.¹¹³

Larciprete et al found a decreased arm fat and lean mass in growth-restricted compared with normally-grown fetuses at 30-31 weeks;¹¹⁴ he also found that gestational diabetics had an increased fetal fat mass compared with non-diabetics at 31-37 weeks.¹¹⁶ Paretti et al found similar results in women with impaired glucose tolerance (IGT) from 25 weeks.⁹⁶ More recently, Liotto et al assessed FGR infants, showing moderate correlation of *in utero* fetal arm fat area on the day of delivery with neonatal biceps and triceps skinfold thickness measurements on the first day of life.¹¹⁵

3D ultrasound was first used in 1993 to assess fetal arm volume, using circumferences, and to identify the mid-point of the humerus.^{107, 141} Liang et al and Schild et al incorporated fetal arm volume into EFW calculations,¹²⁰⁻¹²² and showed no difference between right or left arms.¹²⁰ Chang et al calculated normal ranges for arm volume¹¹⁹ and used arm soft tissue volume (limb volume minus bone volume) to predict SGA fetuses.¹²⁵

Most recently, normal ranges of fetal arm volume have been reported using different methods for 3D volume measurement: XI-VOCAL (eXtended Imaging Virtual Organ Computer-aided AnaLysis) with 5, 10, 15 or 20 computer-generated planes; and multiplanar 5mm slices.^{1,2}

Fractional arm volume (AVol) [Figure 15] measurement is a recent 3D development by Lee et al.¹²⁶ A cylindrical arm volume based on 50% of total humeral length is measured; this includes all tissues (fat, muscle and bone) but avoids measurements at the epiphyses, where definition may be difficult if the external arm outline becomes indistinct.



Fractional arm volume (AVol) is based on 50% of the humeral diaphysis length. Mid-limb measurement eliminates the need for tracing soft tissue borders near the ends of the bone shaft, where acoustic shadowing is more likely to be encountered.

Figure 15: Fractional arm volume (AVol) as described by Lee et al (from Lee 2009³).

AVol appears to be reliable.^{126,142} Lee et al have calculated normal ranges,³ showed significant correlation between AVol and ADP infant body composition,^{128,129} assessed the contribution to variance in percentage body fat,¹³⁰ and used AVol in EFW calculations.¹²⁷

iii. Fetal thigh assessment

Fetal thigh assessment underwent a similar development to that of the fetal arm; methods were often used in both arms and thighs concurrently. However, some studies only assessed thighs, presumably because they were more familiar due to the use of FL in biometry.

As with arm measurements, research in this field began in 1985 with thigh subcutaneous tissue thickness.⁹⁷ In 1991, Bernstein et al found that anterior thigh thickness [Figure 16] did not improve estimation of newborn body composition.¹¹⁰

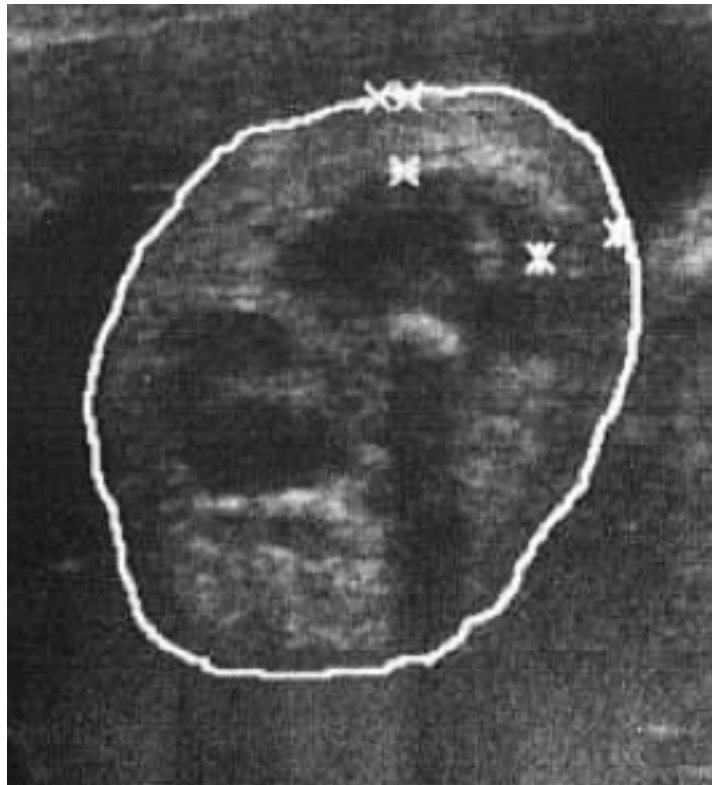


Figure 16: Anterior thigh [fat] thickness as measured at the level of the mid-thigh circumference (from Bernstein 1991¹¹⁰).

In 1992, Hill et al assessed normal, FGR and LGA fetuses, finding that mid-thigh sagittal subcutaneous tissue thickness was not sensitive enough to distinguish between them.¹⁰⁹

Research focus switched to thigh circumference (ThC). Warda et al showed that measurements within 1-2cm of the junction of the middle and upper thirds of the thigh were similar, thus allowing for slight variations in imaging planes.¹⁰² There are no key anatomical landmarks to ensure precise planes as there are in, for example, the AC or HC planes, so this was a useful study. Normal growth curves for ThC¹⁰¹ and incorporation of ThC into EFW calculations¹⁰⁰ followed.

Thigh fat depth was revisited by Kehl et al in 1996, when they showed that LGA fetuses of GDM mothers had a greater growth velocity in this measurement compared with AGA fetuses of diabetic mothers.¹¹¹ Chauhan et al found it unhelpful in diagnosing macrosomia¹⁰⁶ but Garabedian et al found a significant difference in this measurement at 34 weeks between LGA and non-LGA neonates in diabetic pregnancies.¹⁰⁴

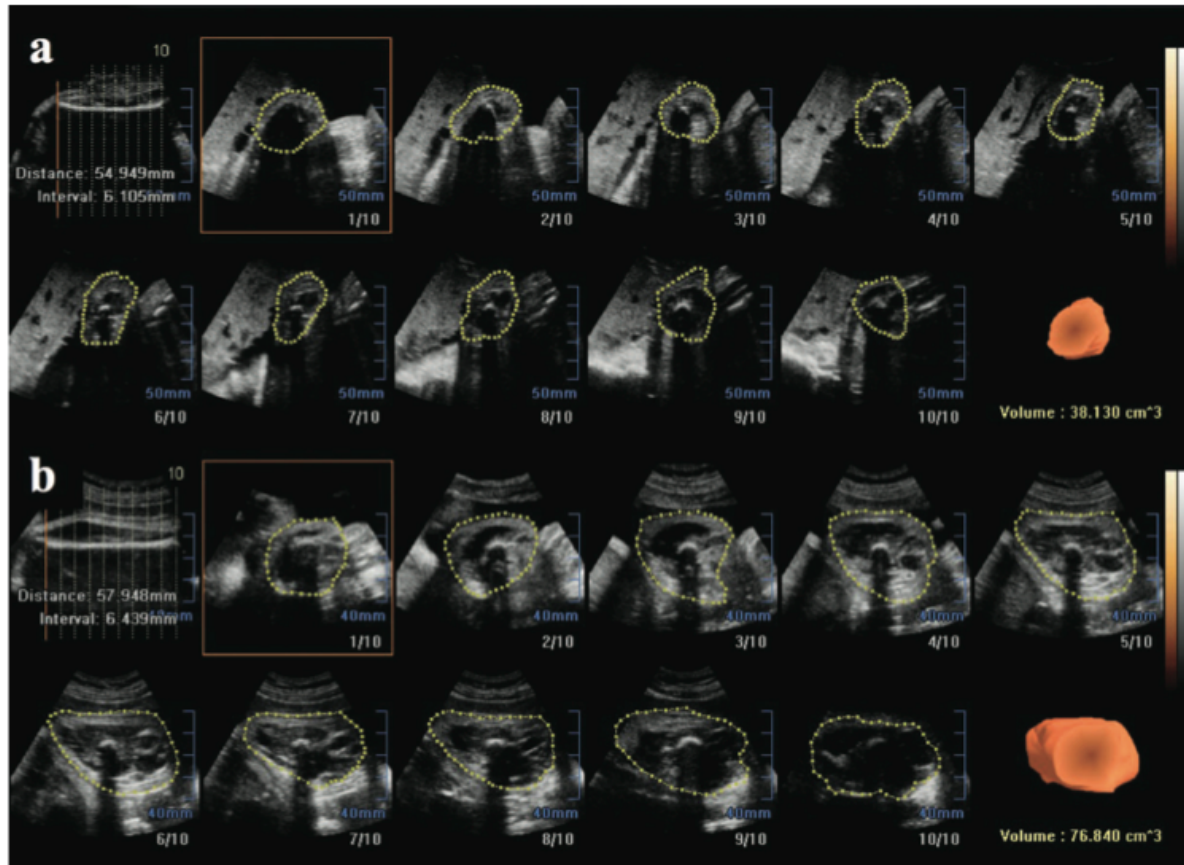
Balouet et al (1992) were the first to outline thigh fat in axial section (for EFW),^{98,99} and Bernstein et al (1997) to measure fetal thigh fat area.¹¹² Many groups who assessed fetal arm fat also assessed thigh fat, showing smaller babies with decreased antenatal thigh fat area at higher altitude (1600m) compared with sea level;⁹⁵ and smaller babies with a non-significant decrease in fetal thigh fat area at increasing altitudes (1600m compared with 3100m);¹¹³ greater thigh fat in fetuses of gestational diabetics at 37-40 weeks of gestation;¹¹⁶ and greater thigh fat in fetuses of women with impaired glucose tolerance.⁹⁶

However, Larciprete found no difference in thigh fat or lean areas in FGR compared with AGA fetuses at 30-31 weeks (whereas there was a difference in the equivalent arm measurements);¹¹⁴ nor did they find that fetal thigh measurements were helpful in estimating fetal weight (whereas fetal arm measurements were incorporated in the EFW calculations).¹⁰⁸ Padoan et al assessed fetal thigh fat area (normalised to femur length) in 17 severe FGR fetuses, comparing them with AGA controls, and found a reduction in fat area with a disproportionate reduction when normalised to femur length. They suggested this could be used to distinguish FGR from constitutionally small fetuses.¹¹⁷

3D ultrasound was first used in 1993 to identify precise mid-thigh perpendicular planes for the measurement of ThC, with incorporation into EFW calculations and an increased estimation of SGA fetuses.^{107,141}

Chang et al calculated thigh volume in 1997 by measuring 3mm slices in fetuses less than 48 hours prior to delivery. They generated normal ranges, showed good correlation between thigh volume and birthweight, and assessed both right and left thighs in fetuses, finding no difference.¹²³ Schild et al measured thigh volume with serial axial slices, measuring an additional slice each time the thigh contour changed. They correlated these thigh volume measurements at 96 hours¹²¹ and at 7 days¹²² prior to delivery with birthweight, and also used them in EFW calculations.¹²² Song et al used a different technique, measuring the fetal thigh area at the proximal, middle and distal diaphysis, and then calculating a thigh volume. This technique required less measurement time and showed good correlation with birthweight, and to a more accurate degree than two-dimensional biometry.¹²⁴

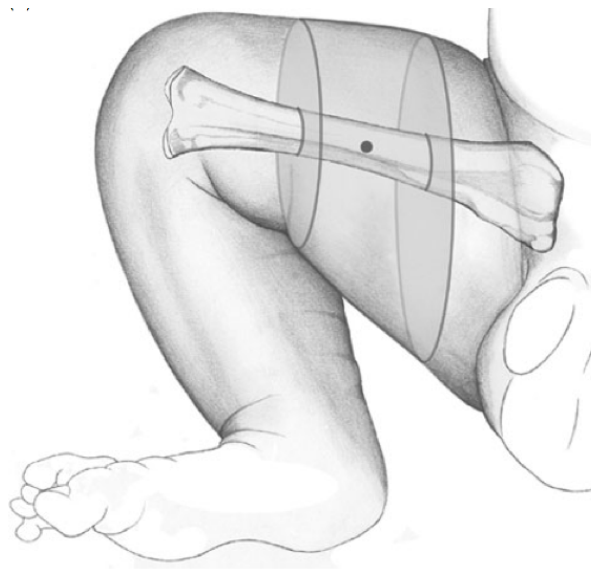
More recently, two groups have compared 3D VOCAL (5 and 10 slices) and 5mm multiplanar techniques, finding no significant differences between the two methods^{1,2,137,138}, [Figure 17].



Fetal upper arm and thigh volume measurements using XI VOCAL method. The initial and last section planes are positioned at the proximal and distal epiphyses, from the upper arm (a) and thigh (b). Afterward, 10 limb areas are delimited in the axial view. Finishing the last area delimitation, the equipment automatically provides a 3D reconstructed image of the limb with its volume.

Figure 17: XI VOCAL approach to arm and thigh volume measurement (from Nardoza 2011^{1,2}).

Lee et al explored the concepts behind fractional thigh volume (TVol) with a pilot study in 1997, assessing a 2cm-cylinder in the mid-thigh region of the fetus^{131,132} and then introduced TVol in 2001, at the same time as AVol.¹²⁶ This uses the same principle of free-hand tracing the outline of an approximately cylindrical thigh volume, based on 50% of total femoral length [Figure 18].



Fractional thigh volume (TVol) is based on 50% of the femoral diaphysis length. Mid-limb measurement eliminates the need for tracing soft tissue borders near the ends of the bone shaft, where acoustic shadowing is more likely to be encountered.

Figure 18: Fractional thigh volume (TVol) as described by Lee et al (from Lee 2009³).

Similar to AVol, TVol shows good reliability and reproducibility.^{3,143} Lee et al calculated normal ranges,³ predicted third trimester TVol from second trimester measurements,¹⁴³ incorporated TVol into EFW equations,^{126,127} and showed that third trimester TVol correlated best with ADP infant body composition, accounting for 46.1% of the variation in neonatal whole body fat percentage.¹²⁸⁻¹³⁰ They also revisited ThC, comparing upper and mid ThC acquired from volumes, and finding that the latter correlated best with neonatal ThC.¹¹⁸

Other groups have used Lee's TVol measurement. Srisantiroi et al used TVol in Thailand in 38 week fetuses to predict EFW¹³³ and Lindell et al assessed EFW in post-dates pregnancies, comparing 2D biometry calculations with two of Lee's EFW calculations using TVol, concluding that they predicted EFW with similar levels of accuracy.¹³⁴ The same group also used the EFW calculations in LGA fetuses in the third trimester, finding that the methods using 3D measurements resulted in a greater detection of macrosomic fetuses.¹³⁶ Moore et al found that TVol at 28 weeks correlated with neonatal body fat measured by ADP at 2 weeks of age.¹³⁵

1h. Fetal magnetic resonance imaging

Magnetic resonance imaging (MRI) uses proton nuclei of hydrogen atoms within tissues to produce images. The spin, alignment and relaxation responses of these magnetic-sensitive protons to the varying electromagnetic fields of the scanner release energy which is used to generate the image.^{144,145} Different relaxation times are measured, to generate T1- and T2-weighted images. The latter are the mainstay of fetal MRI as fluid is bright, demonstrating fetal anatomy well.¹⁴⁴ The advantages of MRI are that it does not use ionising radiation, provides excellent soft tissue imaging and anatomical detail, and enables multiplanar image analysis; the disadvantages are that it is expensive, not suitable for patients with magnetic-sensitive metal implants, requires stillness for optimal images, and usually involves a narrow-bore chamber which can exclude large patients and induce claustrophobia.¹⁴⁶⁻¹⁴⁸

i. Use of MRI in pregnancy

Fetal MRI was first described in 1983¹⁴⁹ and has been used clinically since the 1990s and in the UK since 2000. The development of the single-shot rapid acquisition sequence in the 1990s was particularly useful as it enabled the acquisition of a slice in 1 second, overcoming many of the issues due to fetal movement and rendering fetal sedation unnecessary.^{144,148}

The advantages of fetal MRI compared with ultrasound are that good images can be obtained despite maternal obesity, fetal position, increasing gestational age (increasing ossification of the fetal bones can make ultrasound imaging, particularly of the brain, challenging) and/or oligohydramnios.^{148,150} Challenges include artefacts generated by fetal movement and maternal breathing, and imaging and assessment of small fetal structures.¹⁴⁴

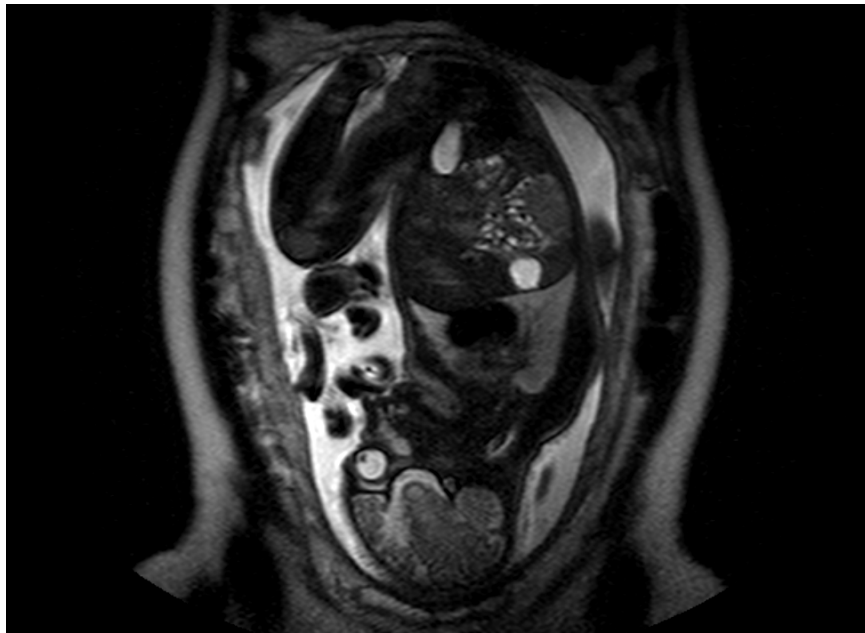


Figure 19: Sagittal fetal MR image at 35 weeks of gestation, demonstrating anatomical detail and clarity.

MRI is a valuable clinical tool for aiding ultrasound diagnosis of fetal abnormalities of the brain and spine, and increasingly of other structures, and is now used for clinical reasons in many units across the world.¹⁵¹⁻¹⁵⁴ It may also be used for maternal imaging in pregnancy.

Fetal neurological conditions in which MRI can provide beneficial information include cerebral ventriculomegaly, agenesis of the corpus callosum, posterior fossa anomalies, vascular brain abnormalities, cortical migrational abnormalities (for example, after fetal CMV or toxoplasmosis infection) and to assess CNS damage in monochorionic twin pregnancies after possible ischaemic episodes (such as the death of one twin, or after laser treatment in twin-to-twin transfusion syndrome). It can also be useful to assess sacrococcygeal teratomas, and to screen for CNS abnormalities in fetuses with neural tube defects.^{144,152,155}

Other fetal conditions in which MRI can be helpful include congenital cystic adenomatoid malformation of the lung, neck tumours which can cause neonatal airway obstruction at birth, some cases of congenital diaphragmatic hernia, and conjoined twins.^{144,152,155}

ii Safety of MRI

Millions of adults and children across the world have had MRI scans since the 1970s and no adverse effects associated with clinical MRI scanning have been demonstrated, including during pregnancy. There have been no reported harmful effects from fetal MRI, which has been used as a clinical tool for over ten years.

No large studies have been carried out to judge specifically whether intrauterine MRI is safe, but epidemiological studies have been carried out to assess possible deleterious effects of MRI on the reproductive health of female MRI technicians.¹⁵⁶ They recorded no significant effects of working with MRI on rates of miscarriage, stillbirth or ectopic pregnancy among the 1915 women responding to the survey. In addition, there was no effect on rates of premature delivery, infertility or low birth weight.

Several studies have also been carried out to try and assess the effects of MRI on human cell development and these have failed to find evidence of abnormalities. Regular exposure of fetal lung fibroblast cells to a 1.5 Tesla (1.5T) scanner over three weeks resulted in no changes in proliferation between imaged and controlled cell cultures.¹⁵⁷ This study was repeated by others using time varied magnetic fields such as those produced by gradient coils and again no difference was found. They also found that there was no evidence of teratogenic effects.¹⁵⁸

Poutamo et al assessed fetal heart rate patterns with CTG patterns of the fetus before and after MRI scan. They found no evidence of stress to the unborn fetus during MRI acquisition, as there was no increase or decrease in either fetal heart rate or fetal movements.¹⁵⁹

Several longitudinal studies have also been done to assess the postnatal effects of prenatal imaging. The largest study, completed by Myers et al, followed 74 women who had undergone imaging during the second and third trimesters of pregnancy and found no effect on imaging on either premature delivery or intrauterine growth restriction.¹⁶⁰ Further studies, including one by Clements et al (2000), found no neurological abnormalities on clinical examination of these infants at nine months of age, and these infants had been imaged four times each *in utero*.¹⁶¹

More invasive studies have been performed on animals, showing that a ten week exposure of rats to a 9.4T static magnetic field has no significant effect on memory, incidence of death, changes in heart rate, body weight, food and water consumption, blood and urine composition, and physical pathology. The conclusion has been that there are no adverse biological effects of exposure in male and female rats, nor in their progeny.¹⁶²

There are thermal effects of radiofrequency for the body as a result of magnetic resonance imaging. This is as a result of the use of radio frequency electromagnetic fields which are used to produce the image. The excess energy can penetrate the superficial surfaces of the body and could possibly be absorbed by the body's tissues. It is dissipated in the form of heat. The fetus is protected by the mother's tissues and amniotic fluid from the potential effects of this heat, and fans and air-conditioning are used to cool women undergoing the scans. The machines also have limits on the energy and absorption rates to ensure there are no adverse thermal effects.

Acoustic noise is another potential side effect of MRI. The risks associated with acoustic noise are a function of both the intensity and duration of exposure. The U.S Food and Drug Administration (FDA) has stated that all magnetic resonance diagnostic devices should not produce noise having unweighted sound peak pressure level higher than 140dB.¹⁶³ This is the maximum allowed for impulse noise. Stricter noise standards have been proposed by UK Medicines and Healthcare Products Regulatory Agency (MHRA)¹⁶⁴ which limit average noise in the MRI scanner to 80dB. In addition, the use of hearing protection devices is mandatory for all patients. There is a theoretical risk that the noise could have an effect on the fetus. To date, there has been no large study assessing this but no research has been published to show that noise in the scanner causes a problem to unborn babies. Many preterm neonates are imaged on a regular basis and there have been no concerns expressed regarding hearing development in that patient population.

In the UK, the National Radiation Protection Board and the MHRA do not recommend MRI in the first trimester due to theoretical risks to the developing fetus.¹⁶⁵ Scans are usually therefore obtained in the second and third trimesters. The American College of Radiology–Society for Pediatric Radiology guidelines for the use of fetal MRI in the USA state that “present data have not conclusively documented any deleterious effects of MR imaging at 1.5T on the developing fetus. Therefore, no special consideration is recommended for any trimester in pregnancy.”¹⁶⁶

1i. Neonatal anthropometry

Even in developed countries the majority of babies have only birthweight as a measure of their nutritional status. Additional indicators include length, other anthropometric measurements, and body composition assessment.

Newborn anthropometry acts as a surrogate marker for weight in developing countries, identifying low and very low birthweight (LBW and VLBW, <2500g and <1500g) babies when scales are not available. Mid-thigh (MTC), mid-calf, mid-upper arm (MUAC), head and chest circumferences have been investigated.¹⁶⁷⁻¹⁷⁷

MUAC <9.5cm had the best sensitivity and specificity in predicting LBW in Cameroon¹⁶⁹ and Tanzania;¹⁷¹ <9cm performed best in an Indian study¹⁷³ and in a Bangladeshi study, where it was found to be a more reliable measurement than chest circumference.¹⁷⁵ MTC <14.5cm had the best sensitivity, specificity and predictive value for identifying LBW babies in Indian studies,^{168,174} and performed well in a study from Bangladesh,¹⁷⁵ whilst <15.5cm provided the best cut-off in a Nigerian population.¹⁷⁶ There have also been studies which have included soft tissue and bony markers – for example, assessing the ratio of MUAC/HC, which has been shown to identify a higher proportion of infants who are symptomatic of FGR.¹⁷⁸⁻¹⁸⁰ A WHO multicentre study showed high correlation of MUAC and chest circumference, as well as calf circumference, with birth weight; however, they suggested using chest circumference as it was the simplest of the measurements to perform.¹⁷⁷

These studies provide biological plausibility for the potential usefulness of arm and thigh measurements as *in utero* markers of body composition.

1j. Neonatal body composition

Body composition assessment produces more information than anthropometry, but requires more complicated equipment and measurements. Methods which have been used in infants include: bioelectrical impedance analysis, dual-energy X-ray absorptiometry (DXA), isotope dilution methods (using labelled water), total body potassium counting, air displacement plethysmography (ADP),¹⁸¹ and quantitative nuclear magnetic resonance imaging (MRI).¹⁸²

DXA and ADP are the most commonly used. Both have good reproducibility and correlation when compared with 4-compartment models (fat mass and 3 fat-free mass components: water, protein and mineral) and animal reference models.^{183,184} DXA and ADP show good reliability and accuracy^{185,186} and positive correlation with other methods: DXA with total body electrical conductivity and total body potassium;¹⁸⁷ ADP with deuterium dilution.¹⁸⁶

DXA is a well-established method of body composition assessment. It measures the degree of attenuation (R) of high and low energy X-ray beams, caused by absorption and scattering of the X-rays as they pass through different tissues (bone, soft tissue). The denser the tissue, the greater the degree of attenuation. The mass of the bone (M_B) can be calculated from this degree of attenuation and a tissue-specific attenuation coefficient (R_B), using the equation $R = M_B(R_B)$.¹⁸⁸ Similar principles are applied to fat and lean tissues.

DXA produces fat, lean, soft tissue and bone mineral data, and is not affected by variations in hydration; however, it involves radiation (albeit equivalent to only 1-10% of a chest X-ray¹⁸⁹) and requires radiography staff. It can also show varying results from different software¹⁹⁰ and machines,¹⁹¹ and can be affected by movement artefact.¹⁹²

ADP uses the same principles as underwater weighing, but in air, which thus makes it a feasible tool for use in infants. Pressure-volume and mass-volume relationships are used to assess whole body density which then enables calculation of fat and fat-free mass using standard densitometric equations.¹⁹³ Boyle's law states that the pressure and volume of a gas have an inverse relationship, when temperature is constant. ADP uses these principles to calculate an unknown volume by directly measuring pressure. The only ADP machine for assessing neonates is the PEA POD infant body composition system (COSMED, Rome, Italy).

The volume of air in the PEA POD test chamber is known, and the volume of air in the test chamber is calculated once the baby is placed inside: small volume changes are generated between the test chamber and a reference chamber, and the resulting pressure changes measured. The difference between the original volume and the new calculated volume generates the baby's volume. Automatic allowances are made to account for the air within the lungs (which is not part of the body volume) and for surface area artefacts (which would otherwise overestimate the air volume in close proximity to the baby's body surface).¹⁹⁴

Density is derived by dividing mass by volume. The mass is provided by measuring the baby's weight on carefully calibrated scales (these form an integral part of the PEA POD machine), and the volume is calculated by ADP, using the PEA POD infant body composition system.¹⁹⁴

Densitometric principles are then used to estimate body composition from body density, under the assumption that the density of any material is the function of the proportion and density of its components, and that such densities are relatively constant and do not vary between individuals.¹⁹⁵ Lean tissue is more dense than fat tissue, so a higher density suggests a higher proportion of lean, and a lower proportion of fat, tissue.

The calculated density is inserted into a standard formula for estimating percentage body fat based on a two compartment model, such as the Siri equation:¹⁹⁴

$$\% \text{ fat} = [(4.95 / \text{density}) - 4.5] \times 100$$

$$\% \text{ fat free} = 100 - \% \text{ fat}$$

The advantages of ADP are that it is quick, safe, non invasive, does not require the subject to lie completely still, does not involve radiation,⁹³ and it is easy to train staff in its use.

Li et al carried out a literature review to assess the research uses of the PEA POD infant body composition system, which can be used from birth until the infant weighs 8kg. They identified six research themes: PEA POD validation, establishing reference body composition data, comparison of body composition between different populations of infants, the effect of infant diet on body composition, the effect of maternal weight / diet on infant body composition, and studies using infant body composition as a reference method. Data from over 2500 infants have been collected with the majority being involved in establishing reference body composition changes and comparison data between different populations.¹⁹⁶

Only two studies have compared antenatal growth evaluation with ultrasound with postnatal infant body composition.^{93,130} Lee et al scanned women up to 4 days prior to delivery, measuring standard 2D fetal biometry, and 2D and 3D limb measurements, and compared these with neonatal body composition within 48 hours of delivery (n=87), assessing the contribution of different parameters to variance in body fat. Their results showed that TVol accounted for 46% of the variance, birthweight 45%, MTC 44%, and AVol 39%; EFW and AC, by comparison, only accounted for 30% and 25% respectively.¹³⁰

Moyer-Mileur et al assessed fetuses at 35 weeks with standard 2D ultrasound biometry and assessed neonatal body composition within 72 hours of birth (n=47). They found that EFW and AC had the highest correlation with neonatal body fat percentage, but only with correlation coefficients of 0.14 and 0.10 (correlation coefficients were not stated in Lee et al's paper). Unlike Lee et al, they did not find that stepwise linear regression modelling identified any fetal biometric parameters as a significant predictor of newborn percentage body fat.⁹³ This may be due to their earlier gestational age of fetal assessment.

There have also been studies using ADP in adults and children. These use the BOD POD machine, in use since the mid-1990s and built by the same company as the PEA POD. This can be used for adults and for children over the age of 2 years. A review by Fields et al showed that the BOD POD has reasonable reliability in measuring body volume and body fat for both adults and children.¹⁹⁴ As well as validation and comparison studies, there have been more than 35 studies on clinical conditions, more than 30 in populations participating in a wide range of sports, and more than 15 in paediatric populations. Clinical conditions in adults include those based directly around nutrition (obesity, eating disorders, cachexia, post-bariatric surgery, food supplements, diabetes, cystic fibrosis) as well as less obvious conditions (degenerative neuromuscular diseases, cardiac and stroke rehabilitation, chronic obstructive pulmonary disease, cancer, orthopaedics, elderly patients). The paediatric studies again include those based around nutrition (children of overweight mothers, children who were breastfed, obese children, those with eating disorders) but also include other clinical conditions (cerebral palsy, cancer, developmental coordination disorders).¹⁹⁷

1k. Discussion

This chapter has provided background information on 2D and 3D ultrasound, and MRI, as a context for this thesis. It has shown the importance of identification of FGR fetuses and their increased morbidity and mortality risks; more accurate identification would allow targeted interventions to fetuses in the greatest need whilst avoiding unnecessary medical interventions in non-FGR fetuses (whether SGA or not). FGR babies may also benefit from healthy lifestyle advice and from screening for cardiovascular and metabolic diseases as adults to enable earlier detection and treatment of any metabolic or cardiovascular diseases.

Fetal fat is laid down during the second and third trimesters.⁸³⁻⁸⁵ Arm and thigh fat measurements are promising *in utero* markers of body composition; 3D fractional limb volumes in particular appear to correlate with neonatal body composition. Work has been carried out by a relatively small number of research groups and only one published study assessed populations in different (two) countries.⁹⁵ The measurement of limb fat (depth or area) has not been incorporated into clinical practice despite more than 25 years of research, probably due to the time-consuming nature of current measurement techniques and the lack of standardised measurements. Studies into the use of arm or thigh fat volume measurements using 3D ultrasound have not been published in the scientific literature.

The neonatal anthropometry studies provide biological plausibility for the potential usefulness of arm and thigh measurements as an *in utero* marker of body composition. Neonatal body composition assessment is a useful adjunct to birthweight, and superior to anthropometry in terms of the information it provides, but requires specialist equipment. ADP might be a useful standard to validate antenatal estimation of body composition.

1I. Contributions to the thesis: chapter 1

Other than where indicated below, and acknowledged in appendix A, all the work in this chapter is that of the author.

The clinical photo of the baby with undiagnosed FGR [Figure 7] was obtained by the author with written parental consent, as per NHS policy.

G.Q. provided the MR image [Figure 19] from the John Radcliffe Hospital, Oxford.

Images taken from journal papers and textbooks are individually cited in the figure captions, by name and year, and with a reference to the full bibliographic details.

Chapter 2. HYPOTHESIS AND AIMS

HYPOTHESIS

Measurement of fetal limb fat can be used to identify fetuses that are nutritionally deprived or growth restricted *in utero*, and distinguish growth restricted fetuses from those that are constitutionally small.

In order to test this hypothesis, I will examine whether fetal limb fat area and/or volume provides a reliable ultrasound marker which reflects fetal nutritional state and, indirectly, fetal body composition. Such a marker is currently lacking in clinical practice. This could be used to identify fetuses with late onset growth restriction, a group that is not diagnosed reliably with current techniques. Distinguishing growth restricted fetuses from small but well nourished fetuses will allow targeted interventions, such as early delivery. In addition, such a marker would enable us to identify a particular sub-group of growth restricted fetuses, who currently escape detection: those whose size is within the normal range of estimated fetal weight (10th-90th percentile), but who are nutritionally deprived, i.e. those who have failed to meet their growth potential. These fetuses may benefit from increased monitoring and possibly modified neonatal management.

AIMS

1. To develop an ultrasound scanning protocol to acquire 2D images and 3D volumes of fetal arms and thighs, which will enable assessment of fat.
2. To develop method(s), suitable for use in clinical practice, to measure fat in these images and volumes.
3. To assess the reproducibility of these methods by measuring systematic and random error from single and multiple assessors.
4. To assess the validity of these methods by comparing and correlating them with MRI images of fetal limb fat.
5. To use these methods in a healthy population to develop reference ranges.
6. To correlate these methods with validated neonatal measurements (whole body fat from body composition, and anthropometry) to assess whether the antenatal methods reflect neonatal body composition accurately.
7. To apply these methods to longitudinal prospective ultrasound images from multiple countries (INTERGROWTH-21st study databank) to assess SGA and FGR fetuses (to see whether we can identify these babies using the fetal limb fat measurement) as well as subtle changes in growth velocity such as fetuses who fail to maintain their growth centile whilst remaining within the 10th-90th centile range.

Chapter 3. METHODS AND MATERIALS: GENERAL PRINCIPLES

In this chapter the populations, ultrasound methods and analytical strategies used in this thesis are described in detail. A number of the sub-studies were based on similar methodology and these are described here; additional study-specific information is provided in individual chapters where appropriate.

3a. Overview of study structure by populations

The work in this thesis recruited women from a number of studies at, or undertaken in coordination with, the University of Oxford, summarised below [Table 3].

Table 3: Summary of study populations used in this thesis.

Thesis chapter(s)	Workstream	Study population
4, 5, 6	Assessment of normal development of limb fat	INTERGROWTH-21 st (Oxford)
4, 6	MRI validation study on limb fat	INTERBIO-21 st (Oxford)
7	Limb fat vs. neonatal body composition (PEAPOD)	INTERGROWTH-21 st (Oxford)
7	Limb fat vs. neonatal body composition (DXA)	MAVIDOS (Oxford)
7	Fat assessment in sagittally-acquired volumes (proof of concept prior to SGA and AGA babies)	INTERBIO-21 st (Thailand)
7	Comparison between SGA and AGA babies	INTERGROWTH-21 st (all sites)
7	Comparison between fetal and neonatal arm fat areas	INTERGROWTH-21 st (Oxford)

3b. Populations: detailed descriptions

The majority of scans used in this thesis were acquired at the Nuffield Department of Obstetrics and Gynaecology (NDOG), John Radcliffe Hospital, Oxford, UK. Some scans from international INTERGROWTH-21st cohorts were also used (chapter 7), acquired at the INTERGROWTH-21st or INTERBIO-21st centres specified below.

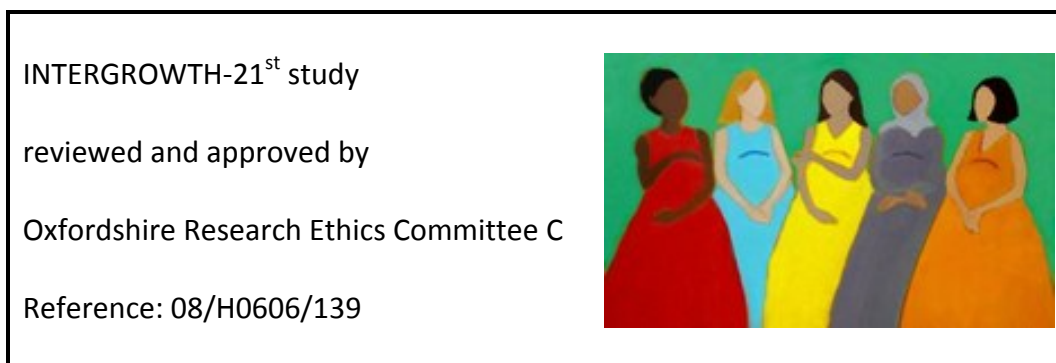
The participants were part of the INTERGROWTH-21st cohort,¹⁹⁸ the INTERBIO-21st cohort,¹⁹⁹ or the MAVIDOS cohort.²⁰⁰ The INTERGROWTH-21st and INTERBIO-21st women were scanned at 5-weekly intervals (+/-1 week), with the first scan at 14-18 weeks of gestation. The MAVIDOS women were scanned twice, at 18-21 weeks and at 34 weeks (+/-1 week). Images and volumes were acquired as per study protocols, outlined below; research images and volumes specific to this thesis were also obtained, as detailed in the relevant chapters.

INTERGROWTH-21st Project¹⁹⁸

The International Fetal and Newborn Growth Consortium for the 21st Century

Women from the Oxford arm of the INTERGROWTH-21st project were included in chapters 4, 5 and 6, whilst women from all sites were included in chapter 7. INTERGROWTH-21st is a large-scale, population-based, multicentre project involving health institutions from eight geographically diverse countries, which aims to assess fetal, newborn and preterm growth under optimal conditions, in a manner similar to that adopted by the WHO Multicentre Growth Reference Study.²⁰¹

The INTERGROWTH-21st Project has three major components, which were designed to create: 1) Longitudinally derived, prescriptive, international, fetal growth standards using both clinical and ultrasound measures (the Fetal Growth Longitudinal Study (FGLS)); 2) Preterm, postnatal growth standards for those infants born $<37^{+0}$ weeks' gestation in the longitudinal cohort, and 3) Birth weight for gestational age standards derived from all newborns delivering at the study sites over an approximately 12 month period.



The eight centres involved in the study are: Universidade Federal de Pelotas, Pelotas, Brazil; Beijing O&G Hospital, Beijing, China; Ketkar Nursing Home, Nagpur, India; Università di Torino, Turin, Italy; Aga Khan University Hospital, Nairobi, Kenya; Ministry of Health, Muscat, Oman; University of Oxford Nuffield Department of O&G, Oxford, UK; and University of Washington Department of O&G, Seattle, USA. These all met the study criteria for a “healthy environment”, serving a population at low risk for impaired fetal growth: low morbidity and perinatal mortality, adequate nutritional status, and with no socio-economic constraints on growth.

In addition, women in this study were all optimally healthy, at an individual level, as determined by detailed screening. The selection characteristics are listed below [Figure 20]:

Recruited women must have the following characteristics at booking:

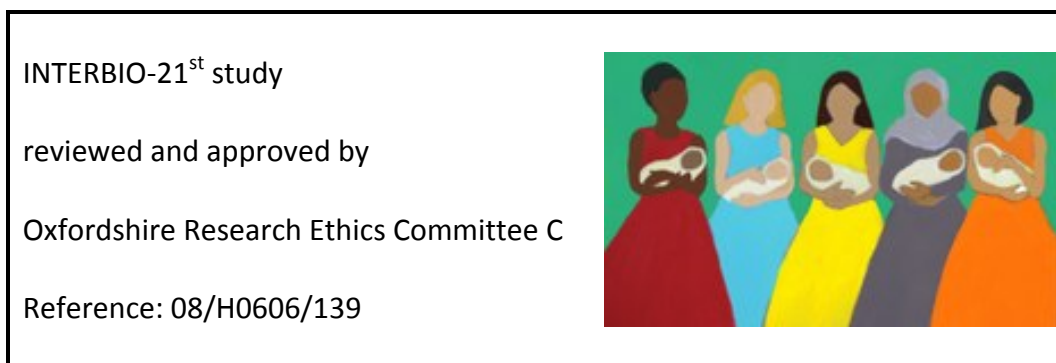
- a) aged ≥ 18 and < 35 years;
- b) BMI ≥ 18.5 and $< 30 \text{ kg/m}^2$;
- c) height $\geq 153 \text{ cm}$;
- d) singleton pregnancy;
- e) a known LMP with regular cycles (defined as 28 days ± 4 days) without hormonal contraceptive use, or breastfeeding in the 2 months before pregnancy;
- f) natural conception
- g) no relevant past medical history (refer to screening form), with no need for long-term medication (including fertility treatment and over-the-counter medicines, but excluding routine iron, folate, calcium, iodine or multivitamin supplements);
- h) no evidence of socio-economic constraints likely to impede fetal growth identified using local definitions of social risk;
- i) no use of tobacco or recreational drugs such as cannabis in the 3 months before or after becoming pregnant;
- j) no heavy alcohol use (defined as > 5 units (50ml pure alcohol) per week) since becoming pregnant;
- k) no more than one miscarriage in the 2 previous consecutive pregnancies;
- l) no previous baby delivered pre-term (< 37 weeks) or with a birth weight $< 2500\text{g}$ or $> 4500\text{g}$;
- m) no previous neonatal or fetal death, previous baby with any congenital malformations, and no evidence in present pregnancy of congenital disease or fetal anomaly;
- n) no previous pregnancy affected by pre-eclampsia/eclampsia, HELLP syndrome or a related pregnancy-associated condition;
- o) no clinically significant atypical red cell alloantibodies;
- p) negative urinalysis;
- q) systolic blood pressure $< 140 \text{ mmHg}$ and diastolic blood pressure $< 90 \text{ mmHg}$;
- r) no diagnosis or treatment for anaemia during this pregnancy (Hb levels will be monitored throughout pregnancy)
- s) no clinical evidence of any other sexually transmitted diseases, including syphilis and clinical Trichomoniasis;
- t) not in an occupation with risk of exposure to chemicals or toxic substances, or very physically demanding activity to be evaluated by local standards. Also women should not be conducting vigorous or contact sports, as well as scuba diving or similar activities

Figure 20: INTERGROWTH-21st recruitment characteristics for study participants.¹⁹⁸

Gestational age was estimated based on the first day of the woman's last menstrual period (LMP), with confirmation by crown-rump length (CRL) at ultrasound scan at 9⁺⁰ to 14⁺⁰ weeks. If the gestational age calculations by LMP and CRL were within 7 days of each other then the women were eligible to participate in the study.

INTERBIO-21st Study (extension to INTERGROWTH-21st)¹⁹⁹
The Functional Classification of Abnormal Fetal and Neonatal Growth Phenotypes

Women from the INTERBIO-21st study were included in chapter 4, 6 (Oxford) and 7 (Thailand). The aims of INTERBIO-21st are to improve the phenotypic characterisation of the intrauterine growth restriction/small for gestational age (SGA) and preterm birth syndromes at molecular, biochemical and clinical levels so as to develop better strategies to correct the short and long-term effects of an adverse intrauterine environment. [Intrauterine growth restriction is referred to as Fetal Growth Restriction (FGR) in this thesis.]



The seven centres involved in the study are: Universidade Federal de Pelotas, Pelotas, Brazil; Aga Khan University Hospital, Nairobi, Kenya; KEMRI/Wellcome Trust, Kilifi, Kenya; Aga Khan University Medical Centre, Karachi, Pakistan; Baragwanath Hospital, Soweto, South Africa; Shoklo Malaria Research Unit, Maesot, Thailand; and University of Oxford Nuffield Department of O&G, Oxford, UK. Brazil, Nairobi and Oxford were all INTERGROWTH-21st centres; the other four centres are in resource-poor settings with high-risk populations.

The INTERBIO-21st study has less restrictive inclusion and exclusion criteria than the INTERGROWTH-21st project as it aims to include women with both optimally and non-optimally healthy pregnancies [Figure 21]:

Women from the entire population attending for antenatal care from <14 weeks' gestation, irrespective of their risk profile for adverse pregnancy/neonatal outcomes, should be recruited for INTERBIO-Bank. However, to participate, women must be at least 18 years old and their pregnancy must have been conceived naturally. Women who have a BMI over 35 must also be excluded from the study as their weight will be a barrier to accurate ultrasound scans. All other women are eligible.

Figure 21: INTERBIO-21st recruitment characteristics for study participants.¹⁹⁹

Gestational age was estimated based on crown-rump length (CRL) at ultrasound scan at <14⁺⁰ weeks. When LMP is available, this was also recorded. This takes into consideration that in a large proportion of very high risk pregnancies the LMP may not be known.¹⁹⁹

MAVIDOS²⁰⁰

Maternal Vitamin D Osteoporosis Study

Women from the Oxford arm of this study were included in chapter 7. This study was a double-blind randomised placebo-controlled trial of vitamin D supplements for pregnant women with low levels of vitamin D in early pregnancy. The aim was to test whether offspring of mothers supplemented with vitamin D in pregnancy have higher bone mass at birth than those whose mothers did not receive vitamin D supplements.

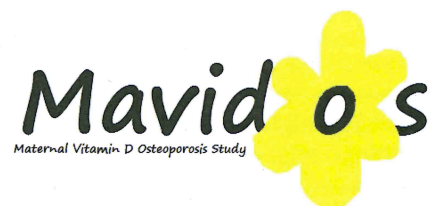
MAVIDOS study

reviewed and approved by

Southampton & South West Hampshire

Research Ethics Committee

Reference: 07/H0502/113



The three centres involved in the study were Southampton, Sheffield, and Oxford.

Women who fulfilled the criteria below [Table 4] were recruited at their first-trimester scan, and a blood sample taken. Those with circulating 25(OH)-vitamin D levels between 25-100 nmol/l were entered into the study and randomised to take an oral vitamin D supplement or matched placebo from 14 weeks.

Table 4: MAVIDOS study criteria.²⁰⁰

Inclusion criteria	Exclusion criteria
• Less than 17 weeks gestation at first assessment (based on last menstrual period (LMP) and dating scan) • Serum 25(OH)-vitamin D concentration is 25-100 nmol/l at nuchal fold/dating scan (10 to 17 weeks gestation) • Aged over 18 years • Singleton pregnancy • Aiming to give birth at local maternity hospital	• Known metabolic bone disease or chronic disease known to be associated with bone abnormalities • Current medication likely to interfere with intrauterine growth (corticosteroids, enzyme-inducing anticonvulsants, PTH, bisphosphonates, more than 6 months GnRH analogues) • Current use of supplement containing vitamin D with daily dose > 400 IU • Foetal physical anomalies on the initial or 18-21 weeks scan (likely to influence bone measurement by DXA or interfere with skeletal development) • Inability to provide informed consent or comply with trial protocol • History of renal stones, hyperparathyroidism, hypercalciuria • A diagnosis of cancer in the last 10 years (other than basal cell carcinoma) • Serum calcium > 2.75 mmol/l

Gestational age was based on LMP and dating scan (from 8 weeks of gestation).

3c. Ultrasound (2D and 3D): image acquisition

All ultrasound scans (whether at Oxford, or at any of the INTERGROWTH-21st or INTERBIO-21st centres) were performed on a Philips HD9 ultrasound machine (Philips, Eindhoven, Netherlands). All volumes were acquired using a Philips volume curved array V7-3 (7-3MHz) transducer. The majority of 2D scans were also acquired with this transducer; however in some cases, for example, due to increased fetal maturity or increased maternal adiposity, a Philips curved array C5-2 (5-2MHz) transducer was used instead. The lower frequency ultrasound wave provided by this transducer, which has better tissue penetration, enabled a more optimal 2D image to be obtained.

In all studies, serial 2D measurements were taken of the Biparietal Diameter (BPD), Occipito Frontal Diameter, Head Circumference (HC), Transverse Abdominal Diameter, Antero-Posterior Abdominal Diameter, Abdominal Circumference (AC) and Femur Length (FL). At least three separate images were acquired, to enable three separate sets of measurements to be taken.

An important aspect of the study was that the HD9 machines were modified for the study so that all measurements were “blind” (i.e. numbers were not shown on the screen at the time of measurement). The images were also scored by the operator²⁰² as a means of quality control. Serial 3D volumes were obtained of the fetal head, abdomen and femur (using standard biometry as the acquisition plane), with at least one of each volume being acquired at each scan. Amniotic fluid volume was assessed and measured using the Amniotic Fluid Index (AFI).

3d. Ultrasound image storage

All images were saved to the HD9 machine and then extracted in several digital formats and saved onto a portable hard drive disk for offline analysis. All images and volumes were anonymised in line with good clinical practice. The file types used were jpeg, .jpg (or bitmap, .bmp) files to enable quick visual assessment of the image or volume; DICOM files to enable technical analysis of the image or volume; and additionally, for the 3D volumes, 3D graphic .3dh and .mvl files to enable manipulation and analysis of the volumes. The DICOM (Digital Imaging and Communications in Medicine) format is the international standard for medical images and related information. It defines the formats for medical images that can be exchanged with the data and quality necessary for clinical use, and is implemented in almost all imaging devices and modalities (ultrasound, MRI, X-ray, CT).

The files were saved in a structured format to allow easy retrieval of scans or volumes. The initial level of organisation was a folder labelled with the date of the scan to enable chronological display of the files. Sub-folders contained each individual patient's scan, labelled with their study ID number and gestational age [Figure 22].

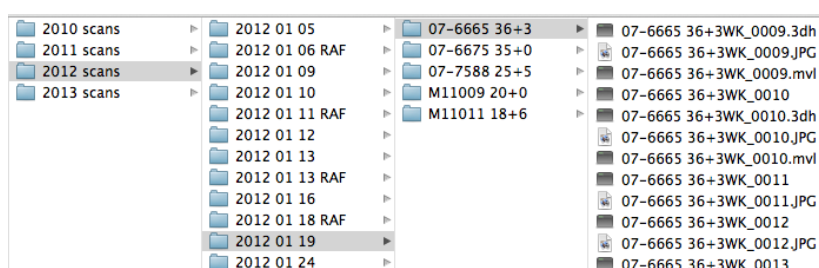


Figure 22: File structure for saving ultrasound scans.

The ultrasound data ultimately comprised >210GB data in >380 date folders. Each contained 1-10 patient scan folders, with 70-100 files. Images and volumes were also copied from the HD9 machine and stored for their respective studies for future reference as needed.

3e. Magnetic Resonance Imaging (MRI): image acquisition and storage

A subset of 50 women within the Oxford cohort for the INTERBIO-21st study underwent fetal MRI imaging for validation of fetal ultrasound images (see Chapter 4). These images were all acquired in the Neuroradiology Department of the John Radcliffe Hospital, Oxford, using a Philips 1.5T Achieva machine (system ID 111001.47; Philips, The Netherlands) with a pelvic phased array coil. Further details are given in Chapter 4. All images were anonymised in line with good clinical practice and saved in DICOM format for offline analysis. Paired MRI and ultrasound images were stored in a databank for future reference.

3f. Ultrasound and MRI image measurement techniques

Images were analysed offline using a MATLAB (MathWorks, Natick, MA, USA) tool designed by collaborating biomedical engineers at the University of Oxford. Additional offline software used included MITK (German Cancer Research Centre, Heidelberg, Germany) for image analysis; RegisterAndConvert (Mark Gooding, University of Oxford, UK) for converting volumes into a format which is readable by the MATLAB tool (Meta Image or .mha file); and the Philips DICOM Viewer PmsDView (Philips, Eindhoven, The Netherlands) for analysis of some 2D DICOM ultrasound images and all MRI images.

A small subset of 2D ultrasound images were analysed using the measurement tools provided in the Philips HD9 ultrasound machine: 2D distance with line trace, and 2D area with ellipse. A small subset of 3D ultrasound thigh volumes were analysed using 4D View imaging analysis software (GE Healthcare, Amersham, UK).

Details of the relevant techniques and tools are provided in individual chapters.

3g. Neonatal anthropometry and body composition analysis

All babies in the INTERGROWTH-21st, INTERBIO-21st and MAVIDOS studies had parental consent for standardised birthweight.

A subset of the babies underwent neonatal body composition analysis either by ADP using the PEA POD Infant Body Composition System within the first 3 days after birth (subset of the Oxford cohorts of the INTERGROWTH-21st and INTERBIO-21st studies); or by DXA using the Lunar iDXA (GE Healthcare, Amersham, UK) or Discovery A DXA (Hologic, Inc., Bedford, MA, USA) within the first 14 days after birth (MAVIDOS).

A subset of babies also had additional anthropometric measurements taken: mid-upper arm circumference, abdominal circumference, and mid-thigh circumference (INTERGROWTH-21st and INTERBIO-21st, within the first 3 days after birth).

Details of the relevant techniques are provided in individual chapters.

3h. Statistical analysis

Statistical analyses were undertaken using Stata IC for Mac (StataCorp LP, College Station, TX, USA) and Microsoft Excel for Mac (Microsoft Corporation, Redmond, WA, USA). Graphs were produced using Microsoft Excel for Mac.

Details of the relevant techniques are provided in individual chapters.

3i. Product specifications

Imaging machines:

Ultrasound – HD9 machine	Philips, Eindhoven, The Netherlands
MRI – 1.5T Achieva machine	Philips, Eindhoven, The Netherlands

Imaging analysis software:

MATLAB	MathWorks, Natick, MA, USA
Philips DICOM Viewer PmsDView R2.6	Philips, Eindhoven, The Netherlands
Philips HD9 measurement tools	Philips, Eindhoven, The Netherlands
RegisterAndConvert v1.2.2	Mark Gooding, University of Oxford, UK
Medical Imaging Interaction Toolkit (MITK)	German Cancer Research Centre, Heidelberg, Germany
itk-SNAP	University of Pennsylvania, PA, USA
4D View	GE Healthcare, Amersham, UK

Neonatal body composition analysis machines:

PEA POD	COSMED, Rome, Italy
Lunar iDXA	GE Healthcare, Amersham, UK
Discovery A DXA	Hologic, Inc., Bedford, MA, USA

Statistical software:

Stata IC 13	StataCorp LP, College Station, TX, USA
Microsoft Excel for Mac 2011	Microsoft Corporation, Redmond, WA, USA

3j. Contributions to the thesis: chapter 3

Other than where indicated below, and acknowledged in appendix A, all the work in this chapter is that of the author.

Images taken from journal papers and textbooks are individually cited in the figure captions, by name and year, and with a reference to the full bibliographic details.

Chapter 4. FETAL ARM FAT ASSESSMENT USING 2D ULTRASOUND: ESTABLISHING NORMALITY

This chapter includes the development of relevant protocols and techniques (including novel approaches); their application to 2D ultrasound imaging to enable measurement of fetal arm fat; and work assessing validation and reproducibility.

Sections 4a and 4b explains the development of the 2D and 3D limb ultrasound protocols, including feasibility and quality studies; and the segmentation techniques used to measure the arm areas, including challenges, the MATLAB tool, and protocol.

Sections 4c and 4d describe measurement of fetal arm fat and lean tissue areas, including scatterplots and centiles describing normal development throughout gestation in an optimally healthy population, the reproducibility of the measurements, and an exploration of longitudinal studies of arm fat areas within individual fetuses.

Section 4e explains a sub-study using MR to validate ultrasound images, including the development of the MRI protocol, acquisition of paired MRI and ultrasound images, validation of the fetal arm ultrasound images, and a comparison of the Philips area measurement tool with the MATLAB area measurement tool developed by the engineers.

Section 4f explores the first of two alternative – and novel – approaches to arm fat measurement: ellipses. This section includes proof-of-concept work, the development of a MATLAB manual ellipse tool, and its application to fetal arm images of varying gestational ages, including longitudinal work in individual fetuses.

Section 4g explores the second novel approach to arm fat measurement: depth, in the form of a simple, three-thickness sampling technique I have named the Fetal Fat Index. This section includes some automated measurement work in collaboration with biomedical engineering, and the development of the manual clinical technique and its application to fetuses of varying gestational ages.

Section 4h lists the publications and presentations arising from the work in this chapter, and **section 4i** lists the contributions to this chapter.

4a. Developing the ultrasound 2D and 3D limb scanning protocols

METHODS

As outlined in chapter 1, basic techniques using 2D imaging have previously been undertaken to measure fetal limb fat; however, no previous studies have used 3D imaging to assess fetal fat; this novel approach is important as fat is distributed along and around limbs in a three-dimensional manner. Several image acquisition methods were therefore attempted, and a protocol produced for arm and thigh fat imaging (see appendix B). This protocol was used to acquire limb images and volumes from subsets of babies within the INTERGROWTH-21st, INTERBIO-21st and MAVIDOS studies at Oxford [see section 3b. for study details, and 3c. for ultrasound equipment details].

Previous work has captured fractional arm and thigh volume (AVol and TVol)¹²⁶ in the sagittal acquisition plane ie. that which is used to acquire FL measurement. Whilst this plane is more familiar, image resolution is always greatest in the original acquisition plane^{34, 38} when compared with the other, reconstructed, planes. As fat measurements were planned in the axial view, a protocol was developed to obtain 3D limb volumes using an axial acquisition plane so as to maximise image quality.

Well-magnified sagittal views of the fetal limb (arm or thigh) were obtained with the 3D V7-3 probe. The harmonic imaging was turned off; this enabled better post-imaging processing. The probe was adjusted until a full-length longitudinal view of the humerus / femur, ideally horizontal, in the centre of the screen was obtained [Figure 23].

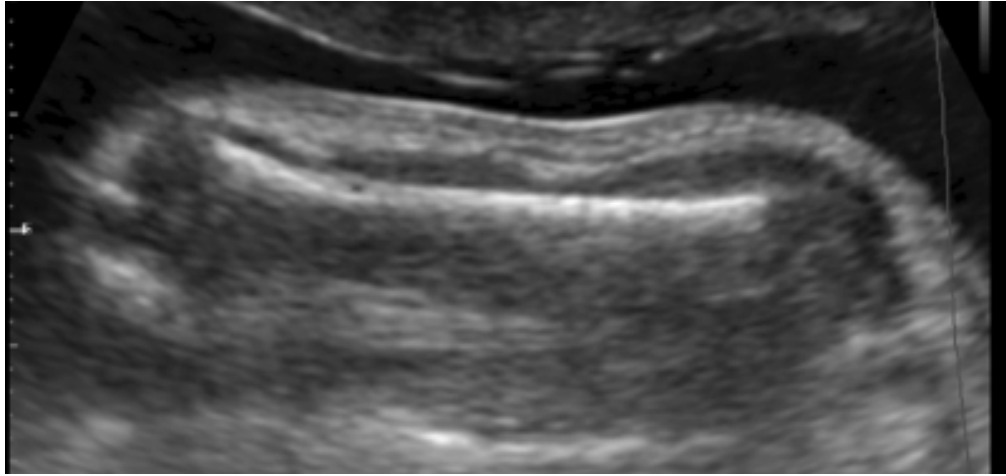
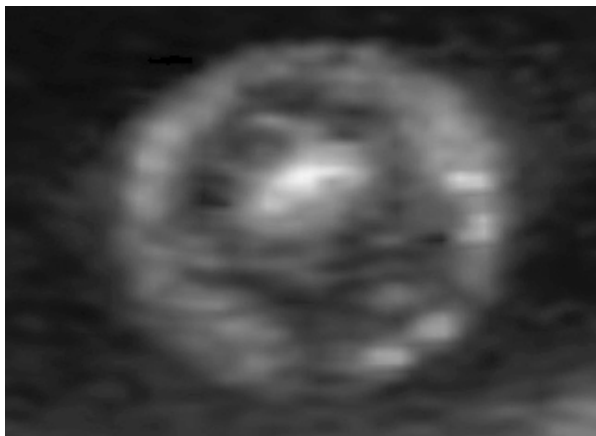
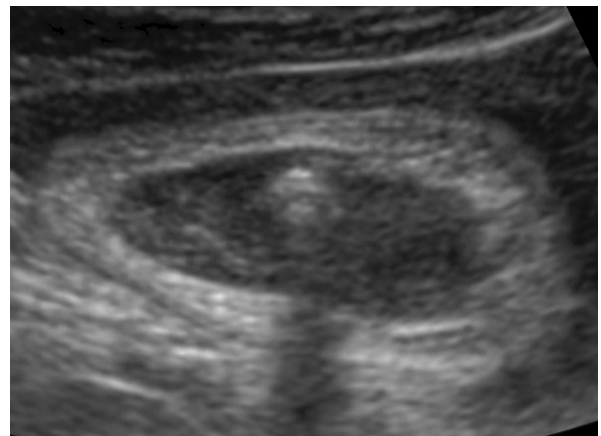


Figure 23: Fetal arm in sagittal section, showing horizontal humerus (29 weeks' gestation).

The probe was rotated through 90° to obtain an axial cross-section of the limb at mid-humeral or mid-femoral level. This has a target-like appearance: a central hyperechoic bone surrounded by hypoechoic muscle and then by an echodense fat layer [Figure 24, Figure 25].



(a)



(b)

Figure 24: Fetal arms, axial mid-humeral view showing (inner to outer) bone, muscle and fat, at (a) 21 weeks and (b) 39 weeks gestation.

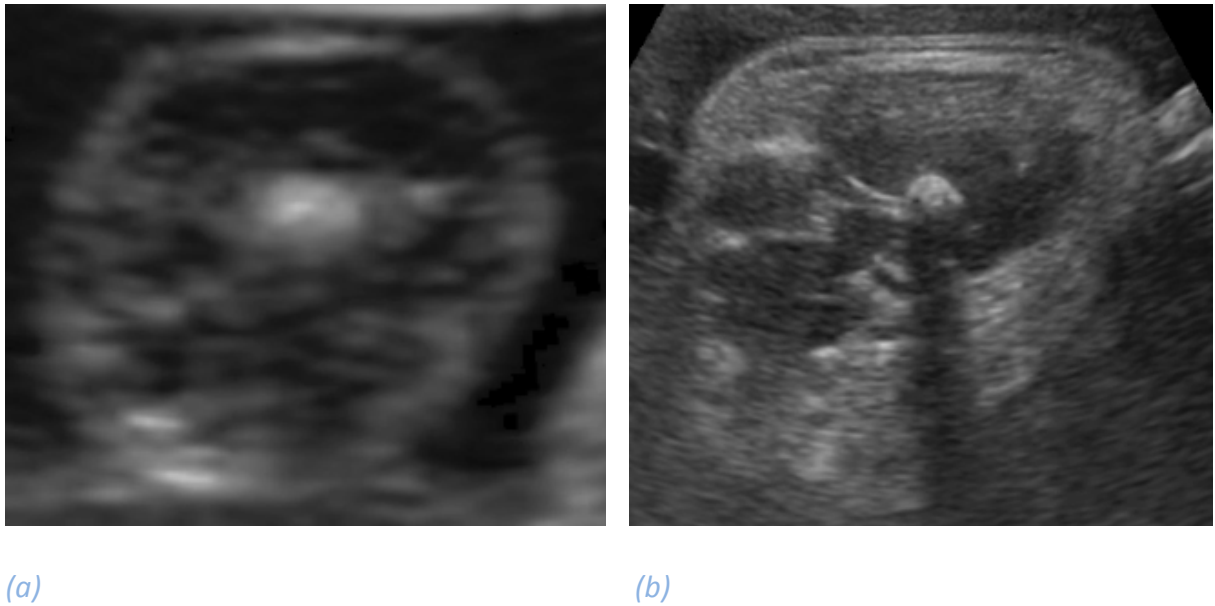


Figure 25: Fetal thighs, axial mid-femoral view showing (inner to outer) bone, muscle and fat, at (a) 21 weeks and (b) 39 weeks gestation.

To ensure that the cross-section of the limb was perpendicular to the bone, the probe was swept along the longitudinal axis of the bone. If the axial view was at right angles to the longitudinal axis then the image of the bone remained in the centre of the screen as the probe was moved. Adjustments were made (for example: rotation, or reacquiring the axial plane from the sagittal view) until this was achieved.

Returning to the mid-humeral / mid-femoral point in the axial view, a 3D 70° sweep was taken of the arm / leg. The sweep speed (normal / fast / fastest) was customised to fetal activity. The 3D reference image was checked to ensure there was no movement artefact, and that the B plane image showed a recreated sagittal humeral / femoral view with the complete length of the bone visible, and ideally horizontal. This was repeated to obtain two good-quality volumes [Figure 26, Figure 27].

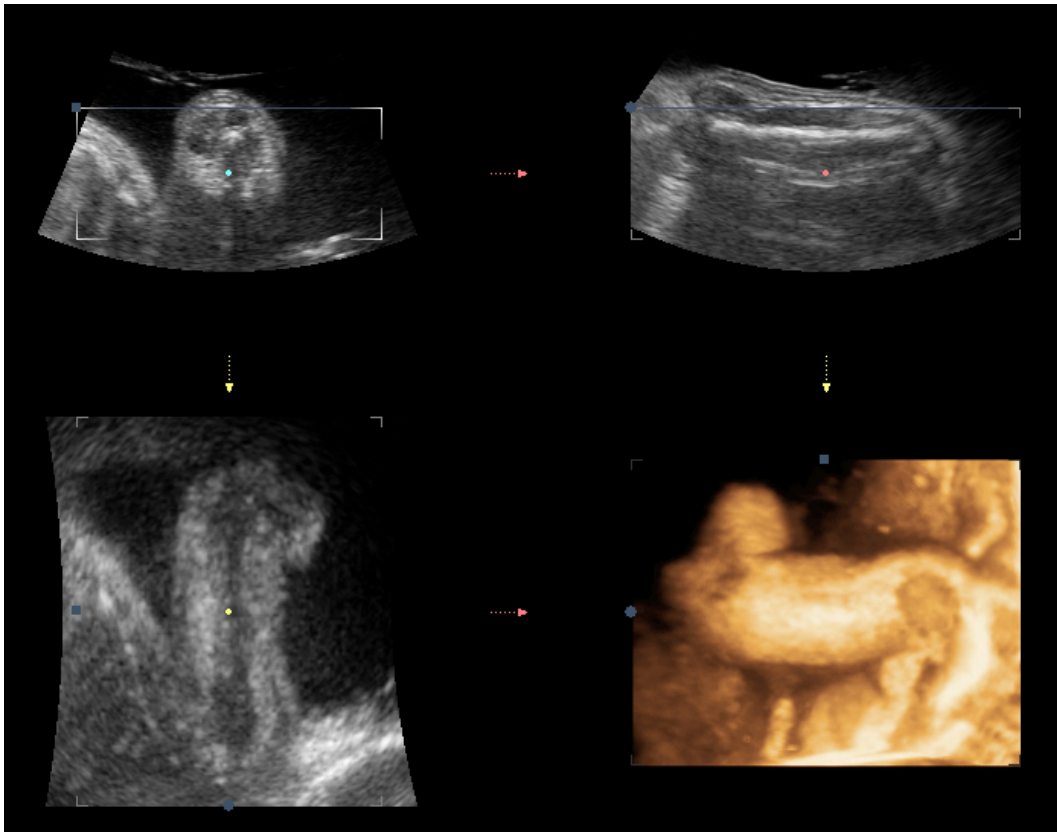


Figure 26: 3D image capture of fetal arm (34 weeks gestation). Note the longitudinal B-plane view of the humerus (top right), showing horizontal orientation and the whole bone length.

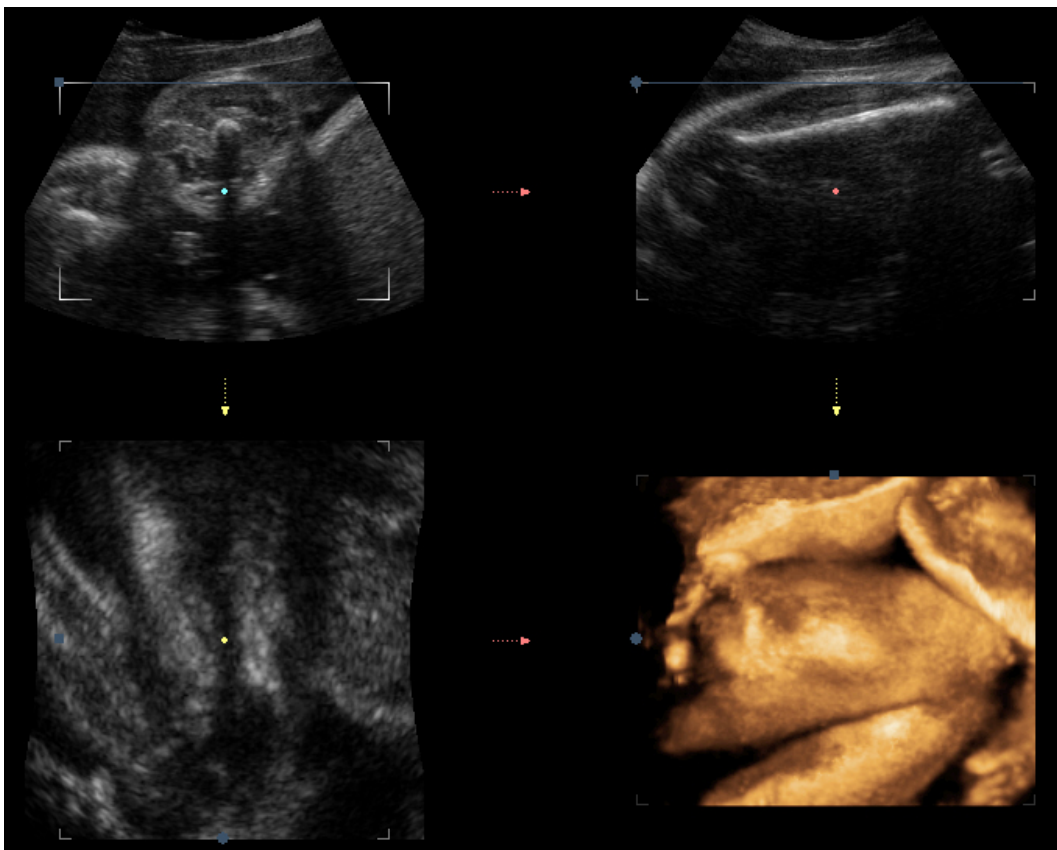


Figure 27: 3D image capture of fetal thigh (35 weeks gestation). Note the longitudinal B-plane view of the femur (top right), showing near-horizontal orientation and the whole bone length.

Immediately after 3D volume acquisition, the V7-3 probe was switched from 3D to 2D mode. The image was optimised to maximise the axial limb size on the screen and then captured, keeping the probe still so as to maintain the perpendicular plane previously identified by 3D scanning. This was repeated until two good quality 2D images were obtained [Figure 28]. The images were labelled “ARM” or “HUM” (humerus), and “LEG” or “FEM” (femur) respectively to avoid confusion at the time of offline segmentation.

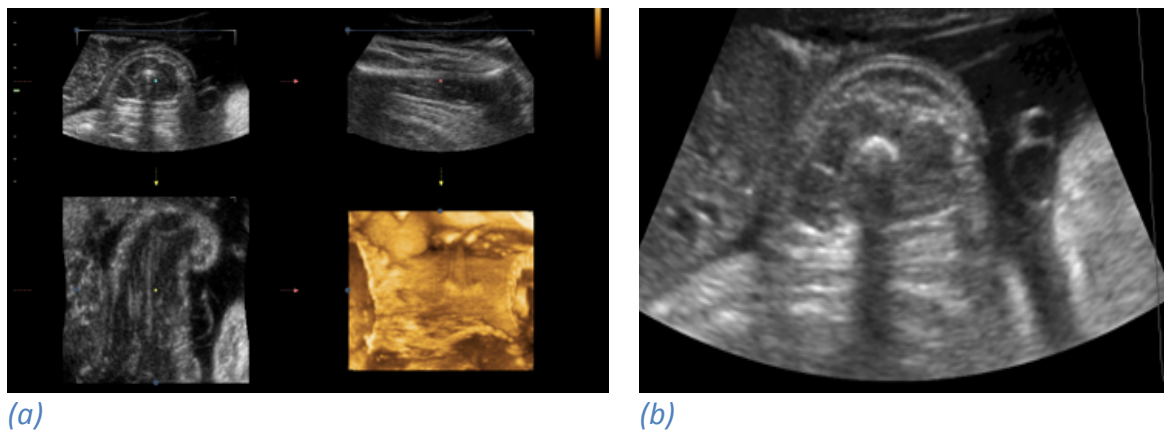


Figure 28: (a) 3D ultrasound of arm at 39+6 weeks of gestation, (b) 2D axial view of arm from same fetus, taken immediately after the 3D image to the left.

The 3D volumes and 2D images were downloaded onto an external hard drive disk for offline analysis [as described in Chapter 3c.] as well as being stored in the respective study image databanks.

RESULTS

i. Feasibility study

Aim

To assess feasibility of obtaining fetal arm, thigh and cheek images and volumes (all potential measurements of fetal nutritional status) across varying gestational ages, presentations and positions and in varying subjects.

Method

50 consecutive patients attending for the INTERGROWTH-21st or MAVIDOS study scans were scanned by the author, using the respective study protocols. Acquisition of arm and thigh images and volumes were attempted. Images and volumes of the fetal cheeks were also attempted (this was being considered, at the time, as an additional ultrasound nutritional marker). Gestational age and fetal position / presentation were recorded, to be able to identify whether these were factors in being able to obtain images.

Results

50 consecutive scans were undertaken between 16⁺⁶ and 40⁺³ weeks of gestation by the author over a total of 16 days [Figure 29].

The 2D images and 3D volumes of fetal arms and thighs could be obtained in all fetuses across the full range of gestational ages, however those of fetal cheeks could only be obtained in 16 of the scans (32%).

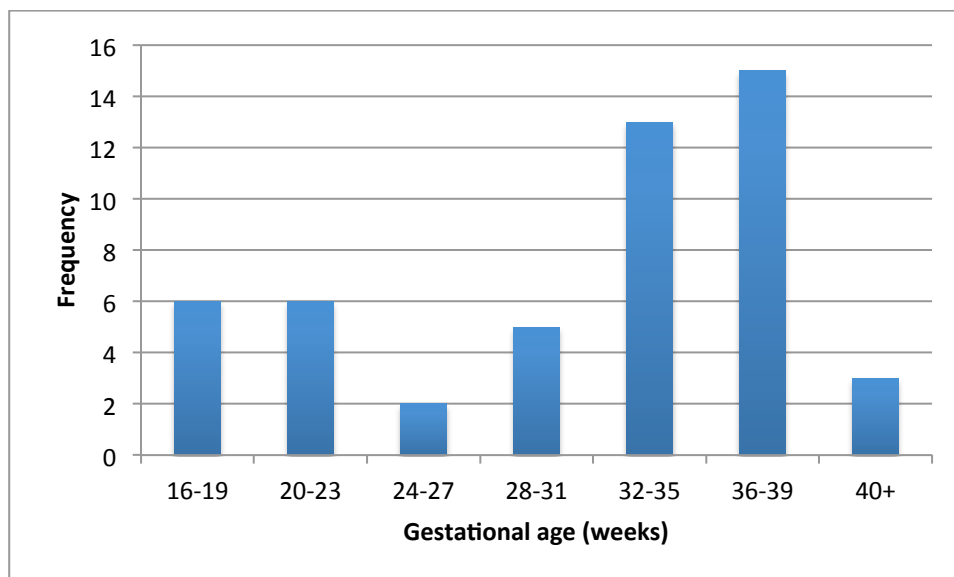


Figure 29: Frequency distribution of gestational ages of fetuses in the feasibility study (n=50).

The 2D images and 3D volumes of fetal arms and thighs could also be obtained from fetuses in all presentations (cephalic, breech, transverse) and positions [Figure 30]:

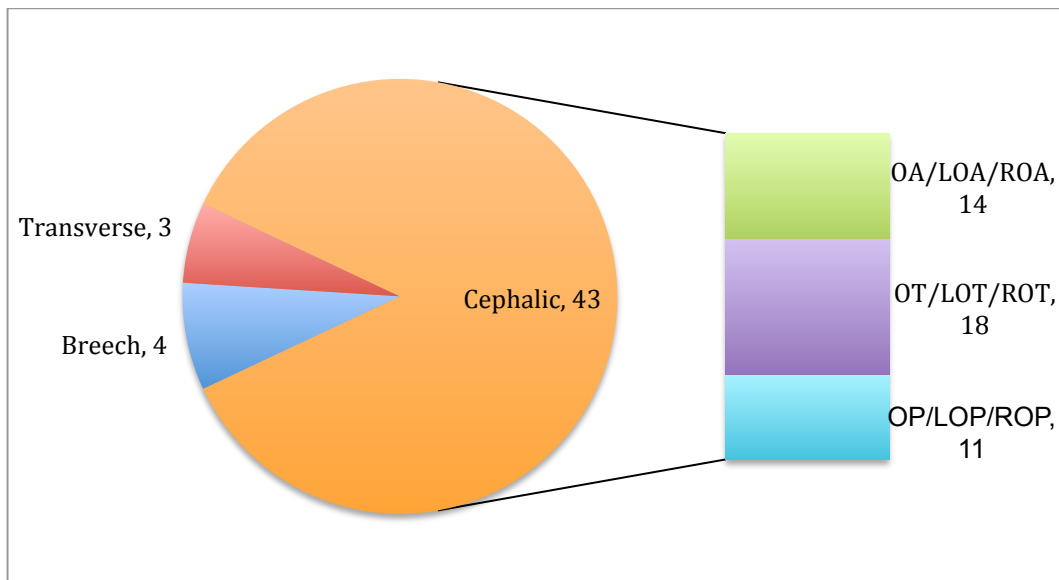


Figure 30: Distribution of fetal presentation (pie chart) and positions (subsidiary bar chart) in the feasibility study (n=50) (OA/OT/OP – occipito-anterior/transverse/posterior).

Images and volumes of the fetal cheeks were mostly obtained in babies in an occipito-posterior (OP) position, across a range of gestational ages; however it was not feasible in all OP babies, for example, if the face was pressed up against an anterior placenta. It was also not possible to visualise the cheeks in babies lying in many of the other positions.

Conclusion

This pilot study demonstrates that it is feasible to acquire arm and thigh images and volumes at different gestational ages, in a range of patients, and in various presentations / positions. Cheek images and volumes could only be acquired in less than a third of fetuses within the feasibility study, and were therefore discarded as being impractical as markers of fetal nutritional status.

ii. Scoring images

Aim

To score each image based on objective quality criteria (similar to that used for quality control of fetal biometry²⁰²), in order to begin our analyses with the highest quality images as these were likely to be the easiest to measure and should yield the most reliable results.

Methods and results

Images were assessed and discussed, and a list of criteria developed by the author and S.R., collaborating postdoctoral biomedical engineer:

- circular plane
- fat layer visible all around (except for humerus acoustic shadow)
- bone surrounded by muscle, ideally centrally placed
- inner edge of fat layer clearly visible (at least 2/3 of perimeter)
- outer edge of fat layer clearly visible (at least 2/3 of perimeter)
- arm occupies at least 30% of image width

Each criterion was given equal weight and 20 sample images, selected by random number generation (www.random.org), were scored from 0-6. These were from 20 different patients, and ranging from 17⁺² to 38⁺⁵ weeks of gestation [Table 5]:

Table 5: Arm image scoring results, 1st set of criteria (n=20).

Score	Frequency
6	1
5	5
4	5
3	3
2	1
1	3
0	2

This system was able to distinguish the very good from the very bad images [Figure 31] but did not appear to be suitably discriminatory for the intervening images, when the scores were compared with the images' appearance.

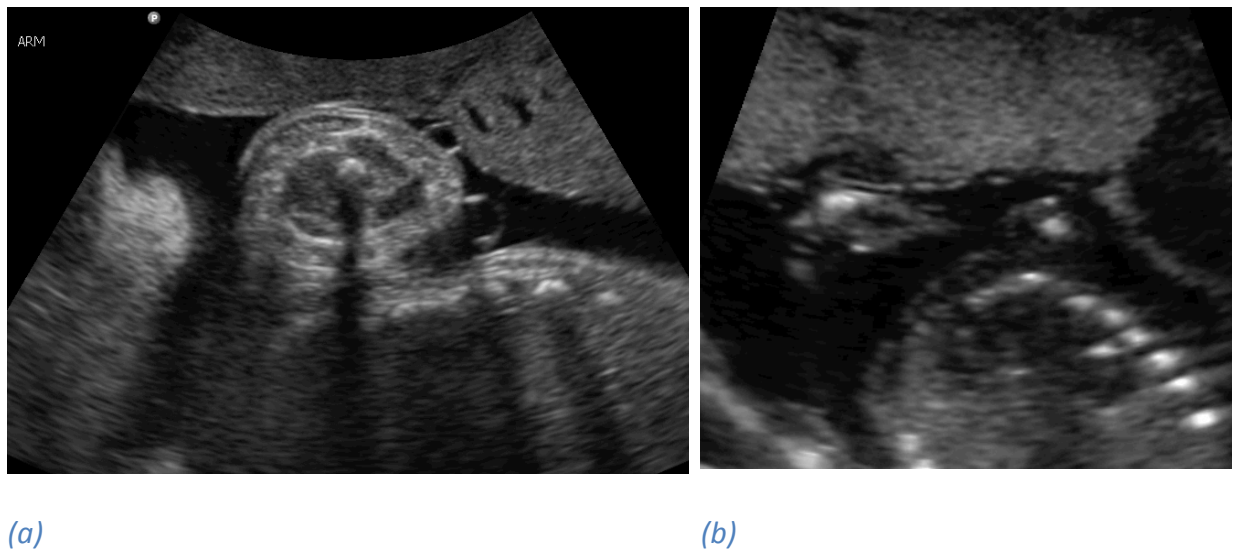


Figure 31: Examples of good and bad images distinguished by scoring: (a) good image, scored 6/6 (b) bad image, scored 1/6 for circular plane (arm is right of centre, above the coronal view of the fetal ribs)

The same criteria were thus modified using “weighted” scoring, whereby varying fat-related criteria were given increased weighting in terms of scoring, with the remaining criteria continuing to score 0 if absent and 1 if present [Table 6]:

Table 6: Arm image scoring, introducing four variations of weighting to 1st set of criteria.

Adjusted criteria (others unchanged)	Weighting	Total maximum score for quality
Fat all around (except humerus acoustic shadow)	Absent: 0 Present: 2	7
Fat all around Outer edge clearly visible	Each criterion: Absent: 0 Present: 2	8
Fat all around Outer edge clearly visible Inner edge clearly visible	Each criterion: Absent: 0 Present: 2	9
Fat all around Outer edge clearly visible Inner edge clearly visible	Fat all around: Absent: 0 Present: 3 Edges clearly visible, each: Absent: 0 Present: 2	10

A further 20 images, again randomly generated, and ranging from 17⁺¹ to 40⁺⁰ weeks of gestation, were scored independently by the author and S.R. using the scoring system above (maximum score 10) [Figure 32] and results then compared:

Table 7: Arm image scoring results, scored out of 10 – comparison of scores (n=20).

Score difference	Frequency	Notes
Identical scores	7	6: identical scoring 1: differing sub-scores
Scores different by 1 mark	8	
Score different by 2 marks	3	
Score different by 3 marks	2	

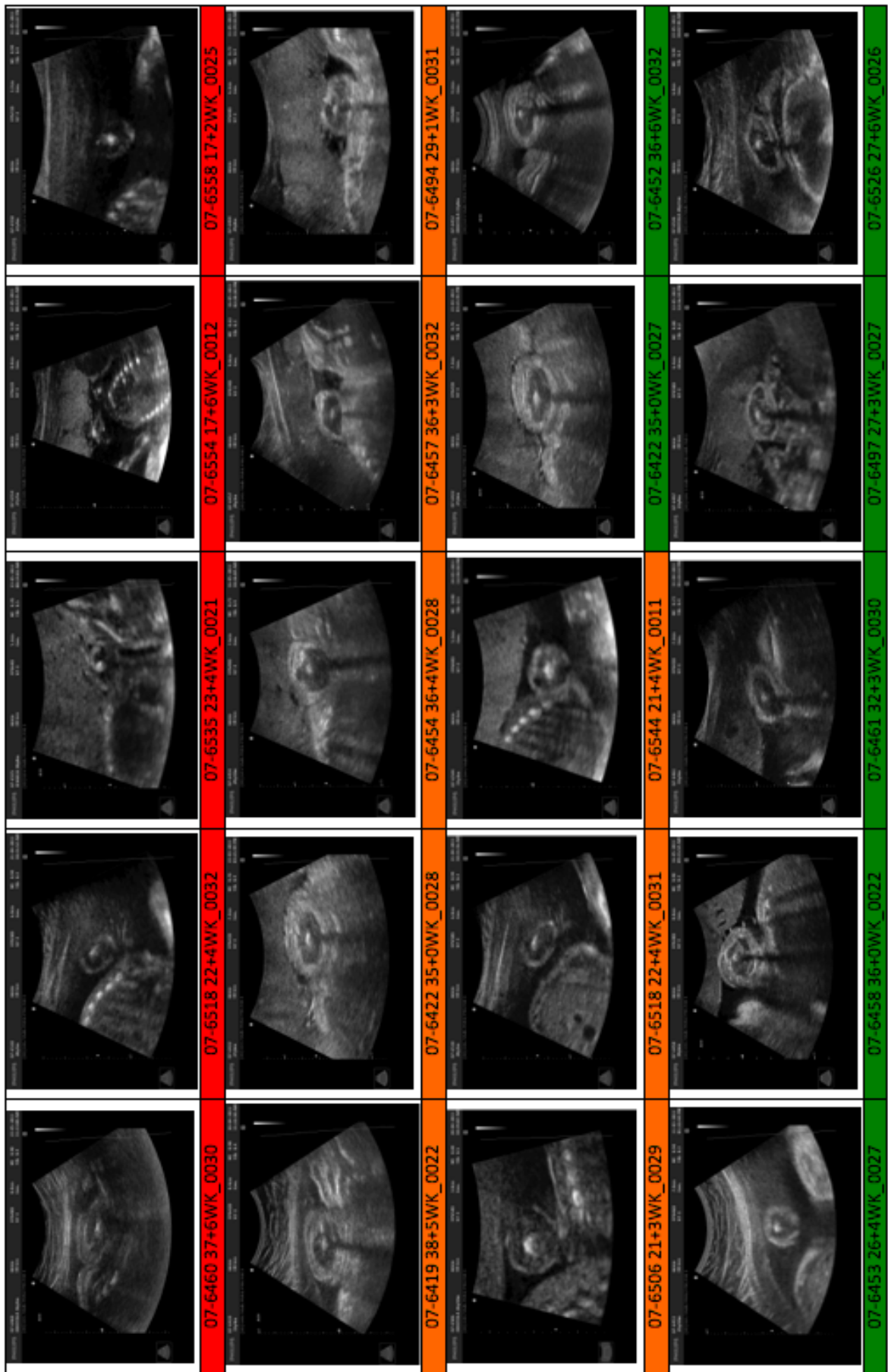


Figure 32: Arm images, scored and sorted into good (green), intermediate (orange), and bad (red) groups.

On reviewing the results, the best agreements were again seen at the extremes – i.e. the very good or very bad images, with poorer agreement for images of intermediate quality. It was decided to divide the scans into groups: any scan scoring 0-3 (“bad”) or 8-10 (“good”) would be accepted as such, but those scoring between 4-7 (“intermediate”) would be reviewed and discussed by us both and a final score then awarded.

A further 98 randomly generated images were scored by both the author and S.R. using the 10-point system, and the results compared. Agreement in three groups (good / intermediate / bad) occurred in 55/98 images; this dropped to 32/98 images matching in terms of individual scores. There were some marked differences in image scoring, with 13 cases having a discrepancy of more than 3 between the two scores; for example, the same images scoring 9 vs. 5, 4 vs. 10, 2 vs. 7, or 5 vs. 1.

Analysis of the scoring criteria revealed that there was a difference of opinion in a third of cases as to: whether the axial image was circular or not; whether the outer edge was clearly visible; and whether the size of the arm occupied at least 30% of the width of the image. There were no clear patterns between those assessing the images – it was not that one person was consistently scoring higher than the other, for example.

After reviewing the images, it was felt that the size criterion, previously 30% of the width of the image, should be changed to 30% of the height of the image, as the curvilinear angle of the image may have been causing some of the differences in the interpretation of this criterion. Removing the circular plane criterion was also considered, as fetal arms approaching term are not necessarily circular in axial section.

Alternative scoring systems were then considered, including methods of subdividing the weighted criteria. This would still generate the same final score (out of 9, if the circular plane criterion was removed) but it was hoped they would prove more robust and less subject to variation in scoring. For example:

Outer edge visible:	<50%	score 0
(max score 2)	<2/3	score 1
	all	score 2
Inner edge visible:	<50%	score 0
(max score 2)	<2/3	score 1
	all	score 2
Fat visible:	all around	score 3
(max score 3)	all around apart from small portion	score 2
	at least 2/3 seen	score 1
	less than 2/3 seen	score 0

--or--

Fat visible:	divide arm into thirds (for example, 12-4, 4-8, 8-12 o'clock
(max score 3)	positions) and score 1 point for each third in which fat is
	clearly visible

We began re-scoring the previous arm images using these newer approaches but they did not result in any improvement in agreement of scoring, particularly for the images of intermediate quality.

Discussion

We performed scoring of images in order to start this project by analysing those images of highest quality. The process allowed us to gain a better understanding of the factors that promote or prohibit good segmentation of fetal fat. However, inter-observer repeatability of this objective scoring was limited, in particular for the intermediate images. It also became evident that the scoring system was becoming increasingly complex. More importantly, on reviewing the images, it seemed likely that many of the 'bad' images would still be able to be segmented, hence negating the need for detailed scoring. Finally there was also a concern that by limiting the analysis to the best images only, we would be limiting overall validity; and that we would unnecessarily eliminate potentially usable and valuable data.

Conclusion

After attempts to develop a robust objective scoring system for the quality of the arm images, we chose to adopt a subjective and practical approach, whereby each image would be assessed visually by the person doing the measuring, and a decision taken as to whether the image was 'segmentable' or 'non-segmentable'.

4b. Developing the techniques to measure arm fat and lean areas

i. Initial segmentations: identifying the main challenges

Offline image segmentation was initially undertaken using a graphics tablet with open source software applications MITK 3M3 v1.1 and itk-SNAP (University of Pennsylvania, PA, USA) for medical image analysis.

Developing the segmentation method took some time. Different approaches were taken to outlining the fat, with variations including: outlining in an extremely detailed manner, only including fat which could be seen, and disregarding shadows / irregularities; outlining in an extremely broad, generalised manner; and outlining in a measured way, somewhere between the two methods [Figure 33].

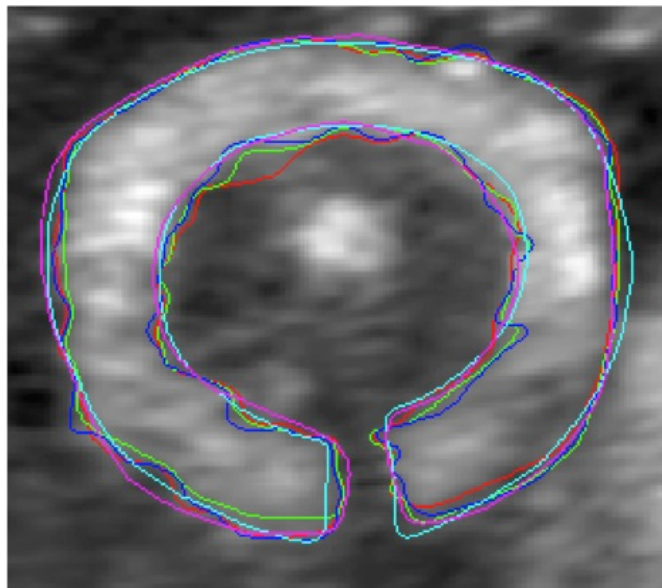


Figure 33: Different methods tried for outlining arm fat showing varying results
pink and turquoise = broad outlining
red and blue = detailed pixel-by-pixel outlining
green = method somewhere between the two other methods.

The first, whilst correct from an imaging and technical point of view, did not take into account that the arm cross-section, if viewed anatomically/histologically, would have a smooth contour. It was also very time-consuming. The last, whilst much quicker, was probably slightly too broad and risked over-estimating the fat, as well as missing subtle differences between arm fat measurements in different fetuses. It was therefore decided that the intermediate method was the most sensible compromise.

There were also challenges in determining the exact location of the inner and outer fat borders. The 'outer border of the fat' actually represents the skin surface of the arm; whilst the skin-fat interface was sometimes visible on late third-trimester fetal scans [Figure 34], it was not visible on earlier scans.

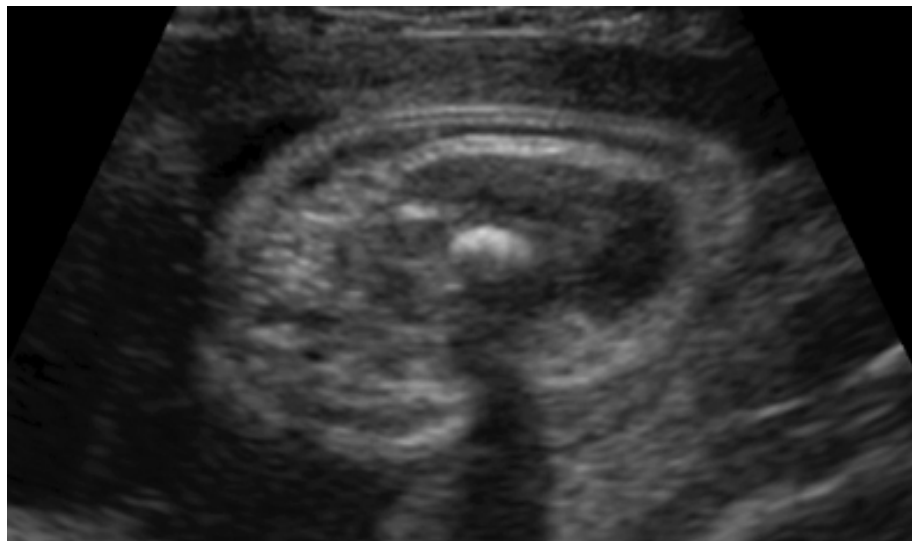
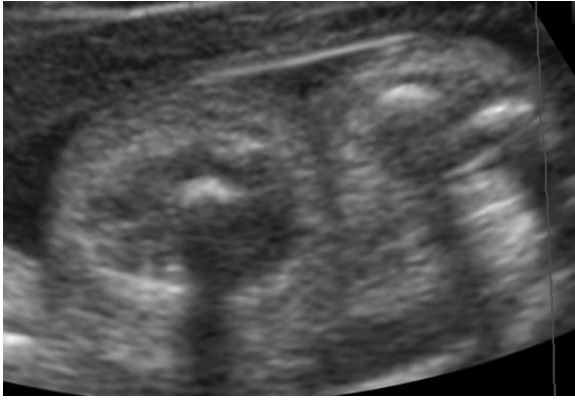
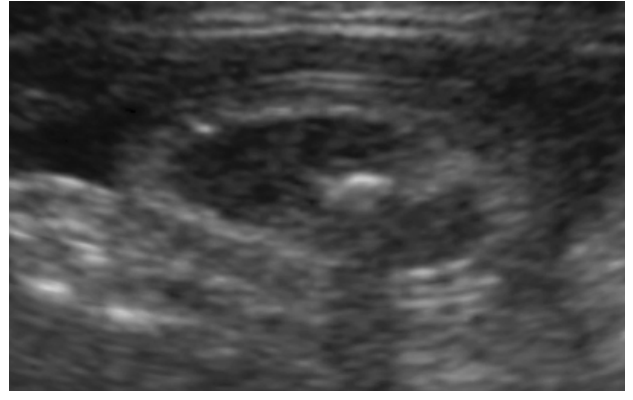


Figure 34: Fetal arm at 38 weeks' gestation, showing distinct fat and skin layers.

The arm could be flexed so that it was pressed up against the forearm, or pressed up against other fetal structures such as the chest or placenta, which made segmenting its outline more difficult. These issues became increasingly common with advancing gestational age.



(a)



(b)

Figure 35: Fetal arm images, showing outer fat border challenges: (a) flexed arm, with arm on left and forearm (two bones, radius and ulna, visible) on right, disrupting outer fat border of the arm; (b) arm pressed up against fetal chest (ribs, in cross-section, visible at lower left of image).

When the arm was against other structures, identification of the outer fat border furthest from the probe (i.e. lowest in the image) was frequently impaired as echoes distort the view. Incorrect segmentation of the border would lead to falsely large fat areas. To avoid this, a visual appraisal of the outlined fat was undertaken before saving. The amount of fat around the fetal arm does show small amounts of variation, but if the lower amount of fat was much greater than the upper then the segmentation was cancelled, the image re-appraised, and the segmentation repeated.

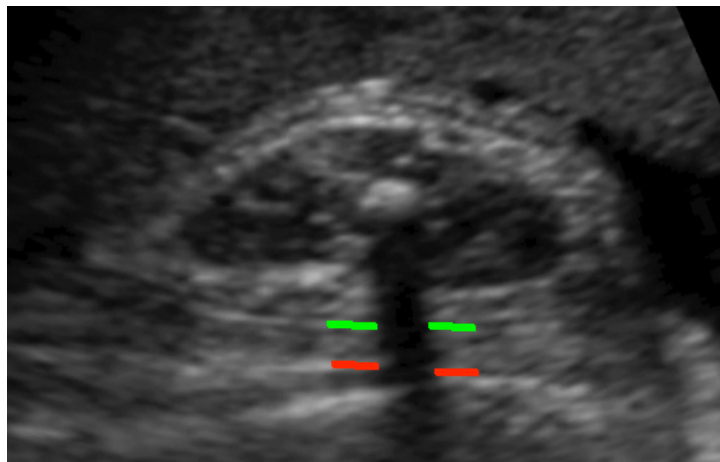


Figure 36: Fetal arm at 35 weeks showing potential difficulties with segmenting the lower outer fat border. Correct segmentation edge is shown in green; incorrect in red. Note the depth/amount of fetal fat in the upper part of the image is similar to the correctly outlined (green) fat in the lower part of the image.

The inner border could also be difficult to distinguish due to variations in echo texture generated by the fetal tissues, particularly as there could be similar shades between the muscle and fat [Figure 37]. Distinguishing the borders could be improved by varying the contrast or by narrowing the eyes when looking at the image. The need for such adjustments decreased as familiarity with the segmentation technique increased.

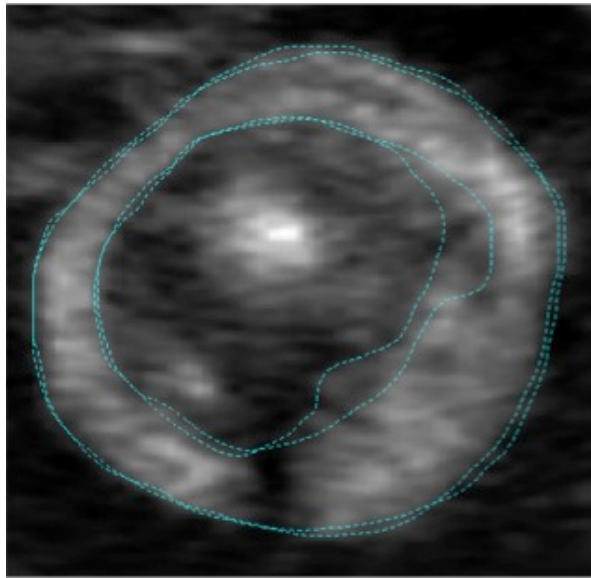


Figure 37: Two segmentations of the same arm image, showing differing interpretation of the inner fat border; the one which gives the larger lean area is correct.

Problems were also posed by the acoustic shadow generated by ultrasound waves reflecting off the humerus. Initially only the visible fat was segmented, with vertical ‘cut off’ lines at the edge of the shadow [Figure 33], but this did not reflect the anatomical reality of the presence of fat around the whole arm. The amount of shadow generated would be expected to increase as the fetus gained maturity, both due to its increasing size but also to its bones becoming more calcified, and thus more reflective to sound waves; however, different fetuses may mature at different rates which meant we found it difficult to justify its exclusion as it would not necessarily be constant between fetuses [Figure 38].

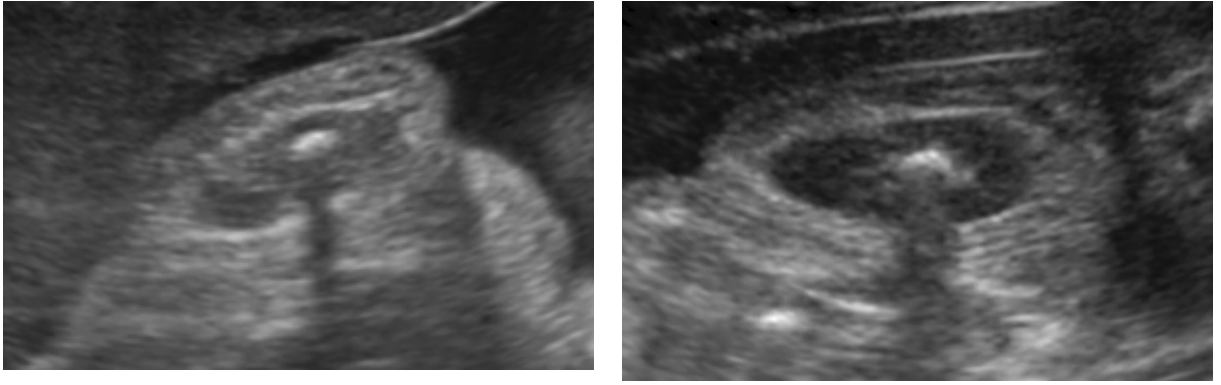
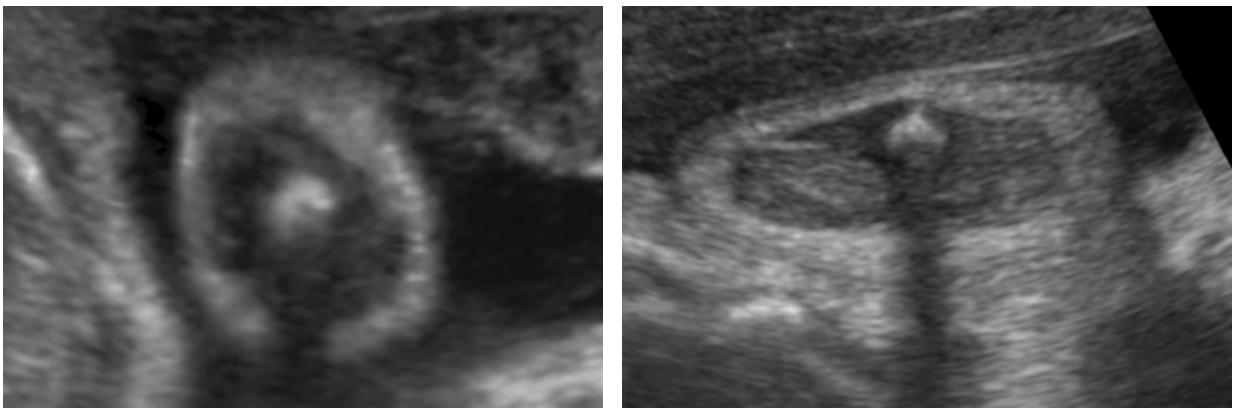


Figure 38: Humeral acoustic shadows of varying width in two different fetuses, both at 36 weeks' gestation.

Whilst many images demonstrated some of the challenges illustrated above, some were straightforward to segment [Figure 39].



(a)

(b)

Figure 39: Examples of good-quality, easily segmented arm images at (a) 24 weeks and (b) 36 weeks.

ii. MATLAB segmentation tool

The commercially available tools did not quite fit our requirements, as their image measurement techniques required additional offline analysis. S.R. (IBME) therefore designed a segmentation tool using MATLAB to fit our precise needs.^{203,204} The advantages of this include its ability to work with images / volumes acquired from any ultrasound machine (as opposed to company-specific tools) and the automatic generation of several results (for example, both arm area and fat area) from one segmentation. She also designed automatic completion of the gap generated by the acoustic shadow, using mathematical modelling.

The MATLAB segmentation tool uses DICOM files of the images to allow area calculations; the images are acquired at different resolutions so jpg files, whilst useful for judging image quality, are not suitable for quantitative analysis. Images were segmented using a mouse or touchpad with a computer or laptop, and could be undertaken on windows or mac machines.

For each scan, image-identifying characteristics (date of scan; anonymised patient ID, gestational age, and scan image number) were automatically acquired from the file name, in the format described in Figure 22. Results were then generated from segmentation: fat area (mm^2); muscle and bone area (i.e. total lean area) (mm^2); axial arm slice total area (mm^2); and fat as a percentage of the arm slice total area. These data were automatically tabulated in a .txt file [Figure 39], which enables easy transfer to excel or a statistical programme. The segmented fat image and related information was stored in a .mat file for future analyses.²⁰³⁻²⁰⁵

20110401	07-6495	0022	23+4WK	119.699813	90.917204	210.295795	56.919737
20110707	07-6554	0011	23+5WK	128.144937	163.024787	290.728711	44.077152
20110620	07-6527	0033	27+2WK	164.725032	181.831383	346.121944	47.591617
20110519	07-6529	0025	24+2WK	151.583678	146.265630	297.426219	50.965136
20110609	07-6536	0014	27+4WK	194.832946	207.286602	401.640561	48.509280
20110804	07-6549	0016	27+3WK	169.887370	199.601741	368.935757	46.047955

Figure 40: Example text file results for segmentation of 2D arm images, showing numerical results (L to R): date, ID number, scan image number, gestational age, fat area, lean area, arm area (all areas in mm²) and fat as a percentage of the arm area.

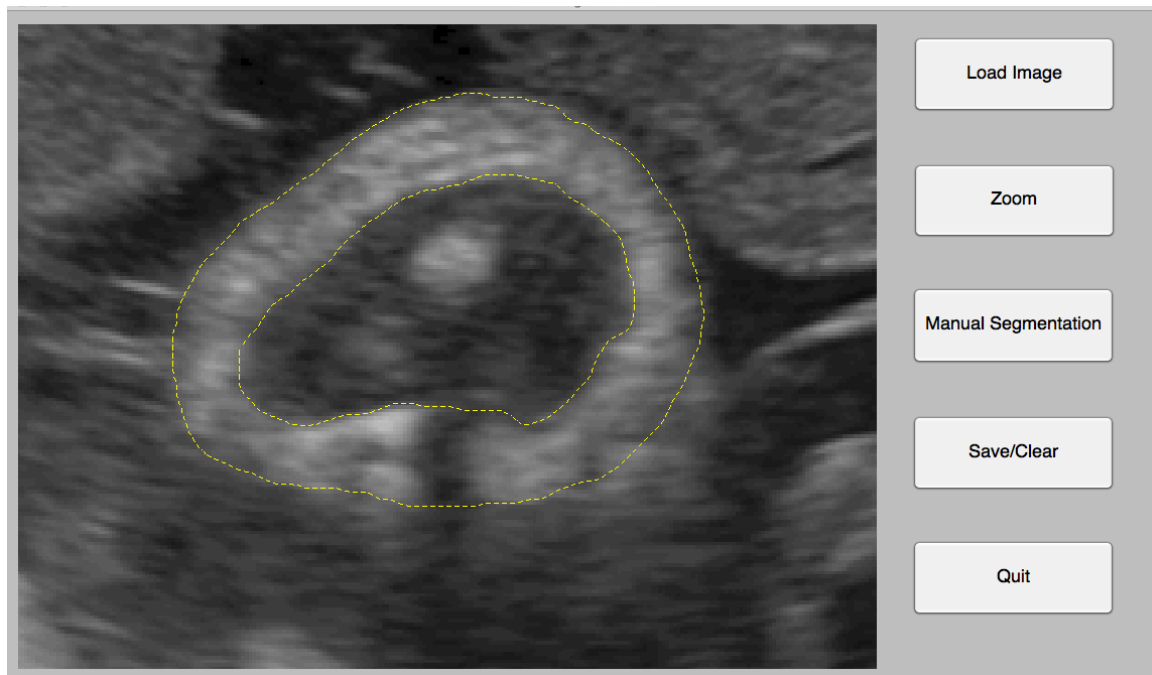
iii. MATLAB segmentation protocol

Within the MATLAB programme, the segmentation tool was opened in a new window. Using the 'load image' button, the appropriate DICOM file, previously identified as the highest-quality image from all available arm images for each patient, was selected and opened. [Figure 41a] The image was magnified, using a hold-and-drag box approach, so that the axial arm image filled at least 50% of the screen.

The inner edge of the fat was outlined in a smooth manner, following the image contours (rather than an irregular, pixel-by-pixel manner); the segmentation was started from one edge of the humeral acoustic shadow and completed at the other edge; with the MATLAB segmentation tool providing automatic completion across the resulting 'gap' left by the acoustic shadow. Any obvious shadows (eg at the lateral aspects) were approximated to a reasonable anatomical shape. This process was then repeated for the outer edge of the fat. [Figure 41b]



(a)



(b)

Figure 41: MATLAB 2D segmentation tool screenshots (a) initial view (b) after magnification and segmentation.

The “Save/Clear” button was clicked. This allowed a final appraisal of the outline, and the option to clear and resegment if it was deemed to be suboptimal. Otherwise the data was saved, generating a new line of results in the text file as well as an individual mat file. The latter was automatically given the same file name as the image, incorporating ID and gestation. Each image was segmented twice, on different days, and mean areas calculated.

4c. Measuring arm fat and lean tissue areas, and establishing reference ranges

i Normal development of fetal arm fat and lean tissues

Aim

To describe the normal development of fetal arm fat in an optimally healthy patient population, and establish a normal range for this population.

Methods

2D axial fetal mid-humeral arm images were obtained from women taking part in the INTERGROWTH-21st study between February 2011 - February 2012. Cross-sectional arm and lean areas were measured twice by the author as per the segmentation protocol, and the mean fat, lean and arm areas plotted against gestational age. Statistical analyses were carried out using STATA using fractional polynomial regression. Fat area data underwent log transformation (natural log) to enable calculation of centiles (reverse transformed to enable easier visualisation); lean area and fat as percentage did not require transformation

Results

180 scans were measured from 106 women, with gestational ages between 16⁺² and 40⁺⁴ weeks. The mean values for fat, lean, and arm areas (mid-humeral axial slice) were converted from mm² to cm² and plotted against gestational age [Figure 42-Figure 45].

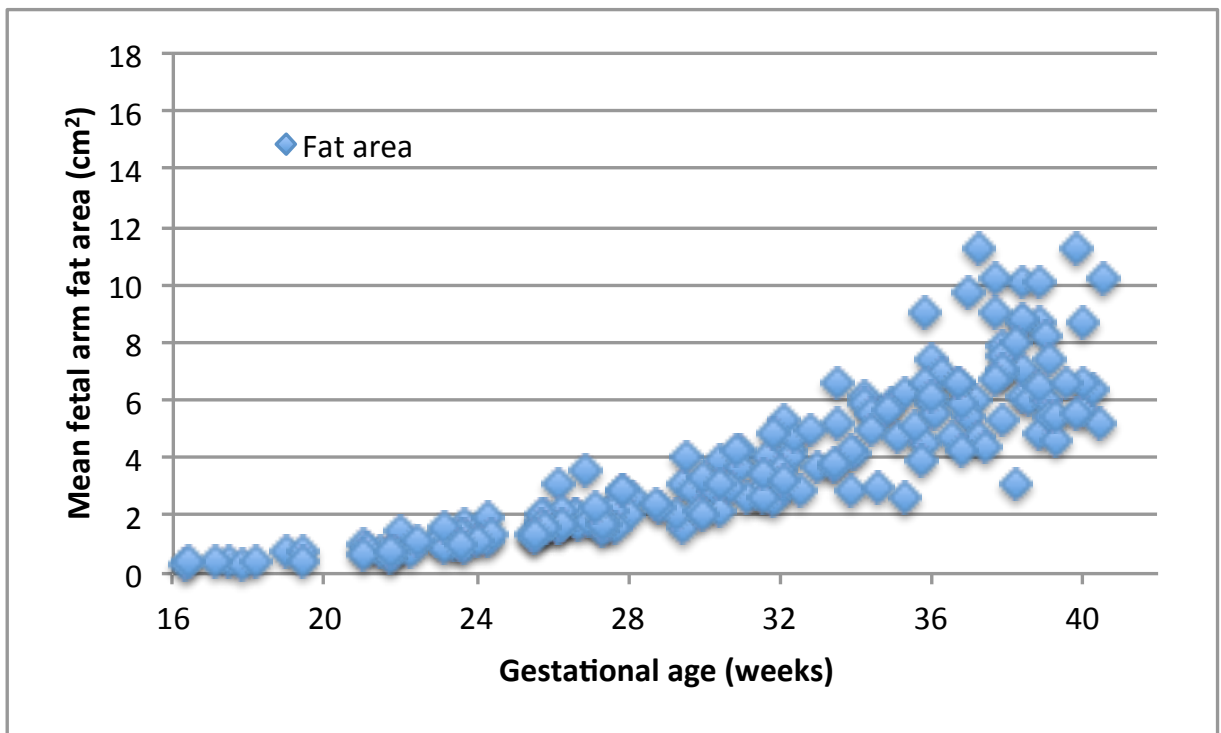


Figure 42: Mean fetal arm fat area (mid-humeral axial slice) (n=180).

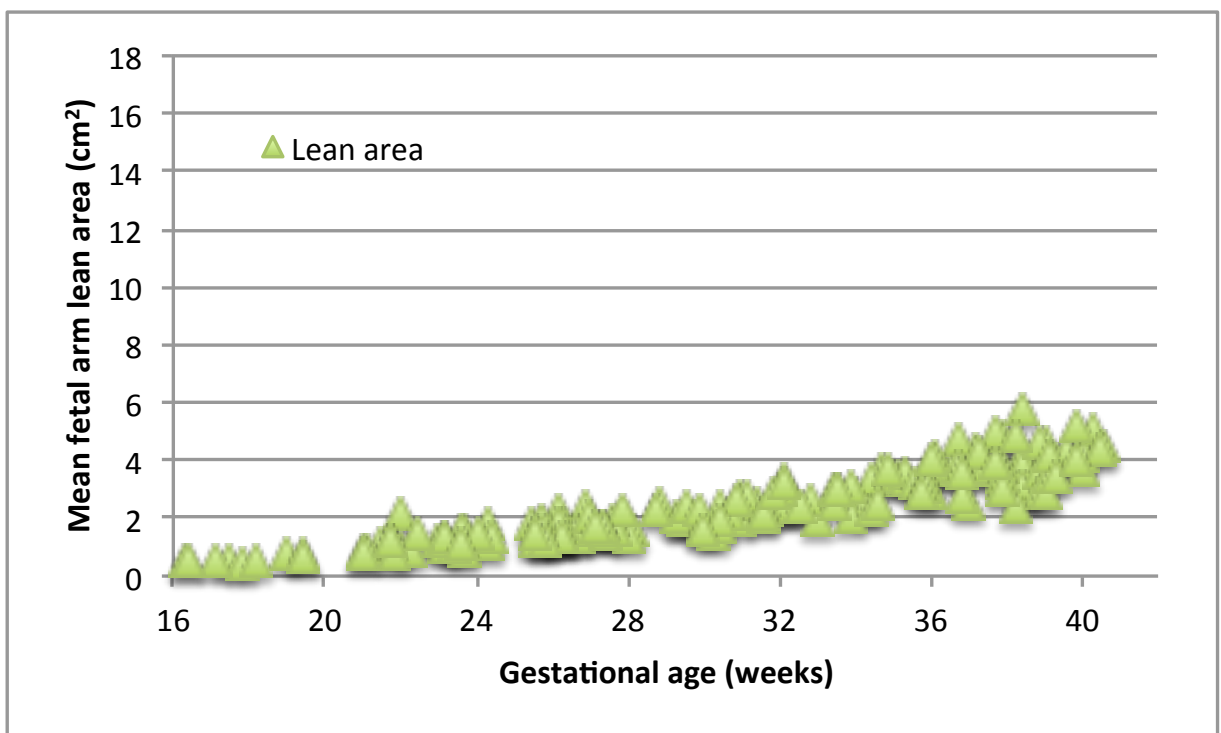


Figure 43: Mean fetal arm lean area (mid-humeral axial slice) (n=180).

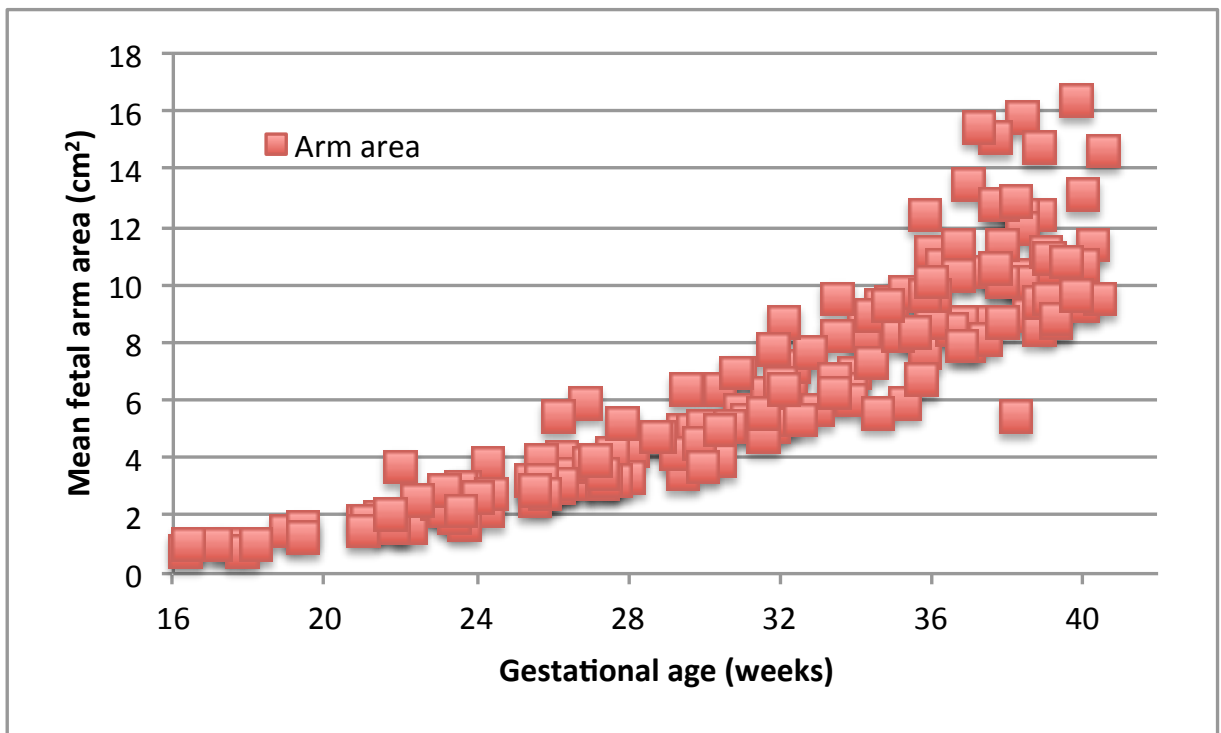


Figure 44: Mean fetal arm area (mid-humeral axial slice) (n=180).

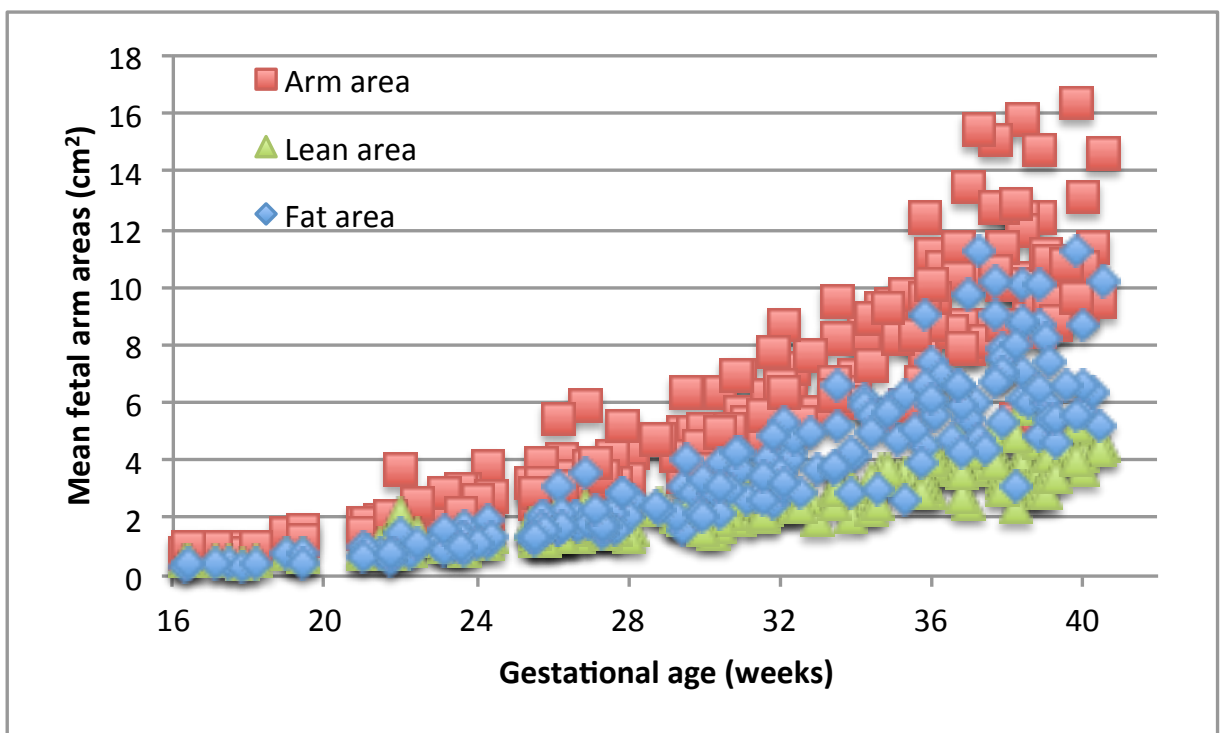


Figure 45: Mean fetal arm, fat and lean areas (mid-humeral axial slice) superimposed (n=180).

Fractional polynomial regression^{206,207} was used to generate equations to calculate the mean / 50th centile (C50) values for fat area and lean area for a given gestational age (GA):

$$\text{Log (arm fat area) C50} = ((\ln(\text{GA}/10)) \times 3.573999) + 1.727577$$

$$\text{Arm lean area C50} = ((\text{GA}/10)^2 \times 27.3902) - 23.51686$$

The relationship with standard deviation (SD) was also generated using the same approach.

$$\text{SD of log (arm fat area) C50} = (\text{GA} \times 0.0043701) + 0.1194121$$

$$\text{SD of arm lean area C50} = (\text{GA} \times 2.670115) - 38.56833$$

This enabled calculation of the centiles for both sets of data:

$$3^{\text{rd}} / 97^{\text{th}} \text{ centiles} = \text{mean} \pm 1.88 \text{ SD}$$

$$5^{\text{th}} / 95^{\text{th}} \text{ centiles} = \text{mean} \pm 1.64 \text{ SD}$$

$$10^{\text{th}} / 90^{\text{th}} \text{ centiles} = \text{mean} \pm 1.28 \text{ SD}$$

For the arm fat area, SD was used to calculate the log-transformed centiles, and the results then transformed back to generate results in cm² [Table 8]. The arm lean area data did not require log transformation [Table 9].

Table 8: Centile values for mean arm fat areas for each week of gestation.

GA (weeks)	Mean fetal arm fat area (cm ²) centiles						
	3 rd (mean -1.88 SD)	5 th (mean - 1.64 SD)	10 th (mean - 1.28 SD)	50 th (mean)	90 th (mean + 1.28 SD)	95 th (mean + 1.64 SD)	97 th (mean +1.88 SD)
16	0.21	0.22	0.24	0.30	0.38	0.41	0.43
17	0.26	0.27	0.29	0.37	0.48	0.52	0.54
18	0.32	0.33	0.36	0.46	0.59	0.64	0.67
19	0.38	0.40	0.43	0.56	0.72	0.78	0.82
20	0.45	0.48	0.51	0.67	0.87	0.94	0.99
21	0.54	0.56	0.61	0.80	1.05	1.13	1.19
22	0.63	0.66	0.71	0.94	1.24	1.34	1.41
23	0.73	0.77	0.83	1.10	1.46	1.58	1.67
24	0.84	0.89	0.96	1.29	1.71	1.86	1.96
25	0.97	1.02	1.11	1.49	1.99	2.16	2.29
26	1.10	1.17	1.27	1.71	2.31	2.51	2.65
27	1.25	1.33	1.45	1.96	2.65	2.89	3.06
28	1.42	1.50	1.64	2.23	3.04	3.32	3.51
29	1.59	1.69	1.85	2.53	3.47	3.79	4.02
30	1.78	1.89	2.07	2.85	3.93	4.30	4.57
31	1.99	2.11	2.32	3.21	4.45	4.87	5.18
32	2.21	2.35	2.58	3.59	5.01	5.50	5.85
33	2.44	2.60	2.86	4.01	5.62	6.18	6.59
34	2.70	2.88	3.17	4.46	6.29	6.93	7.39
35	2.97	3.17	3.49	4.95	7.02	7.74	8.26
36	3.26	3.48	3.84	5.48	7.80	8.62	9.21
37	3.56	3.81	4.21	6.04	8.66	9.58	10.25
38	3.88	4.16	4.61	6.64	9.57	10.61	11.36
39	4.23	4.53	5.03	7.29	10.56	11.73	12.57
40	4.59	4.93	5.48	7.98	11.63	12.93	13.88
41	4.97	5.34	5.95	8.72	12.77	14.22	15.28

Table 9: Centile values for mean arm lean areas for each week of gestation.

GA (weeks)	Mean fetal arm lean area (cm ²) centiles						
	3 rd (mean -1.88 SD)	5 th (mean - 1.64 SD)	10 th (mean - 1.28 SD)	50 th (mean)	90 th (mean + 1.28 SD)	95 th (mean + 1.64 SD)	97 th (mean +1.88 SD)
16	0.39	0.40	0.41	0.47	0.52	0.53	0.54
17	0.43	0.44	0.47	0.56	0.64	0.67	0.68
18	0.47	0.50	0.53	0.65	0.77	0.81	0.83
19	0.52	0.55	0.60	0.75	0.91	0.95	0.98
20	0.58	0.62	0.67	0.86	1.05	1.10	1.14
21	0.64	0.69	0.75	0.97	1.20	1.26	1.30
22	0.71	0.76	0.83	1.09	1.35	1.42	1.47
23	0.78	0.84	0.92	1.21	1.51	1.59	1.64
24	0.86	0.92	1.02	1.34	1.67	1.76	1.82
25	0.95	1.01	1.12	1.48	1.84	1.94	2.01
26	1.04	1.11	1.22	1.62	2.01	2.12	2.20
27	1.13	1.21	1.33	1.76	2.19	2.31	2.39
28	1.23	1.32	1.45	1.91	2.38	2.51	2.59
29	1.34	1.43	1.57	2.07	2.57	2.71	2.80
30	1.45	1.55	1.70	2.23	2.76	2.91	3.01
31	1.57	1.67	1.83	2.40	2.96	3.12	3.23
32	1.69	1.80	1.97	2.57	3.17	3.34	3.45
33	1.82	1.94	2.11	2.75	3.38	3.56	3.68
34	1.95	2.07	2.26	2.93	3.60	3.79	3.91
35	2.09	2.22	2.42	3.12	3.82	4.02	4.15
36	2.23	2.37	2.58	3.31	4.05	4.26	4.40
37	2.38	2.53	2.74	3.51	4.29	4.50	4.65
38	2.54	2.69	2.91	3.72	4.53	4.75	4.90
39	2.70	2.86	3.09	3.93	4.77	5.01	5.16
40	2.86	3.03	3.27	4.15	5.02	5.27	5.43
41	3.04	3.21	3.46	4.37	5.28	5.53	5.70

Table 7 and Table 8 are shown in graph form below for fat [Figure 45] and lean area [Figure 46] (50th centile coloured line; 10th / 90th centiles dashed lines; 3rd / 97th centiles dotted lines):

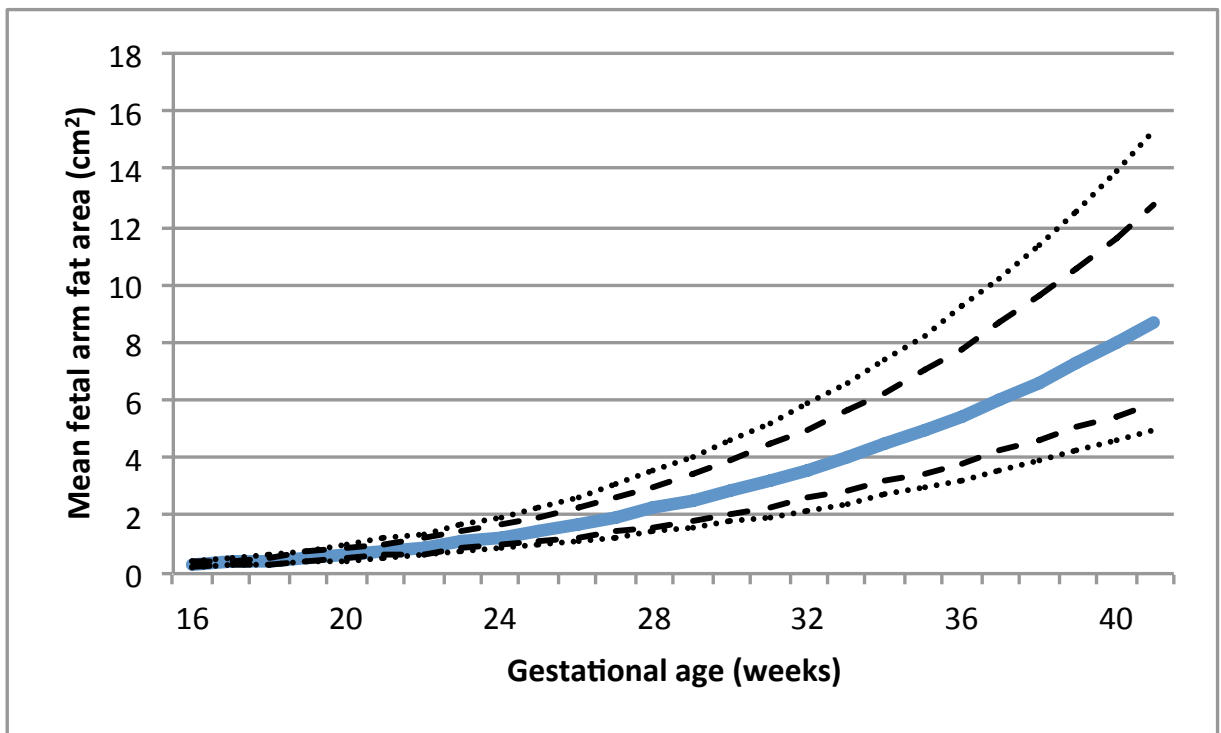


Figure 46: Fetal arm fat area (3rd, 10th, 50th, 90th and 97th centiles).

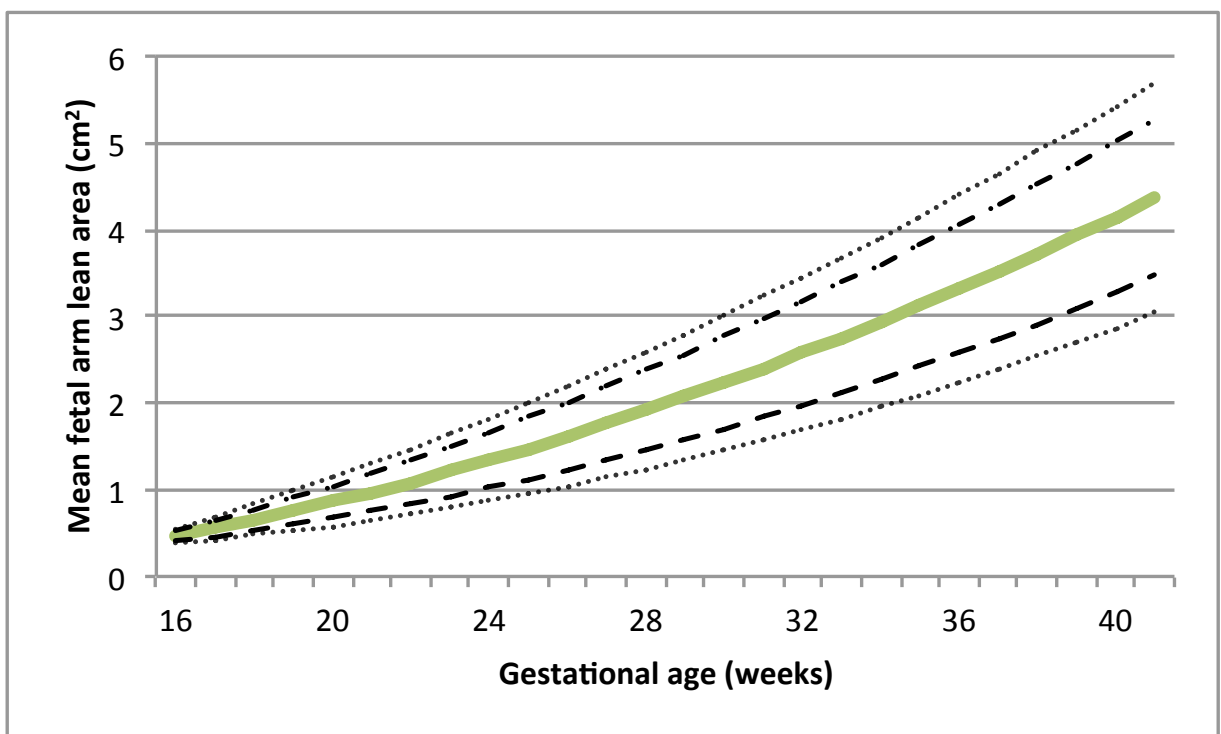


Figure 47: Fetal arm lean area (3rd, 10th, 50th, 90th and 97th centiles).

Arm fat was also assessed in the form of the percentage of the whole arm area it occupied:

$$\text{Fat area as \% arm area C50} = ((\ln(\text{GA}/10)) \times 30.15639) + 23.64476$$

$$\text{SD of fat area as \% arm area C50} = (\text{GA} \times 0.1304905) + 1.938664$$

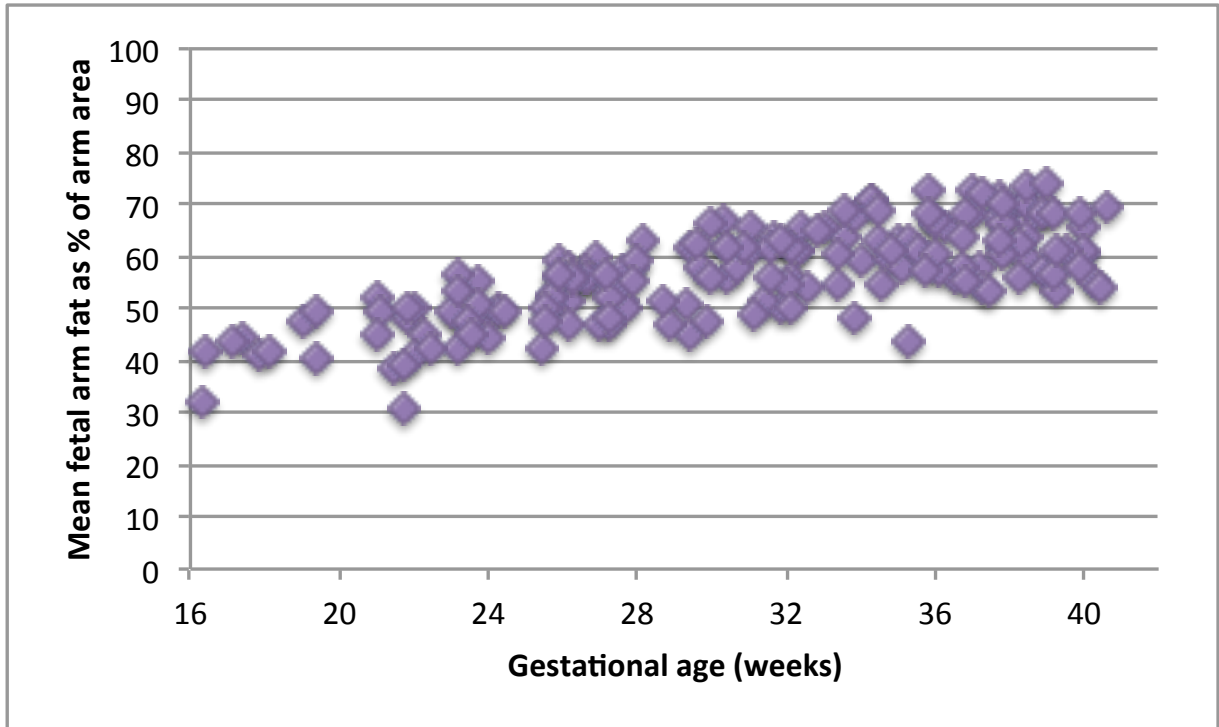


Figure 48: Fetal arm fat as percentage of arm area (mid-humeral axial slice) (n=180).

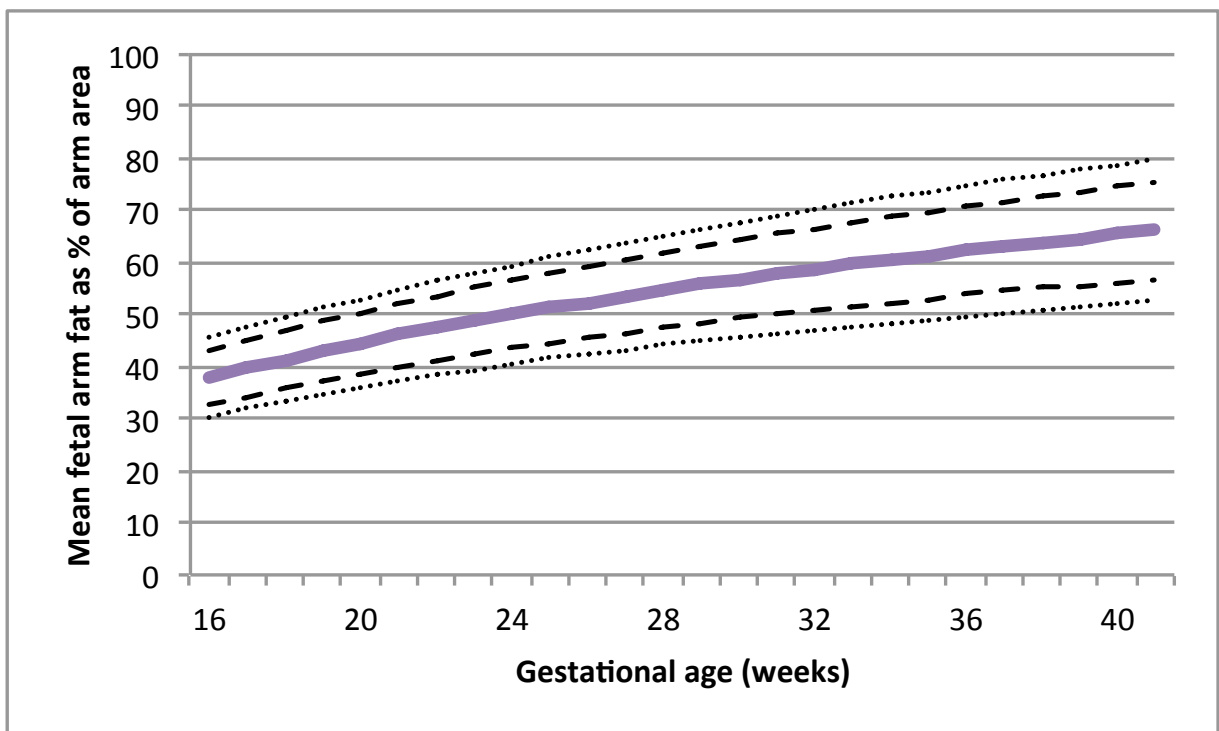


Figure 49: Fetal arm fat as percentage of arm area (3rd, 10th, 50th, 90th and 97th centiles).

Table 10: Centile values for mean arm fat as a percentage of arm area for each week of gestation.

GA (weeks)	Mean fetal arm fat (as a percentage of arm area) centiles						
	3 rd (mean -1.88 SD)	5 th (mean - 1.64 SD)	10 th (mean - 1.28 SD)	50 th (mean)	90 th (mean + 1.28 SD)	95 th (mean + 1.64 SD)	97 th (mean +1.88 SD)
16	30.2	31.2	32.7	37.8	43.0	44.4	45.4
17	31.8	32.8	34.3	39.6	45.0	46.5	47.5
18	33.3	34.3	35.9	41.4	46.9	48.4	49.4
19	34.7	35.8	37.3	43.0	48.7	50.2	51.3
20	36.0	37.1	38.7	44.5	50.4	52.0	53.1
21	37.2	38.3	40.0	46.0	52.0	53.7	54.8
22	38.4	39.5	41.3	47.4	53.6	55.3	56.5
23	39.5	40.7	42.4	48.8	55.1	56.9	58.0
24	40.5	41.7	43.6	50.0	56.5	58.4	59.6
25	41.5	42.7	44.6	51.3	57.9	59.8	61.1
26	42.4	43.7	45.6	52.5	59.3	61.2	62.5
27	43.3	44.6	46.6	53.6	60.6	62.6	63.9
28	44.2	45.5	47.5	54.7	61.9	63.9	65.2
29	45.0	46.4	48.4	55.8	63.1	65.1	66.5
30	45.8	47.2	49.3	56.8	64.3	66.4	67.8
31	46.5	48.0	50.1	57.8	65.4	67.6	69.0
32	47.2	48.7	50.9	58.7	66.5	68.7	70.2
33	47.9	49.4	51.7	59.6	67.6	69.9	71.4
34	48.6	50.1	52.4	60.5	68.7	71.0	72.5
35	49.2	50.8	53.1	61.4	69.8	72.1	73.7
36	49.8	51.4	53.8	62.3	70.8	73.2	74.7
37	50.4	52.0	54.4	63.1	71.8	74.2	75.8
38	50.9	52.6	55.1	63.9	72.7	75.2	76.9
39	51.5	53.2	55.7	64.7	73.7	76.2	77.9
40	52.0	53.7	56.3	65.5	74.6	77.2	78.9
41	52.5	54.2	56.9	66.2	75.5	78.1	79.9

Arm area centiles were also calculated using log-transformed data:

$$\text{Log (arm area) C50} = ((\ln(\text{GA}/10) \times 2.99995) + 2.936137)$$

$$\text{SD of log (arm area) C50} = 0.19$$

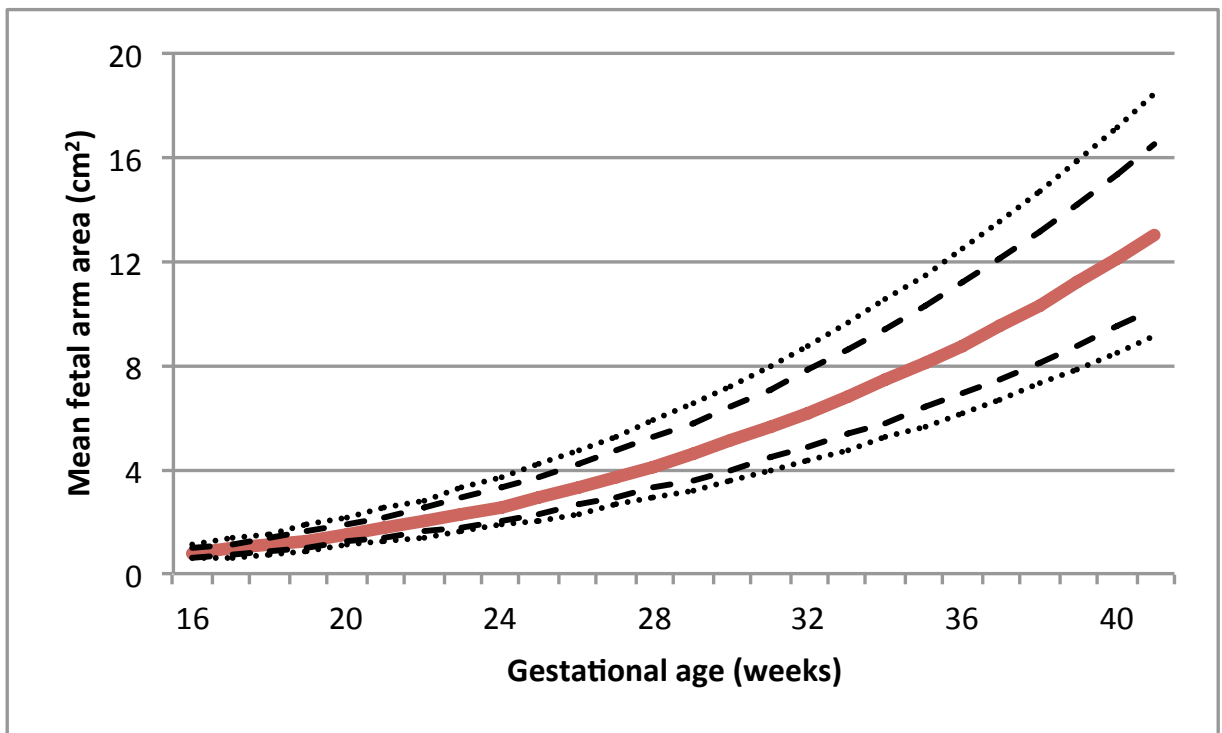


Figure 50: Fetal arm area (3rd, 10th, 50th, 90th and 97th centiles).

The goodness of fit of each set of centiles was checked visually against the scatterplots (arm area below [Figure 51]) and by counting the values above and below the 3rd-97th centiles to ensure they were within the expected range (i.e. approximately 6% of data points).

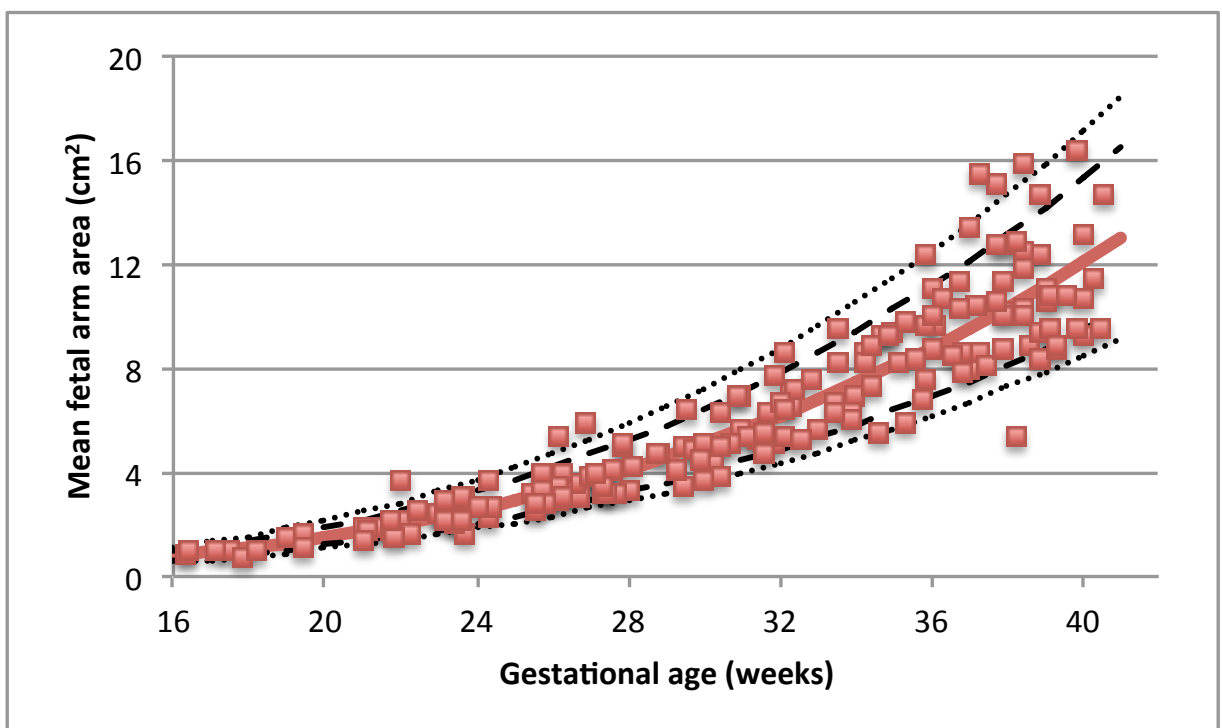


Figure 51: Scatterplot of arm area superimposed over centiles.

Table 11: Centile values for mean arm areas for each week of gestation.

GA (weeks)	Mean fetal arm area (cm ²) centiles						
	3 rd (mean -1.88 SD)	5 th (mean - 1.64 SD)	10 th (mean - 1.28 SD)	50 th (mean)	90 th (mean + 1.28 SD)	95 th (mean + 1.64 SD)	97 th (mean +1.88 SD)
16	0.54	0.57	0.61	0.77	0.98	1.05	1.10
17	0.65	0.68	0.73	0.93	1.18	1.26	1.31
18	0.77	0.81	0.87	1.10	1.40	1.49	1.56
19	0.91	0.95	1.02	1.29	1.64	1.75	1.84
20	1.06	1.11	1.19	1.51	1.91	2.05	2.14
21	1.23	1.29	1.37	1.74	2.22	2.37	2.48
22	1.41	1.48	1.58	2.01	2.55	2.72	2.85
23	1.61	1.69	1.81	2.29	2.91	3.11	3.26
24	1.83	1.92	2.05	2.60	3.31	3.54	3.70
25	2.07	2.17	2.32	2.94	3.74	4.00	4.18
26	2.33	2.44	2.61	3.31	4.20	4.50	4.70
27	2.61	2.73	2.92	3.71	4.71	5.04	5.27
28	2.91	3.05	3.26	4.14	5.25	5.62	5.87
29	3.24	3.38	3.62	4.60	5.83	6.24	6.53
30	3.58	3.75	4.01	5.09	6.46	6.91	7.22
31	3.95	4.13	4.42	5.61	7.13	7.62	7.97
32	4.35	4.55	4.86	6.17	7.84	8.38	8.77
33	4.77	4.99	5.33	6.77	8.60	9.19	9.62
34	5.21	5.45	5.83	7.41	9.40	10.06	10.52
35	5.69	5.95	6.36	8.08	10.26	10.97	11.47
36	6.19	6.47	6.92	8.79	11.16	11.94	12.48
37	6.72	7.03	7.52	9.54	12.12	12.96	13.55
38	7.28	7.61	8.14	10.34	13.13	14.04	14.68
39	7.87	8.23	8.80	11.18	14.19	15.18	15.87
40	8.49	8.88	9.50	12.06	15.31	16.37	17.12
41	9.14	9.56	10.23	12.99	16.49	17.63	18.44

Discussion

In this study, it was found that fetal arm fat increased 29-fold from 16 weeks (50th centile 0.30cm²) to 41 weeks (8.72cm²) This is in line with the chemical composition studies by Widdowson in 1951,⁸⁸ and with more recent work,⁸⁵ which suggested in excess of a 30-fold increase in fetal whole body fat from second trimester to term (0.5% to >16%). In the third trimester, this study showed a 4-fold increase in fetal arm fat from 28 weeks to 41 weeks (2.23cm² to 8.72cm²) which reflects Widdowson's 4.5-fold increase in fetal whole body fat (3.5% to >16%).⁸⁵ This therefore provides preliminary evidence that changes in fetal arm fat are representative of fetal whole body fat changes *in utero*; and that fetal arm fat can be reliably measured using non-invasive tools.

The lean tissue in the fetal arm increased 9-fold from 16 weeks (50th centile value 0.47cm²) to 41 weeks (4.37cm²) thus showing a slower rate of increase that was near linear, rather than the accelerated deposition of fat [Figure 52]. This is harder to relate to chemical composition studies as the whole body fat-free mass includes protein (muscles and organs) as well as bone and water.⁸⁸ There was a 2-fold increase in lean tissue from 28 to 41 weeks.

Comparison of 50th centiles shows more lean tissue until 24 weeks, similar amounts at 25 weeks, and more fat from 26 weeks. Parretti's study of normal and impaired glucose tolerance (IGT) pregnancies showed differences in arm fat at 25 weeks;⁹⁶ Galan found differences at 26 weeks in fetuses at different altitudes.⁹⁵ Lee showed an increasing rate of fat deposition from 27-28 weeks.¹⁴² These suggest that late second / early third trimester is an important time for increasing deposition of fetal arm fat. Larciprete's study found a difference in arm fat from 31 weeks in normal and GDM pregnancies.¹¹⁶

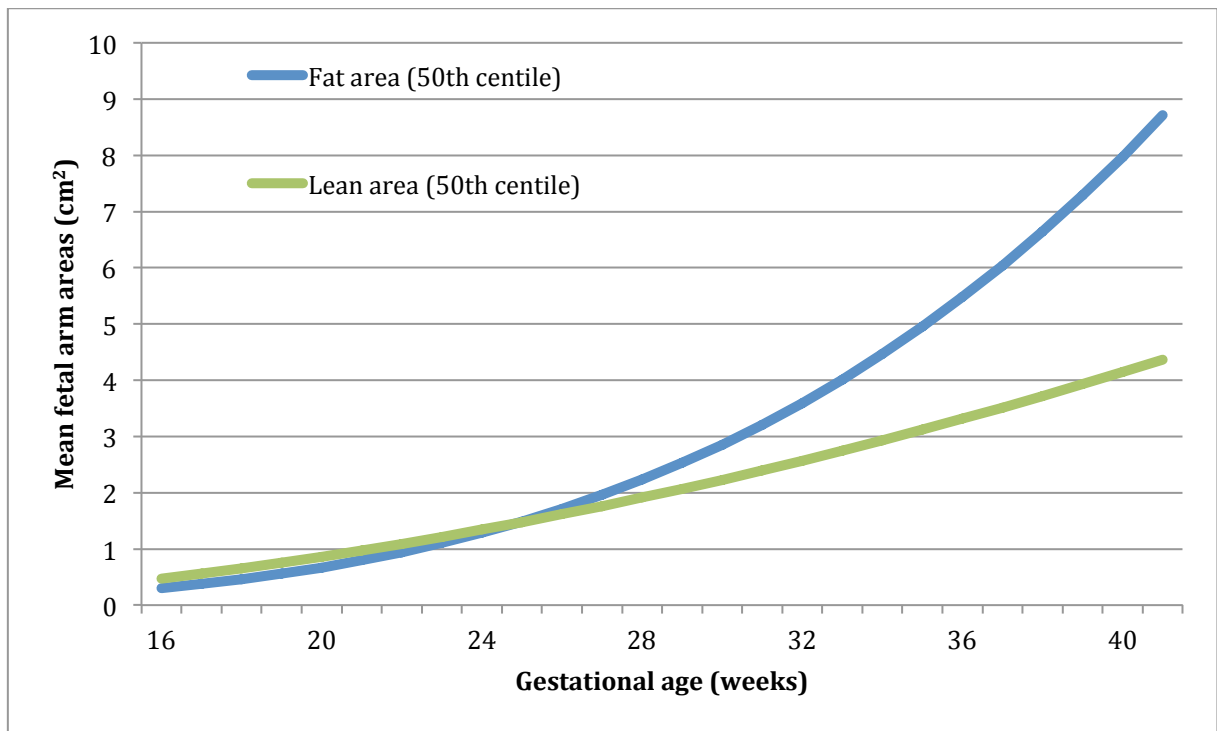


Figure 52: 50th centile lines for mean fetal arm fat and lean areas (mid-humeral axial slice).

This thesis showed a wider distribution of fetal arm fat area values in the third trimester than the second trimester, consistent with variations in nutrition and growth [Figure 42]. The lean area values only showed a very slight change during the same period [Figure 43].

This research adds to the small body of published work on normal population ranges of fetal arm fat and lean areas [Table 12], and for the first time, provides this across a wide gestational age range (16-40 weeks). Importantly, this is the first study to assess women prospectively selected to be of optimal health and nutrition, and therefore shows the potential for normal fetal fat deposition in fetuses under optimal conditions (prescriptive standard, rather than descriptive range).

Table 12: Comparison of papers providing values / graphs for fetal arm fat and lean area in healthy subjects.

	Bernstein 1997¹¹²	Galan 2001⁹⁵	Larciprete 2003¹¹⁶	Parretti 2003⁹⁶	Larciprete 2005¹¹⁴	This thesis 2014
Patients	36	48	218	123	14	106
PMHx	None	None	No HT/DM	No HT/GDM	'Healthy'	None
Smoking	No	No	---	No	---	No
Age	---	24.1	27.4	32.5	28.4	29.7
BMI	Normal	---	24.3	22.7	26.8	23.3
POHx	None; singleton	None; singleton	None; singleton	None; singleton	None; P0, singleton	None; singleton
GA	LMP, 2 nd trim scan	LMP, 2 nd trim scan	'Certain' (?method)	1 st trim scan	LMP, 1 st /2 nd trim scan	LMP, 1 st trim scan
Country	USA (Vermont)	Italy (Milan)	Italy(Rome Florence)	Italy (Florence)	Italy (Rome)	UK (Oxford)
Other	---	Sea level	Controls for GDM	Controls for IGT	Controls for FGR	Optimally healthy
GTT	Normal	---	Normal	Normal	---	---
GA range	19-40wk	19-38wk	20-40wk	24-38wk	30-31wk	16-40wk
Scan freq	4-weekly	3-4-weekly	3-weekly	3-weekly	Once	4-6-weekly
Total scans	135	---	---	---	14	180
ARM FAT 29-31wk	225mm ² (30wk)*	2.51cm ² # (30wk)	3.00cm ² (29-31wk)	3.5cm ² (30wk)*	3.5cm ² (30-31wk)	2.85cm ² (30wk)
ARM FAT 38-40wk	475mm ² (38wk)* 550mm ² (40wk)*	5.52cm ² # (38wk) 6.55cm ² # (40wk)	6.32cm ² (38-40wk)	6cm ² (38wk)*	n/a	6.64cm ² (38wk) 7.98cm ² (40wk)
ARM LEAN 29-31wk	247mm ² (30wk)	2.15cm ² # (30wk)	2.24cm ² (29-31wk)	3cm ² (30wk)*	2.1cm ² (30-31wk)	2.23cm ² (30 wk)
ARM LEAN 38-40wk	424mm ² (38wk) 475mm ² (40wk)	3.85cm ² # (38wk) 4.36cm ² # (40wk)	4.08cm ² (38-40wk)	5.5cm ² (38wk)*	n/a	3.72cm ² (38wk) 4.15cm ² (40wk)

Values listed are 50th centile, or mean values if centiles not provided

--- not stated

* from graph (otherwise from tables / calculated from provided equations)

documented as mm² on graph (eg "3.85mm2") but values evidently in cm²

NB the Bernstein equation for fetal arm fat incorporated maternal age and maternal pre-pregnancy weight – these values could not be reproduced, therefore graphs were used.

This prescriptive approach in optimally healthy pregnancies may explain why, although a similar growth curve of increasing arm fat is seen, our babies had a somewhat higher overall amount of fat at term: fetal arm fat area at 38-40 weeks was 4.08-5.5cm² (50th centile) in previous studies, compared with 6.64cm², 7.29cm², and 7.98cm² at 38, 39 and 40 weeks respectively in this study [Table 12]. Overall, the lean measurements in this study are similar to measurements from previous papers.

Our women were carefully selected to be optimally healthy, and represent the optimal environment for fetal growth. Previous studies were conducted 10-18 years ago so some of the differences in fetal arm fat could have arisen from changes in population nutrition and growth during this time. Diets may have changed, and world populations are getting taller and heavier; European adults are approximately 10-30mm taller per decade,²⁰⁸ whilst the WHO has reported that worldwide obesity has nearly doubled since 1980.²⁰⁹

There have also been increases in birth weight, which the differences in arm fat may reflect. A study from Ohio, USA showed an increasing average birth weight of 3360g (1930-49), 3490g (1970-89), and 3520g (1990-2008); the percentage of obese or overweight mothers also increased from 17.9% to 47.6% (1930-2008).²¹⁰ UK births showed an upwards trend from 1970 to 2008, for both male (3345g to 3401g) and female infants (3246g to 3288g).²¹¹ Many developing countries have shown a similar birthweight increase of 50-60g over a 10-20 year period [Figure 53].²¹² There are also a greater number of babies weighing >4000g at birth: 8.5% to 11.5% in the UK (1984-2000),²¹³ and 9.2% to 10.9% in Australia (1990-2005).²¹⁴ Contributing factors include increasing maternal age, increasing gestational diabetes (partly related to maternal obesity) and decreasing smoking rates.^{213,214}

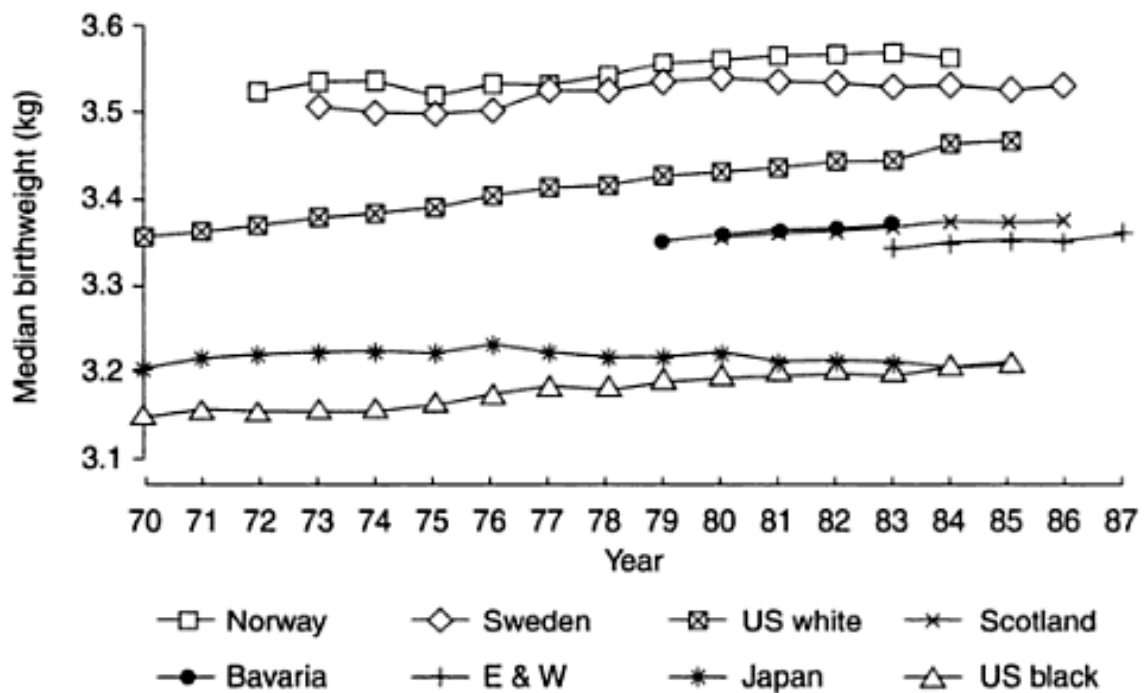


Figure 53: Trends in median birthweight by selected countries (taken from Spencer 2003²¹²).

Variations in the fetal arm fat study designs may have also influenced the results or increased the risk of bias. A recent systematic review showed a wide range of methodological approaches in studies of ultrasound biometry, many of which could have introduced bias.²¹⁵ For example, all of the fetal arm fat studies above made efforts to confirm gestational age but the methods used were variable – some used a 1st trimester scan to confirm EDD by LMP whilst others used a 2nd trimester scan – which could have introduced variation in the results.

In general, attempts were made in the studies of fetal arm area to control for factors known to affect growth (whether SGA or LGA), including avoiding medical conditions such as hypertension or diabetes (for controls). Mean age and BMI (where stated) were also within a healthy range in most of the studies (other than mean BMI >25 in Larciprete 2005^{114,116}).

There may also have been methodological differences. The measurement techniques employed in this research appear to be similar to those used by other groups, based on published images by Larciprete et al.^{114,116} and Parretti et al.⁹⁶ Galan et al.'s images show a slight variation in the tracing of the inner fat border with some disregarding of septae / fat-muscle border irregularities,⁹⁵ but this is unlikely to be enough to account for the differences. In addition, the extra increase in fat area in this research (1.22-1.95cm²) is greater than the decrease in lean area (0.41-0.81cm²) at 38-40 weeks, so the variation is not simply due to a difference in fat-muscle border interpretation. It is also possible that improvements in ultrasound technology have contributed to the different results, enabling more accurate measurement of fetal fat.

The work presented in this chapter also considered two other measurements of the fetal arm: whole fetal arm area of a mid-humeral axial slice [Figure 44] and fetal arm fat as a percentage of whole arm area [Figure 48 - Figure 49]. These are both novel measurements which have not been reported in the scientific literature.

Fetal whole mid-axial slice arm area has been measured previously by the groups assessing fetal fat areas [Table 12] – the lean area is subtracted from the arm area to give the fat area – but has not been reported as a separate entity. I focused on fetal arm and lean areas as the more important measurements, potentially representative of fetal body composition *in utero*.

The arm area represents both lean and fat compartments of the arm. If the increase in lean measurements across gestation was a purely linear relationship, then arm area could be taken as a surrogate for arm fat; however the lean area, whilst not so striking in its changes as arm area, still has a curvilinear relationship with gestational age [Figure 47]. It therefore seems more clinically relevant to study arm fat as an individual measurement.

We undertook to assess fetal arm fat as a percentage of arm area to normalise the data for size; a larger baby and a smaller baby of the same gestational age would have different absolute areas of arm fat but may have similar percentages. This was suggested by visual assessment of the limbs during ultrasound scanning, where the arm appeared to have a higher percentage of fat than the thigh [Figure 13]. Arm fat increased from 37.8% of arm area at 16 weeks to 66.2% at 41 weeks, and the scatterplots and centiles show less variation than those of the absolute fat areas. As might have been expected, the week-by-week change in percentage was quite small (an increase of 1.8 from week 16 to 17 but only 0.7 from week 40 to 41) which could suggest it might be more difficult to distinguish subtle changes in fat deposition using the percentage approach.

Conclusion

This work has defined, for the first time, the amount of fetal arm fat measured by ultrasound in an optimally healthy population. Whilst in general the ranges are similar to those produced by previous studies, we have shown an increased amount of fetal arm fat near term. This normal range will be useful as a reference range for comparison studies involving non-optimally healthy populations. Lean and whole arm areas were also assessed; we also introduce the novel concept of arm fat expressed as a percentage of arm area.

ii. Reproducibility of manual segmentation of fetal arm fat

Aim

To assess technique reproducibility (intra- and inter-observer variability) for 2D fetal arm fat area segmentation, and compare these with standard fetal biometry.

Method

Inter-observer variability: 68 fetal arm images were segmented twice (as per the protocol) by two experts (the author and S.R.) and the inter-observer variability was calculated. The segmentations were carried out separately to avoid any bias that may have occurred from segmenting at the same time.

Intra-observer variability: the 180 scans used for the study above (section 4c.i) were segmented twice by the author. The two segmentations were performed several days apart, and with large sets of images at a time, to reduce the risk of recall bias.

The results were displayed visually using Bland-Altman plots, comparing the mean of the two measurements against the difference between them.²¹⁶ Percentages were used rather than absolute numbers as this enabled better comparison across different gestational ages.

Results

The inter-observer variability was calculated on scans ranging from 21-36 weeks of gestation; the intra-observer variability was calculated on scans ranging from 16-40 weeks.

Intra-observer variability was 1.2% with a $\pm 13.9\%$ limit of agreement; inter-observer variability was 6.2% with a $\pm 14.1\%$ limit of agreement [Figure 54]. The intraclass correlation coefficients (ICC) were 0.995 and 0.979, showing a high level of agreement.

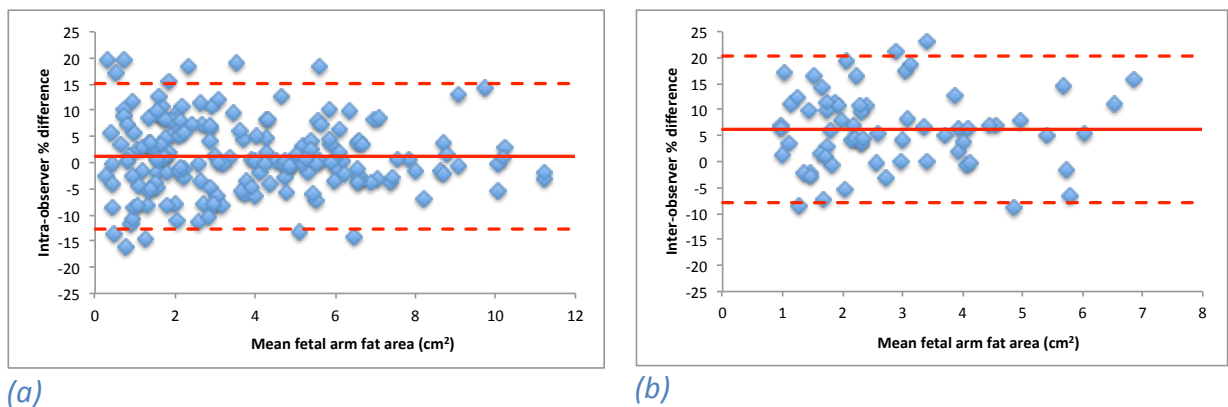


Figure 54: Bland-Altman plots of (a) intra-observer (n=180) and (b) inter-observer (n=68) variability in fetal arm fat area.

Discussion

As is usual when comparing intra- and inter-observer variability, the mean difference in measurements was smaller for intra-observer than for inter-observer variability, and the ICC was a lower value, reflecting that repeatability by the same observer is likely to be better than that between two observers. There may also have been small differences in variability due to the differing backgrounds of the two assessors, as clinicians and engineers may interpret ultrasound images in a slightly different manner.

The random measurement error expressed by the limits of agreement ($\pm 13.9\%$) is larger than errors in linear measurements. It should be noted that part of this is an intrinsic issue, as an area (two dimensions) has the potential for larger error than a linear measurement; errors in volumetry (three dimensions) are larger still [Table 13].

In addition to the variation provided by the dimensions of measurements, compound errors are introduced as the arm fat area is measured by using a freehand tracing technique (likely to be less reproducible than ellipse or distance callipers), with each being calculated by subtraction of the lean compartment from the whole arm area, i.e. two compartments each subject to freehand tracing errors.

Table 13: Intra- and inter-observer variability (with limits of agreement) for different biometric measurements and different dimensions.

	Intra-observer	Inter-observer	Reference
HC (one dimension)	0.0% ($\pm 3.0\%$)	0.5% ($\pm 4.9\%$)	Sarris et al 2012 ²¹⁷
AC (one dimension)	-0.3% ($\pm 5.3\%$)	1.2% ($\pm 8.7\%$)	Sarris et al 2012 ²¹⁷
FL (one dimension)	0.2% ($\pm 6.5\%$)	0.0% ($\pm 11.1\%$)	Sarris et al 2012 ²¹⁷
Arm fat area (two dimensions)	1.2% ($\pm 13.9\%$)	6.2% ($\pm 14.1\%$)	Presented in this thesis 2014
Femur volume (three dimensions)	0.9% ($\pm 32\%$)	0.2% ($\pm 58\%$)	Ioannou 2012 ²¹⁸

The intra-observer mean difference in measurement decreased with a higher number of segmented scans: 3.7% after the first 68 sets of segmentations, 1.2% overall after 180 sets and 0.5% for the last 50 sets (not shown separately). This reflects increased expertise with the segmentation technique, and shows a low systematic bias. It is also at a similar level to intra-observer variability measurements for standard fetal biometry from a recent Australian study (1.1-1.7%).²¹⁹

The limits of agreement did not show the same changes with increasing expertise, but remained at about the same level (+/-14.7%, +/-13.9%, +/-14.9%), suggesting this may reflect the true limits of agreement for fat area measurement. This is an acceptable level for an ultrasound derived measurement, and is similar to the 95% confidence interval of 15-18% of Hadlock's various EFW formulae (which involve combining two to four different measurements).²¹⁹ It should be noted that the results in this study represent variation of re-measuring the same scan image; measuring two different images from the same scan would introduce additional variation.

I have focused on fat area in this section, as this is the key measurement in terms of fetal body composition; however it is of interest to compare the intra-observer variability of the other measurements [Table 14]:

Table 14: Intra-observer differences in arm measurements (expressed as percentages).

Measurement	Mean difference	Limit of agreement
Arm fat area	1.2%	+/-13.9%
Arm lean area	-0.2%	+/-9.1%
Whole arm area	0.7%	+/-7.2%
Fat expressed as % of arm area	0.6%	+/-8.6%

This shows a clear difference, with the arm fat area having the highest mean difference and limits of agreement. This may be due to the fact that it is calculated from two freehand tracing methods (the total arm area minus the lean area), whereas the whole arm and arm lean areas are calculated from a single tracing method. The limits of agreement for the non-fat area measurements are similar to those shown for abdominal circumference (+/-8.7%) by Sarris et al.²¹⁷

Conclusion

This reproducibility study provides evidence of a low systematic bias in the measurement of fetal arm fat area, and limits of agreement which are in line with that of other fetal biometric measurements. As a secondary measure, it also provides evidence (through a decreasing mean difference measurement) for improved technique as an assessor gains experience in segmenting the fat in fetal arm images.

4d. Longitudinal studies (2D) in individual fetuses

Aim

To show longitudinal results for individual fetuses – rather than a population cross-section – to demonstrate the possibility of individual growth curve assessment.

Methods

This was a sub-analysis of the fetal arm scans used in the previous experiment. The scans were assessed, and results from fetuses which had been scanned 3-6 times were extracted. These results were then plotted in a longitudinal manner, and results from individual fetuses compared.

Results

Of the 106 women whose 180 scans had been measured, 16 had results from 3-6 scans. This resulted in a total of 73 scans. The mean arm fat area measurements were then plotted in a longitudinal manner for each fetus, and the graphs superimposed [Figure 55].

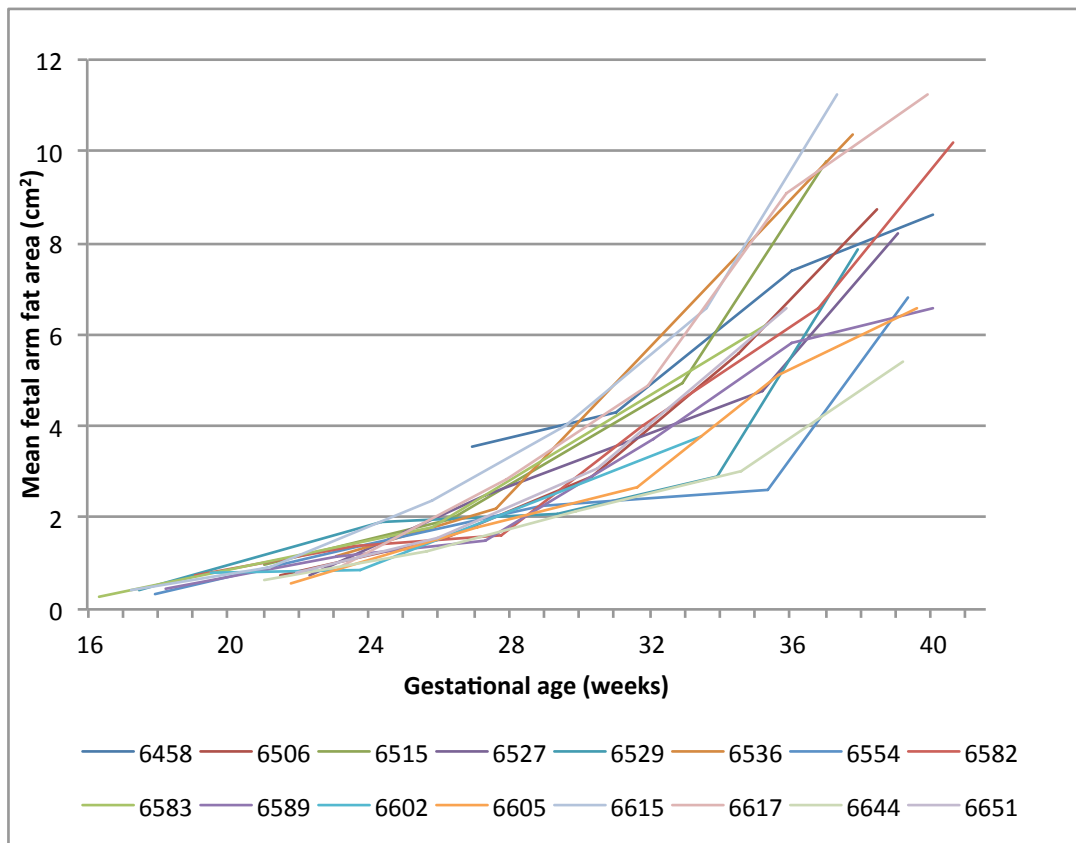


Figure 55: Longitudinal development of fetal arm fat demonstrated in 16 fetuses.

Discussion

These plots of fetal arm fat in individual fetuses show relative uniformity of growth from 16 weeks until about 28-30 weeks of pregnancy, with a wider range of mid-humeral fetal arm fat areas towards term. This reflects variations in individual growth trajectories, and mirrors the cross-sectional data from this cohort [Figure 42].

These are pregnancies in optimally healthy women (the INTERGROWTH-21st study cohort) so it would be expected that the majority of these fetuses would be optimally healthy and meet their growth potential. When curves of individual fetuses were assessed, however, they did show some variation in pattern [Figure 56 - Figure 57]:

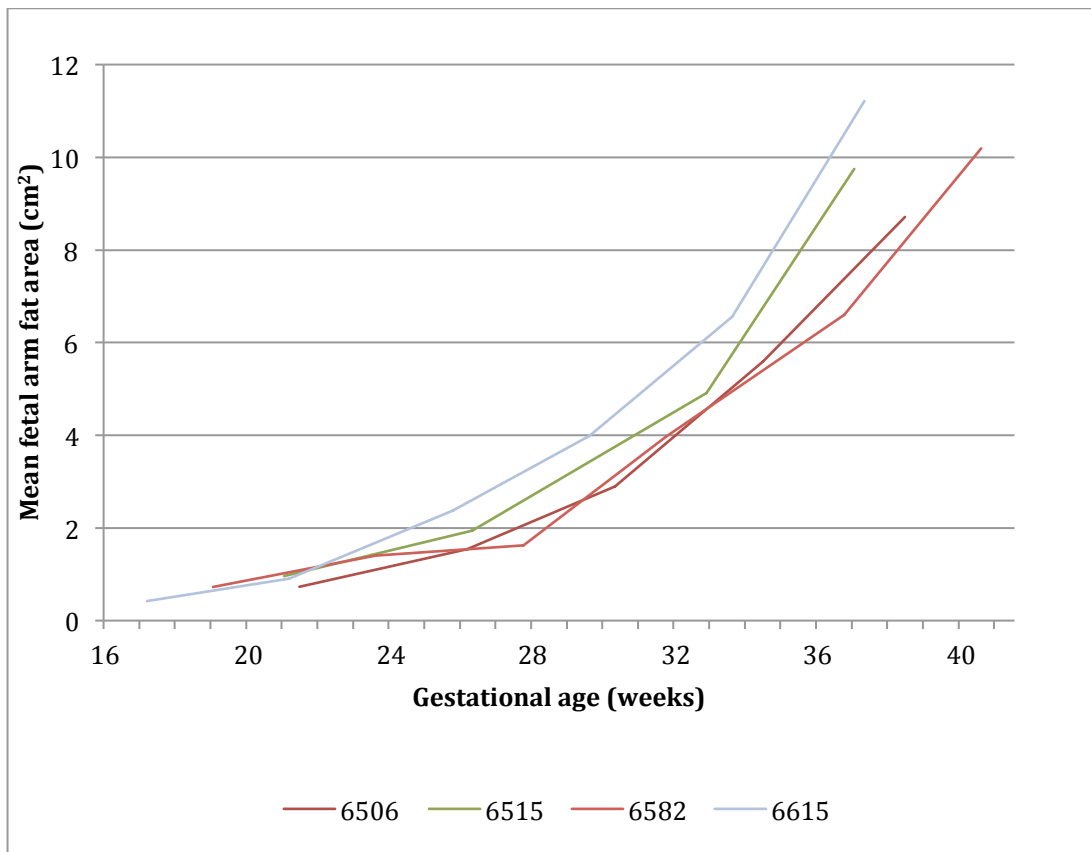


Figure 56: Graph showing increasing fetal arm fat area across gestational age, following the curve of the population 50th centile [Figure 52].

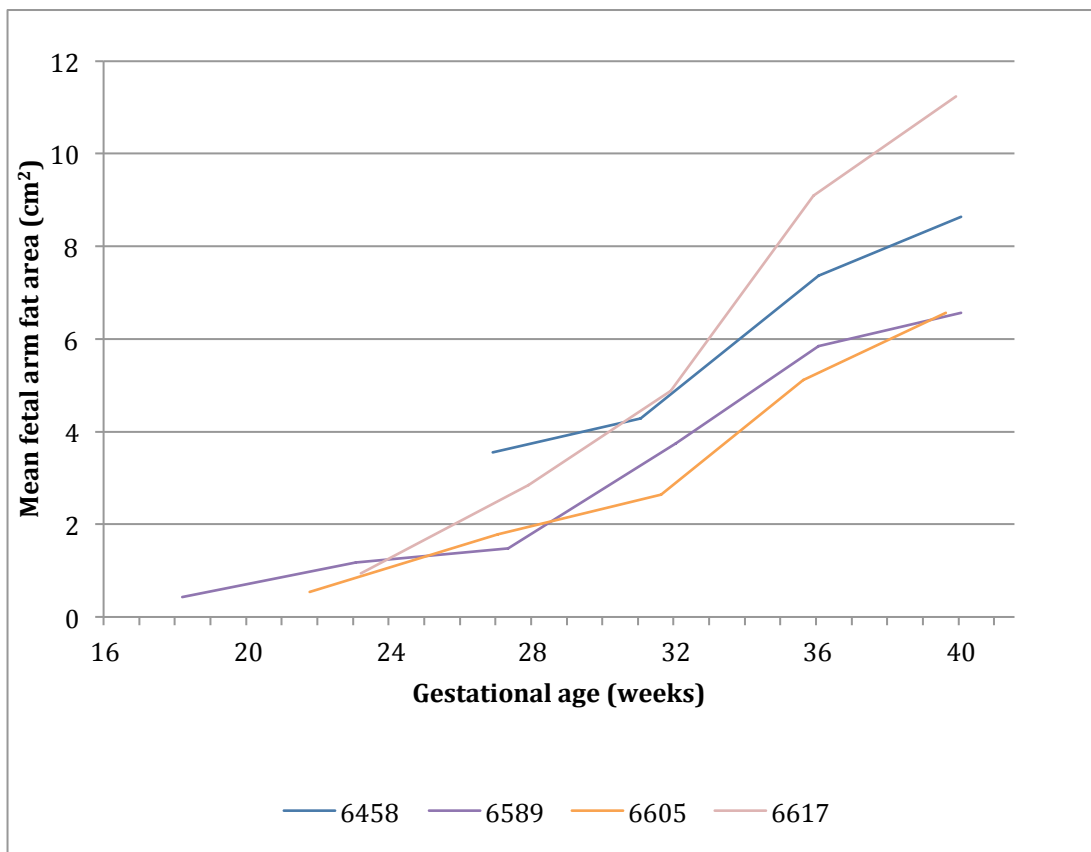


Figure 57: Graph showing slowing velocity of the rate of increase of fat area in the third trimester, when compared with the curve of the population 50th centile [Figure 52].

All fetuses showed an initial upwards fat area trajectory and some continued to grow healthily, following the expected curve and demonstrating an increasing laying down of fat, particularly in the third trimester, with accelerated growth until the last scan [Figure 56].

Others, after the initial upwards fat area trajectory, showed a slowing of velocity of the rate of increase of arm fat area in the mid to late third trimester [Figure 57] – the period of key interest for the assessment of late-onset FGR. It is possible that this may reflect subtle changes in placental function affecting fetal nutrition.

In order to investigate this further, I examined the birthweight and newborn percentage body fat (both absolute values and centiles) [Table 15]:

Table 15: Neonatal birthweight and body fat for fetuses showing different patterns of arm fat growth.

	ID	GA at birth	Sex	Birthweight	Weight centile ²²⁰	PEA POD %body fat	PEA POD centile ²²¹
Normal arm fat growth 3 rd trimester	6506	40 ⁺⁶	m	4178g	91 th	17.3%	95-97.5 th
	6515	40 ⁺³	f	3669g	74 th	13.0%	50-75 th
	6582	41⁺¹	m	3262g	32nd	10.0%	50-75th
	6615	42 ⁺¹	f	4354g	95 th	16.0%	75-90 th
	<i>Mean</i>	<i>41⁺¹</i>	---	<i>3866g</i>	<i>73rd</i>	<i>14.1%</i>	<i>75th (median)</i>
Slow arm fat growth 3 rd trimester	6458	39 ⁺⁶	f	3736g	79 th	15.6%	75-90 th
	6589	41 ⁺⁶	m	3407g	43 th	9.7%	25-50 th
	6605	41⁺⁶	m	3632g	60th	6.2%	10-25th
	6617	40 ⁺⁶	m	4114g	89 th	12.5%	50-75 th
	<i>Mean</i>	<i>41⁺¹</i>	---	<i>3722g</i>	<i>68th</i>	<i>11.0%</i>	<i>50th (median)</i>

At first glance, these results look rather unremarkable. As would be expected from an optimally healthy pregnant population, all eight babies were born at term and were of normal birthweight (ranges of 3262-4354g, within 32nd-95th centiles, taken from the UK-WHO 0-4 years sex-specific weight charts²²⁰). A closer examination, however, shows some differences. Whilst most babies' weight centiles are reflected in their whole body fat centiles, there are two babies (shown in bold in the table) where this is clearly not the case.

Baby 6582 (normal arm fat growth), whose birthweight was on the 32nd centile, had a body fat range in the 50-75th centile; thus whilst his weight was below average, his body fat was above average. This would not make a particular difference in this case, but could be important if a similar difference were to be demonstrated in a <10th centile baby. Meanwhile, baby 6605 (slow arm fat growth), had a birthweight on the 60th centile but body fat within the 10-25th centile range. Whereas baby 6582's measurements were only one centile range bracket apart, baby 6605's measurements are two brackets apart, reflecting a more serious discrepancy between the measurements of birthweight and whole body fat, and evidence that birthweight does not necessarily reflect body composition / body fat.

Conclusion

This section provides preliminary evidence for the use of individual growth curves in the assessment of arm fat in individual fetuses, including variations in the development of arm fat in the third trimester. It also provides individual case examples of babies with discrepant birthweight and body fat measurements, reflected in individual arm fat growth patterns. This could be useful for detecting late-onset FGR; it might also be possible that arm fat growth may plateau or fall in FGR, and may markedly accelerate in macrosomic babies.

4e. MRI validation of ultrasound images

Aim

To develop appropriate MRI scanning protocols to obtain good images of fetal limb fat; to obtain a databank of paired ultrasound and MR images for use in future research; and to validate 2D and 3D ultrasound imaging of fetal arm fat using these MR images.

Background

Although the use of ultrasound is well established in pregnancy, it nevertheless has some disadvantages (including shadows and imaging artefacts) when compared with other imaging modalities such as CT or MRI scanning, which provide well-defined anatomical images without acoustic shadows from bones. The measurement of fetal arm fat on ultrasound images, whilst seeming straightforward, provides some challenges including the delineation of the fat borders, and the presence or absence of fat within the intermuscular septae. MRI, with its detailed demonstration of anatomy, may help to resolve these challenges; it could also help to ensure accuracy by validating measurements using different imaging modalities. [See section 1h for additional background information on fetal MRI scanning.]

Method

Ethical approval for this study was obtained from the NRES Committee South Central – Oxford C (REC reference 12/SC/0141). (See appendix C.)

Women were recruited from the INTERBIO-21st cohort at Oxford,¹⁹⁹ following a normal 20-week anomaly scan, and provided they met the inclusion criteria [Table 16]. The women were given verbal and written information about the MRI study (see information leaflet, appendix C) by the INTERBIO-21st research midwife F.R.. Women who were interested, and met the inclusion criteria, were then contacted by the author to answer any questions. The women who agreed were asked to complete a consent form (appendix C) and attended an MRI scan, immediately followed by an ultrasound scan (or vice versa). Monthly recruitment data were uploaded to the UK Clinical Research Network (UKCRN) Portfolio Database.

Table 16: MRI study inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
• Pregnant • Normal routine anomaly ultrasound scan at 18-22 weeks • 18 years of age or older • Weight less than 150kg and abdominal girth of less than 58 inches (the limits of the MRI machine) • Able to understand the information and give written informed consent • No standard contraindications to having an MRI scan	• Fetal abnormality at the anomaly scan • Maternal weight exceeds 150kg or abdominal girth exceeds 58 inches (the limits for the MRI scan machine) • Under 18 years of age • Presence of objects contraindicated in MRI scanning, including: ○ Permanent pacemaker ○ Metal in the eye(s) ○ Any other electronic, mechanical or magnet-sensitive metal implants

The MRI scans were obtained by G.Q. (with radiographers and radiologists) with a Philips 1.5T Achieva machine in the neuroradiology department of the John Radcliffe Hospital, Oxford. Women were asked to complete the standard hospital MRI information and consent form, and to remove all metal-containing clothing, jewellery and accessories. They were scanned feet-first to minimise the risk of claustrophobia, and in a left-lateral or left-tilted position to minimise the risk of aortocaval compression by the gravid uterus. Ear protection was provided as standard, together with a 'panic button' for communication with the imaging team. The total scan time for all required sequences was 20-30 minutes, but with the option of ending the scan early should the woman feel distressed or unable to continue.

The ultrasound scan, immediately preceding or following the MRI scan, was as per the standard INTERBIO-21st protocol, plus the fetal limb scanning protocol (see section 4a.). Additional 2D images and 3D volumes from non-standard biometric planes were obtained of the fetal head and abdomen in order to capture as much ultrasonic detail as possible to enable comparison with the MRI images and to provide a databank of images for future research. The scans had to be timetabled to fit with the requirements of the INTERBIO-21st study, but wherever possible the aim was to collect data from a wide range and even spread of gestational ages, originally from 20 to 41 weeks.

MR images were anonymised and stored, together with the paired ultrasound images, in a databank. Offline analysis of the MRI images was carried out using the Philips programme PmsDView; comparable ultrasound images from the same fetuses were then analysed using the same programme but at a later date, and with no reference to the paired MR images or results, so as to reduce bias.

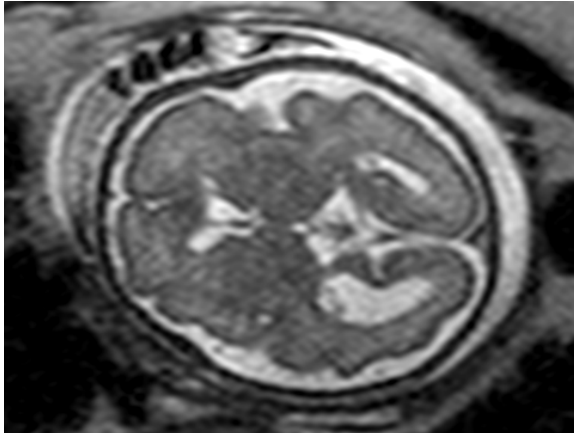
The MRI images were surveyed within the programme, and the best mid-humeral axial arm image selected. The image was magnified to 500% and placed in the central of the screen. The arm skin and arm fat-muscle borders were then outlined, using the smoothed polygon tool, to generate arm and lean areas. Fat area was calculated by subtracting the lean area from the arm area.

Ultrasound images were measured using the same programme. The appropriate DICOM file was loaded and magnified to 150% so as to occupy the central third of the height of the screen. The arm and arm fat-muscle interfaces were then outlined, again using the smoothed polygon tool, in a similar manner to the MR images.

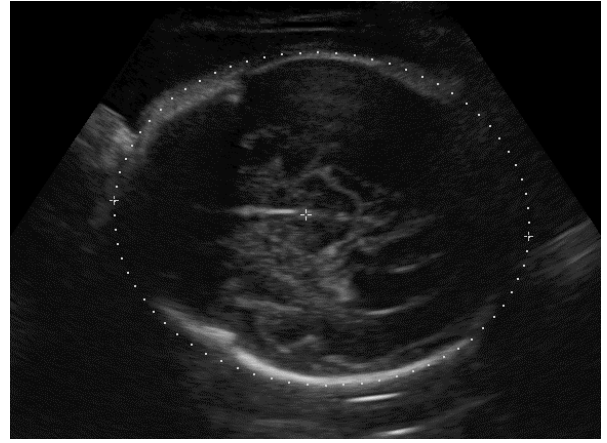
Results and discussion

i. MRI scanning protocol

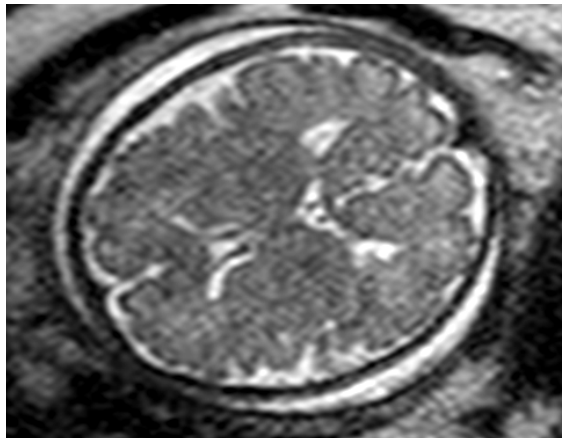
The MRI scanning protocol (see appendix C) was developed through discussion with a consultant neuroradiologist (G.Q.), fetal medicine specialist (A.P.), and biomedical engineer (S.R.), including assessment of images from various standard fetal imaging sequences. At each study, sequences were obtained of the fetal arm (axial), thigh (axial), abdomen (sagittal) and brain (coronal, sagittal and axial) [Figure 58]. These were mostly T2-weighted images (3mm or 4mm), which provided the best anatomical detail, but e-THRIVE (enhanced T1-weighted High Resolution Isotropic Volume Examination) sequences were used to image the fetal liver. This is a fat-suppression technique optimised to provide good contrast and resolution with a fast scan time.²²² Screenshots of the slice selections (setting up the sequence based on localiser or planning scans) were also obtained, as these are helpful for the biomedical engineering techniques of registering and aligning images.



(ai) 31 wk, MRI axial image of brain.



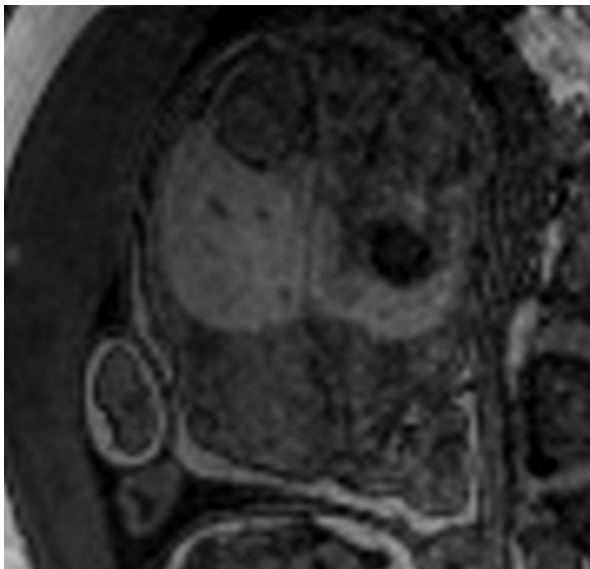
(aii) 31wk, ultrasound transthalamic plane
[same fetus as shown in (ai)].



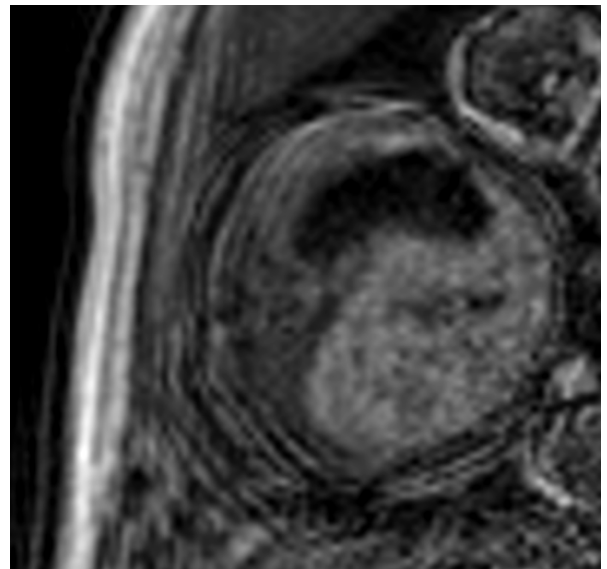
(b) 35 wk, MRI axial image of brain.



(c) 35 wk, MRI sagittal image of brain.



(d) 38 wk, MRI e-THRIVE image of liver
(arm also shown).



(e) 40wk, MRI showing maternal breathing artefact.

Figure 58: Sample fetal MR images – (a-c) T2-weighted, (d-e) T1-weighted e-THRIVE.

ii. Acquisition of MR images for the databank

46 valid pairs of ultrasound and MRI scans were obtained from the 300 women originally given information about the study. [Figure 60] This was better than our pilot enquiries, which suggested that 1 in 8 women would consider taking part in an altruistic MRI study.

Although the aim had been to scan from 20 to 41 weeks, the first obtained at 24 weeks showed that it would be difficult to measure the limb fat even at that gestation, so women were then recruited above 24 weeks. Several scans were arranged for patients at 41 weeks of gestation but they unfortunately all delivered prior to their MRI appointment. Overall, however, MRI scans were obtained at a range of gestational ages, as per the aim:

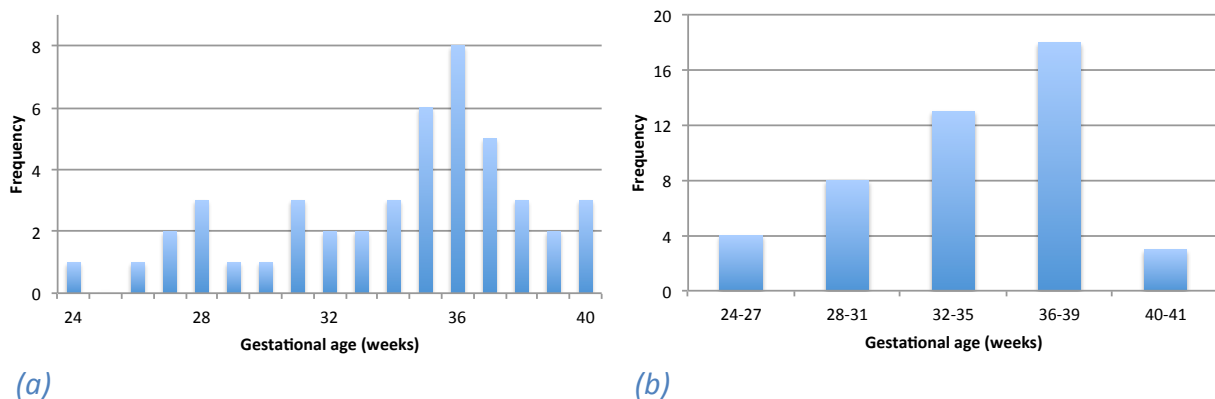


Figure 59: Frequency distribution of fetal MRI scans by gestational age in (a) weeks and (b) 4-weekly groups.

The scan images themselves varied in quantity and quality; some women were not able to tolerate the full scan duration, others had difficulty with the breath-holding required for some sequences; whilst in others, the fetus was very active which caused movement artefacts. Some images thus only had brain and abdomen, or limb and brain, sequences rather than all the protocol images; others had the full set of images but of varying quality.

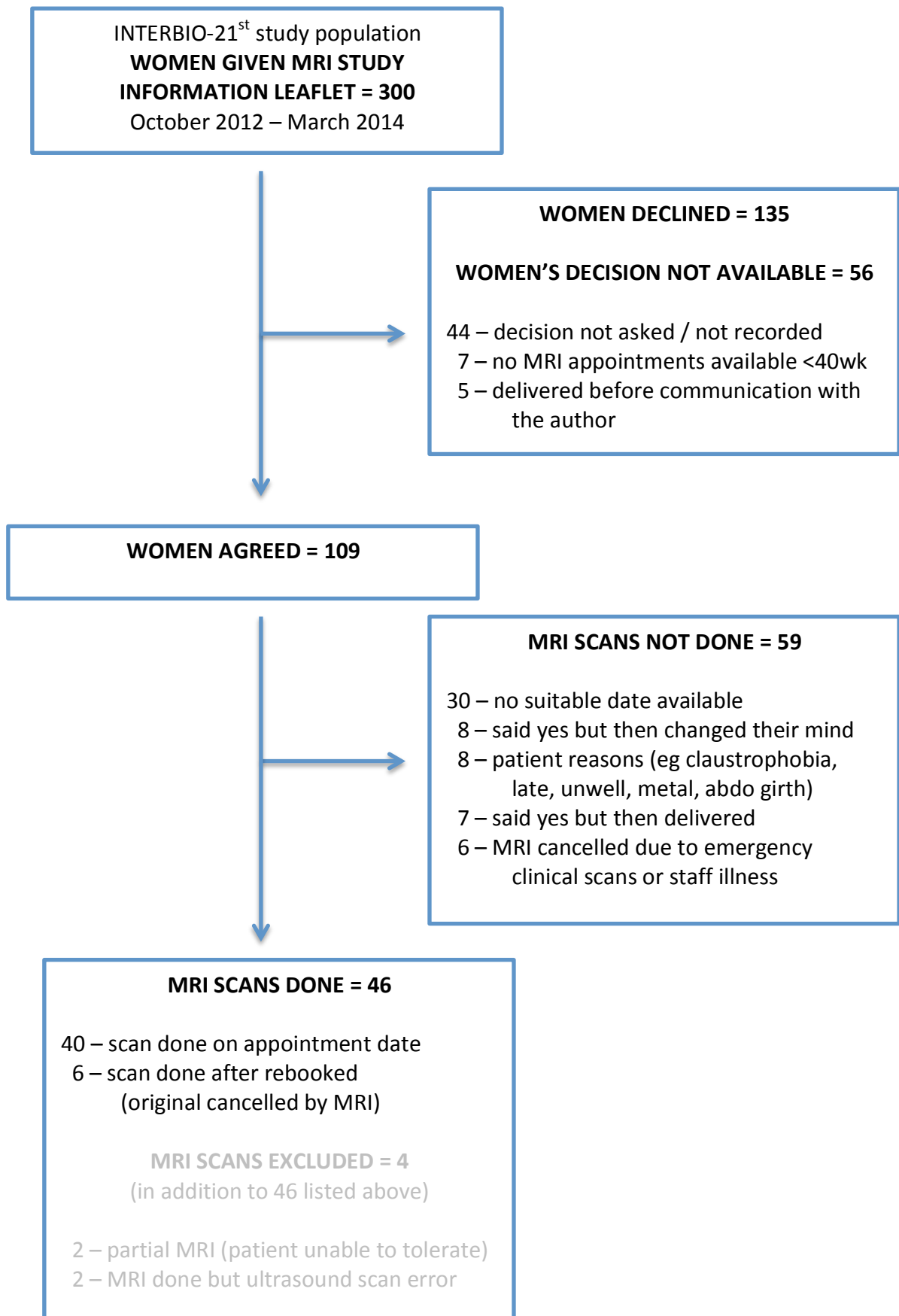
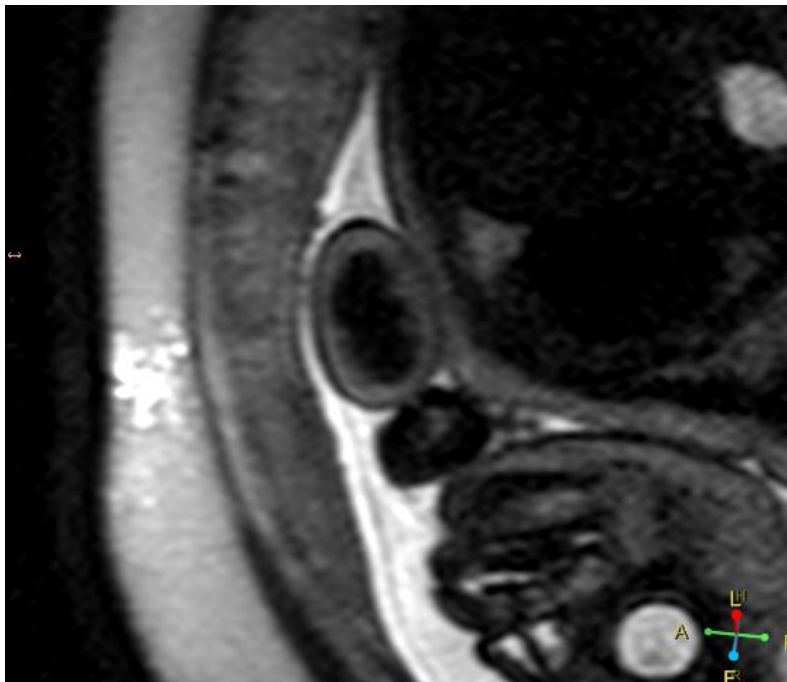


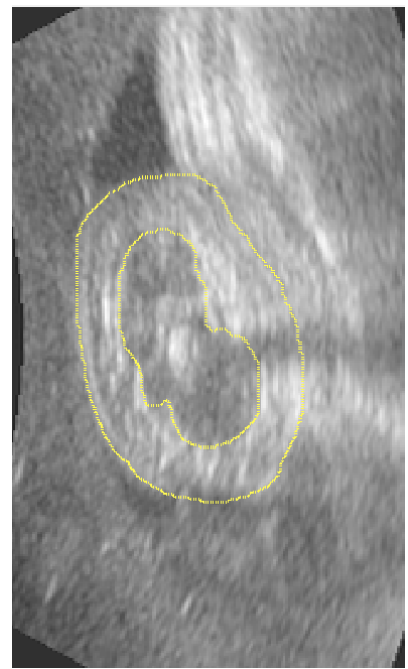
Figure 60: Flow diagram of MRI study recruitment and participants.

iii. Validation of ultrasound images using MRI

33 of the 46 pairs of MRI-ultrasound images had suitable arm images for analysis. As expected, the anatomical detail available with the MRI scans was much greater – for example, the separate delineation of arm skin from arm fat [Figure 61]. Ultrasound images do not show this amount of detail, so the segmentation of the “fat” area includes the skin.



(a) Original MRI, showing cephalic fetus with chest and arm.



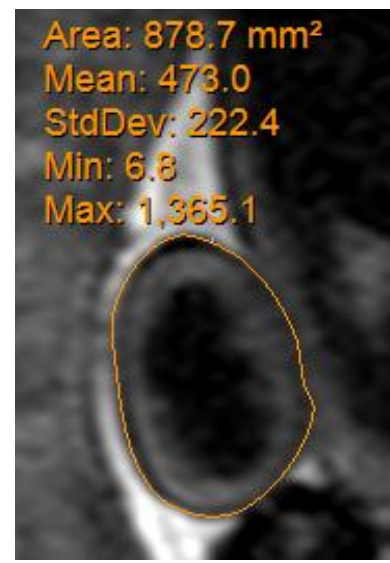
(b) Ultrasound for comparison.



(c) Lean area measurement.



(d) Fat-skin border (error).



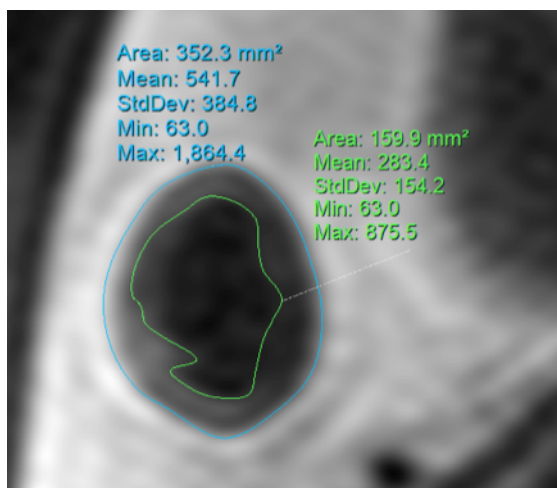
(e) Arm area measurement.

Figure 61: Fetal arm axial images (35 weeks gestation) showing measurement with smoothed polygon.

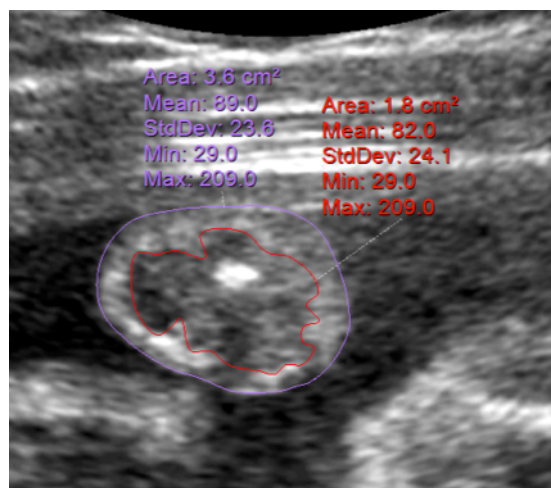
The scans measured were from 28⁺² to 40⁺² weeks of gestation. Some images showed excellent correlation, both visually (for anatomical structure) and in terms of measured areas [Figure 62], whilst others showed considerable variation. This was to be expected in the visual assessment, as although the scans followed each other, some fetuses were still very active so had changed their positions in the intervening time.

In terms of the measurements themselves, some variation was expected due to the acquisition of the mid-humeral slice. For the ultrasound images, the probe was rotated by 90 degrees from a full sagittal length of the humerus to obtain the axial view, and errors could have arisen due to incomplete rotation, or the probe not being completely centred around the mid-point of the humerus. For the MRI images, the central slice was taken from those showing the axial images of the fetal arm; again, this may not have been precisely central and the arm images may not have been completely perpendicular. The fetus may also have moved between the planning and acquisition.

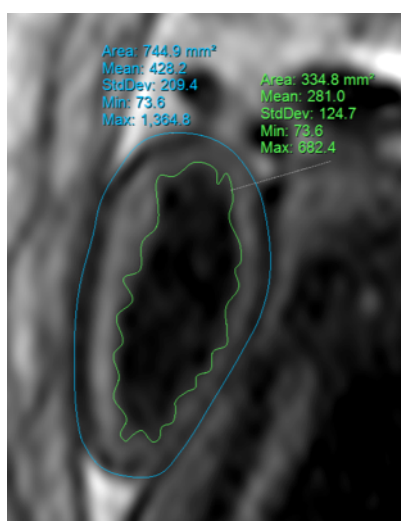
The differences in the measurements of fetal arm fat and lean areas were compared visually using scatterplots, with both MRI and ultrasound measurements plotted on the same axes [Figure 63, Figure 65]. The MRI results were then plotted on the centiles derived from the 2D measurements of arm fat and lean tissue [Figure 46, Figure 47] to compare the results against the previously calculated reference ranges [Figure 64, Figure 66]. Finally, the differences were assessed statistically for each pair of images using Bland-Altman plots [Figure 67] and ICCs.



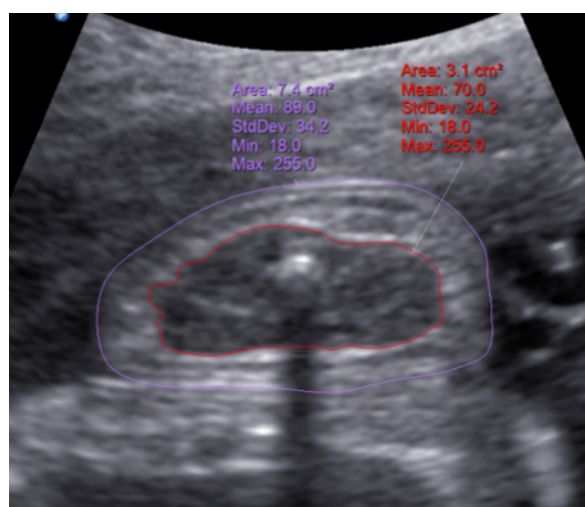
(a) Fetal arm, 28 weeks (MRI).



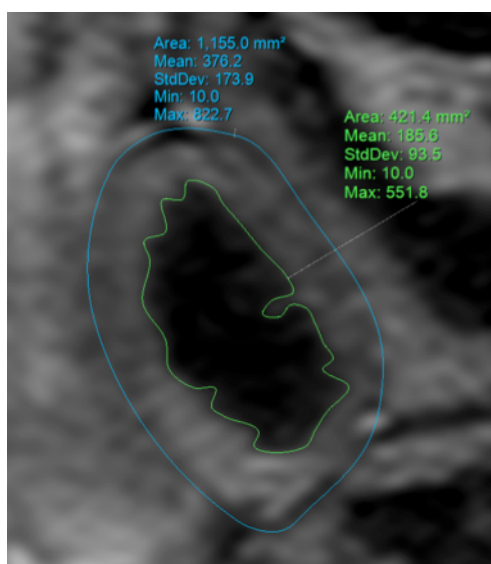
(b) Fetal arm, 28 weeks (ultrasound).



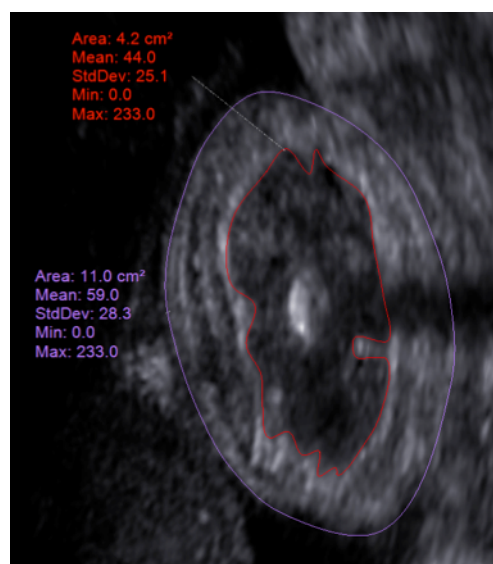
(c) Fetal arm, 34 weeks (MRI).



(d) Fetal arm, 34 weeks (ultrasound).



(e) Fetal arm, 40 weeks (MRI).



(f) Fetal arm, 40 weeks (ultrasound, rotated).

Figure 62: Paired MRI and ultrasound images showing good correlation of anatomy and areas.

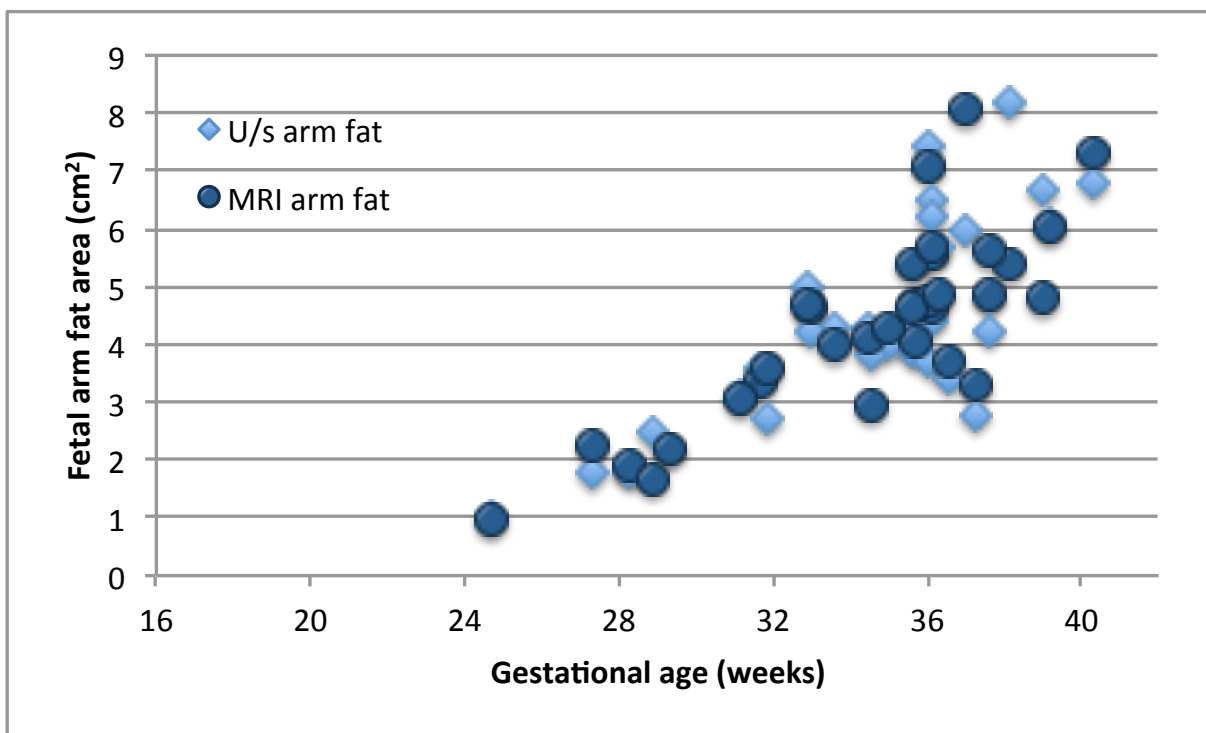


Figure 63: Fetal arm fat area calculated on pairs of MR and ultrasound images from the same fetus.

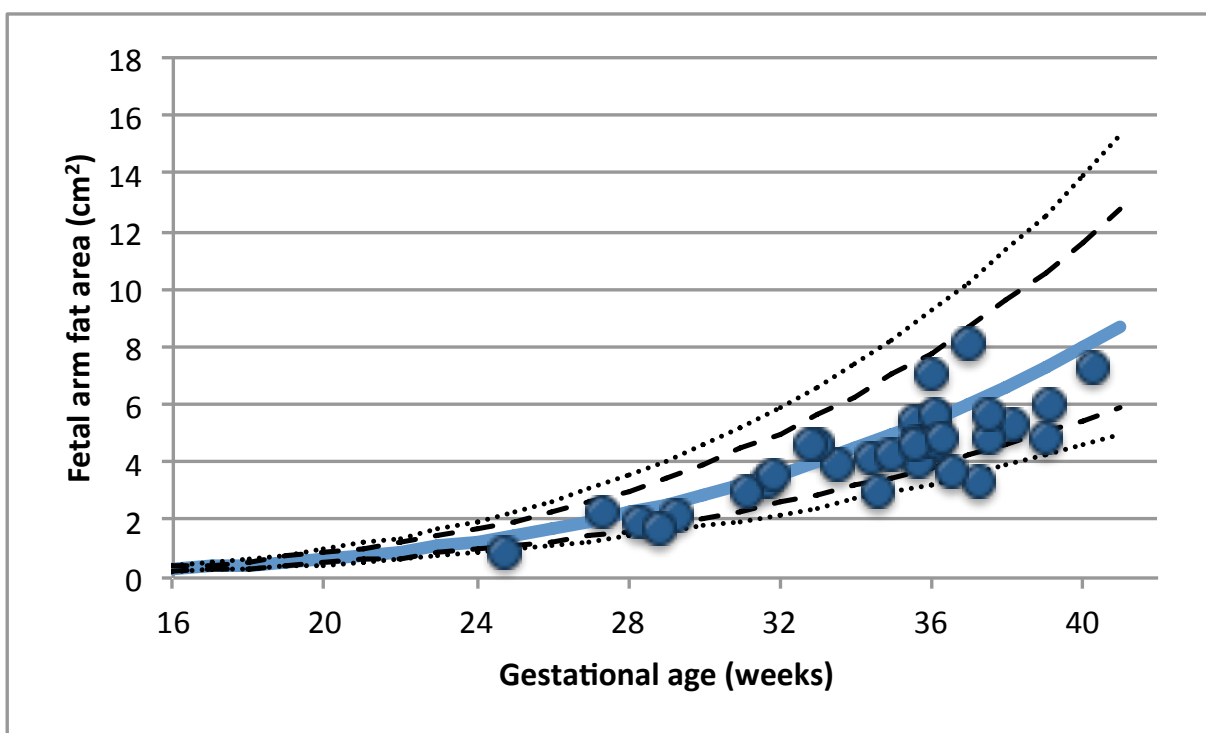


Figure 64: Fetal arm fat area measured on MRI superimposed against centile charts from 2D ultrasound.

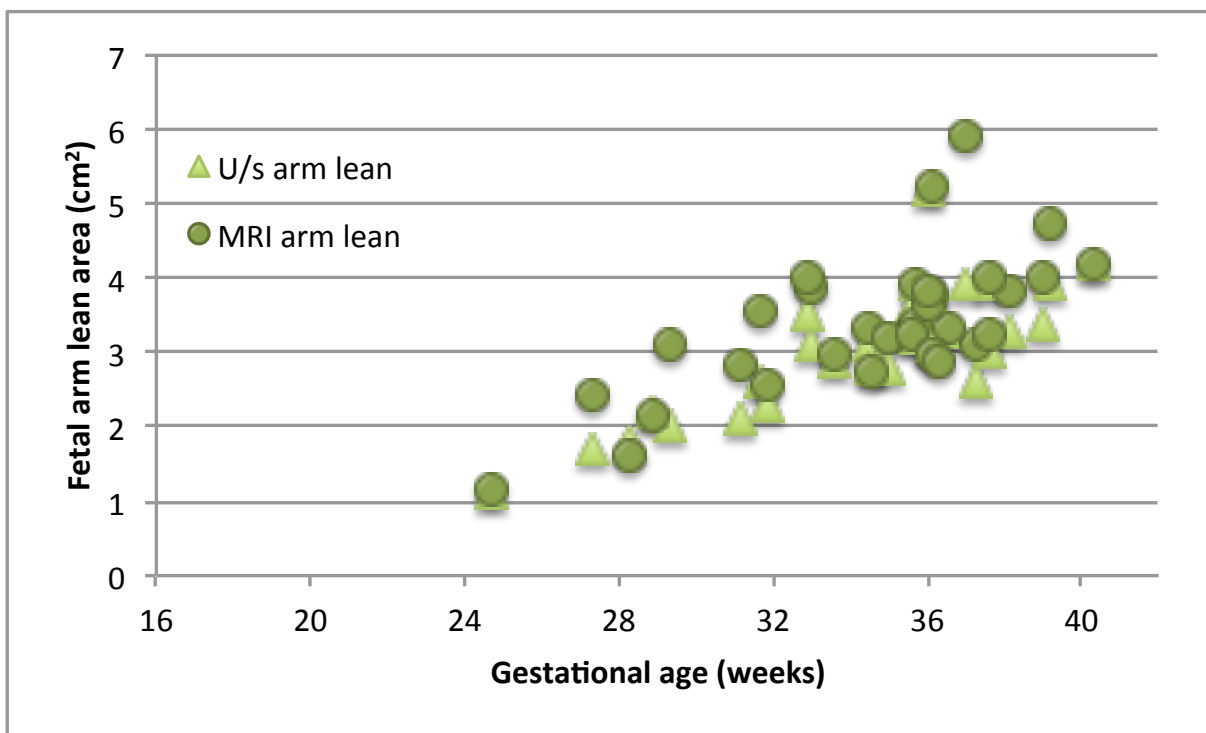


Figure 65: Fetal arm lean area calculated on pairs of MR and ultrasound images from the same fetus.

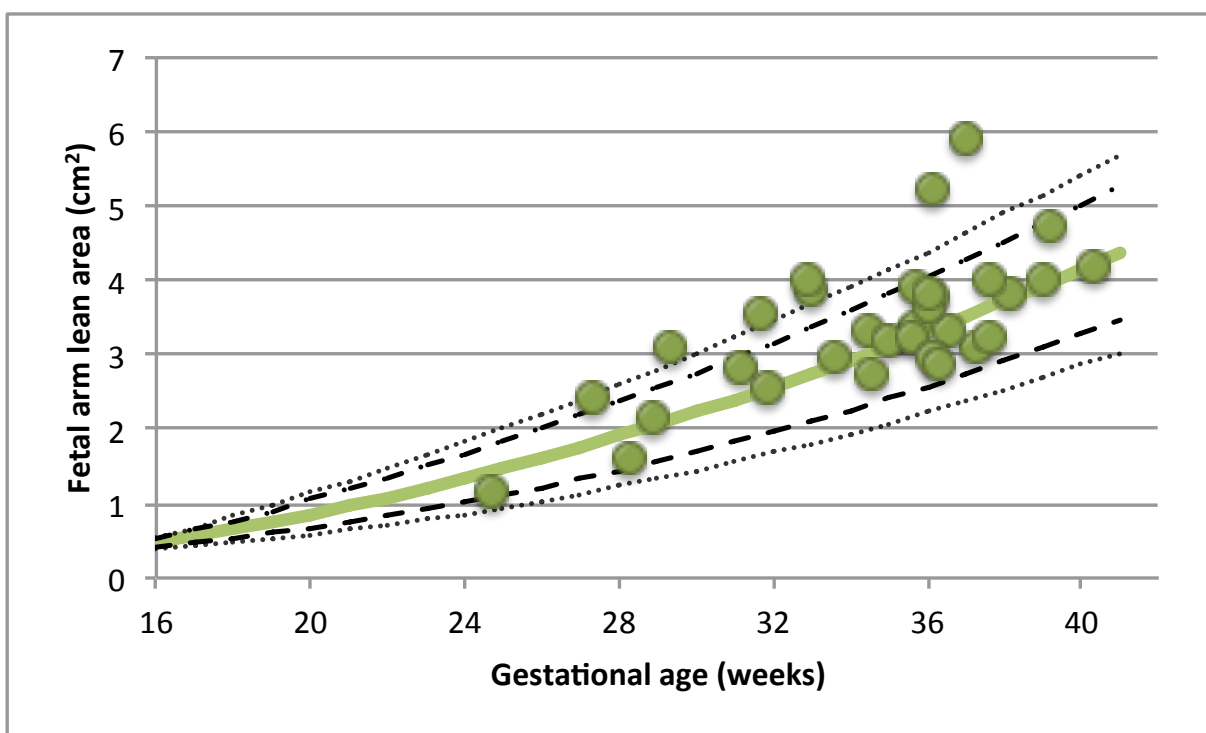


Figure 66: Fetal arm lean area measured on MRI superimposed against centile charts from 2D ultrasound.

Visually, ultrasound images gave a reasonably good demonstration of fetal arm anatomy although, as expected, the MR images showed finer detail, particularly with respect to the fat-muscle border [Figure 62]. The MRI scans also seem to confirm the presence of fat within the intermuscular septae, suggesting that our approach to include these in our segmentations was correct.

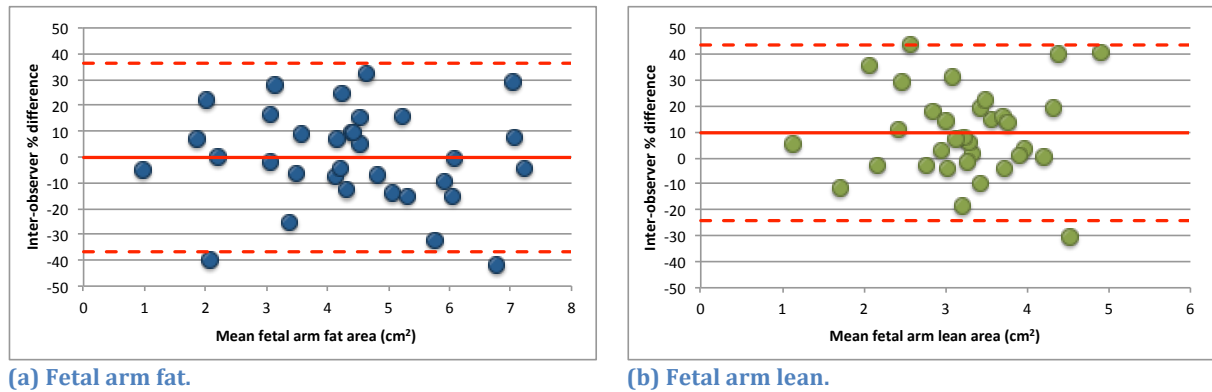


Figure 67: Bland-Altman plots showing the inter-observer percentage variation between measurements from MR and ultrasound images.

The Bland-Altman plots comparing the two measurements showed an inter-observer variability of -0.2% for fat and 9.6% for lean, with limits of agreement of $\pm 36.5\%$ and $\pm 33.8\%$, and ICCs of 0.853 and 0.692, respectively. The arm area measurements (not shown) had an inter-observer variability of 4.2% with $\pm 27.2\%$ limits of agreement and ICC 0.883. The ICCs show reasonable agreement, given that the scans were from two modalities.

The limits of agreement, whilst similar between fat and lean measurements, are considerably larger than the $\pm 14.1\%$ for inter-observer variability of 2D fat measurement of ultrasound images. However, the MRI and ultrasound results involved measuring scans obtained with two different modalities and in a moving subject measured an hour apart. There is also the issue of the variation in definition of the mid-axial slice, as discussed above. All of these factors would be expected to introduce additional variation.

The positive value of the variability for the lean and arm measurements shows that the MRI images gave larger areas when compared with the ultrasound. This is unexpected; it might have been thought that ultrasound, with its 'fuzziness' and sometimes hard-to-identify borders, would produce the larger areas. It may be that omitting the slight fuzziness on the outer surface of the ultrasound images and concentrating on where the border becomes clearer actually slightly underestimates the true area(s) of the arm [Figure 41, Figure 62].

The lean areas may have also been measured as larger due to the increased anatomical detail on the MRI scans when compared with the ultrasound images. The same approach was attempted in segmenting all images, tracing the fat-muscle border as clearly as possible, but the clarity of the MR images when compared with ultrasound [Figure 62, images c and d] may have enabled a more precise outline in the MR images versus a 'smoother' approach to the more grainy ultrasound images, which may underestimate the lean area.

As the arm areas did not yield such large discrepancies as the lean areas, it is unsurprising that the larger lean areas [Figure 66] are compensated for by smaller calculated fat areas [Figure 64]. Overall, however, the majority of both the fat and lean areas do correlate with the centile ranges previously calculated from 2D ultrasound images.

Conclusion

An MRI scanning protocol was developed to enable acquisition of suitable images in 46 fetuses, who also underwent ultrasound scanning. Measurement of fetal arm MR images enabled validation of the ultrasound fat segmentation technique. The paired MR and ultrasound images have been stored in a databank for future research.

iv. Comparison of Philips and MATLAB tools to measure arm areas.

Aim

To compare the fetal arm fat areas generated by using MATLAB segmentation tool with those obtained from the commercially-available Philips tool.

Methods

20 of the fetal arm ultrasound images used in the MRI-ultrasound segmentation study were further segmented with the MATLAB tool, and the resulting areas compared.

Results

	Inter-observer variability	Limits of agreement	ICC
Arm area	-2.7%	+/-9.1%	0.982
Lean area	-3.2%	+/-10.0%	0.972
Fat area (arm–lean)	-1.8%	+/-16.7%	0.973

Discussion and conclusion

The Philips tool gave a larger area on the same image. This is not surprising, as it uses a smoothed polygon approximation whereas MATLAB uses manual outlining. The variability is similar between the two techniques, ICCs show good agreement, and limits of agreement are similar to those of many ultrasound measurements.

4f. Other measurement methods: ellipses

Although manual segmentation allows a detailed measurement of fetal arm fat, I was keen, as a practising clinician, to develop more clinically practical tools. These would also be easier to use with more basic ultrasound machines, for example, in the developing world.

i. Proof of concept: ellipses.

Aim

To develop a measurement tool to provide a reasonable approximation of fetal arm fat but in less time than manual segmentation, which may thus be more suited to clinical practice.

Background and method

This approach was based upon fetal HC, whereby the head is approximated by an ellipse.²²³ It seemed likely that ellipses would work well on earlier gestation arm images (e.g. Figure 24(a)), which are usually circular in cross section with clear edges. Instead, the ellipses were tested on the difficult, but clinically-useful, 36-39 weeks scans. Images at this gestation were often difficult to segment manually, due to bone maturity, shadows and close approximation to other fetal structures, and thus an approximate ellipse tool could be a practical solution.

The ellipse tool in the Philips HD9 machine was used to outline the mid-arm circumference and the inner fat-muscle border in 15 fetuses at 36-39 weeks. Lean area was subtracted from whole arm area to give fat area. The results were compared with manual segmentations of the same images [Figure 68].

Results

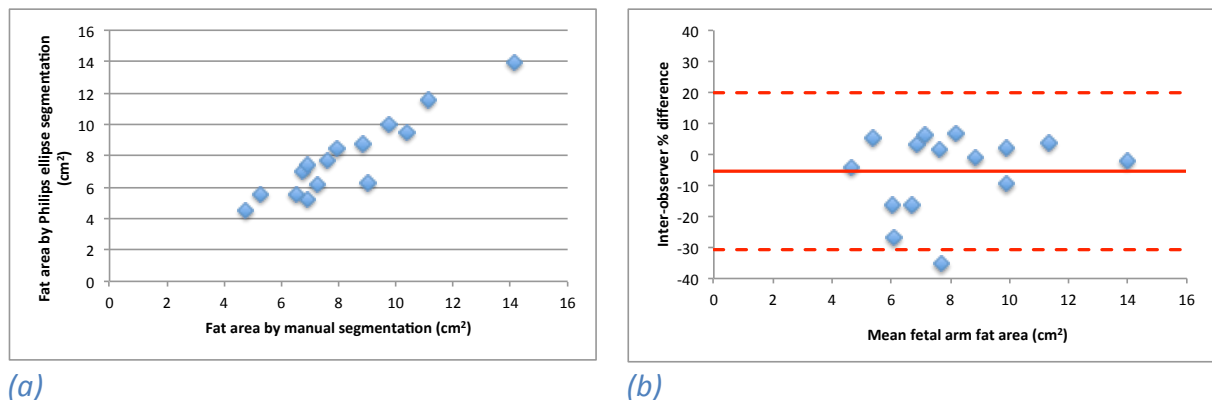


Figure 68: Comparison of manual segmentation and ellipses to measure fat area in 15 images (a) scatterplot (b) Bland-Altman plot comparing fetal arm fat areas measured using the ellipse tool with those measured using manual segmentation.

This trial sample showed a good correlation, as demonstrated by the scatterplot and a correlation coefficient (r) of 0.93 and a coefficient (r^2) of determination of 0.87. Ellipse measurements were, on average, 5.4% or 0.3cm^2 smaller than manual segmentation [Figure 68b]. This mean percentage difference is similar to the inter-observer variability demonstrated on assessment of manual segmentation [Figure 54].

The ellipse tool was faster to use than the manual segmentation, taking 15-45 seconds per segmentation rather than 60-120 seconds.

Conclusion

The ellipse measurements seemed to perform adequately compared with the manual segmentation tool, and enabled measurements to be obtained more quickly. We therefore went on to develop a MATLAB ellipse tool.

ii. MATLAB manual ellipse tool

Aim

To develop a MATLAB manual ellipse tool to enable easy measurement of images and comparison with manual segmentation results.

Method

This was developed collaboratively between S.R. (engineering) and the author (clinical input), by adaptation of the previously-designed manual segmentation tool. The ellipse tool [Figure 69] uses placement of two ellipses, one outer (measuring the arm area by tracing the skin surface) and one inner (measuring the lean area by tracing the fat-muscle border). Each can be enlarged in two dimensions, rotated, and moved (through use of the centre point) to allow the best possible fit onto the fat in the image. Results are generated in a text file and a mat file, in a similar way to those previously described [Figure 40].

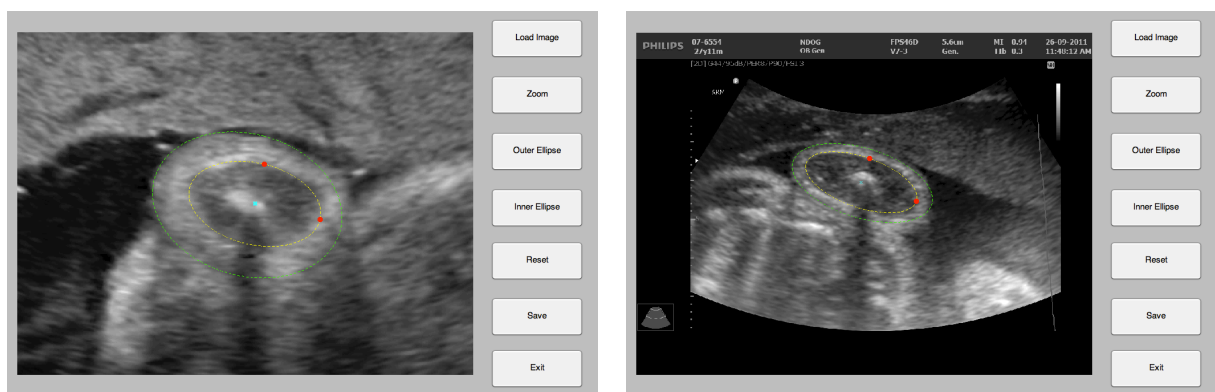


Figure 69: MATLAB manual ellipse tool screenshots showing outer (green) and inner (yellow) ellipses applied to two different 2D arm images. The blue cross marks the centre of the active ellipse; the red dots enable enlargement of the active ellipse.

Ellipse measurements were compared with manual segmentations of the same image. Measurements were also undertaken in different scans in the same fetus (in those having had at least 4 scans), to assess the technique's suitability in assessing longitudinal data.

Results: correlation

36 fetal arm images, from 16-40 weeks gestation, were measured with the ellipse tool and the results compared with those generated by manual segmentation of the fat area [Figure 70]. These were different images to those used for the proof of concept study.

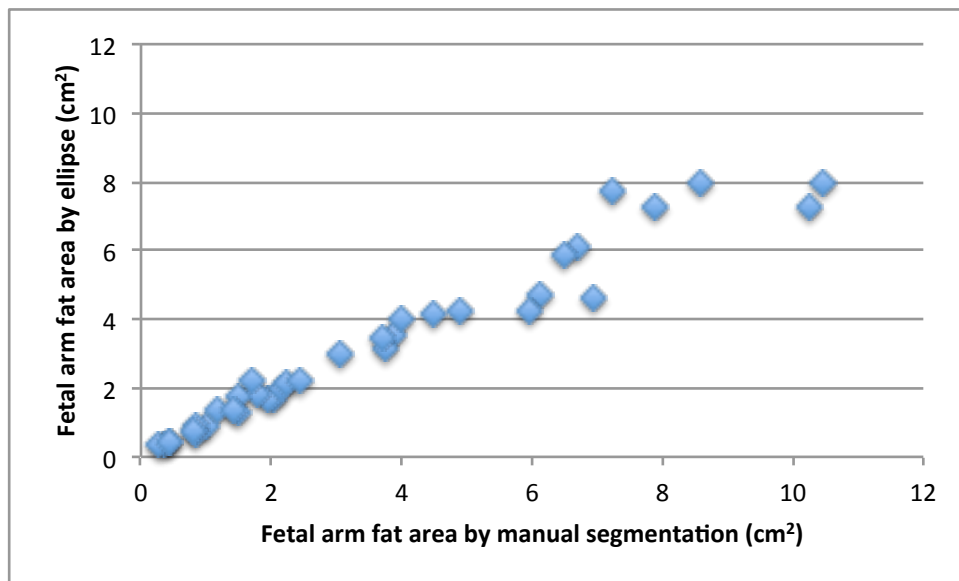


Figure 70: Fat segmentation using MATLAB ellipse tool vs. manual segmentation.

The scatterplot illustrates the strong correlation between ellipse measurements and manual segmentation ($r = 0.97$; $r^2 = 0.95$). The measurements appear to be following a linear progression until about 5cm² (equivalent to the 50th centile at 35 weeks, [Table 8]) when it appears that there is more variation in the measurements, with less strong correlation.

Results and discussion: reproducibility

The intra- and inter-observer variability were calculated for the 36 fetal arm fat measurements [Figure 71].

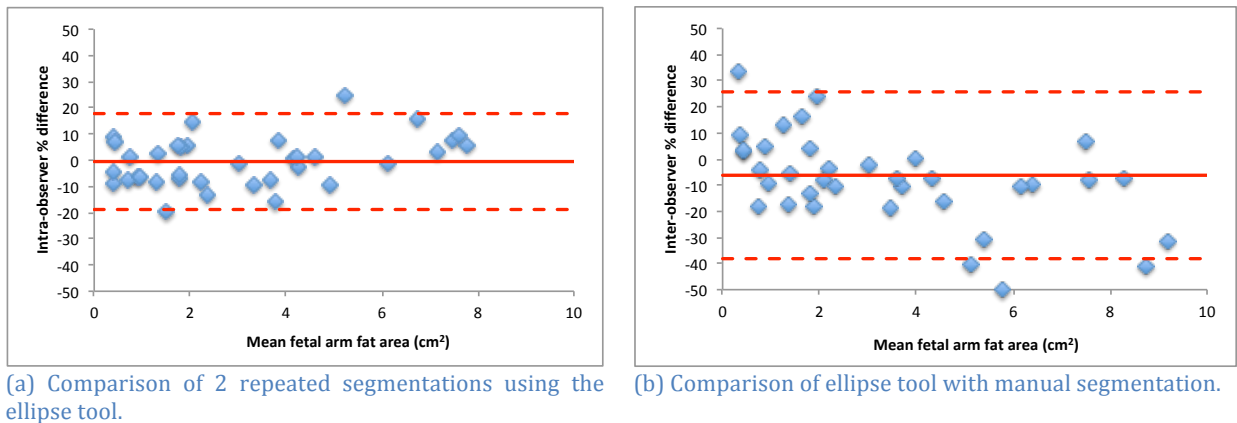


Figure 71: Bland-Altman plots of (a) intra- and (b) inter-observer variability (%) in fetal arm fat area.

The intra-observer variability showed that two segmentations of the same images with the ellipse tool are, on average, very similar at 0.5% difference (with the second measurement being fractionally larger). The limits of agreement were similar to those for manual segmentation [Figure 54] (+/-18% vs. +/-14%).

The inter-observer variability confirmed the proof of concept results: ellipses tend to underestimate the area when compared with manual segmentation (in this case, by 6.2%). It appears that more of the large discrepancies occur at later gestations, plus one occurring at a very early gestation. The former reflects the change in cross-sectional shape of the fetal arm, as it tends to be non-elliptical at later gestations, making the ellipse measurements less accurate. The latter reflects the challenge of accurate measurement of early gestations.

Results and discussion: longitudinal data

These results [Figure 72] confirm the suggestions of the Bland-Altman plots, showing that ellipses tend to underestimate fetal arm fat area at higher gestational ages, for the reasons discussed above.

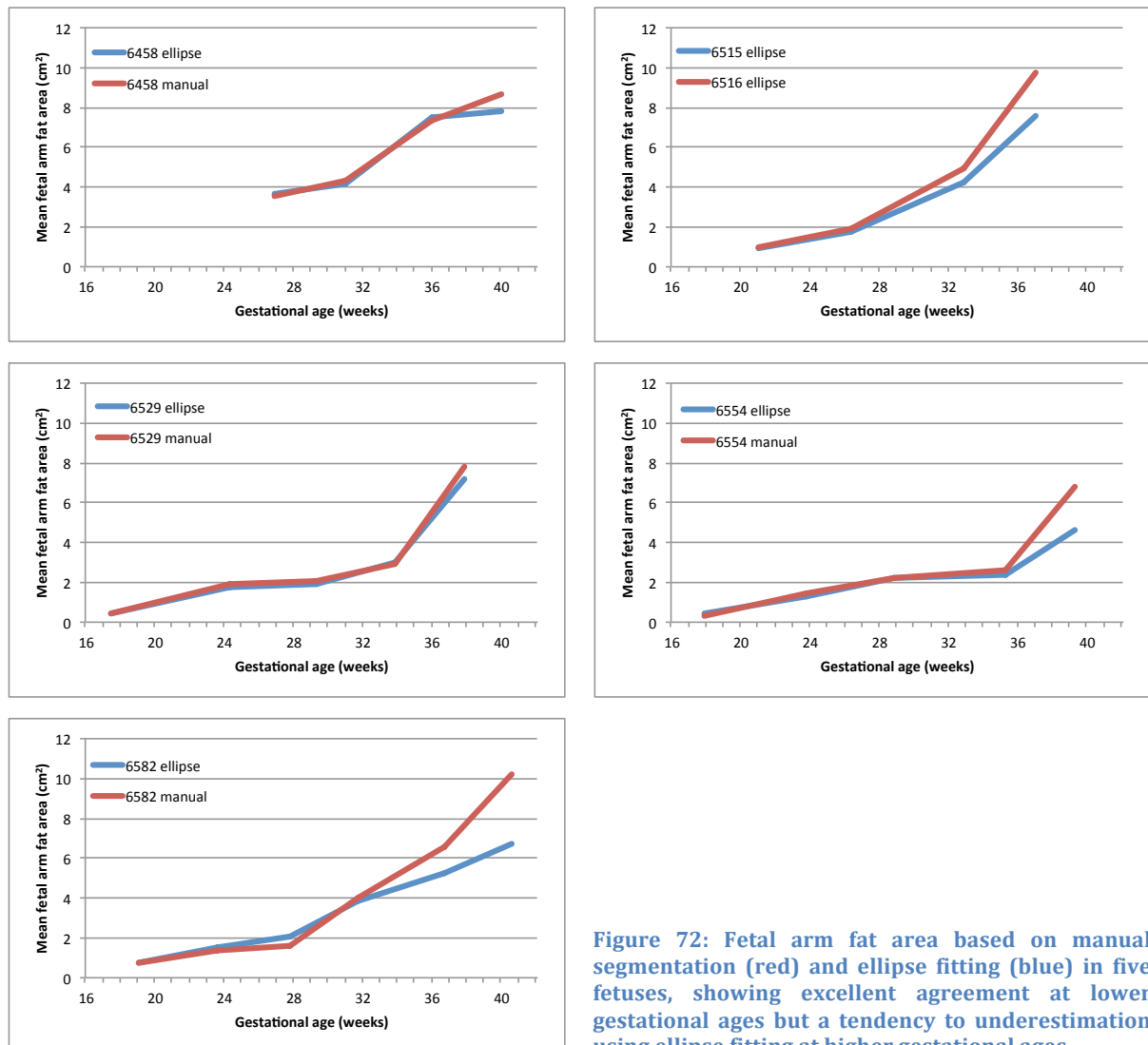


Figure 72: Fetal arm fat area based on manual segmentation (red) and ellipse fitting (blue) in five fetuses, showing excellent agreement at lower gestational ages but a tendency to underestimation using ellipse fitting at higher gestational ages.

Conclusion

Although the ellipse technique is quicker than manual segmentation, it shows inaccuracies at later gestational ages, when the assessment of fetal fat might be expected to aid with assessment of late-onset FGR. This technique was therefore not developed further.

4g. Other measurement methods: depth (Fetal Fat Index)

i. Automatic measurement of fat thickness parameters.

Aim

A proof-of-concept study to show fat variation around the arm and whether results based on automatic measurement of arm fat thickness will correlate with arm fat area.

Background

Single measurements of fetal arm fat thickness have shown varying results in diabetic pregnancies and macrosomic babies.^{103,105} I hypothesised that this was because a single measurement of arm fat does not represent all fat, and thought other parameters might show better correlation.

Method

Fetal fat was assessed automatically, using a method developed by S.R.,²⁰³⁻²⁰⁵ in 20 fetal arm images from 21-31 weeks of gestation. The fat profile was assessed and maximum, minimum, median and mean thicknesses automatically calculated [Figure 73] and plotted against gestational age. The mean and maximum were also plotted against the corresponding fetal arm fat area, the latter measured by automatic segmentation.

Results and discussion

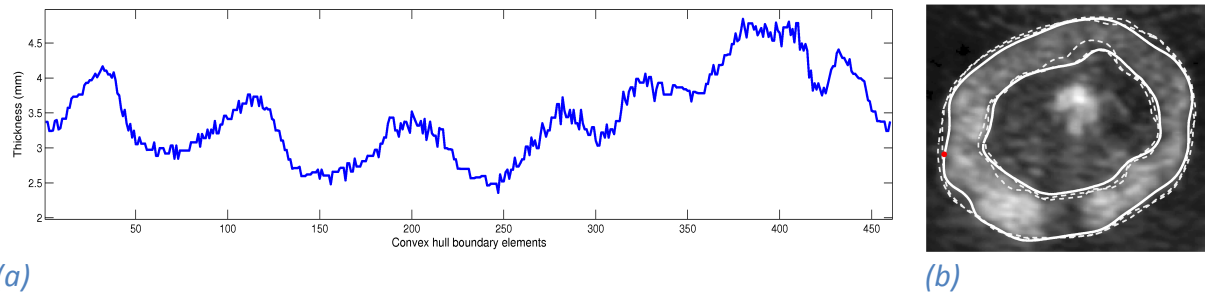


Figure 73: (a) Graph showing varying fat thickness in mm for (b) segmented arm image, starting from the red dot at 9 o'clock and proceeding in a clockwise direction (taken from Rueda²²⁴).

As expected, the fat thickness varied around the arm [Figure 73]. Thus a single measurement of thickness cannot consistently represent the fat thickness around the whole arm.

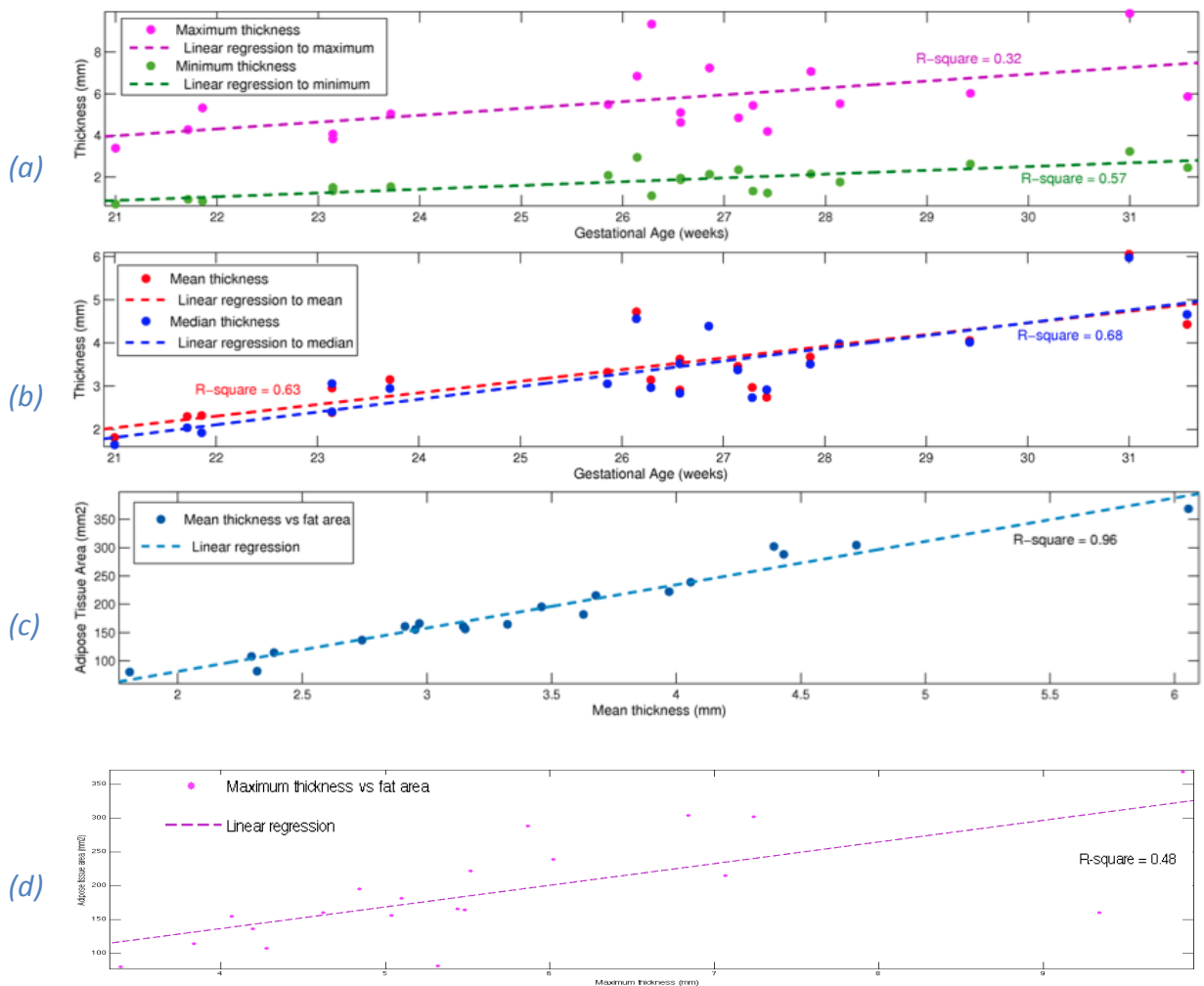


Figure 74: Fetal arm fat thickness parameters (n=20) (a) maximum and minimum thickness, vs. gestation; (b) median and mean thickness, vs. gestation; (c) mean thickness, vs. corresponding fat area; (d) maximum thickness, vs. corresponding fat area (taken from Rueda²²⁴).

The maximum and minimum thicknesses diverged during gestation [Figure 74a]; this correlates with the increased laying down of fat seen in the third trimester. We thought maximum thickness, rather than an arbitrary thickness measurement, might show good correlation with adipose tissue area, however this was not the case ($r^2=0.48$) [Figure 74d].

The median and mean were very similar across the gestational range, suggesting that the data are normally distributed [Figure 74b]. As would be expected, the mean shows excellent correlation ($r^2= 0.96$) with adipose tissue area [Figure 74c]. Calculation of the mean in this way is, however, only possible from assessing the whole area first (whether by manual or automatic segmentation).

Conclusion

This sub-study showed that, as we had suspected from our observations, fat thickness varies around the arm, explaining why single-sampling methods had been unsuccessful. Of the fat thickness measurements assessed using automatic methods, the mean showed the best correlation with adipose tissue area.

ii. Fetal fat index (FFI): three-thickness sampling of fat

Aim

A proof-of-concept experiment to assess whether a three-thickness fetal fat index (FFI) sampling approach would correlate with fetal arm fat area.

Background

The automatic approach shows potential but is not yet ready for clinical practice. I hypothesised that a sampling approach, using several measurements of arm fat thickness, may provide a more representative measure of fetal arm fat. This is similar to the amniotic fluid index⁷¹ concept, whereby liquor volume is assessed by maximum vertical depth in four quadrants of the uterus. The measurements would be obtained with the distance calliper (standard on ultrasound machines), and would be a quick and easily-understood technique.

Method

A common-sense approach seemed to measure at each of the 90° aspects from either the bone or central point of the arm [Table 17], equivalent to 3, 6, 9 and 12 o'clock positions on a clock face. The 6 o'clock measurement is not possible due to acoustic shadows; measurements were taken at the other three positions [Figure 75]. The different reference points – bone or central point – were chosen as the latter may sample fat in a more consistent place, for example if the bone is non-centrally situated.

Four different measurement strategies were employed in 23 images across a range of gestations from 16-40 weeks.

Table 17: Explanation of different FFI measurement strategies for each image.

	Centre point	9 o'clock	12 o'clock	3 o'clock
1a	humerus	horizontal	vertical	horizontal
1b	humerus	< - - - - perpendicular to inner surface of fat - - - - >		
2a	centre of arm	horizontal	vertical	horizontal
2b	centre of arm	< - - - - perpendicular to inner surface of fat - - - - >		

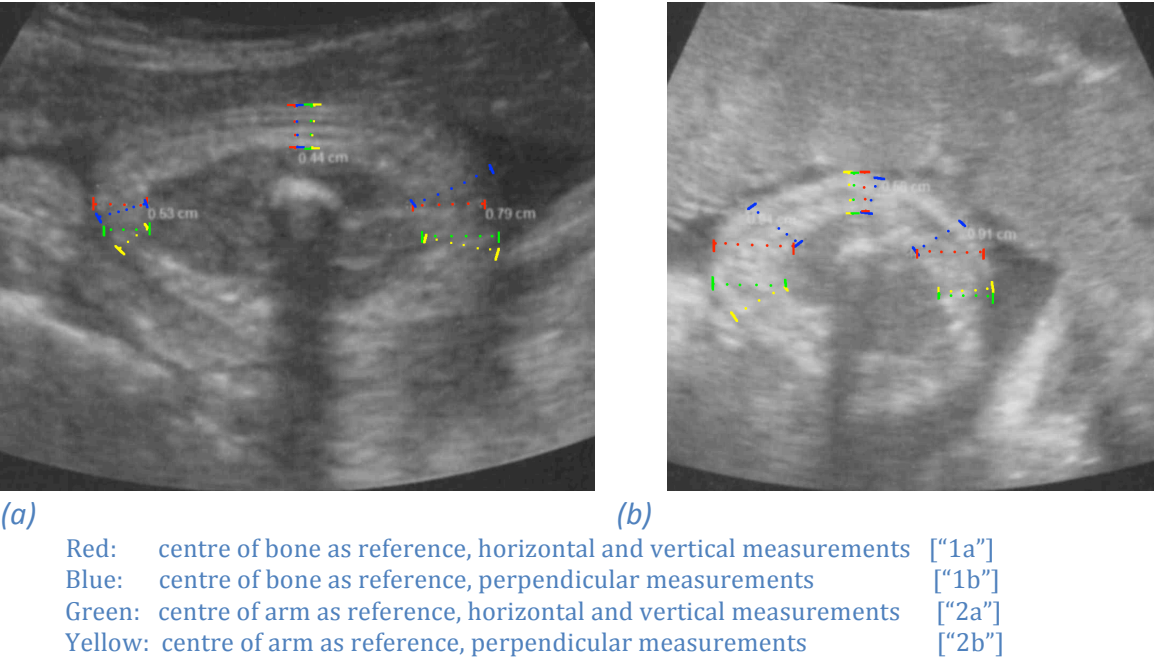


Figure 75: Different approaches to fat measurement, on (a) 35 week and (b) 39 week images.

The total FFI of the three measurements was calculated and plotted against gestational age and fetal arm fat area. The 12 o'clock measurement was also assessed for comparison, as this was a similar approach to that taken by previous authors.^{103,105}

Results and discussion

The measurements were possible in all cases.

It took 8-13 seconds (total) to measure the FFI (i.e. 3 thicknesses) in each of 10 scans. This compares favourably with 15-45 seconds for the ellipse method and 60-120 seconds for manual segmentation.

Paired T-tests revealed no statistical differences between any of the FFI measurement groups except between group 1a and 1b ($p < 0.001$). Sub-analyses of the individual measurement positions only showed statistical difference at 3 o'clock ($p = 0.003$). Assessment of the data suggested both significant differences were due to several large variations in 3 o'clock thicknesses in some images. Otherwise horizontal/vertical and perpendicular measurements were not significantly different; the former were more straightforward to obtain.

For purposes of comparison, a single measurement at the 12 o'clock position for each of the groups gave correlations of $r = 0.75-0.79$ and $r^2 = 0.57-0.62$ with mean fat area (by manual segmentation). There was an improved correlation between the FFI and fat area ($r = 0.93-0.94$; $r^2 = 0.87-0.89$), with group 1a showing the strongest correlation [Figure 76]. Whilst not quite as strong as the 0.96 correlation showed by the automatic mean measurement, this is nevertheless a strong correlation and better than a single measurement.

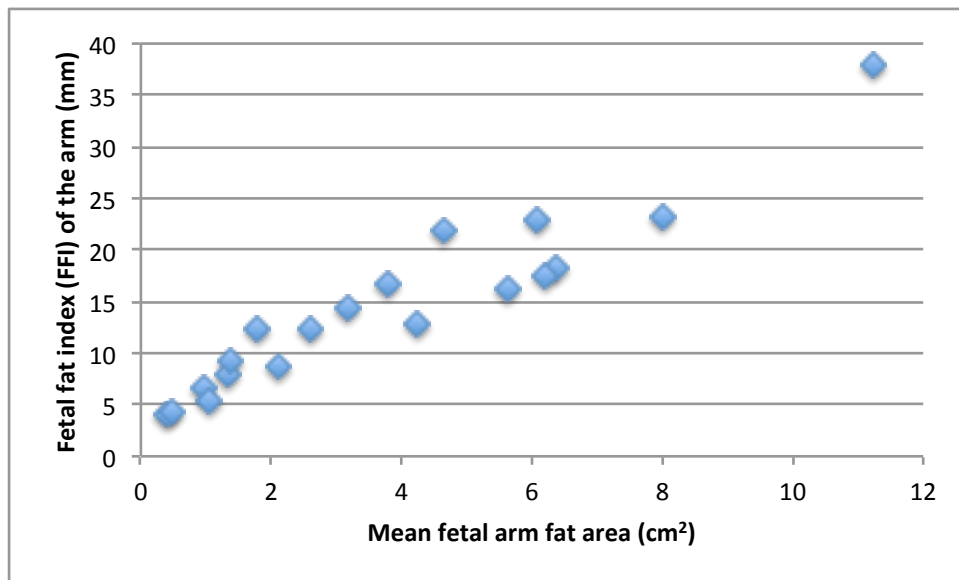


Figure 76: Fetal arm fat index showing good correlation ($r^2=0.94$) with arm fat area.

The 1a FFI measurements also showed a similar pattern to fat area [Figure 42] when plotted against gestation age [Figure 77].

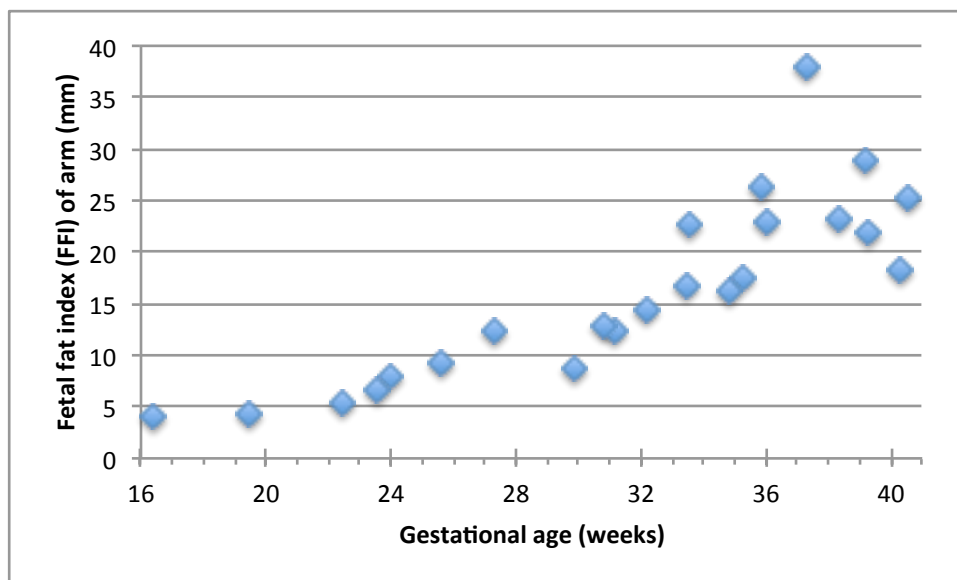


Figure 77: Fetal arm fat index plotted against gestational age.

Assessing the 1a group measurements showed a 0.1-12.2mm difference between the smallest and largest of the three measurements making up each FFI, accounting for 2.5-48% of the FFI. It is thus evident that a single measurement cannot reflect the different distribution of thicknesses, whereas three-thickness sampling is more representative, as reflected in the correlation coefficients.

Conclusion

These experiments suggest FFI may be a helpful tool in assessing fetal arm fat. The use of the humerus as the central point, with measurements being taken at 9, 12 and 3 o'clock positions (not necessarily perpendicular to the inner surface of the fat) yielded the strongest correlation; however all four methods gave good results and it may be that using the central point of the image as the reference point would be a more practical approach.

4h. Publications and presentations arising from this chapter

OP02.07 Fetal fat index: a novel technique to assess fetal arm fat²²⁵

C.L.Knight, S.Rueda, J.A.Noble, A.T.Papageorghiou

Ultrasound Obstet Gynecol 2013; 42 (s1): 52

Oct 2013: ISUOG 23rd World Congress, Sydney, Australia

[oral presentation]

P08.16 Systematic thickness analysis of the adipose tissue around the fetal arm across gestation using 2D ultrasound images²²⁶

S.Rueda, **C.L.Knight**, A.T.Papageorghiou, J.A.Noble

Ultrasound Obstet Gynecol 2013; 42 (s1): 145

Oct 2013: ISUOG 23rd World Congress, Sydney, Australia

[poster presentation]

P11.04 Novel automatic coupled ellipse fitting tool for fetal arm adipose tissue quantification in 2D ultrasound images across gestation²²⁷

S.Rueda, **C.L.Knight**, J.A.Noble, A.T.Papageorghiou,

Ultrasound Obstet Gynecol 2013; 42 (s1): 152

Oct 2013: ISUOG 23rd World Congress, Sydney, Australia

[poster presentation]

Fetal arm fat as an ultrasound marker of nutritional status

Aug 2013: listed in full in Chapter 7

Neonatal peripheral adiposity measurements using 2D ultrasound in premature infants and comparison with fetal adiposity measures – a novel marker for nutritional status²²⁸

A. Kopuri, S. Rueda, **C. Knight**, K. McCormick, A. Noble, A. Papageorghiou, P. Sullivan

JPGN 2013; 56 (s2): 148

May 2013: European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) 46th Annual Meeting, London, UK

[oral presentation: poster of distinction]

Oriented feature-based coupled ellipse fitting for soft tissue quantification in ultrasound images²²⁹

S. Rueda, **C.L. Knight**, A.T. Papageorghiou, J.A. Noble

April 2013: IEEE 10th International Symposium on Biomedical Imaging (ISBI), San Francisco, USA

[paper and presentation]

Fetal arm ultrasound as a marker of fetal body composition
Dec 2012: listed in full in Chapter 7

Ultrasound-based fetal arm soft tissue thickness quantification across gestation²²⁴
Sylvia Rueda, **Caroline L. Knight**, Aris T. Papageorghiou and J. Alison Noble
October 2012: 15th International Medical Image Computing and Computer Assisted
Intervention(MICCAI), Nice, France
[Workshop: Perinatal & Paediatric Imaging; paper and presentation]

OP17.04: Fetal arm fat: development throughout pregnancy in selectively screened
optimally healthy patients.²³⁰
Knight, C. L., Rueda, S., Noble, J. A., and Papageorghiou, A. T.
Ultrasound Obstet Gynecol 2012; 40 (s1): 105-106
Sept 2012: ISUOG 22nd World Congress, Copenhagen, Denmark
[oral presentation]

OP17.05: Fetal arm fat: an *in utero* marker of body composition?²³¹
Sept 2012: listed in full in Chapter 7

P28.09: Novel automatic method for measuring fetal arm adipose tissue in 2D ultrasound
images across gestation.²⁰⁵
Rueda, S., **Knight, C. L.**, Papageorghiou, A. T. and Noble, J. A.
Ultrasound Obstet Gynecol 2012; 40 (s1): 281
Sept 2012: ISUOG 22nd World Congress, Copenhagen, Denmark
[poster presentation]

Regularised feature-based fuzzy connectedness segmentation of ultrasound images for
fetal soft tissue quantification across gestation²⁰⁴
S.Rueda, **C.Knight**, A.Papageorghiou, J.A.Noble
May 2012: IEEE 9th International Symposium on Biomedical Imaging (ISBI), Barcelona, Spain
[paper and presentation]

Local phase-based fuzzy connectedness segmentation of ultrasound images²⁰³
S.Rueda, **C.Knight**, A.Papageorghiou, J.A.Noble
July 2011: Medical Image Understanding and Analysis (MIUA), London, UK
[paper and presentation]

4i. Contributions to the thesis: chapter 4

Other than where indicated below, and acknowledged in appendix A, all the work in this chapter (including concepts and hypotheses, protocols, acquisition and segmentation of scans, analyses, production of graphs and figures) is that of the author.

S.R. and the author developed the image scoring criteria; both scored the scan images. S.R. developed the MATLAB segmentation tool (2D) with clinical input from the author; S.R. adapted this tool to produce the MATLAB ellipse tool. S.R. segmented 68 images to enable calculation of inter-observer reproducibility. S.R. designed the tool to automatically measure fat thickness, and produced the related graphs and analyses [Figure 73, Figure 74]. B.K. provided statistical advice about fractional polynomials and centiles. MRI study ethics application paperwork and process undertaken by the author with the support of A.P.; additional input from G.Q., S.R., A.N. and J.B.. All other supporting paperwork (patient information leaflet, GP letter, consent form, study protocol), study administration, and UKCRN portfolio database management, was undertaken by the author. MRI scanning protocol developed by G.Q. and the author with input from S.R. and A.P.. Most MRI study leaflets given to patients by F.R.; the rest by the author. MRI study scans acquired by G.Q. with radiologists and radiographers at the John Radcliffe Hospital, Oxford. All ultrasound scans for the MRI study were acquired by the author.

Images taken from journal papers and textbooks are individually cited in the figure captions, by name and year, and with a reference to the full bibliographic details. Neonatal birthweight centiles from www.paediatrics.co.uk/nicu/growth-charts (using the UK-WHO growth charts, 0-4 years²²⁰).

5. FETAL ARM FAT ASSESSMENT USING 3D ULTRASOUND

This second results chapter considers 3D ultrasound assessment of the fetal arm. It includes segmentation protocols and techniques; the novel measurement of fetal arm fat and lean volumes; work assessing validation and reproducibility; and further exploration of the novel Fetal Fat Index (FFI) measurement.

Sections 5a and 5b explore the methodology, describing the 3D volume segmentation tool and protocol; and assessing fractional volume variables (angle and number of slices).

Section 5c and 5d describe measurement of fetal arm fat and lean tissue volumes, including scatterplots and centiles describing normal development throughout gestation in an optimally healthy population, reproducibility (intra- and inter-observer), and an exploration of longitudinal studies of arm fat volumes within individual fetuses who had at least 4 scans.

Section 5e explores the use of a perpendicular central slice from the fractional volume to generate 2D data. Scatterplots and centiles were produced, and original 2D images were compared with those from the 3D volumes.

Section 5f builds on section 4g, assessing FFI through central slice extractions from all arm volumes in this chapter and comparing the results with the corresponding arm fat volume measurements. Reference centiles for FFI are presented and reproducibility assessed.

Section 5g lists the publications and presentations arising from the work in this chapter, and **section 5h** lists the contributions to this chapter.

5a. MATLAB 3D volume segmentation tool and protocol

The general methods are described in chapter 3. In addition, we needed a tool that would enable segmentation of limb fat volumes; this was developed by M.A., supervised by S.R., allowing AVol to be measured in a similar manner to that described by Lee et al.¹²⁶ Importantly, this tool also enabled segmentation of fractional fat, lean and limb volumes, rather than just fractional limb volumes.

Philips 3D ultrasound volumes were converted to the appropriate .mha format using a programme developed at the IBME, RegisterAndConvert. The segmentation protocol is outlined below, and provided in full in appendix D.

MATLAB was opened and a converted axial volume file uploaded; the reference images were then manipulated to give a clear sagittal view of the complete humerus / femur. The bone length was delineated and the programme automatically selected the central 50% for analysis. An odd number (to ensure the presence of a central slice) of 3 or more equally-spaced slices were selected, and perpendicular (to bone) or parallel (in line with vertical on the original image) slice generation chosen. Exploration of both of these variables (number of slices and angle) is outlined in sections 5b and 5c.

The generated slices were then segmented individually in the same way as the 2D method (section 4b.iii). Once all five slices had been segmented, the images were reviewed and could be cleared and re-measured if there were any doubts or discrepancies. The volume was then interpolated from the five slices [Figure 78].

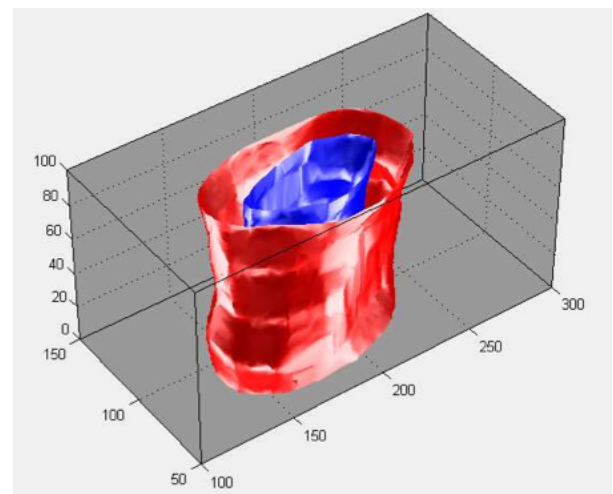
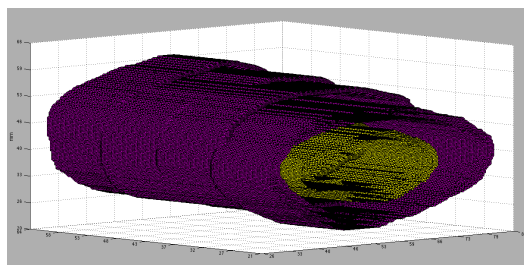
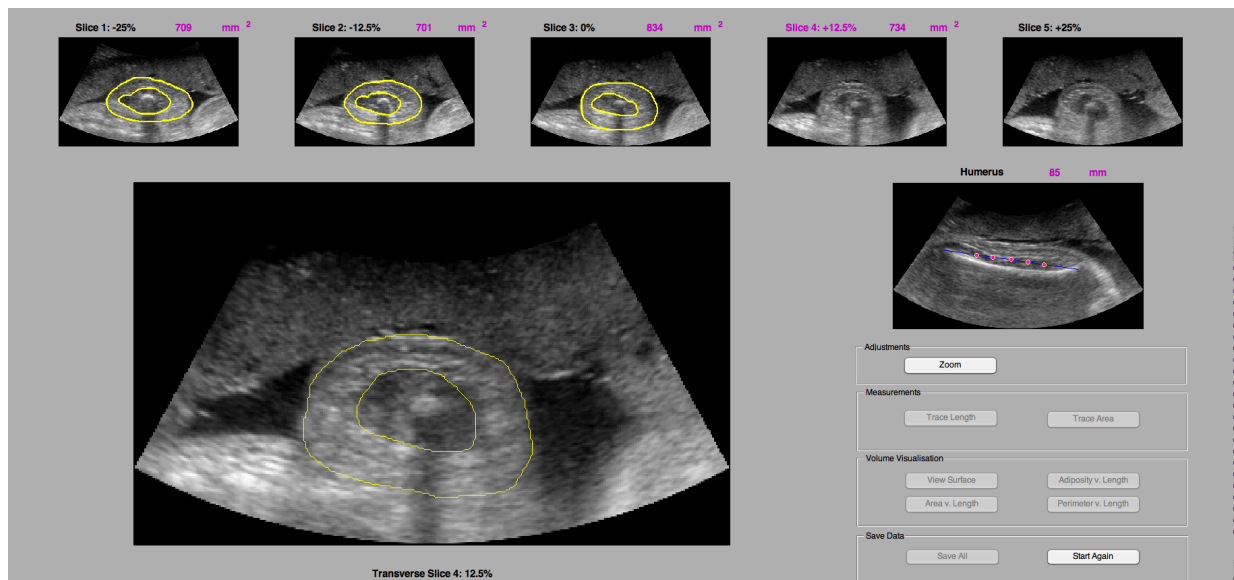


Figure 78: MATLAB 3D volume segmentation tool screenshot showing 5-slice 50% AVol. The sagittal view of the humerus demonstrates slice positions (pink dots). 3 of the 5 generated slices (top) have been segmented; the 4th has just been segmented, shown by the yellow outline on the large image. 3D impressions of fractional arm volumes are also shown (above and right).

Results were generated in a text and excel file in a similar way to the 2D images: identifying characteristics (date of scan, anonymised patient ID, gestational age, scan number), 3D volume results (fat, lean and arm 50% fractional volumes in cm^3 , plus fat volume as a percentage of the arm volume), and 2D area results for the central slice (fat, lean and arm 50% fractional volumes in mm^2 , plus fat area as a percentage of the arm slice area).

Versions of the tool were developed to allow measurement of arm and thigh fat both from axially- and sagittally-acquired volumes.

5b. Variables affecting fractional limb fat volume

i. The effect of the angle of slices: perpendicular vs. parallel

Aim

To evaluate the impact of angle of slice plane acquisition on fetal arm fat volume, and whether this impact varied if the humerus was more angled.

Background

Based on ultrasound knowledge we wanted to ascertain the effect on volumes of taking slices perpendicular to the longitudinal axis of the humerus, versus those taken more parallel to the vertical axis of the ultrasound transducer. We hypothesised that the “parallel” slices would produce larger, overestimated, volumes.

Methods

20 volumes were assessed: one horizontal (0.2-2.7°) and one angled (3.0-24.7°) humerus at each of 30-39 weeks of gestation (i.e. 10 per group). Each was segmented twice, once with parallel and once with perpendicular slice acquisition [Figure 79], and the volumes compared.

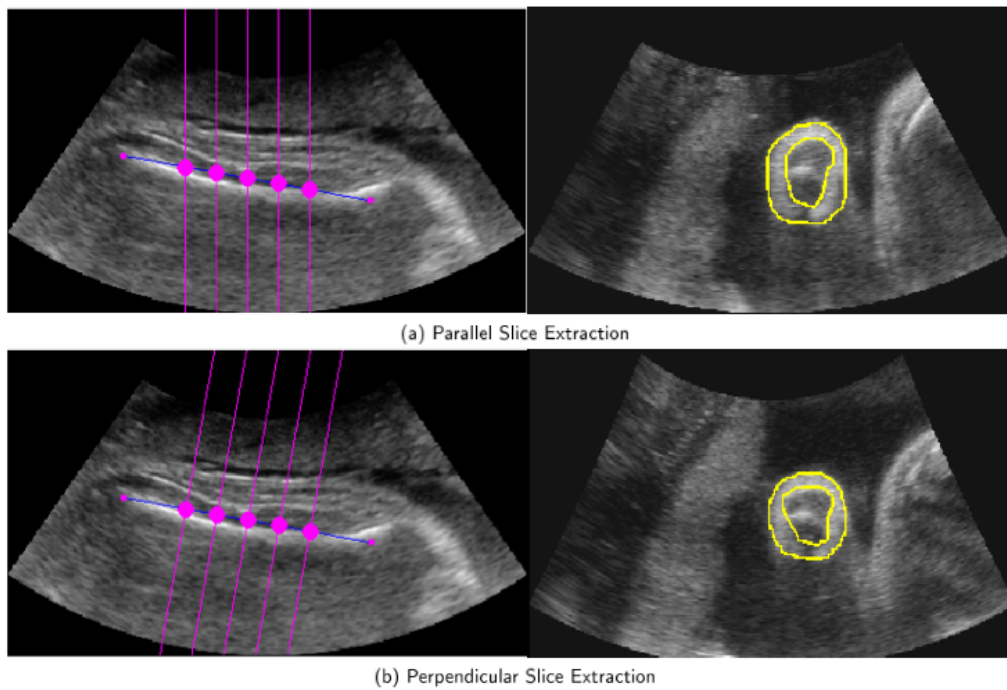


Figure 79: Demonstration of (a) parallel (to vertical axis of ultrasound transducer) vs. (b) perpendicular (to bone) slice extraction from the same 3D fetal arm scan, and the different axial slice generated.

Results

Overall the volumes from the more parallel slices were 3.5% larger than the perpendicular; as expected this effect was most pronounced in volumes with the greatest angle between humerus and segmentation. Thus for a horizontal humerus (0°) the parallel slice-acquired mean volume was 2.4% larger, whilst for the most “angled” humerus (25°) it was 27% larger.

Conclusion

These data suggest a significant effect of angulation and demonstrate the importance of performing segmentation (2D/3D) as perpendicular to the humerus as possible: a more parallel angle of segmentation tends to increase area or volume measurements, suggesting that perpendicular slices should be used for segmentation. Acquiring the volume with the humerus as horizontal as possible makes such segmentations easier.

ii. The effect of the number of slices

Aim

To assess the effect of increasing the number of slices on the generated volume, and to ascertain the optimal number of slices to be used.

Background

Lee's work on fractional limb volumes used a 5-slice technique.¹⁴³ Whilst this is a practical number (for example, in terms of time taken to measure), we wanted to assess whether adjusting the number of slices could have a significant impact on the resulting volume.

Method

Five fetal arm volumes from 26 to 33 weeks of gestation were selected as being of good quality in order to minimise the effect of image quality on volume calculation. Each was segmented once using a 17-slice perpendicular technique. Volumes were calculated from odd numbers of slices [Figure 80] to maintain the presence of a central, mid-humeral slice.

<u>1</u> ---- 2---- 3---- 4---- 5---- 6---- 7---- 8---- <u>9</u> ----10----11----12----13----14----15----16---- <u>17</u>																
3-slice volume		used	slices 1,9,17													
5-slice volume		used	slices 1,5,9,13,17													
9-slice volume		used	slices 1,3,5,7,9,11,13,15,17													
17-slice volumes		used	all slices													

Figure 80: Different multi-slice approaches to volume calculation; the central and end slices were used in all.

Results and discussion

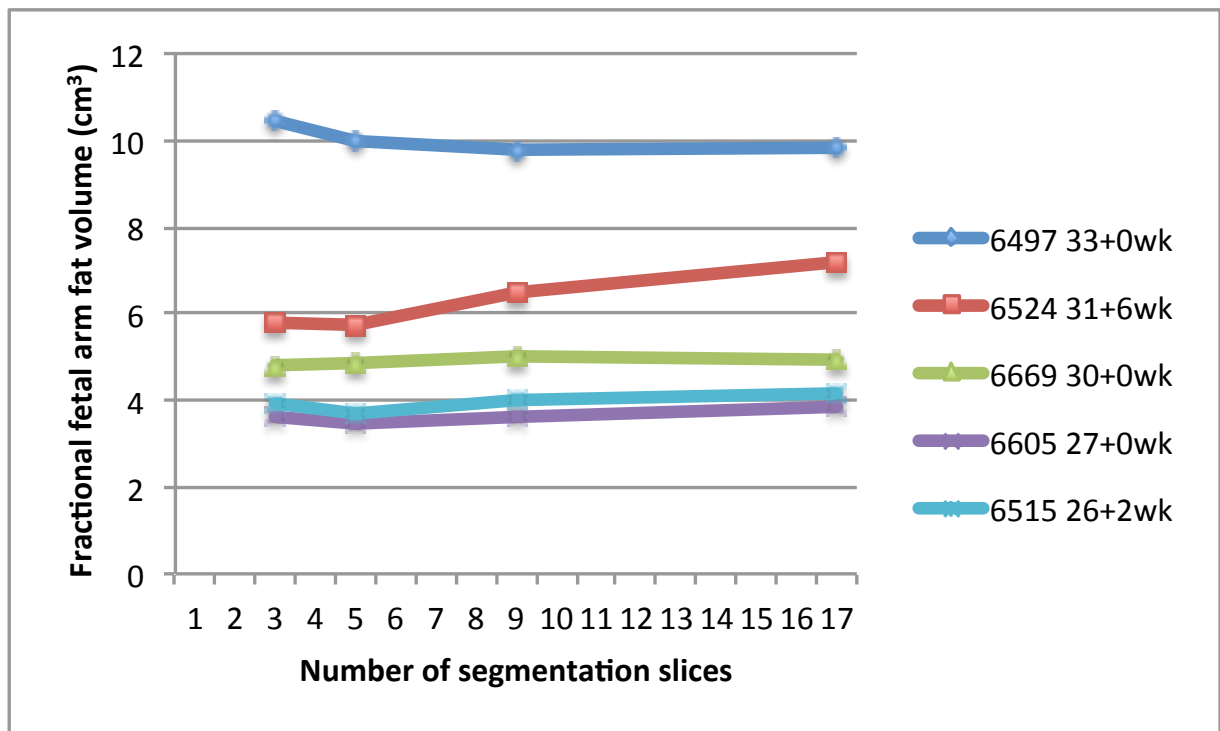


Figure 81: Graph showing effect of increasing numbers of slices on fetal fractional arm fat volume.

On average, there was a 3.5% decrease (range: -6.1 to 0.7%) in arm fat volume from a 3- to 5-slice volume; a 6% increase (-1.9 to 13.2%) from 5- to 9-slice volume; and a 3.6% increase (-2.2 to 10.3%) from 9- to 17-slice volume [Figure 81]. It thus seems likely that the 3-slice overestimates the true volume somewhat, as might be anticipated from interpolating a volume from so few slices. The extra information added by an additional number of slices, however, does not markedly increase the volume (at least, not beyond the accepted percentage variability of ultrasound measurements) whilst adding segmentation time.

Conclusion

A 5-slice volume seems a reasonable compromise between ensuring an accurate volume and having a practical measurement which does not take an excessive amount of time.

5c. Measuring arm fat and lean tissue volumes, and establishing reference ranges

i. Normal development of fetal arm fat and lean volumes

Aim

To assess the fetal arm using 3D ultrasound and produce reference ranges for fat and lean volumes, from an optimally healthy population.

Method

Axial mid-humeral volumes from 16-41 weeks' gestation were acquired from women in the INTERGROWTH-21st study between January 2011 and May 2012. The best volume was selected from each scan based on subjective visual assessment.

These volumes were measured (segmented) using the specifically-designed MATLAB tool (section 5a) by three people: the author, M.A. (biomedical engineer), and K.E. (research midwife). M.A. and K.E. underwent training in the segmentation tool and techniques by the author. A perpendicular-slice, 5-slice method was used, determined as being optimal in the work of the previous section.

The patients and scans were divided between the operators to ensure a balanced distribution and try to minimise the effect of any bias.

First, the scans were subdivided into two cohorts: patients whose infants had later undergone PEA POD body composition analysis (see section 7a) and those which had not. The former would comprise a separate cohort for later analyses although both cohorts' data contributed to the analyses in this section of the thesis.

Within each cohort, the patients were divided into groups based on the number of scans (1-6) available for analysis. Scans in each group were listed in chronological order by their study ID number, and were allocated individually in turn to each operator. This was to account for the possibility that the quality of the scans may have improved with time as those scanning became more familiar with the techniques.

A 50% fractional volume model was used.¹⁴³ Lean volume (muscle and bone) was then subtracted from arm volume to generate fat volume. The volumes (in cm³) were plotted against gestational age; fat was also expressed as a percentage of arm volume. Statistical analyses were carried out using STATA to calculate centiles (3rd, 5th, 10th, 50th, 90th, 95th and 97th), using fractional polynomial regression, with log transformation where necessary.

Results

638 arm volumes were obtained from 212 women taking part in the INTERGROWTH-21st study; each woman was scanned 1-6 times (median 4, mean 3 scans). The gestational ages ranged from 16⁺³ weeks to 41⁺⁰ weeks. Fat and lean volumes could be measured in 556 (87%) [Figure 82].

The ease of segmentation was recorded in 420/556 cases, of which 71% were stated to be “easily segmentable”. In those which could not be segmented, a reason had been recorded in 58/82 cases. Of these, 29 (50%) were not acquired according to the protocol (14 were too rotated to be corrected and then measured; 14 did not acquire the full sagittal length of the humerus; and 1 did not capture all of the arm fat within the volume). In 27 cases it was not possible to segment the fat due to problems of visualisation. This was particularly evident at lower gestational ages (19/27 were <21 weeks). Of the 8 scans which could not be segmented at gestational ages of 21 weeks or later (i.e. after the usual timing of the anomaly scan): 4 were due to difficulties with the outer border, and 4 were due to difficulties with both the outer and inner fat borders.

K.E. had received training but was the least experienced in the segmentation technique, and was only able to segment 56% of the first set of volumes (64/113). This was considerably lower than the 86% (98/114, C.K.) and 82% (94/114, M.A.) of the other, more experienced, segmenters. The author therefore reassessed K.E.’s 49 rejected volumes, determining that a further 33 were segmentable; retraining followed and segmentation rates were comparable between all three people undertaking the measurements thereafter.

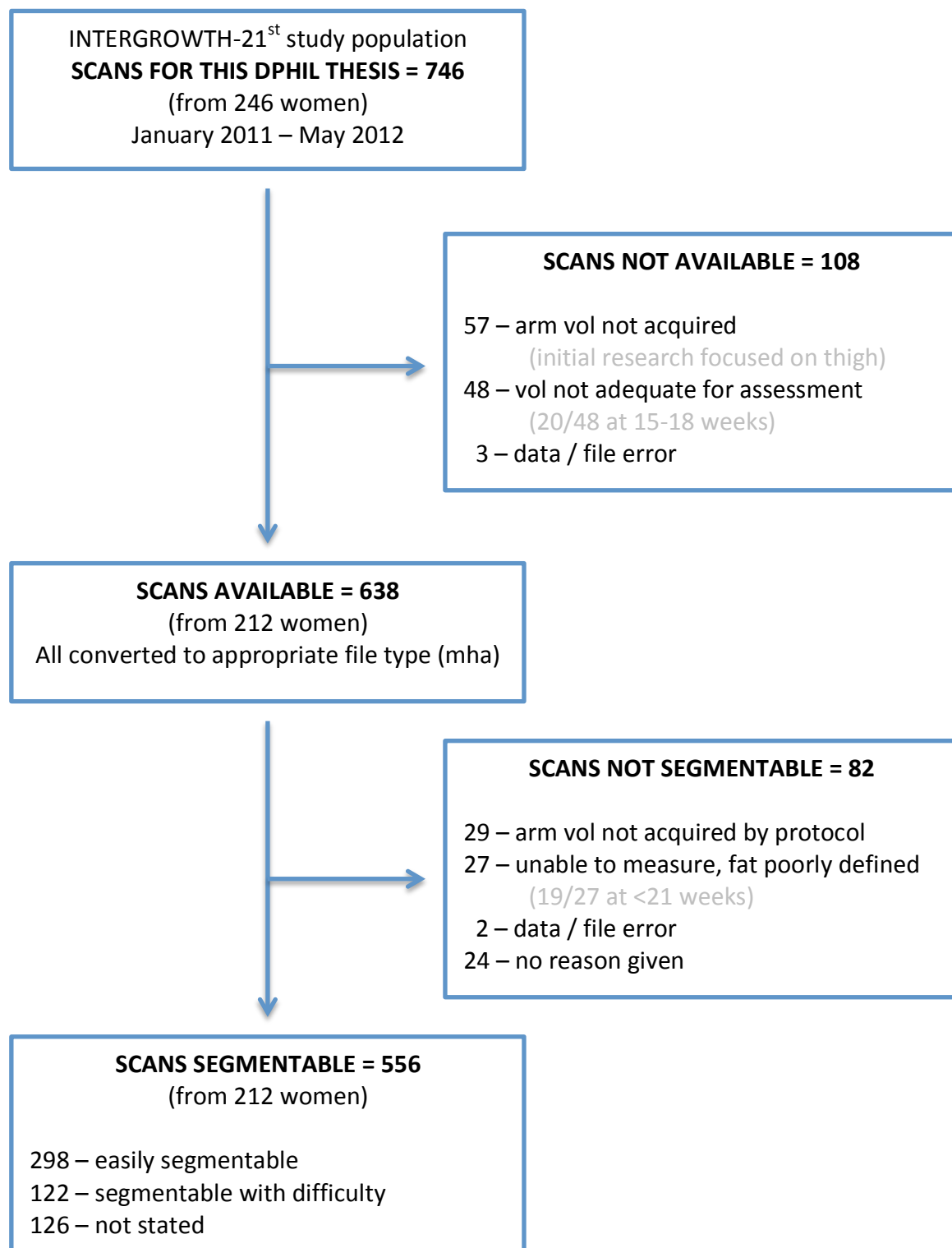


Figure 82: Flow diagram showing generation of fetal arm volume results.

Humeral length (HL) was the first measurement of the segmentation process, as the central 50% of the length was used to generate the 50% fractional volume. This was measured with the reconstructed sagittal plane running parallel to the long humeral axis and perpendicular to the axial plane (after slice selection +/- rotation of the volume if necessary), for optimal accuracy.

If a volume is not rotated so as to obtain the correct, maximal, HL, this can result in a falsely shortened HL and – as the volume is interpolated between the equidistant slices within the fractional volume – corresponding falsely small fractional volume measurements. A falsely short HL measurement can also occur if the sagittal length of the bone is not fully captured, and if this is not correctly identified (and the volume rejected) on visual inspection of the volume prior to segmentation.

It is also possible to overestimate the HL (for example by including part of the acromion) which can result in a falsely large volume measurement. This occurs much less frequently.

The measured volumes were assessed visually, and inadequately rotated volumes excluded from the analyses. To aid with the identification of such cases, the HL measurements were compared with Chitty's reference centiles for HL.²³²

Chitty's studies to generate reference centiles for fetal biometry were found to be the highest quality in a 2012 systematic review.²¹⁵ As the INTERGROWTH-21st study participants were optimally healthy, it would be expected that the majority of the fetal HL results should fall within the mean $\pm$ 3SD on the Chitty chart (which would be expected to encompass 99.7% of the population). This wide range of $\pm$ 3SD was used to allow for possible variations between the chart and the optimally healthy INTERGROWTH-21st population, and to try to avoid exclusion of biological, rather than methodological, outliers.

Overall, this resulted in the exclusion of 55 (9.9%) cases (46 below 3SD and 9 above 3SD), leaving 501 cases for analysis from 208 women. The excluded cases ranged from 18⁺¹ weeks to 40⁺⁶ weeks, with more at later gestational ages (median 35⁺² weeks, inter-quartile range 31⁺⁵ to 37⁺¹ weeks). The final segmentation rate, after excluding inadequately rotated volumes, was an average of 79% of arm volumes being able to be segmented.

The calculated fat, lean and arm 50% fractional volumes (in cm³) were plotted against gestational age [Figure 83 - Figure 86].

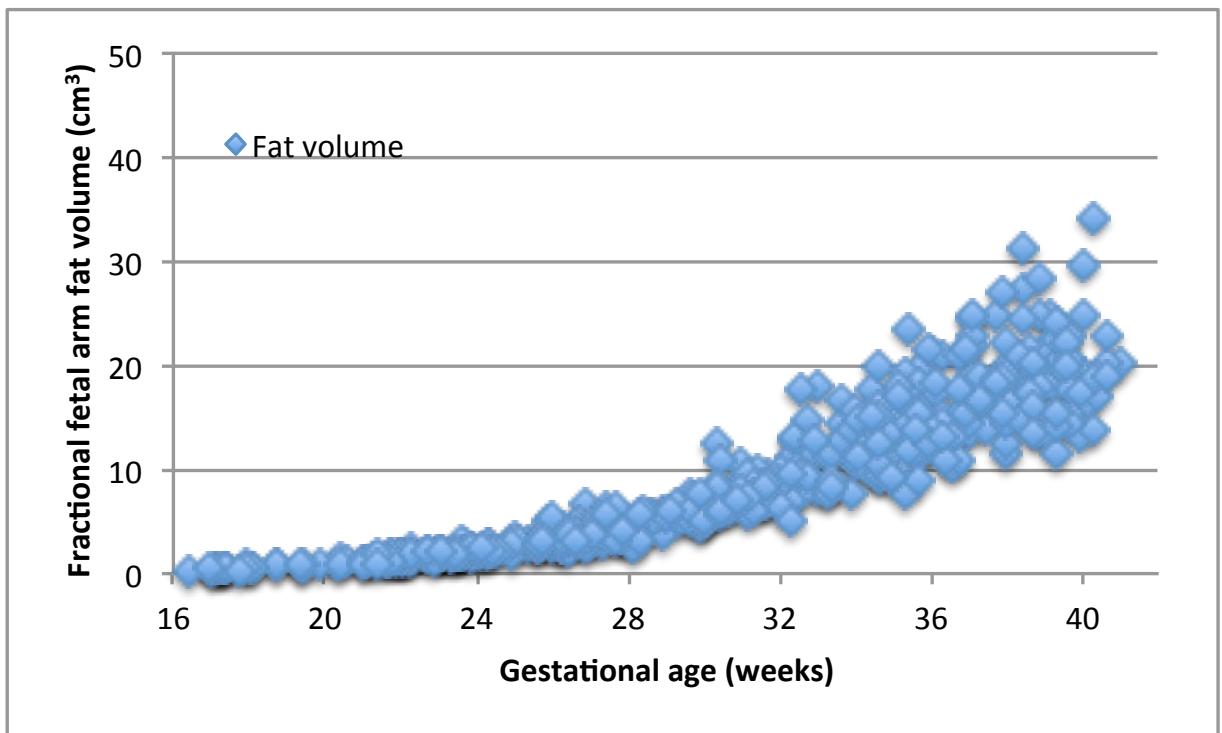


Figure 83: Fractional fetal arm fat volume (50% fractional volume, acquired axially) (n=501).

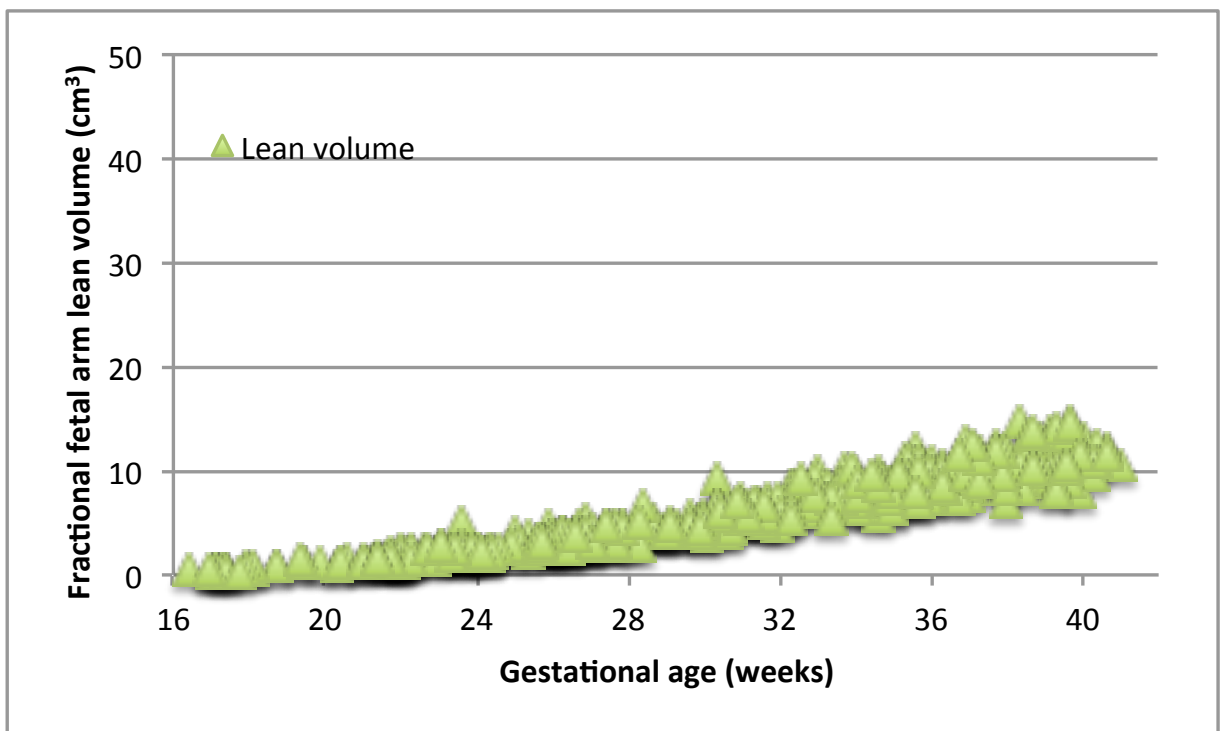


Figure 84: Fractional fetal arm lean volume (50% fractional volume, acquired axially) (n=501).

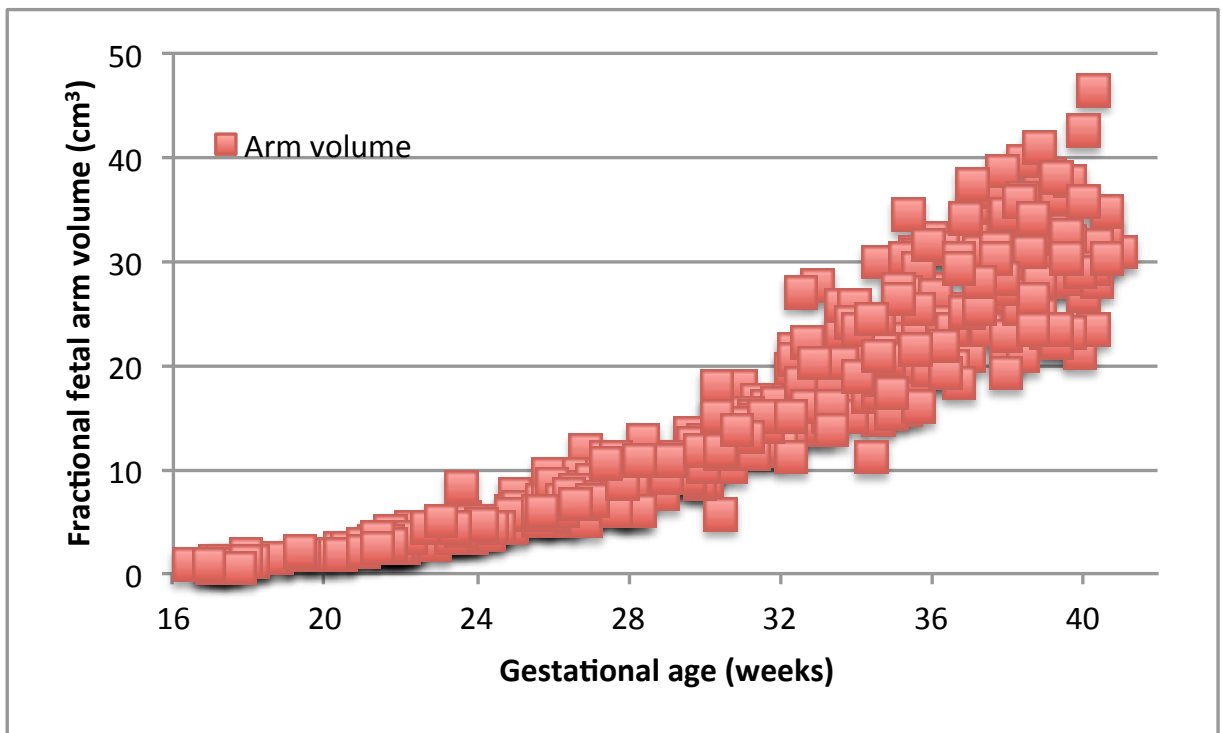


Figure 85: Fractional fetal arm volume (50% fractional volume, acquired axially) (n=501).

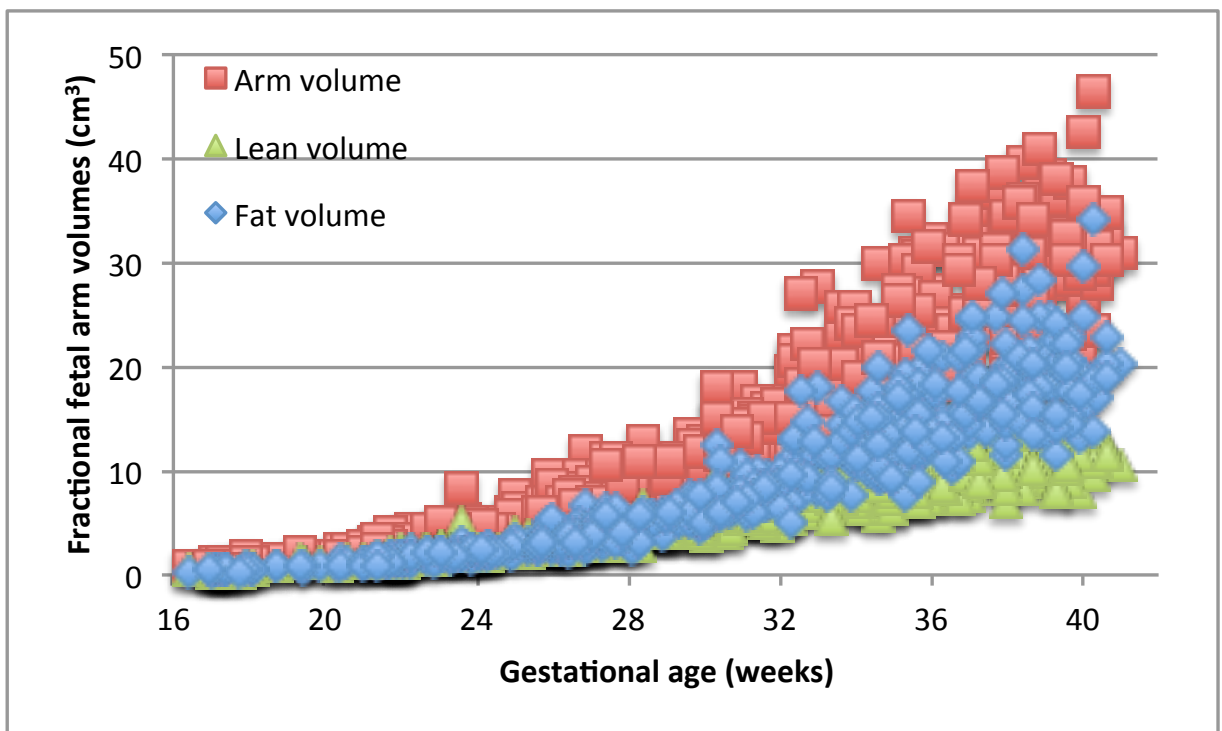


Figure 86: Fractional fetal arm, fat and lean volumes (50% fractional volumes, acquired axially) superimposed (n=501).

Fractional polynomial regression^{206,207} was used to generate equations to calculate the mean / 50th centile (C50) values for arm fat volume and lean volume (50% fractional volumes) for a given gestational age (GA):

$$\text{Log (arm fat volume) C50} = ((\text{GA}/10) \times 2.811848) + ((\text{GA}/10)^3 \times -0.0455122) - 5.316413$$

$$\text{Log (arm lean volume) C50} = ((\text{GA}/10)^{-1} \times -9.506378) + 4.838558$$

The relationship with standard deviation (SD) was also generated using the same approach.

$$\text{SD of log (arm fat vol) C50} = 0.21$$

$$\text{SD of log (arm lean vol) C50} = 0.16$$

This enabled calculation of the centiles for both sets of data:

$$3^{\text{rd}} / 97^{\text{th}} \text{ centiles} = \text{mean} \pm 1.88 \text{ SD}$$

$$5^{\text{th}} / 95^{\text{th}} \text{ centiles} = \text{mean} \pm 1.64 \text{ SD}$$

$$10^{\text{th}} / 90^{\text{th}} \text{ centiles} = \text{mean} \pm 1.28 \text{ SD}$$

For both the fractional arm fat volume and the fractional arm lean volume, SD was used to calculate the log-transformed centiles, and the results then transformed back to generate results in cm³ [Table 18, Table 19].

Table 18: Centile values for fractional fetal arm fat volume for each week of gestation.

GA (weeks)	Fractional fetal arm fat volume (cm ³) centiles						
	3 rd (mean -1.88 SD)	5 th (mean - 1.64 SD)	10 th (mean - 1.28 SD)	50 th (mean)	90 th (mean + 1.28 SD)	95 th (mean + 1.64 SD)	97 th (mean +1.88 SD)
16	0.25	0.26	0.28	0.37	0.48	0.52	0.54
17	0.32	0.33	0.36	0.47	0.61	0.66	0.69
18	0.40	0.42	0.45	0.59	0.78	0.84	0.88
19	0.51	0.53	0.57	0.75	0.98	1.06	1.12
20	0.64	0.67	0.72	0.94	1.24	1.33	1.40
21	0.80	0.84	0.90	1.18	1.55	1.67	1.75
22	0.99	1.04	1.12	1.47	1.92	2.07	2.18
23	1.22	1.29	1.39	1.82	2.38	2.56	2.70
24	1.50	1.58	1.71	2.23	2.92	3.15	3.31
25	1.84	1.93	2.08	2.72	3.56	3.84	4.04
26	2.22	2.34	2.52	3.30	4.32	4.66	4.90
27	2.68	2.82	3.04	3.97	5.20	5.61	5.90
28	3.20	3.36	3.63	4.75	6.21	6.70	7.05
29	3.79	3.99	4.30	5.63	7.37	7.94	8.36
30	4.46	4.69	5.06	6.62	8.66	9.35	9.83
31	5.20	5.47	5.90	7.73	10.11	10.90	11.47
32	6.02	6.33	6.83	8.94	11.69	12.61	13.27
33	6.90	7.26	7.83	10.25	13.41	14.46	15.21
34	7.85	8.25	8.90	11.65	15.24	16.44	17.29
35	8.83	9.29	10.02	13.11	17.16	18.51	19.47
36	9.85	10.36	11.18	14.63	19.14	20.64	21.71
37	10.88	11.44	12.34	16.15	21.14	22.80	23.98
38	11.90	12.51	13.49	17.66	23.11	24.92	26.21
39	12.87	13.54	14.60	19.11	25.00	26.97	28.36
40	13.78	14.49	15.63	20.45	26.77	28.87	30.36
41	14.59	15.35	16.55	21.66	28.34	30.57	32.15

Table 19: Centile values for fractional fetal arm lean volume for each week of gestation.

GA (weeks)	Fractional fetal arm lean volume (cm ³) centiles						
	3 rd (mean -1.88 SD)	5 th (mean - 1.64 SD)	10 th (mean - 1.28 SD)	50 th (mean)	90 th (mean + 1.28 SD)	95 th (mean + 1.64 SD)	97 th (mean +1.88 SD)
16	0.24	0.25	0.27	0.33	0.41	0.43	0.45
17	0.35	0.36	0.38	0.47	0.58	0.62	0.64
18	0.47	0.49	0.52	0.64	0.79	0.84	0.87
19	0.62	0.65	0.69	0.85	1.05	1.11	1.15
20	0.80	0.83	0.88	1.09	1.34	1.42	1.48
21	1.00	1.05	1.11	1.37	1.68	1.78	1.86
22	1.23	1.28	1.36	1.68	2.07	2.19	2.28
23	1.49	1.55	1.64	2.02	2.49	2.65	2.75
24	1.77	1.84	1.95	2.41	2.96	3.14	3.27
25	2.07	2.16	2.29	2.82	3.47	3.68	3.83
26	2.40	2.50	2.65	3.26	4.02	4.26	4.43
27	2.75	2.86	3.03	3.73	4.60	4.88	5.08
28	3.12	3.24	3.44	4.24	5.22	5.53	5.76
29	3.50	3.64	3.86	4.76	5.87	6.22	6.47
30	3.91	4.06	4.31	5.31	6.54	6.94	7.22
31	4.33	4.50	4.77	5.88	7.25	7.69	7.99
32	4.76	4.95	5.25	6.47	7.98	8.46	8.80
33	5.21	5.42	5.75	7.08	8.73	9.26	9.63
34	5.67	5.90	6.26	7.71	9.50	10.08	10.48
35	6.15	6.39	6.78	8.35	10.29	10.91	11.35
36	6.63	6.89	7.31	9.01	11.10	11.77	12.24
37	7.12	7.40	7.85	9.67	11.92	12.64	13.14
38	7.62	7.92	8.40	10.35	12.75	13.52	14.06
39	8.12	8.44	8.95	11.03	13.60	14.42	15.00
40	8.63	8.97	9.52	11.73	14.45	15.33	15.94
41	9.14	9.51	10.09	12.43	15.31	16.24	16.89

Table 17 and Table 18 are shown in graph form below for fractional arm fat [Figure 86] and lean volumes [Figure 87] (50th centile coloured line; 10th / 90th centiles dashed lines; 3rd / 97th centiles dotted lines):

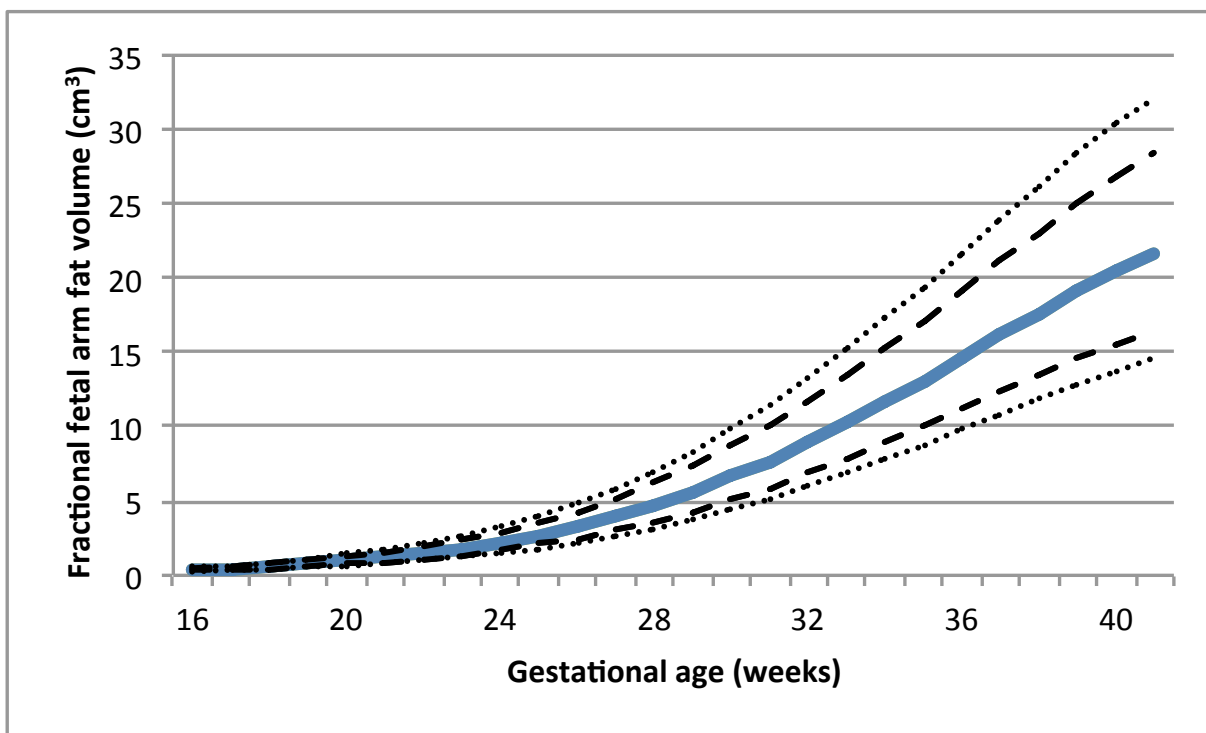


Figure 87: Fractional fetal arm fat volume (3rd, 10th, 50th, 90th and 97th centiles).

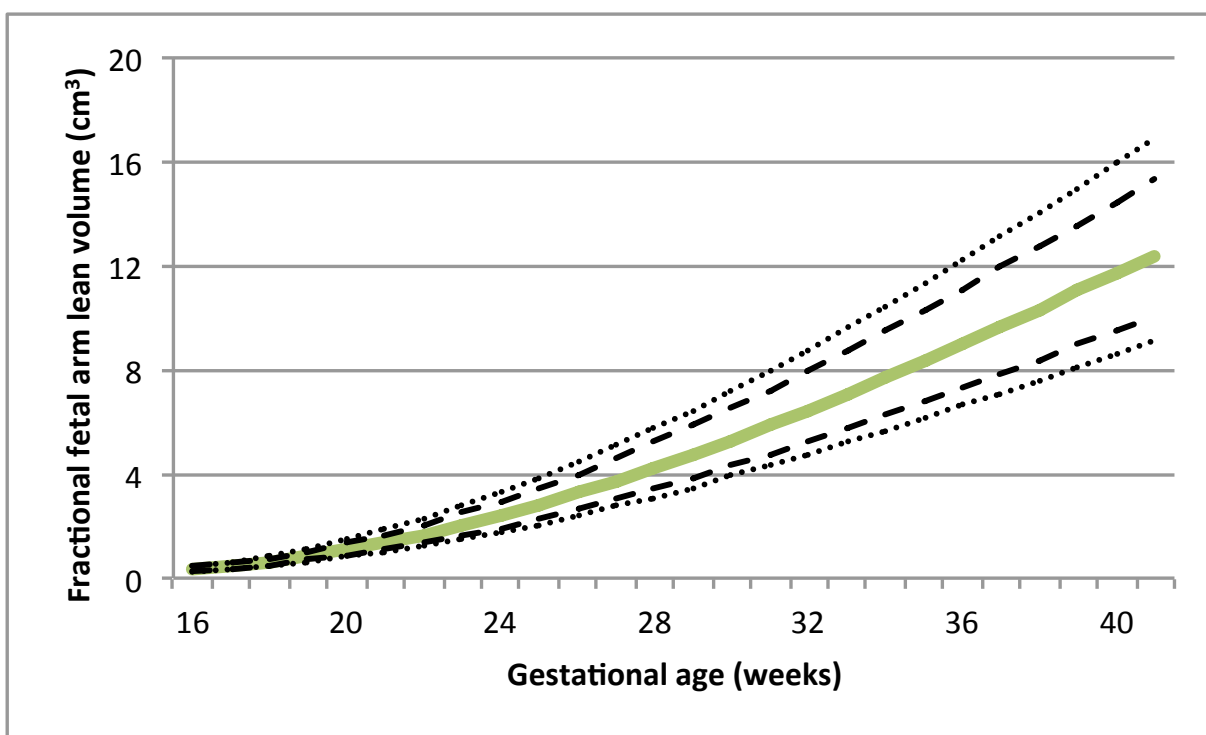


Figure 88: Fractional fetal arm lean volume (3rd, 10th, 50th, 90th and 97th centiles).

Arm fat was also assessed in the form of its percentage of the fractional arm volume:

$$\text{Fat as \% arm volume C50} = ((\text{GA}/10)^3 \times 2.096363) + (((\text{GA}/10)^3 \times \ln(\text{GA}/10)) \times -1.172409) + 33.65732$$

$$\text{SD of fat as \% arm vol C50} = 4.32$$

Table 20: Centile values for fractional fetal arm fat as a percentage of arm volume for each week of gestation.

GA (weeks)	Fractional fetal arm fat (as % of arm volume) centiles						
	3 rd (mean -1.88 SD)	5 th (mean - 1.64 SD)	10 th (mean - 1.28 SD)	50 th (mean)	90 th (mean + 1.28 SD)	95 th (mean + 1.64 SD)	97 th (mean +1.88 SD)
20	35.8	36.8	38.4	43.9	49.5	51.0	52.1
21	36.9	37.9	39.5	45.0	50.5	52.1	53.1
22	38.0	39.0	40.6	46.1	51.7	53.2	54.3
23	39.2	40.2	41.8	47.3	52.8	54.4	55.4
24	40.3	41.4	42.9	48.4	54.0	55.5	56.6
25	41.5	42.5	44.1	49.6	55.2	56.7	57.8
26	42.7	43.7	45.3	50.8	56.3	57.9	58.9
27	43.9	44.9	46.5	52.0	57.5	59.1	60.1
28	45.1	46.1	47.6	53.2	58.7	60.3	61.3
29	46.2	47.3	48.8	54.3	59.9	61.4	62.5
30	47.4	48.4	50.0	55.5	61.0	62.6	63.6
31	48.5	49.5	51.1	56.6	62.1	63.7	64.7
32	49.5	50.6	52.1	57.7	63.2	64.8	65.8
33	50.6	51.6	53.2	58.7	64.2	65.8	66.8
34	51.5	52.6	54.1	59.7	65.2	66.7	67.8
35	52.4	53.5	55.0	60.6	66.1	67.7	68.7
36	53.3	54.3	55.9	61.4	66.9	68.5	69.5
37	54.0	55.1	56.6	62.1	67.7	69.2	70.3
38	54.7	55.7	57.3	62.8	68.3	69.9	70.9
39	55.2	56.3	57.8	63.4	68.9	70.4	71.5
40	55.7	56.7	58.3	63.8	69.3	70.9	71.9
41	56.0	57.0	58.6	64.1	69.7	71.2	72.3

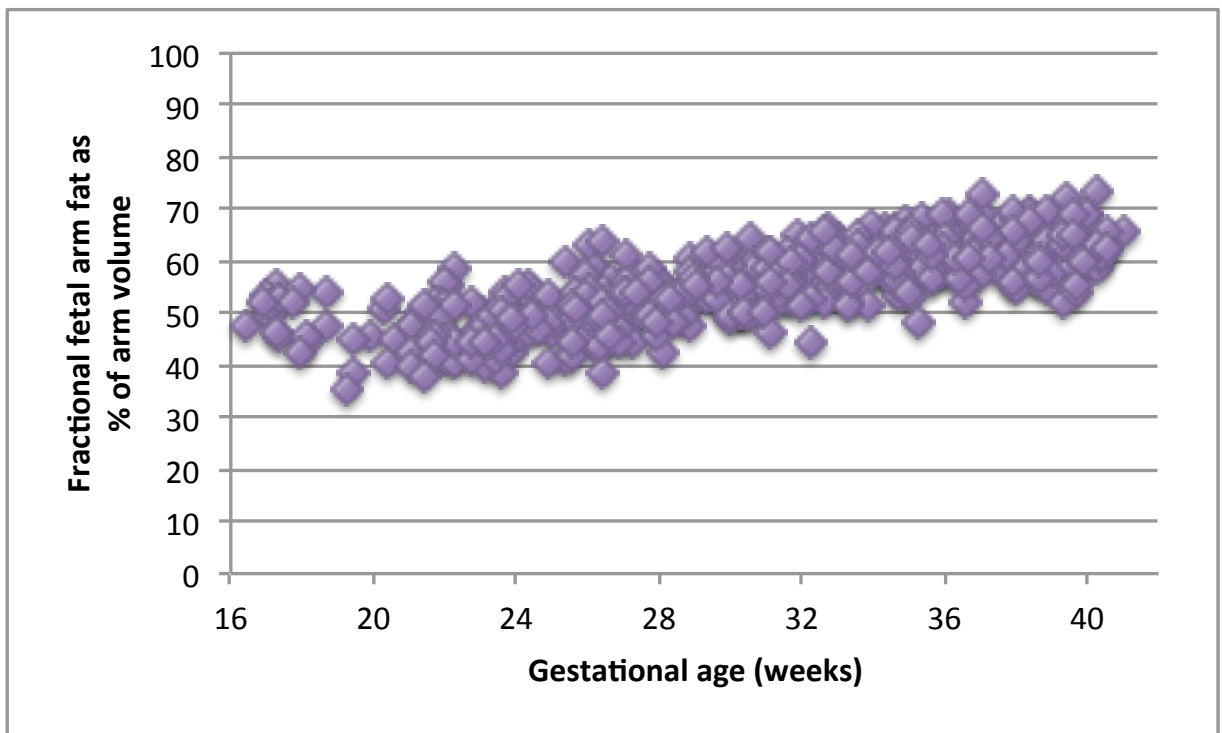


Figure 89: Fractional fetal arm fat as percentage of arm volume (50% fractional volume, axial) (n=501).

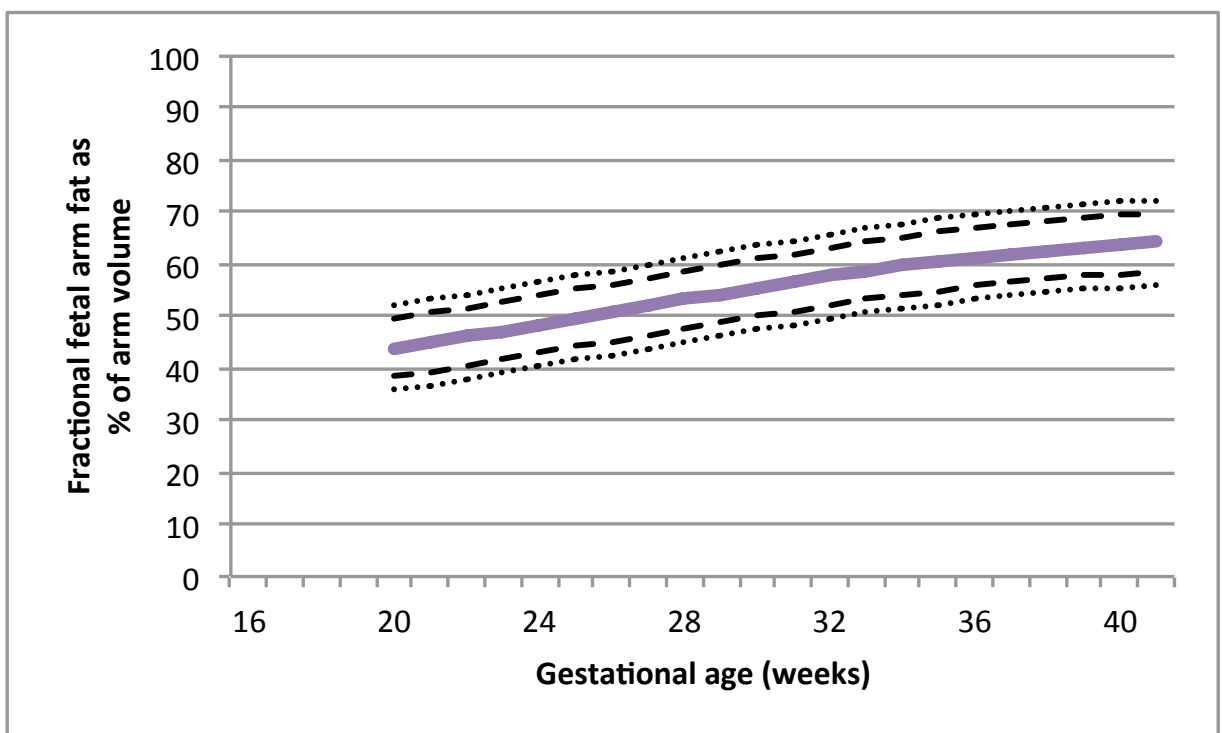


Figure 90: Fractional fetal arm fat as percentage of arm volume (3rd, 10th, 50th, 90th and 97th centiles).

Finally, arm volume [Figure 90, Table 20] centiles were generated:

$$\text{Log (arm volume) C50} = ((\text{GA}/10)^{-0.5} \times -13.46631) + 10.24241$$

$$\text{SD of log (arm volume) C50} = 0.17$$

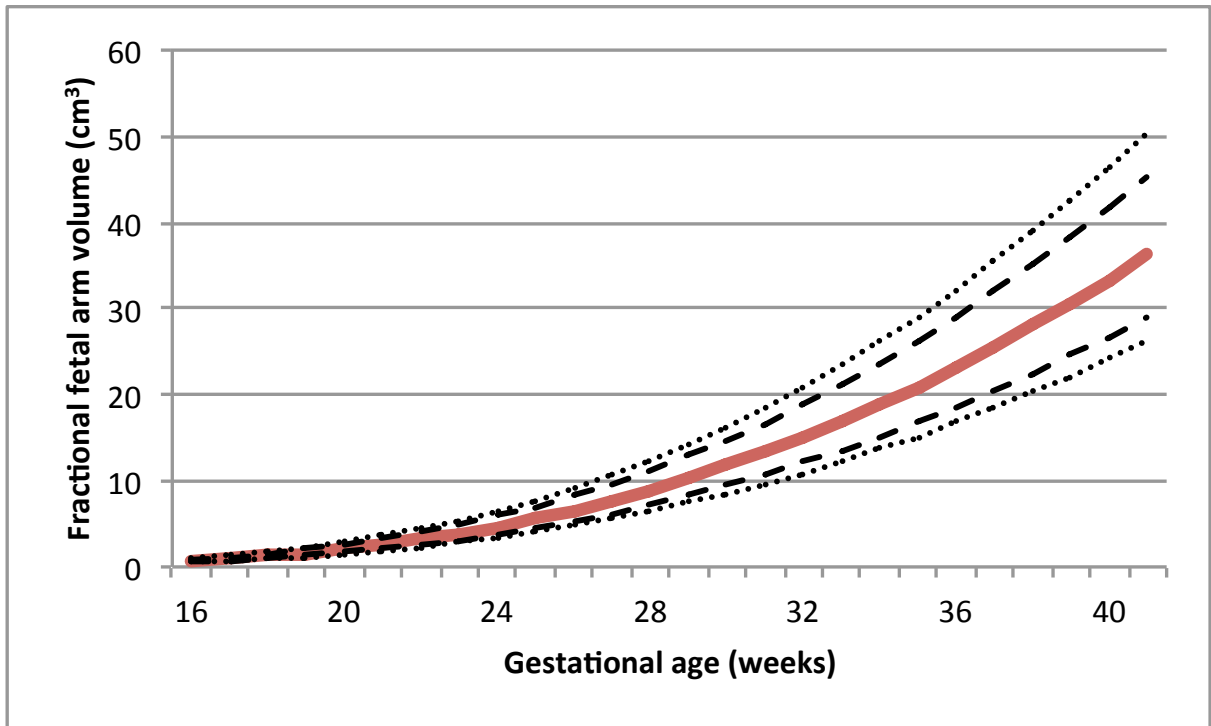


Figure 91: Fractional fetal arm volume (3rd, 10th, 50th, 90th and 97th centiles).

Table 21: Centile values for fractional fetal arm volume for each week of gestation.

GA (weeks)	Fractional fetal arm volume (cm ³) centiles						
	3 rd (mean -1.88 SD)	5 th (mean - 1.64 SD)	10 th (mean - 1.28 SD)	50 th (mean)	90 th (mean + 1.28 SD)	95 th (mean + 1.64 SD)	97 th (mean +1.88 SD)
16	0.48	0.50	0.53	0.67	0.83	0.89	0.93
17	0.66	0.69	0.73	0.92	1.15	1.22	1.27
18	0.89	0.92	0.98	1.23	1.53	1.63	1.70
19	1.16	1.21	1.28	1.60	2.00	2.13	2.22
20	1.48	1.55	1.65	2.06	2.57	2.73	2.85
21	1.87	1.94	2.07	2.59	3.23	3.44	3.58
22	2.31	2.41	2.56	3.20	4.00	4.26	4.44
23	2.82	2.94	3.13	3.91	4.88	5.20	5.42
24	3.40	3.54	3.77	4.71	5.88	6.26	6.53
25	4.05	4.22	4.50	5.62	7.01	7.47	7.78
26	4.78	4.98	5.31	6.63	8.27	8.81	9.18
27	5.59	5.83	6.20	7.75	9.67	10.30	10.73
28	6.48	6.75	7.19	8.98	11.21	11.93	12.44
29	7.45	7.77	8.27	10.33	12.90	13.73	14.31
30	8.51	8.87	9.44	11.79	14.73	15.68	16.35
31	9.66	10.07	10.72	13.38	16.71	17.79	18.55
32	10.89	11.36	12.09	15.10	18.85	20.07	20.92
33	12.22	12.74	13.56	16.94	21.15	22.51	23.47
34	13.64	14.22	15.14	18.90	23.61	25.13	26.20
35	15.15	15.79	16.81	21.00	26.22	27.91	29.10
36	16.75	17.47	18.59	23.22	29.00	30.87	32.18
37	18.45	19.24	20.48	25.57	31.94	34.00	35.45
38	20.25	21.11	22.47	28.06	35.04	37.30	38.89
39	22.13	23.08	24.56	30.68	38.31	40.78	42.52
40	24.12	25.14	26.76	33.42	41.74	44.43	46.32
41	26.19	27.31	29.07	36.30	45.34	48.26	50.31

Discussion

Fetal arm fat volumes [Table 18] showed a 59-fold increase from 16 to 41 weeks (50th centile: 0.37cm³ to 21.66cm³); this is double the 30-fold increase in fetal whole body chemical composition studies of fat,⁸⁸ and the 29-fold increase in 2D arm fat area measurements (section 4c). The third trimester fetal arm fat volumes, however, show a 4.6-fold increase (4.75cm³ to 21.66cm³), similar to the 4.5-fold chemical composition studies,⁸⁵ and to the 4-fold 2D arm fat data.

Fetal arm lean tissue volumes [Table 19] showed a 38-fold increase from 16 to 41 weeks (50th centile: 0.33cm³ to 12.43cm³). As previously discussed, it is difficult to compare these measurements with fetal whole body chemical composition studies of lean tissues, but this increase far exceeds the observed 9-fold changes in arm 2D arm lean area measurements. In a comparable manner, though, the third trimester fetal arm lean volume showed a 2.9-fold increase from 28 to 41 weeks (4.24cm³ to 12.43cm³), which was similar to the 2.3-fold 2D lean increase.

The differences between limb and whole body composition from 16 to 41 weeks might be explained by differential laying down of central and peripheral fat, but the differences between 2D and 3D arm measurements are harder to explain. It is possible that the errors associated with measuring volumes have a more significant role at low gestational ages. All three modalities (2D and 3D ultrasound measurements, and whole body chemical composition studies), however, produce similar results in the third trimester (28 to 41 weeks).

Meanwhile the relative changes in arm fat and lean volumes show a similar pattern to the areas. The arm has comparable amounts of fat and lean tissue at 16-17 weeks; more lean than fat from 18-25 weeks; and more fat than lean from 26 weeks onwards [Figure 92]. This is also the gestational age at which 2D fat area exceeded lean area [Figure 52]. At 40 weeks, there was a greater fat:lean ratio in 2D areas (1.92:1) compared with 3D volumes (1.74:1).

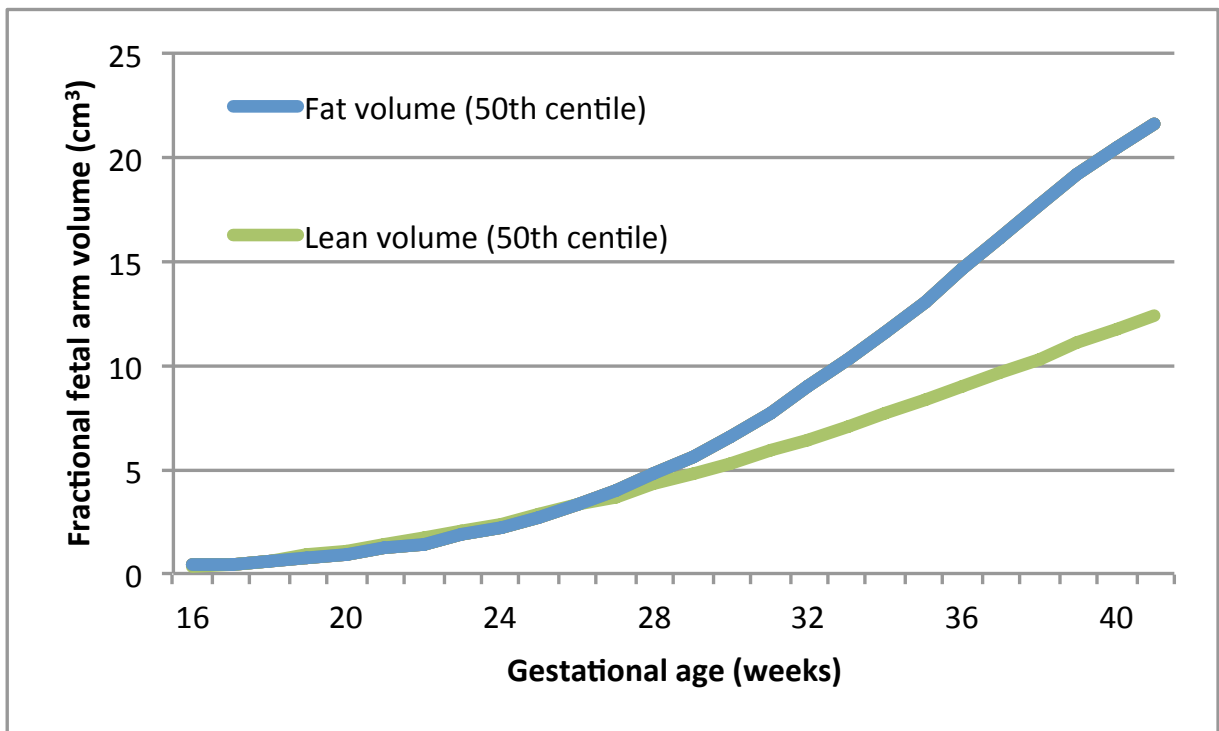


Figure 92: 50th centile lines for fractional fetal arm fat and lean volumes.

Variations in the amount of fat and lean tissue in the five slices which contribute to the fractional volume may result in a different fat:lean ratio; volumes would take account of this variation, but areas (single slice) would not. Such variations are demonstrated, particularly near term, in sample volumes at 2-weekly intervals in 13 different fetuses [Figure 93].

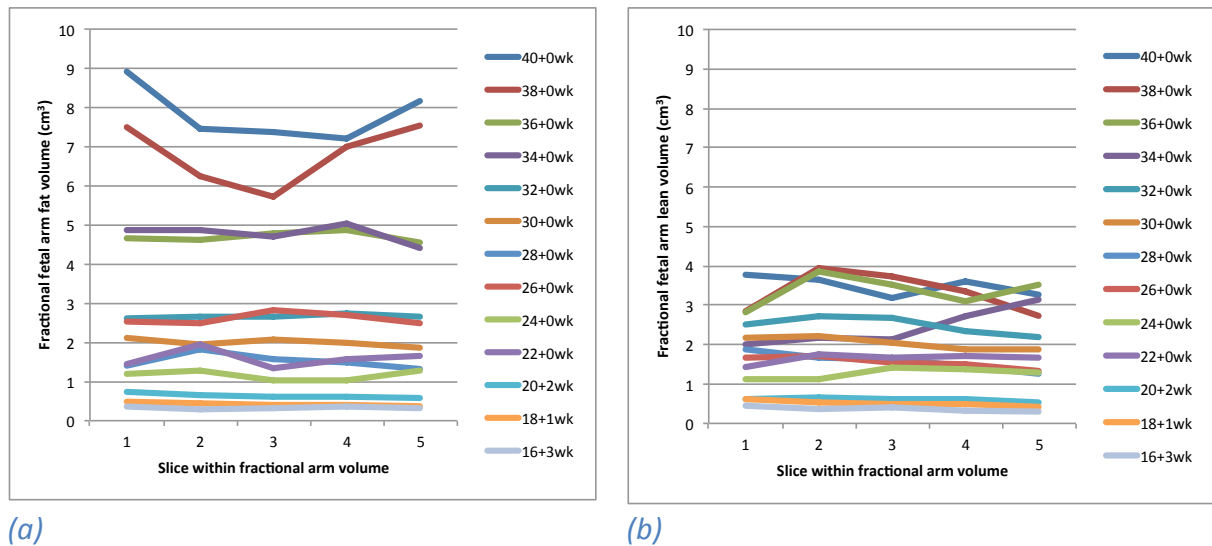
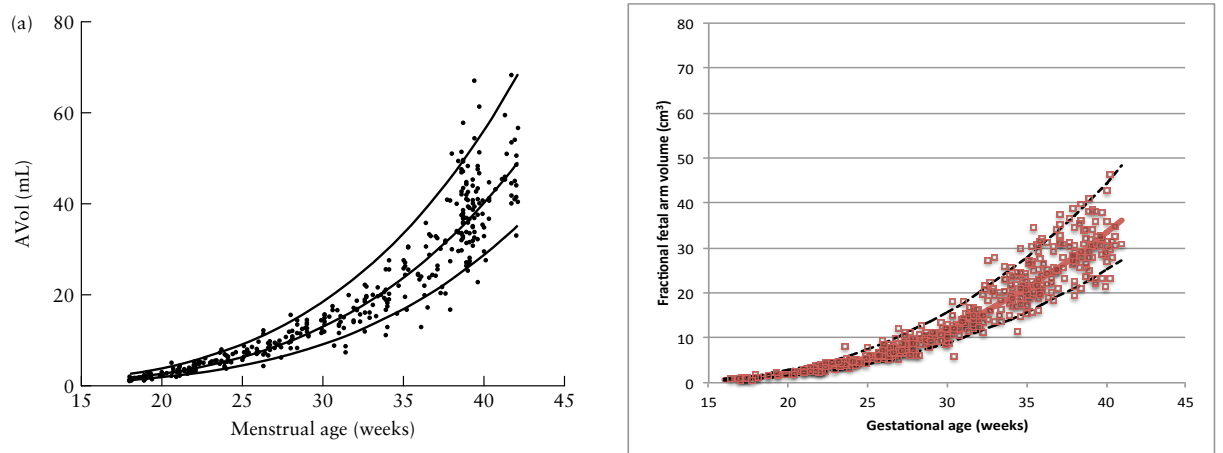


Figure 93: Differences in areas by slice of fractional volume for (a) fat and (b) lean areas in sample volumes.

In a related concept to that of fat:lean ratios, fetal fractional arm fat volume can also be expressed as a percentage of the fetal fractional arm volume. The scatterplot of these measurements [Figure 89] shows percentage fat volume values at 16-18 weeks which seem higher (42-55%) than expected at this gestational age, when compared with the other data points. Indeed, visual assessment of these images confirms that they appear to have only a very small percentage of fat. The discrepancy might well be due to the limitations of the measuring tool, the smallest unit of which is 0.3mm. Taking this into consideration, and the fact that, in clinical terms, fat is unlikely to be calculated at such low gestational ages, the centiles were thus calculated for gestational ages of 20 weeks and above [Figure 90]. The 50th centiles showed a similar rate of increase for both areas and volumes, although the percentage fat area showed more divergent 3rd-97th centiles.

The measurements discussed above are novel, so have no direct point of comparison. Fractional arm volume (AVol) measurements, however, were first described by Lee et al in 2001¹²⁶ and have been explored by his group in several papers and abstracts. Of these, there is only one large study across the second and third trimesters, in which reference ranges are calculated.³ I shall therefore compare my work with this paper in some detail.

The scatterplots, including 5th, 50th and 95th centiles, are shown below [Figure 94]:



(a) Scatterplot of AVol vs. menstrual age, with 5th, 50th and 95th centiles, taken from Lee et al 2009.³

(b) Scatterplot of AVol vs. gestational age, with 5th, 50th and 95th centiles, from this thesis (axes as per Lee et al).

Figure 94: Comparison of AVol scatterplots and reference ranges.

Lee's work uses 379 data points from 18.0 to 42.1 weeks; mine includes 487 data points for a similar gestational age range (18.0 to 41 weeks). The centiles from Lee's paper are recorded as "18-18.9" weeks of menstrual age [Figure 95a], whereas the centiles in my work are calculated by gestational age on a day-by-day basis. I therefore calculated the centiles in the middle of each week of gestational age (18⁺³ etc) to enable comparison [Figure 95b]. Of note, the "1 SD" column of Lee's table expresses the variance of the 50th centile line, rather than the variance of the data points. For my data, 0.17 is the SD of the log-transformed data for the full dataset; back-transformation gives a value of 1.19.

Menstrual age (weeks)	n	5 th	10 th	25 th	50 th	75 th	90 th	95 th	1 SD
18–18.9	16	1.3	1.4	1.6	1.9	2.2	2.6	2.8	1.27
19–19.9	12	1.5	1.7	1.9	2.3	2.7	3.1	3.4	1.27
20–20.9	11	1.9	2.1	2.4	2.9	3.3	3.8	4.2	1.26
21–21.9	17	2.3	2.5	2.9	3.4	4.0	4.6	5.0	1.26
22–22.9	15	2.7	2.9	3.4	3.9	4.6	5.3	5.8	1.26
23–23.9	12	3.4	3.7	4.3	5.0	5.8	6.7	7.3	1.26
24–24.9	10	3.8	4.1	4.8	5.5	6.4	7.4	8.0	1.25
25–25.9	14	4.6	5.0	5.7	6.7	7.8	8.9	9.6	1.25
26–26.9	13	5.4	5.9	6.7	7.8	9.1	10.4	11.3	1.25
27–27.9	12	6.3	6.8	7.8	9.0	10.5	12.0	13.0	1.25
28–28.9	14	7.2	7.8	8.9	10.3	11.9	13.6	14.7	1.24
29–29.9	11	8.2	8.9	10.1	11.7	13.6	15.5	16.8	1.24
30–30.9	14	9.5	10.3	11.7	13.6	15.7	17.9	19.3	1.24
31–31.9	13	10.8	11.7	13.4	15.4	17.8	20.3	22.0	1.24
32–32.9	15	12.5	13.5	15.4	17.8	20.5	23.3	25.2	1.24
33–33.9	14	14.3	15.4	17.5	20.2	23.3	26.5	28.6	1.24
34–34.9	9	15.3	16.5	18.8	21.6	24.9	28.3	30.6	1.23
35–35.9	13	17.3	18.7	21.2	24.4	28.1	32.0	34.5	1.23
36–36.9	13	19.7	21.3	24.1	27.7	31.9	36.2	39.1	1.23
37–37.9	15	22.0	23.7	26.9	30.9	35.6	40.3	43.5	1.23
38–38.9	45	25.0	27.0	30.6	35.1	40.3	45.7	49.2	1.23
39–39.9	49	26.9	28.9	32.8	37.6	43.2	48.9	52.7	1.23
40–40.9	9	29.4	31.6	35.8	41.1	47.1	53.3	57.4	1.23
41–41.9	13	33.6	36.1	40.8	46.8	53.7	60.7	65.3	1.22
42–42.9	8	35.0	37.7	42.6	48.8	55.9	63.2	68.0	1.22

GA	n	5th	10th	50th	90th	95th	log SD
18+3	4	1.0	1.1	1.4	1.7	1.8	0.17
19+3	4	1.3	1.4	1.8	2.2	2.4	0.17
20+3	4	1.7	1.8	2.3	2.8	3.0	0.17
21+3	20	2.1	2.3	2.8	3.5	3.8	0.17
22+3	21	2.6	2.8	3.5	4.4	4.6	0.17
23+3	26	3.2	3.4	4.2	5.3	5.6	0.17
24+3	15	3.8	4.1	5.1	6.4	6.8	0.17
25+3	16	4.5	4.8	6.0	7.5	8.0	0.17
26+3	37	5.3	5.7	7.1	8.9	9.4	0.17
27+3	36	6.2	6.6	8.3	10.3	11.0	0.17
28+3	19	7.2	7.6	9.5	11.9	12.7	0.17
29+3	16	8.2	8.8	10.9	13.7	14.5	0.17
30+3	22	9.4	10.0	12.5	15.6	16.6	0.17
31+3	29	10.6	11.3	14.1	17.6	18.8	0.17
32+3	21	11.9	12.7	15.9	19.8	21.1	0.17
33+3	17	13.4	14.2	17.8	22.2	23.6	0.17
34+3	33	14.9	15.8	19.8	24.7	26.3	0.17
35+3	34	16.5	17.6	21.9	27.4	29.2	0.17
36+3	24	18.2	19.4	24.2	30.2	32.2	0.17
37+3	20	20.0	21.3	26.6	33.3	35.4	0.17
38+3	29	21.9	23.4	29.2	36.4	38.8	0.17
39+3	26	24.0	25.5	31.8	39.8	42.3	0.17
40+3	13	26.1	27.7	34.6	43.3	46.1	0.17
41+3	1	28.3	30.1	37.6	46.9	50.0	0.17

(a)

(b)

Figure 95: AVol centiles from (a) Lee et al 2009³ and (b) this thesis. (The number of observations in (b) relate to the number of scans at that completed week eg 18+3 = 18⁺⁰ to 18⁺⁶.)

The 5th, 50th and 95th centiles were then plotted to enable visual comparison [Figure 96]:

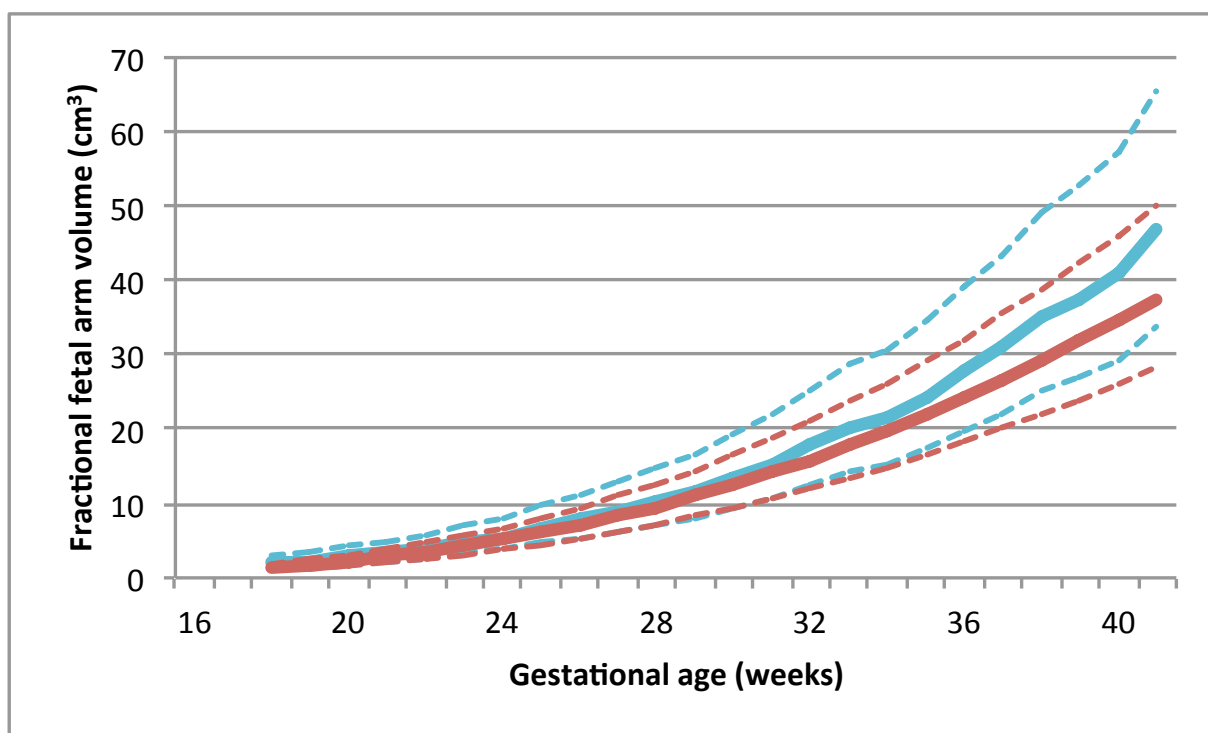


Figure 96: Comparison of AVol centiles: 50th centile solid line, 5th and 95th dashed lines (author's work in red, compared with that of Lee et al 2009³ in turquoise).

It is immediately evident, from the scatterplot and centiles, that Lee's AVol values are higher than those from this thesis. The 50th centiles are comparable until 31 weeks, after which Lee's 50th centile is at a higher value (19% greater at 40 weeks, 41.1 vs. 34.6cm³). A similar pattern is observed for the 5th centiles: similar until 32 weeks, after which Lee's 5th centile is at a higher value (13% greater at 40 weeks, 29.4 vs. 26.1cm³). Lee's 95th centiles, however, are higher than mine throughout, and 25% higher at 40 weeks (57.4 vs. 46.1cm³). The reasons for these differences could be methodological or due to population differences.

Lee's volumes were obtained from a sagittal reference image, whereas mine were axial (for optimal image quality for segmentation). His group used GE machines, and ours Philips, but this should not result in an appreciable difference in volumes; nor should the segmentation using 4D View 5.0 (Lee) or the MATLAB tool (this thesis). Both studies measured the arm using 5 equidistant slices in the middle 50% of the humerus to generate AVol. The representative images in his paper show a similar segmentation approach, with a similar amount of time of a few minutes for each.

Both studies used natural logarithmic transformation of the AVol data to enable centile calculations, with similar methods: weighted least-squares regression (Lee) and generalised least-square regression (this thesis). Lee's centiles are not smooth lines when plotted [Figure 95], which may suggest some methodological variation. His centiles are also given in 7-day ranges (eg "18.0-18.9 weeks"), with no equation provided. My centiles are calculated on a daily basis rather than approximating between completed weeks. A comparison of my raw data, by completed weeks of gestation, and Lee's fitted centiles [Table 21] confirmed a difference between the values, with increases at 30, 36 and particularly 38-40 weeks.

Table 22: Comparison of AVol values: thesis raw data (mean) with fitted centiles (Lee 2009³).

GA (weeks)	Lee n	AVol 50th centile (cm3)	Knight n	AVol mean (cm3)	Difference (Lee- Knight)
18	16	1.9	4	1.4	0.5
19	12	2.3	4	1.9	0.4
20	11	2.9	4	2.2	0.7
21	17	3.4	19	3.0	0.4
22	15	3.9	21	3.6	0.3
23	12	5	26	4.5	0.5
24	10	5.5	15	4.7	0.8
25	14	6.7	16	6.4	0.3
26	14	7.8	37	7.1	0.7
27	12	9	36	8.3	0.7
28	14	10.3	19	9.2	1.1
29	11	11.7	16	10.6	1.1
30	14	13.6	22	12.0	1.6
31	13	15.4	29	14.2	1.2
32	15	17.8	21	16.9	0.9
33	14	20.2	17	19.3	0.9
34	9	21.6	33	20.9	0.7
35	13	24.4	34	22.9	1.5
36	13	27.7	24	24.8	2.9
37	15	30.9	20	29.8	1.1
38	45	35.1	29	29.4	5.7
39	49	37.6	26	30.0	7.6
40	9	41.1	13	32.6	8.5
41	13	46.8	1	n/a	n/a

Whilst Lee's criteria were less exclusive than those of the INTERGROWTH-21st study, they did nevertheless aim for a healthy population, excluding women with hypertension, diabetes, or who smoked; multiple pregnancies; and fetuses with abnormal anomaly scans. All subjects had confirmed dating, though Lee's had a first or second trimester scan, whilst INTERGROWTH-21st used first trimester scan only. The latter is a more accurate method of dating²³³, so this difference could have contributed to some of the variation in results.

The descriptive statistics of the study populations are compared overleaf [Table 23].

Table 23: Comparison of descriptive statistics of study populations for Lee et al 2009³ vs. this thesis.

	Lee et al 2009³	Knight 2014
Study location	Houston and Detroit, USA	Oxford, UK
Median maternal age	29.4	30.0
Interquartile range (IQR)	25.0-34.0	27.0-32.3
Mean maternal BMI	not recorded	23.5
IQR		21.5-25.3
% population White	58.7%	not recorded
% primigravidae	26.9%	66.3%
Median birthweight	3241g	3438g
IQR	2755-3716g	3140-3780g
Gestational age at birth	39.0	40.0
IQR	37.7-39.7	39.0-40.9
Newborn gender	51.2% F : 48.8% M	48.8% F : 51.2% M

Some of the characteristics are similar – for example, the median maternal age is similar between both groups, although the interquartile range (IQR) is wider for Lee’s population. Other factors are less clear. BMI was an inclusion criterion (>18.5 and <30.0) for the INTERGROWTH-21st study [Figure 20]; the subset presented in this thesis reflects this BMI range (18.6-29.9). Of these, the majority were within the “healthy” BMI range of 18.5-24.9, as demonstrated by the IQR. BMI was not stated in Lee’s study but it is recognised that the USA has high levels of obesity. National statistics show that in 2008, 44.3% of 16-44 year old women from the UK had a BMI ≥ 25 so were overweight or obese.²³⁴ In comparison, in 2007-2010 the average BMI in 20-39 year old (non-pregnant) women from the USA was 26.3,²³⁵ meaning that more than 50% of these women were overweight or obese.

The same USA national statistics also show differing average BMI values for 20-39 year old women of different ethnic origins: 26.25 (White, non-Hispanic), 27.7 (Hispanic), 28.4 (Mexican American) and 30.6 (Black, non-Hispanic).²³⁵ With 41.3% of his population being from non-White ethnic groups, it may be that Lee's patients had higher BMI values. Ethnicity was not recorded for INTERGROWTH-21st and whilst it is not possible to directly relate population census data to study data, the American study population (58.7% White) seems likely to have had a higher percentage of non-White ethnic origin women compared with those in Oxford (78% White by 2011 census data).²³⁶

In addition to possible BMI differences, it is well recognised that women of Black African, Black Caribbean, South Asian and Middle Eastern family origin are at an increased risk of gestational and undiagnosed type 2 diabetes.²³⁷ Diabetic pregnancies carry increased risks of raised birthweight, and studies have shown that GDM and impaired glucose tolerance pregnancies have increased fetal arm fat.^{96,116} Higher rates of GDM and undiagnosed type 2 diabetes could have contributed to the AVol differences seen between my study and Lee's.

The parity characteristics of the two studies were quite different. 73.1% of the women in the US study were multiparous compared with 33.7% in my study, and it is recognised that increasing parity is associated with increasing birthweight.²³⁸⁻²⁴¹ Despite this, the Oxford babies had a higher median and IQR birthweight: this may have been due to the optimal health of the INTERGROWTH-21st women. The Oxford babies had smaller fractional arm volumes, in spite of their higher birthweight. One possible reason could be greater body and limb fat in the US study fetuses, causing an increased AVol but, due to the decreased density of fat when compared to lean tissue, not an increased birthweight.¹⁹³

Whilst population differences may have contributed to the differences in the AVol results and calculated centiles, it is likely that there were also remaining methodological differences. A systematic review of fetal ultrasound biometry studies found significant differences between centile charts even in studies which were objectively scored as of high quality: for example, there was up to 10% difference between 50th centiles in AC charts.²¹⁵ This highlights the importance of standardising every aspect of this measurement with great care. Even then, as previously discussed, there are intrinsic increased random measurement errors associated with 3D volumes, and these will have contributed to the differences between the two studies.

Conclusion

This section demonstrates the novel measurements of fractional fetal arm fat and lean volumes, and presents reference ranges from 16 to 41 weeks of gestation. It also adds to the body of work on fractional arm volumes, although our population had a smaller AVol than those studied by Lee et al. This could be due to methodological or population differences. Reference centiles for fractional arm fat volume expressed as a percentage of fractional arm volume are also presented; this is also a novel measurement and will be used as a comparison value with thigh measurements.

ii. 3D volumes: reproducibility

Aim

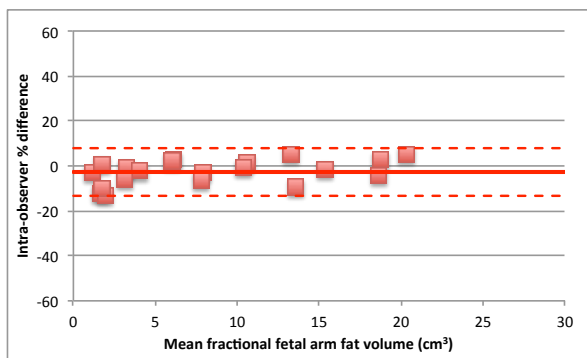
To assess the intra- and inter-observer variability for fat volume measurement.

Method

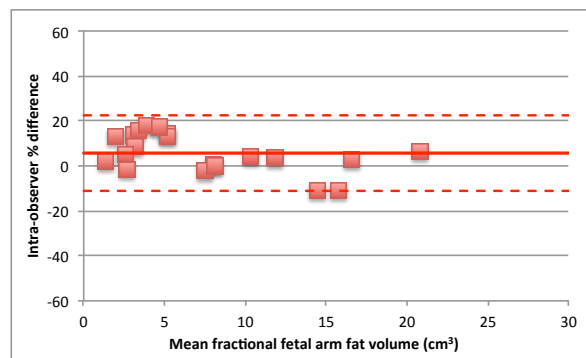
The segmented volumes were listed by the person who segmented them. A random number generator (www.random.org) was used to generate a random selection from each group: 20 for the original segmenter to repeat (intra-observer variability), and 10 for each of the other two segmenters to measure (inter-observer variability). Each person thus measured 40 volumes, and each intra- and inter-observer group had 20 pairs of volumes. The average and range of gestational ages were calculated for each person's 40 volumes, and t-tests performed to ensure the groups were not significantly different. The volumes were then segmented using the MATLAB tool and the results compared using Bland-Altman plots and ICCs.

Results

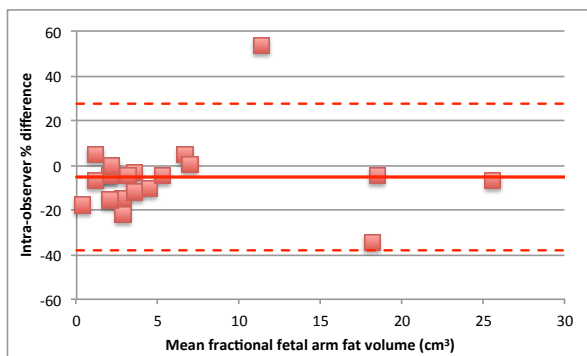
The average gestational ages and ranges for the three groups were: 29^{+5} (20^{+4} - 40^{+4}), 29^{+6} (20^{+3} - 39^{+1}) and 27^{+5} (17^{+0} - 39^{+1}). The three groups were compared in pairs, with non-statistically different t-test results (0.31, 0.93, 0.25) suggesting that the groups were all similar in terms of the gestational ages.



(a) C.K.

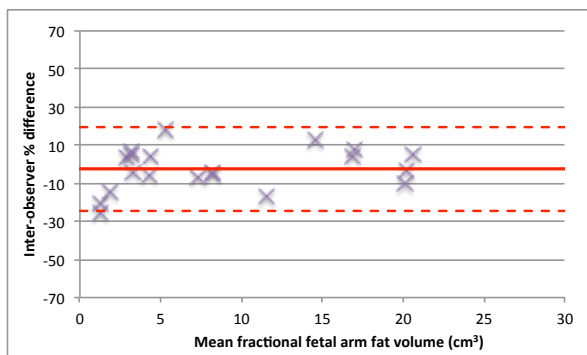


(b) K.E.

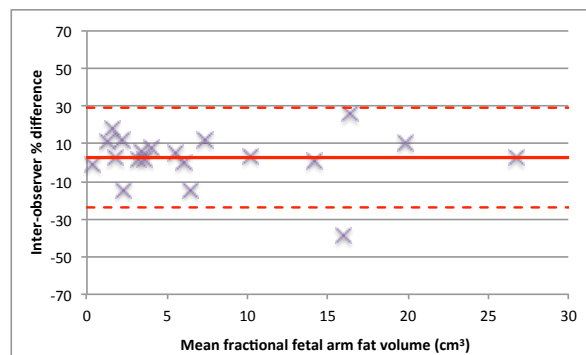


(c) M.A.

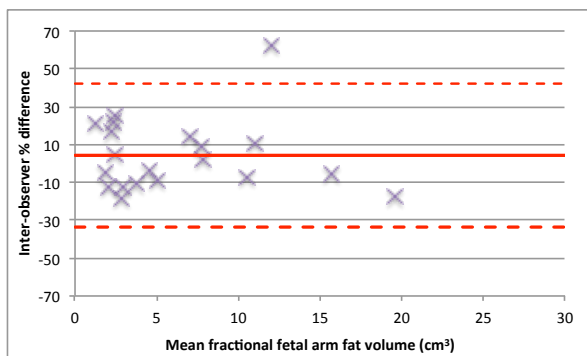
Figure 97: Intra-observer variability for fractional fetal arm fat volume for each segmenter .



(a) C.K. - K.E.



(b) C.K. - M.A.



(c) K.E. - M.A.

Figure 98: Inter-observer variability for fractional fetal arm fat volume for each pair of segmenters.

The intra-and inter-observer differences were calculated and plotted as Bland-Altman plots [Figure 97, Figure 98], and are summarised below, together with the ICCs [Table 24].

Table 24: Intra- and inter-observer differences for fractional fetal arm fat volume measurement.

(a) INTRA-OBSERVER			
Segmenter	Mean difference	Limits of agreement	ICC
C.K.	-2.7%	+/-10.6%	0.997
K.E.	5.7%	+/-16.8%	0.992
M.A.	-5.3%	+/-32.8%	0.958

(b) INTER-OBSERVER			
Segmenters	Mean difference	Limits of agreement	ICC
C.K. – K.E.	-2.5%	+/- 22.0%	0.991
C.K. – M.A.	2.7%	+/-26.5%	0.971
M.A. – K.E.	-4.3%	+/-37.9%	0.932

The mean differences are greater than those for the 2D measurements (section 4c), as might be expected for a 3D measurement. Comparison of the different pairs shows that K.E.'s measurements were the larger in both sets of her pairs, whereas M.A.'s measurements were the smaller in both of her sets of pairs. This results in the mean difference of greatest magnitude in the pairing where M.A. and K.E. are compared, but even then the mean difference is less than 5% between the two, showing a low level of systematic bias.

The limits of agreement were greater for inter-observer than for intra-observer, reflecting the greater random error between two people performing a measurement. There are, however, outliers in one intra- and two inter-observer groups which have skewed the limits of agreement (M.A., C.K.-K.E, and M.A.-K.E.). Removal of the 53.8% and -34.4% measurements from the intra-observer group, gives a mean difference of -7.0% with limits of agreement of $\pm 14.5\%$. Removal of the -38.5% measurement from the inter-observer group (C.K.-K.E.) gives a mean difference of 4.8% with limits of agreement of $\pm 19.0\%$. Removal of the 62.8% measurement from the inter-observer group (M.A.-K.E.) gives a mean difference of 1.3% with limits of agreement of $\pm 27.6\%$. These limits of agreement are similar to those seen in the other intra- and inter-observer pairs.

Given that this is a three-dimensional measurement method, the differences and limits of agreement (even including the outliers) are of an acceptable level when compared with preceding measurements [Table 13].

Conclusion

Although there are a few outliers (4 out of 120 pairs of measurements), overall the technique shows an acceptable level of intra- and inter-observer variability.

5d. Longitudinal scans (3D) in individual fetuses

Aim

To assess the development of fat in individual fetuses by measuring arm fat volume.

Method

The same cohort was used as in section 5c, but only fetuses which had been scanned four, five or six times, and who had then undergone body composition analysis with the PEA POD, were included in this analysis. Of these, those who had had their first scan by 25 weeks gestation, and those with their last scan at 37 weeks or later, were included in the analysis, so as to assess the curves over a wide range of gestational age which included the end of the third trimester. Individual fat volume curves were plotted and assessed visually, building on the work in section 4d. The fetuses were then divided into those where the scans showed a continuous upward trajectory, and those where the scans showed an apparent slowing of the rate of fat volume gain at the end of the third trimester. Birthweight, gender, and whole body fat percentage data were then collated for these fetuses, and the groups compared.

Results

29 fetuses had at least four scans and had also undergone newborn PEA POD body composition analysis. Of these, 19 met the gestational age criteria. The curves were plotted: 7 showed a slowing of rate of fat volume accrual at the last (late third trimester) scan [Figure 100], whilst the remaining 12 showed a continuous upward trend [Figure 99].

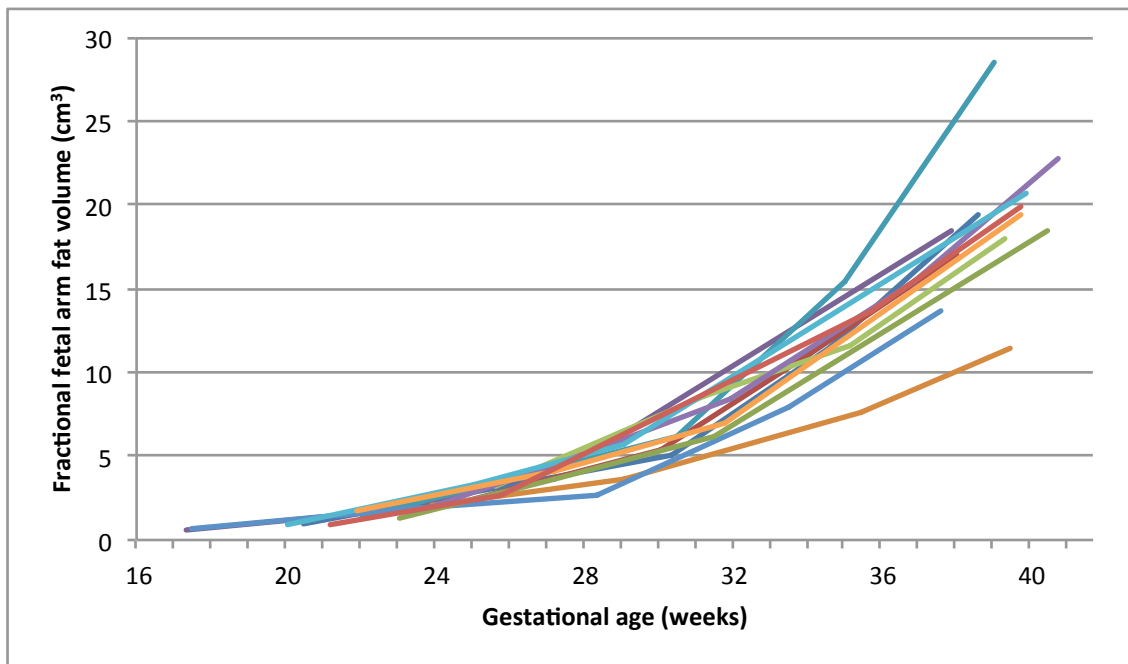


Figure 99: Longitudinal fractional fetal arm fat volumes in individual fetuses, demonstrating continuous upward trend.

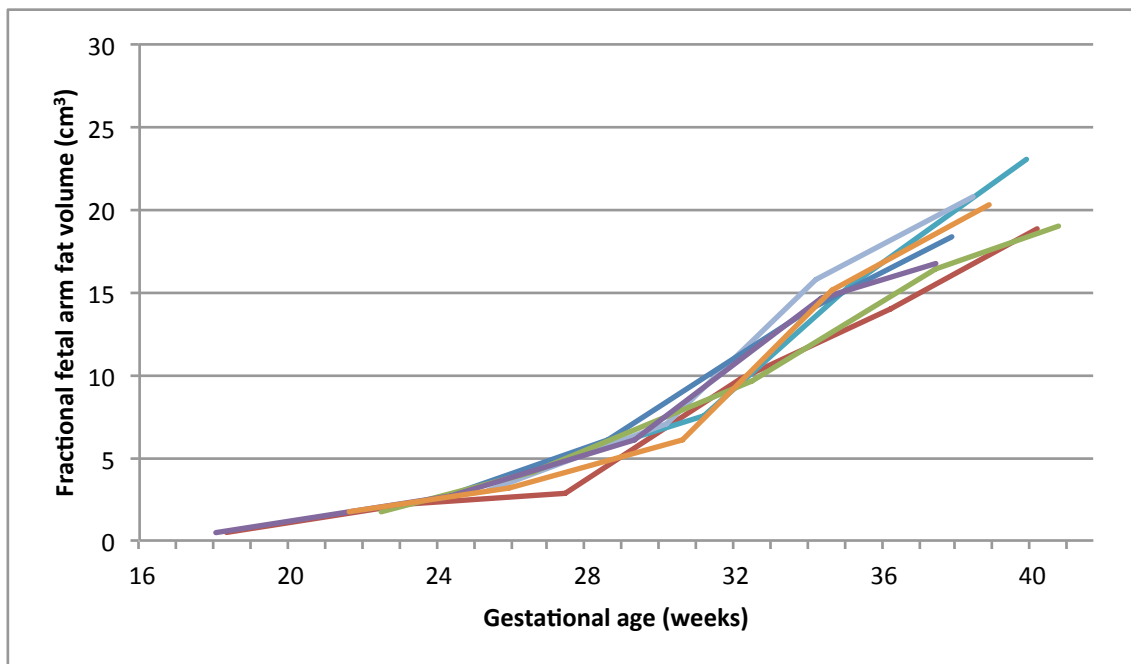


Figure 100: Longitudinal fractional fetal arm fat volumes in individual fetuses, demonstrating slowing of rate of fat volume accrual in the late third trimester.

The gestational age at birth, birthweight (and centile, using RCPCH/WHO year 0-4 data²²⁰), percentage body fat using neonatal PEA POD body composition analysis, and body fat percentile were recorded for each of these infants [Table 25].

Table 25: Neonatal birthweight and body fat, for fetuses showing different patterns of antenatal arm fat volume growth.

	ID	GA at birth	Sex	Birthweight	Weight centile ²²⁰	PEA POD %body fat	PEA POD centile ²²¹
Normal arm fat growth 3 rd trimester (n=12)	6496	40 ⁺⁵	f	3218g	38 th	12.7%	50-75 th
	6498	39 ⁺⁴	f	2766g	11 th	5.9%	5-10 th
	6536	41 ⁺⁶	m	4460g	97 th	15.5%	90-95 th
	6552	40 ⁺³	f	3844g	76 th	19.0%	90-95 th
	6554	39 ⁺³	f	2760g	10 th	5.7%	5-10 th
	6558	40 ⁺³	f	2810g	12 th	7.7%	10-25 th
	6580	40 ⁺⁶	f	3635g	72 nd	17.1%	75-90 th
	6582	41 ⁺¹	m	3262g	32 nd	10.0%	50-75 th
	6597	40 ⁺³	f	3343g	49 th	12.2%	25-50 th
	6605	39 ⁺¹	m	3632g	60 th	13.8%	10-25 th
	6663	41 ⁺⁴	f	3831g	84 th	12.0%	25-50 th
	6687	40 ⁺⁶	f	3657g	74 th	12.4%	25-50 th
	<i>Mean</i>	<i>40⁺⁴</i>	<i>---</i>	<i>3435g</i>	<i>51st</i>	<i>12.0%</i>	<i>25-50 (median)</i>
Slow arm fat growth 3 rd trimester (n=7)	6589	41 ⁺⁶	m	3407g	43 rd	9.7%	25-50 th
	6606	42 ⁺¹	m	3880g	78 th	7.0%	25-50 th
	6626	38 ⁺³	m	3507g	51 st	13.5%	75-90 th
	6677	39 ⁺⁶	f	3144g	32 nd	6.9%	10-25 th
	6678	42 ⁺¹	f	3742g	79 th	14.8%	50-75 th
	6680	39 ⁺⁴	f	3067g	27 th	13.0%	50-75 th
	7588	40 ⁺⁰	m	3678g	64 th	15.9%	90-95 th
	<i>Mean</i>	<i>40⁺⁶</i>	<i>---</i>	<i>3489g</i>	<i>53rd</i>	<i>11.5%</i>	<i>50-75 (median)</i>

In the eight fat area curves assessed in section 4d, 6 showed synchronous birthweight and peapod centiles, irrespective of the pattern of fat curves. The other 2 seemed to support the possibility that the arm fat assessment might reflect underlying whole body fat and provide a more sensitive measurement of body composition than birthweight.

Unfortunately, with the larger number of cases presented in this section, the picture is less clear. Of the 12 fetuses with an upwards trajectory of fat volume graphs: 6 showed synchronous results (same centile bracket, or within 3 centiles of the same bracket eg birthweight 11th, PEA POD 5-10th); 3 showed a PEA POD centile in a higher bracket than birthweight centile; but 3 showed birthweight higher than PEA POD centile. The hypothesis was that this set would show the first two categories of growth, but not the last category. Meanwhile, for the 7 fetuses with a slowing trajectory of fat volume graphs: 2 showed synchronous or near-synchronous results, 2 showed a birthweight centile higher than PEA POD centile, but 3 showed a higher PEA POD than birthweight centile. The latter would not be what we would have anticipated, given the slowing of arm fat volume on the graphs.

These inconsistencies are reflected in the mean and median data for the two groups: whilst the mean gestational age is very similar for both groups, the mean birthweight and centile are also similar (both slightly lower in the 'normal curve' vs. the 'slow curve' group), whilst the median PEA POD centile is lower in the 'normal curve' than the 'slower curve' group (25-50th vs. 50-75th), all of which might not have been expected. The percentage arm fat, however, is slightly lower in the group of babies with the slower curve (11.5% vs. 12.0%), which is more in line with what was anticipated. These difference suggests that arm fat alone may not be a reliable marker of whole body fat. The correlation will be explored further in Chapter 7.

It was possible to identify different patterns of growth when assessing the individual fetuses. These included appropriate growth (following 50th centile), accelerated growth (crossing increasing centiles), small for gestational age (below 10th centile – in this case, below 3rd), and failure to meet growth potential (falling centiles, whilst remaining within 10th-90th range) [Figure 101].

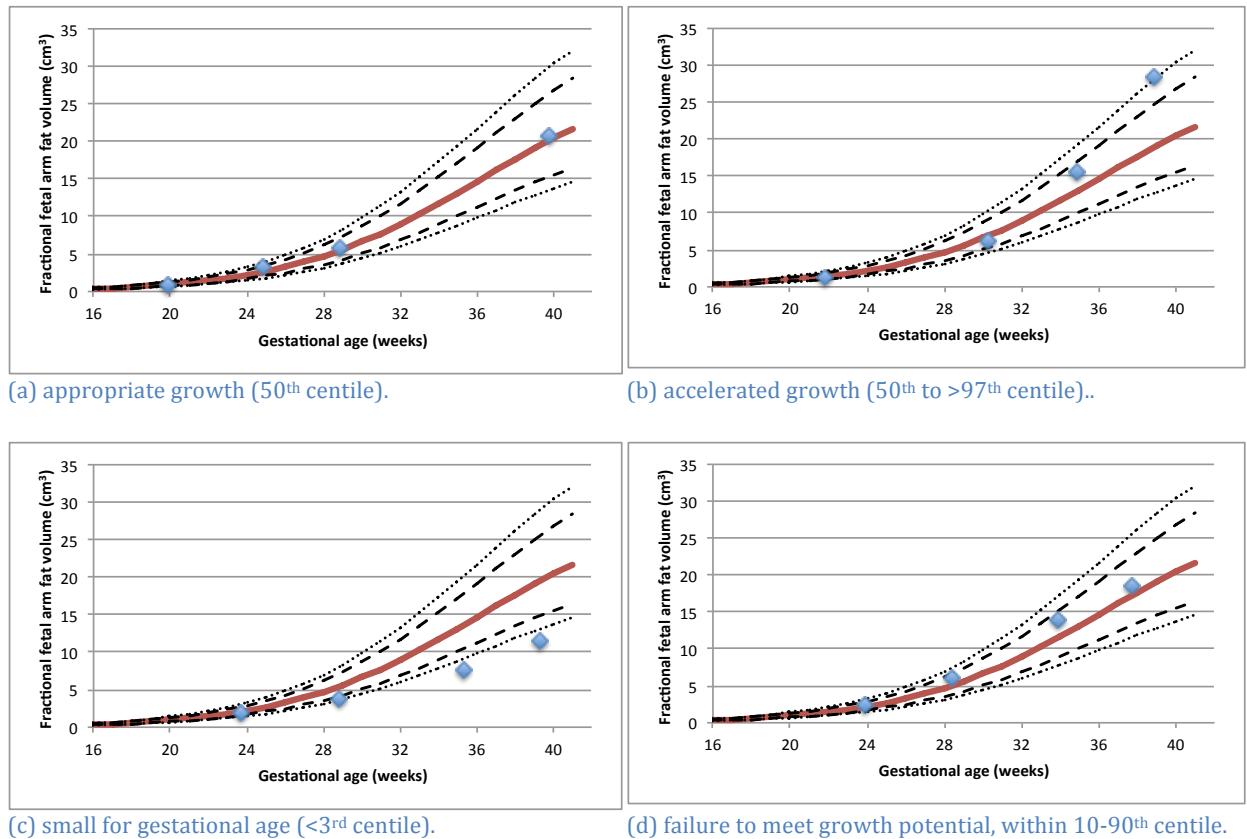


Figure 101: Patterns of fractional fetal arm fat volume graphs in individual fetuses.

Conclusion

This section demonstrates longitudinal changes in arm fat volume in individual fetuses, including different patterns of growth across the second and third trimesters. However, unlike suggestions from the preliminary 2D results, the differing shape of the graphs does not seem to correspond well with variations in neonatal whole body fat.

5e. 2D fat, lean and arm areas: reference ranges (extracted from 3D volumes)

i. Comparison of original 2D images with 2D images extracted from 3D volumes

Aim

To assess the difference in arm fat areas acquired by real-time 2D ultrasound or extracted from 3D volumes using the middle slice, perpendicular to the humerus.

Background

Arm fat area tends to be enlarged if slices are not perpendicular to the humerus, as demonstrated in section 5b.i. Importantly, on 2D ultrasound assessment, the angulation of the humerus in the axial view is not readily visible. This is compounded by a lack of landmarks to ensure a correct slice through the arm, unlike, for example, AC⁶ or HC.²²³

The potential for 3D ultrasound is that arm volume can be examined and manipulated to ensure the humerus is perpendicular to the ultrasound beam, then a correct slice extracted for area measurement. If this is perpendicular, the area would be expected to be smaller than with 2D-acquired images, which may be oblique.

Methods

Arm areas were used from the first round of segmentations of the original 2D arm images (section 4c) and compared with the central slice of the 3D volumes (section 5c) from the same fetuses during the same scan, using Bland-Altman plots.

Results

170 fetuses, from 16⁺³ to 40⁺⁴ weeks, had segmentable fat area from both a 2D image and 3D volume. The mean of the two measurements was taken, and the percentage difference calculated, as shown below [Figure 102].

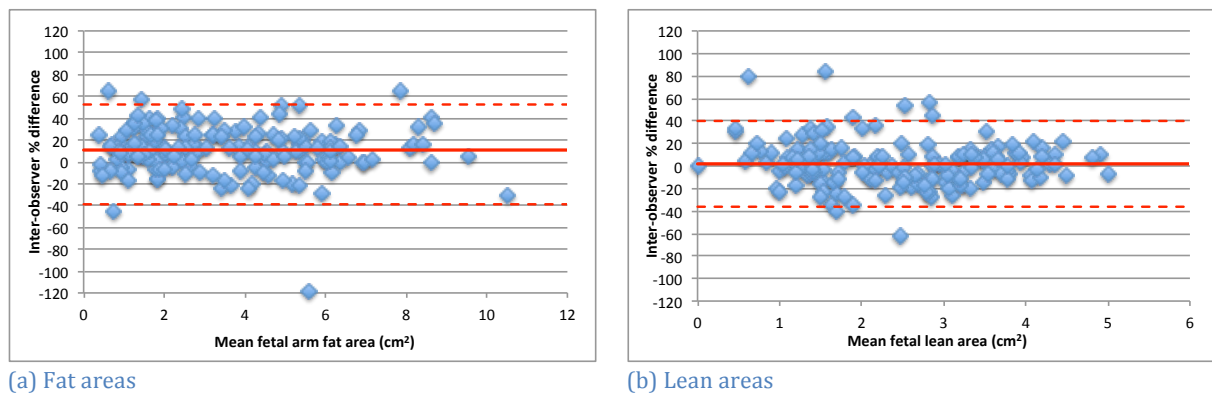


Figure 102: Bland-Altman plot comparing 2D images with slice extracted from 3D volumes (n=170).

These show an average of 10.9% percentage difference for fat area (whereby the 2D areas are the larger) but only a 1.9% difference for lean area between the two methods, with limits of agreement of -38.8 to 52.6% (fat) and -36.2 to 40% (lean).

Non-perpendicularity of the raw 2D image is the most likely factor to explain the reason for the increased area size, although the variations in fat and lean proportions at different levels of the arm may also contribute (i.e. if a 2D image is not mid-humeral).

Even allowing for this difference in fat areas, the 2D and 3D-acquired 2D areas had a strong correlation ($r=0.90$, $r^2=0.82$) [Figure 103], as did the lean areas ($r=0.92$, $r^2=0.85$).

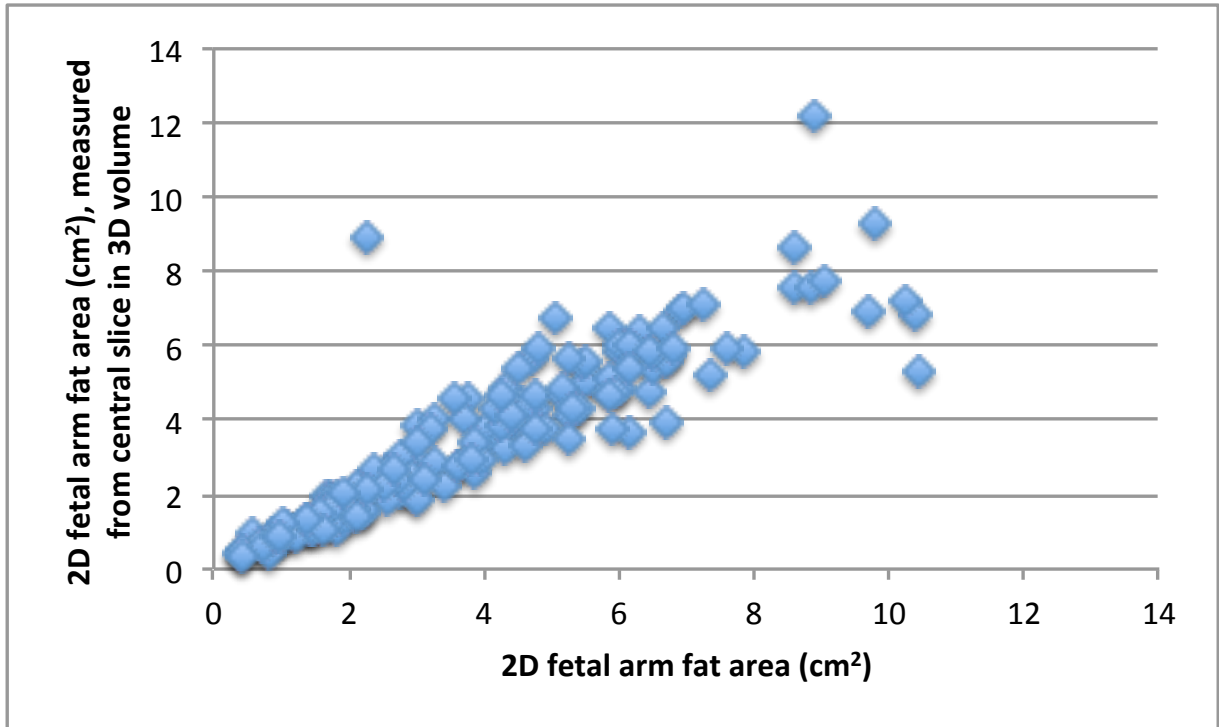


Figure 103: 2D fetal arm fat areas acquired from 2D images and the central slice of 3D volumes.

Conclusion

As expected, 2D raw images yield larger fat area values when compared with areas measured in the central, perpendicular, slices taken from 3D volumes in the same fetuses. This partly reflects the challenges of trying to ensure a mid-humeral, perpendicular slice (without the aid of suitable anatomical landmarks) when acquiring 2D axial images of the fetal arm. It would therefore seem sensible to use the 3D-acquired slices for future analyses, as these will definitely be perpendicular. There is also an efficiency benefit as the 2D images do not have to be segmented separately; the data can be extracted from the segmentation of the 3D volume.

ii. 2D fat, lean and arm areas: reference ranges

Aim

To describe the normal development of fetal arm fat and lean tissues, using optimal 2D images extracted from 3D volumes.

Background

This work built on the original 2D work (section 4c) but using optimal slices acquired from 3D volumes as described above (section 5e). The hypothesis was that the 2D-acquired-from-3D images would show similar results to the 2D images but would be more accurate as they would be perpendicular. This dataset had a larger number of data points which should also provide more accurate growth curves.

Methods

This data was acquired from the same group of INTERGROWTH-21st women as those assessed in section 5c, after incorrectly rotated volumes were excluded. The volumes were segmented in the 5-slice fractional limb volume as described in section 5a and the central slice extracted to provide an optimal 2D image. Fractional polynomials were used to produce centiles, as before.

Results

The data were analysed from 501 scans from 208 women (as per section 5c). Fractional fetal arm, fat and lean volumes were plotted against gestational age [Figure 104 - Figure 107].

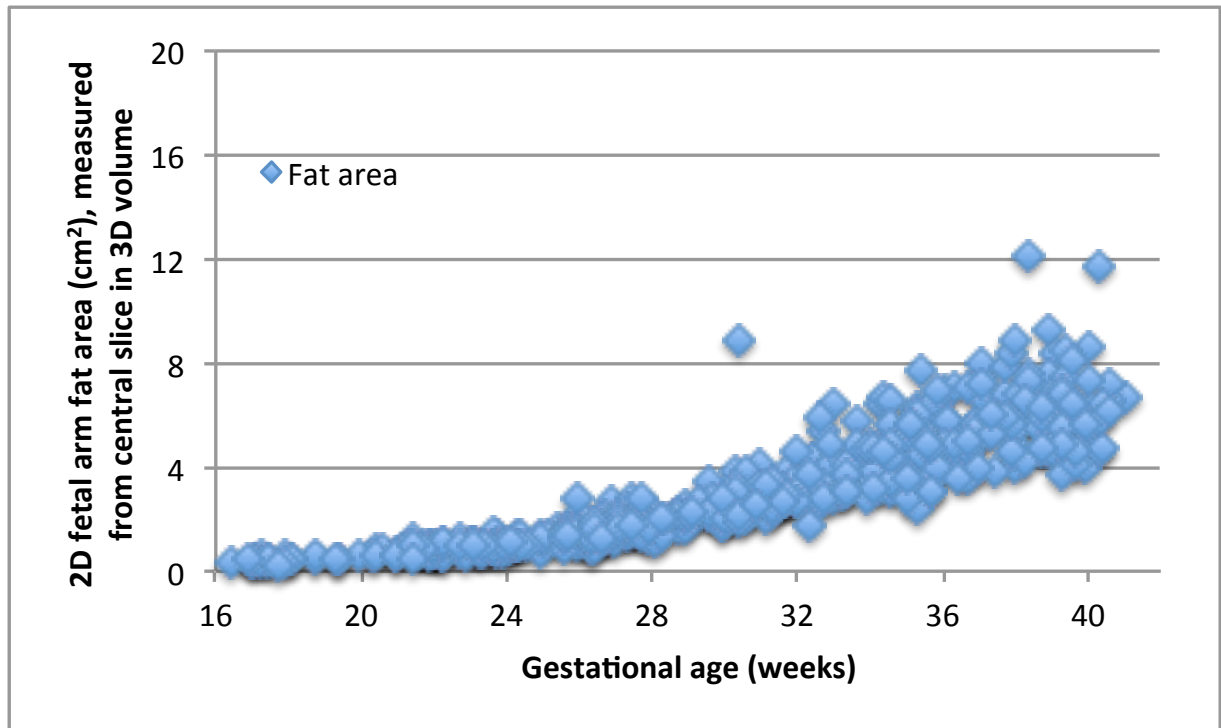


Figure 104: 2D fetal arm fat area, measured from central slice in 3D volume (n=501).

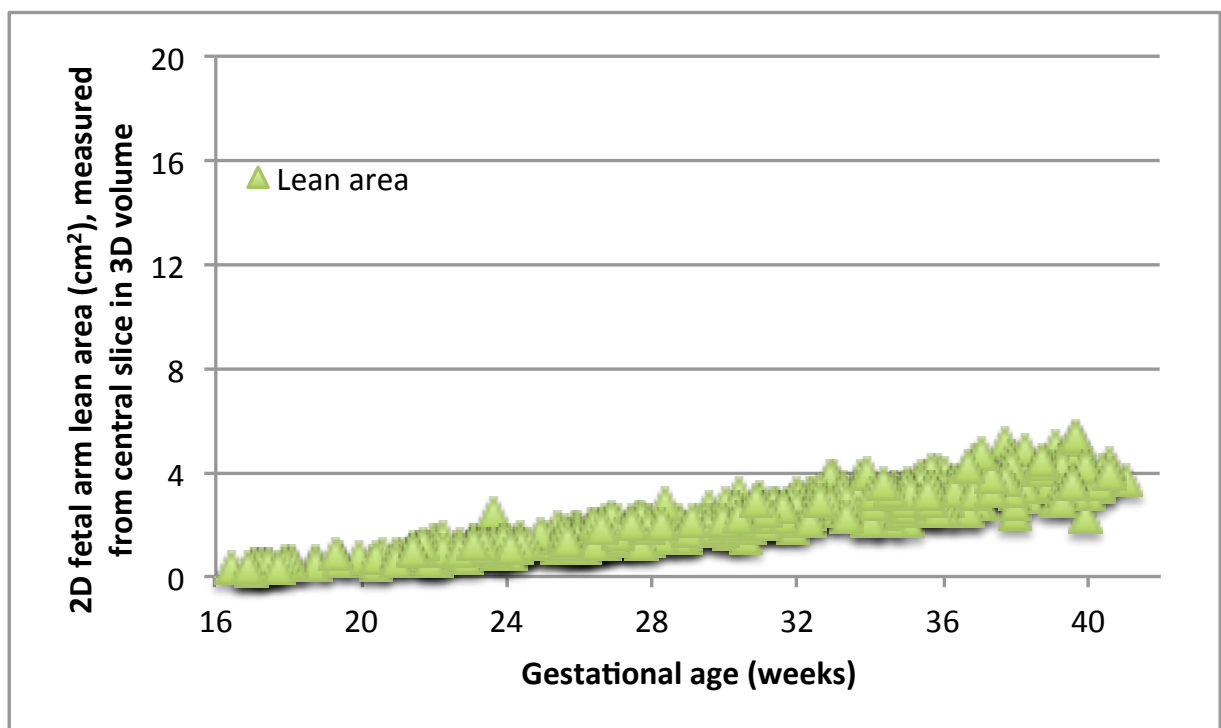


Figure 105: 2D fetal arm lean area, measured from central slice in 3D volume (n=501).

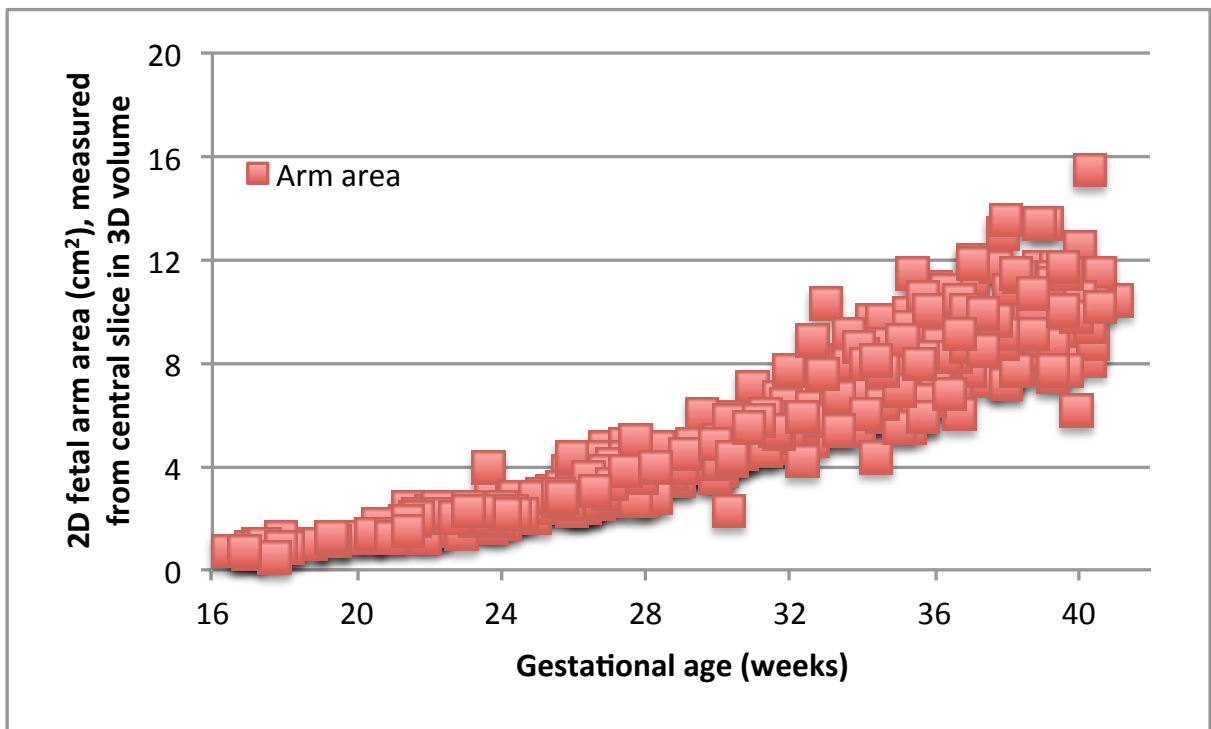


Figure 106: 2D fetal arm area, measured from central slice in 3D volume (n=501).

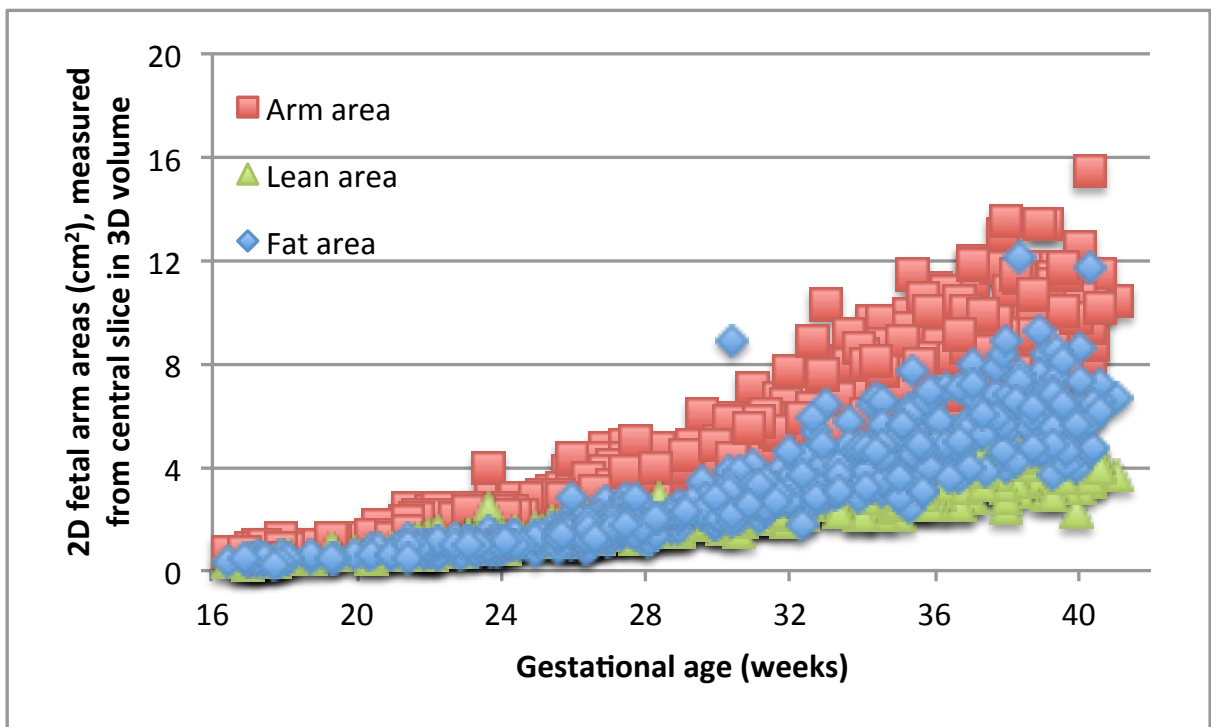


Figure 107: 2D fetal arm, fat and lean areas superimposed, measured from central slice in 3D volume (n=501).

Fractional polynomial regression^{206,207} was used to generate equations to calculate the mean / 50th centile (C50) values for fat area and lean area for a given gestational age (GA):

$$\begin{aligned} \text{Log (arm fat area) C50} &= ((\text{GA}/10)^3 \times 0.2663783) + \\ &((\text{GA}/10)^3 \times (\ln(\text{GA}/10))) \times -0.1514079) + 2.828404 \end{aligned}$$

$$\text{Log (arm lean area) C50} = ((\text{GA}/10)^{-1} \times -6.851155) + 7.687931$$

The relationship with standard deviation (SD) was also generated using the same approach.

$$\text{SD of log (arm fat area) C50} = 0.21$$

$$\text{SD of log (arm lean area) C50} = 0.16$$

This enabled calculation of the centiles for both sets of data:

$$3^{\text{rd}} / 97^{\text{th}} \text{ centiles} = \text{mean} \pm 1.88 \text{ SD}$$

$$5^{\text{th}} / 95^{\text{th}} \text{ centiles} = \text{mean} \pm 1.64 \text{ SD}$$

$$10^{\text{th}} / 90^{\text{th}} \text{ centiles} = \text{mean} \pm 1.28 \text{ SD}$$

For the arm fat and lean areas, SD was used to calculate the log-transformed centiles, and the results then transformed back to mm², and divided by 100 to generate results in cm² [Table 26, Table 27].

Table 26: Centile values for arm fat areas for each week of gestation, from central slice in 3D volume.

GA (weeks)	Fetal arm fat area (cm ²) centiles (from 3D)						
	3 rd (mean -1.88 SD)	5 th (mean - 1.64 SD)	10 th (mean - 1.28 SD)	50 th (mean)	90 th (mean + 1.28 SD)	95 th (mean + 1.64 SD)	97 th (mean +1.88 SD)
16	0.25	0.27	0.29	0.38	0.49	0.53	0.56
17	0.28	0.30	0.32	0.42	0.55	0.60	0.63
18	0.32	0.34	0.36	0.48	0.62	0.67	0.71
19	0.36	0.38	0.41	0.54	0.71	0.76	0.80
20	0.41	0.43	0.47	0.62	0.81	0.87	0.92
21	0.47	0.50	0.54	0.70	0.92	1.00	1.05
22	0.54	0.57	0.62	0.81	1.06	1.15	1.21
23	0.63	0.66	0.71	0.93	1.22	1.32	1.39
24	0.72	0.76	0.82	1.08	1.41	1.52	1.60
25	0.83	0.88	0.95	1.24	1.63	1.76	1.85
26	0.96	1.02	1.10	1.44	1.88	2.03	2.14
27	1.11	1.17	1.27	1.66	2.18	2.35	2.47
28	1.28	1.35	1.46	1.91	2.51	2.71	2.85
29	1.48	1.55	1.68	2.20	2.88	3.11	3.28
30	1.69	1.78	1.92	2.52	3.30	3.57	3.75
31	1.93	2.03	2.19	2.87	3.77	4.07	4.28
32	2.19	2.30	2.48	3.26	4.27	4.61	4.85
33	2.46	2.59	2.80	3.67	4.81	5.19	5.46
34	2.75	2.90	3.12	4.10	5.37	5.80	6.10
35	3.05	3.20	3.46	4.53	5.95	6.42	6.75
36	3.34	3.51	3.79	4.97	6.51	7.03	7.40
37	3.61	3.80	4.10	5.38	7.05	7.61	8.01
38	3.86	4.06	4.38	5.75	7.54	8.13	8.56
39	4.07	4.28	4.62	6.06	7.94	8.57	9.02
40	4.22	4.44	4.79	6.28	8.24	8.89	9.36
41	4.31	4.53	4.89	6.41	8.41	9.08	9.55

Table 27: Centile values for arm lean areas for each week of gestation, from central slice in 3D volume.

GA (weeks)	Fetal arm lean area (cm ²) centiles (from 3D)						
	3 rd (mean -1.88 SD)	5 th (mean - 1.64 SD)	10 th (mean - 1.28 SD)	50 th (mean)	90 th (mean + 1.28 SD)	95 th (mean + 1.64 SD)	97 th (mean +1.88 SD)
16	0.22	0.23	0.25	0.30	0.37	0.39	0.41
17	0.29	0.30	0.32	0.39	0.48	0.50	0.52
18	0.36	0.37	0.40	0.49	0.60	0.63	0.65
19	0.44	0.46	0.48	0.59	0.73	0.77	0.80
20	0.53	0.55	0.58	0.71	0.87	0.92	0.96
21	0.62	0.64	0.68	0.84	1.03	1.09	1.13
22	0.72	0.75	0.79	0.97	1.19	1.26	1.31
23	0.82	0.85	0.90	1.11	1.36	1.44	1.50
24	0.93	0.97	1.02	1.26	1.54	1.63	1.70
25	1.04	1.08	1.15	1.41	1.73	1.83	1.90
26	1.16	1.20	1.28	1.56	1.92	2.03	2.11
27	1.28	1.33	1.41	1.73	2.12	2.24	2.33
28	1.40	1.45	1.54	1.89	2.32	2.45	2.55
29	1.52	1.58	1.68	2.06	2.52	2.67	2.77
30	1.65	1.71	1.81	2.22	2.73	2.89	3.00
31	1.77	1.84	1.95	2.39	2.94	3.11	3.23
32	1.90	1.97	2.09	2.56	3.15	3.33	3.46
33	2.03	2.11	2.23	2.74	3.36	3.56	3.69
34	2.15	2.24	2.37	2.91	3.57	3.78	3.93
35	2.28	2.37	2.51	3.08	3.78	4.00	4.16
36	2.41	2.50	2.65	3.25	3.99	4.23	4.39
37	2.54	2.64	2.79	3.42	4.20	4.45	4.62
38	2.66	2.77	2.93	3.60	4.41	4.67	4.86
39	2.79	2.90	3.07	3.77	4.62	4.89	5.08
40	2.91	3.03	3.21	3.94	4.83	5.11	5.31
41	3.04	3.16	3.34	4.10	5.03	5.33	5.54

The tables above are shown as graphs for fat [Figure 108] and lean areas [Figure 109] (50th centile coloured line; 10th / 90th centiles dashed lines; 3rd / 97th centiles dotted lines):

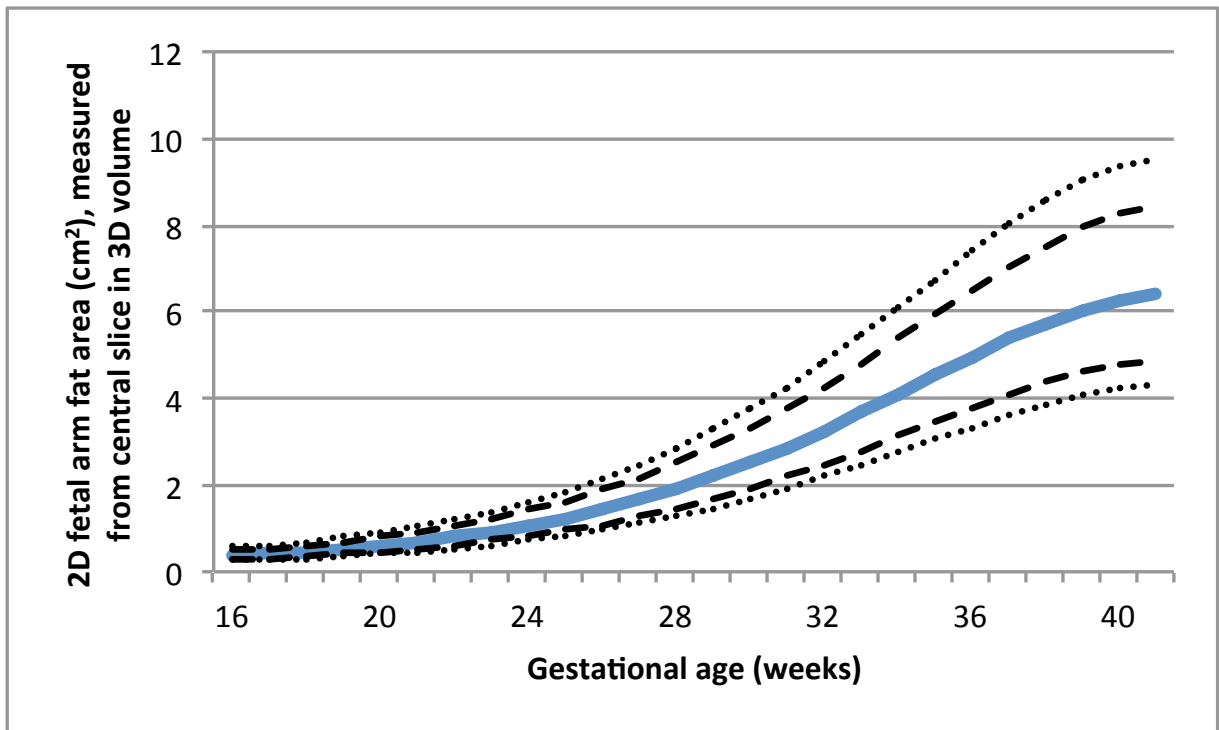


Figure 108: Fetal arm fat area, from central slice in 3D volume (3rd, 10th, 50th, 90th and 97th centiles).

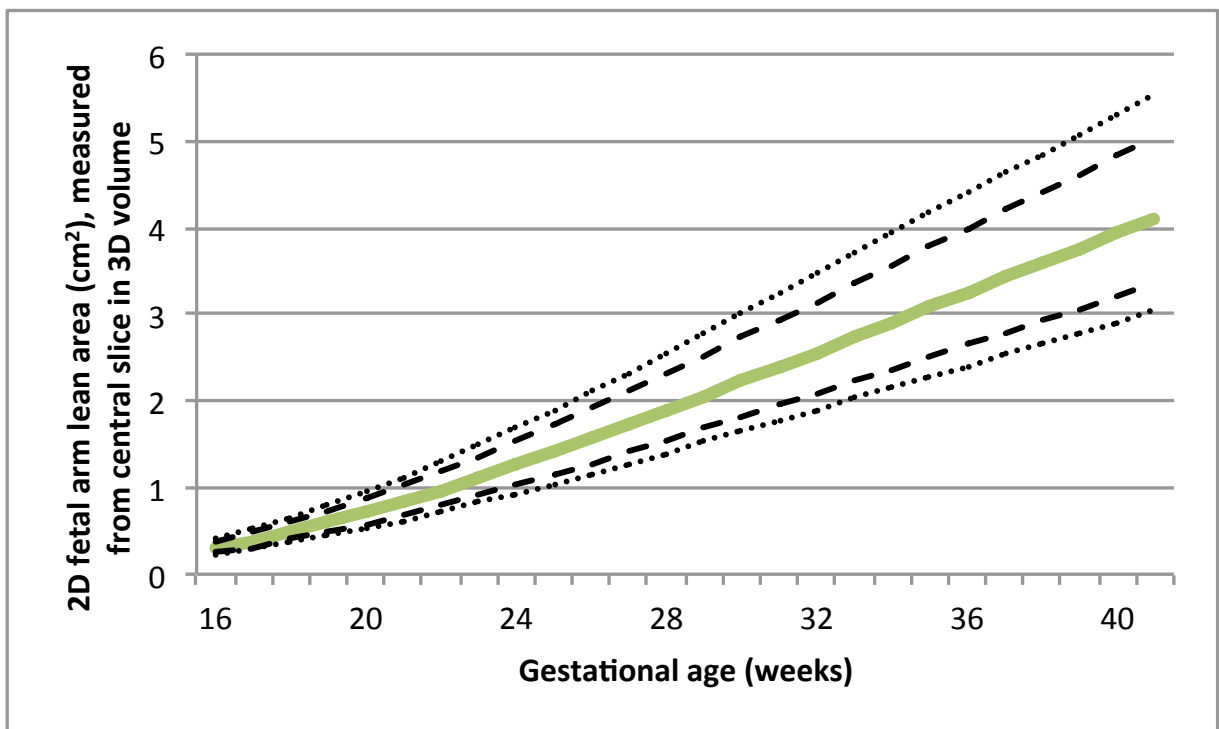


Figure 109: Fetal arm lean area, from central slice in 3D volume (3rd, 10th, 50th, 90th and 97th centiles).

Arm fat was also assessed in the form of the percentage of the whole arm area it occupied:

$$\text{Fat area as \% arm area C50} = (\text{GA} \times 0.9834298) + 23.61524$$

$$\text{SD of fat area as \% arm area C50} = 5.36$$

Table 28: Centile values for 2D fetal arm fat area as a percentage of central slice arm area in 3D volume, for each week of gestation.

GA (weeks)	Fetal arm fat area (as a percentage of central slice arm area) centiles (from 3D)						
	3 rd (mean -1.88 SD)	5 th (mean - 1.64 SD)	10 th (mean - 1.28 SD)	50 th (mean)	90 th (mean + 1.28 SD)	95 th (mean + 1.64 SD)	97 th (mean +1.88 SD)
20	33.2	34.5	36.4	43.3	50.1	52.1	53.4
21	34.2	35.5	37.4	44.3	51.1	53.1	54.3
22	35.2	36.5	38.4	45.3	52.1	54.0	55.3
23	36.2	37.4	39.4	46.2	53.1	55.0	56.3
24	37.1	38.4	40.4	47.2	54.1	56.0	57.3
25	38.1	39.4	41.3	48.2	55.1	57.0	58.3
26	39.1	40.4	42.3	49.2	56.0	58.0	59.3
27	40.1	41.4	43.3	50.2	57.0	59.0	60.2
28	41.1	42.4	44.3	51.2	58.0	59.9	61.2
29	42.1	43.3	45.3	52.1	59.0	60.9	62.2
30	43.0	44.3	46.3	53.1	60.0	61.9	63.2
31	44.0	45.3	47.2	54.1	61.0	62.9	64.2
32	45.0	46.3	48.2	55.1	61.9	63.9	65.2
33	46.0	47.3	49.2	56.1	62.9	64.9	66.1
34	47.0	48.3	50.2	57.1	63.9	65.8	67.1
35	48.0	49.2	51.2	58.0	64.9	66.8	68.1
36	48.9	50.2	52.2	59.0	65.9	67.8	69.1
37	49.9	51.2	53.1	60.0	66.9	68.8	70.1
38	50.9	52.2	54.1	61.0	67.8	69.8	71.1
39	51.9	53.2	55.1	62.0	68.8	70.8	72.0
40	52.9	54.2	56.1	63.0	69.8	71.7	73.0
41	53.9	55.1	57.1	63.9	70.8	72.7	74.0

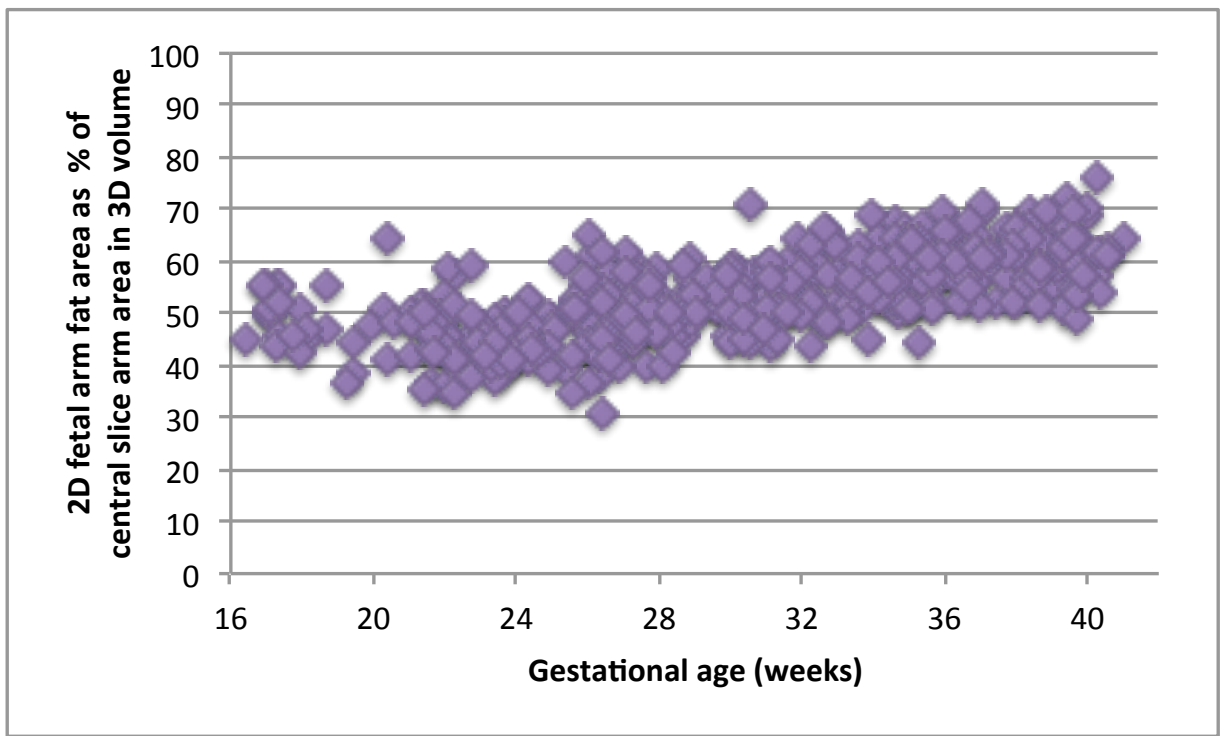


Figure 110: Fetal arm fat as percentage of arm area, from central slice in 3D volume (n=180).

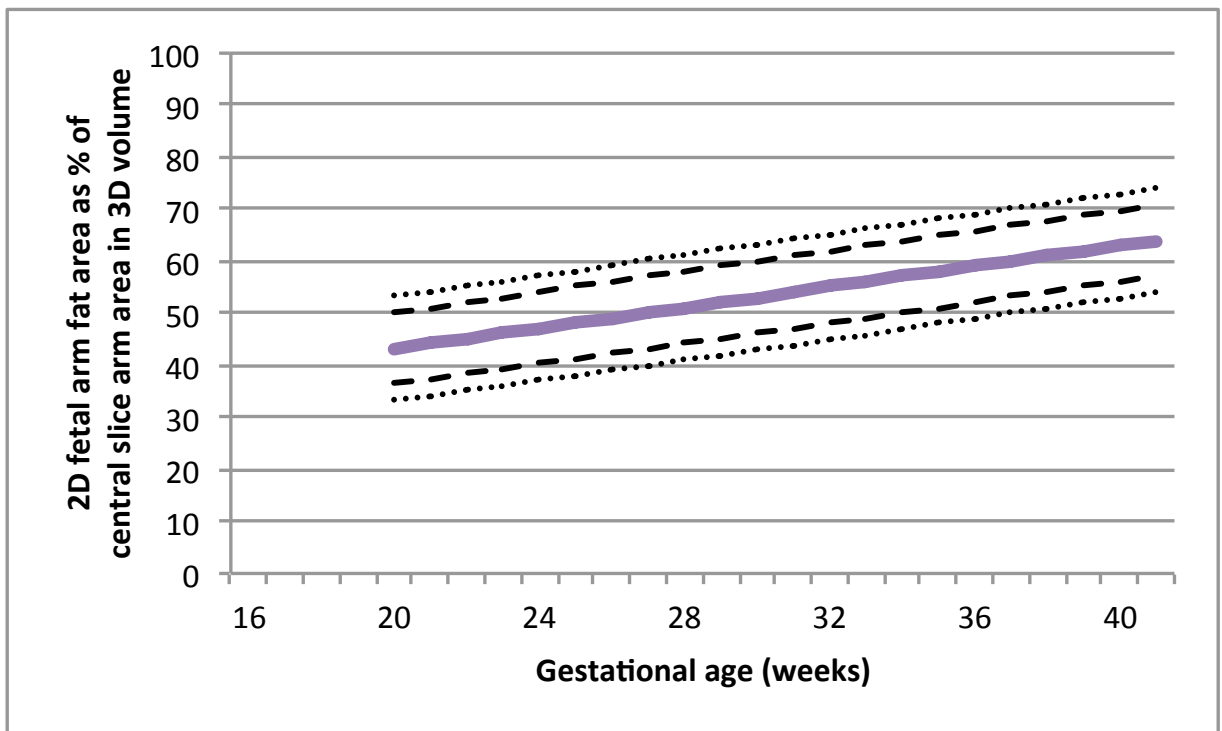


Figure 111: Fetal arm fat as percentage of arm area, from central slice in 3D volume (3rd, 10th, 50th, 90th and 97th centiles).

Finally, arm area centiles were calculated using fractional polynomial regression, with centiles calculated from SD:

$$\text{Log (arm area) C50} = ((\text{GA}/10)^{0.5} \times 4.787621) + ((\text{GA}/10)^3 \times -0.0131027) + -1.777371$$

$$\text{SD of log (arm area) C50} = 0.15$$

These are shown below [Figure 112, Table 29].

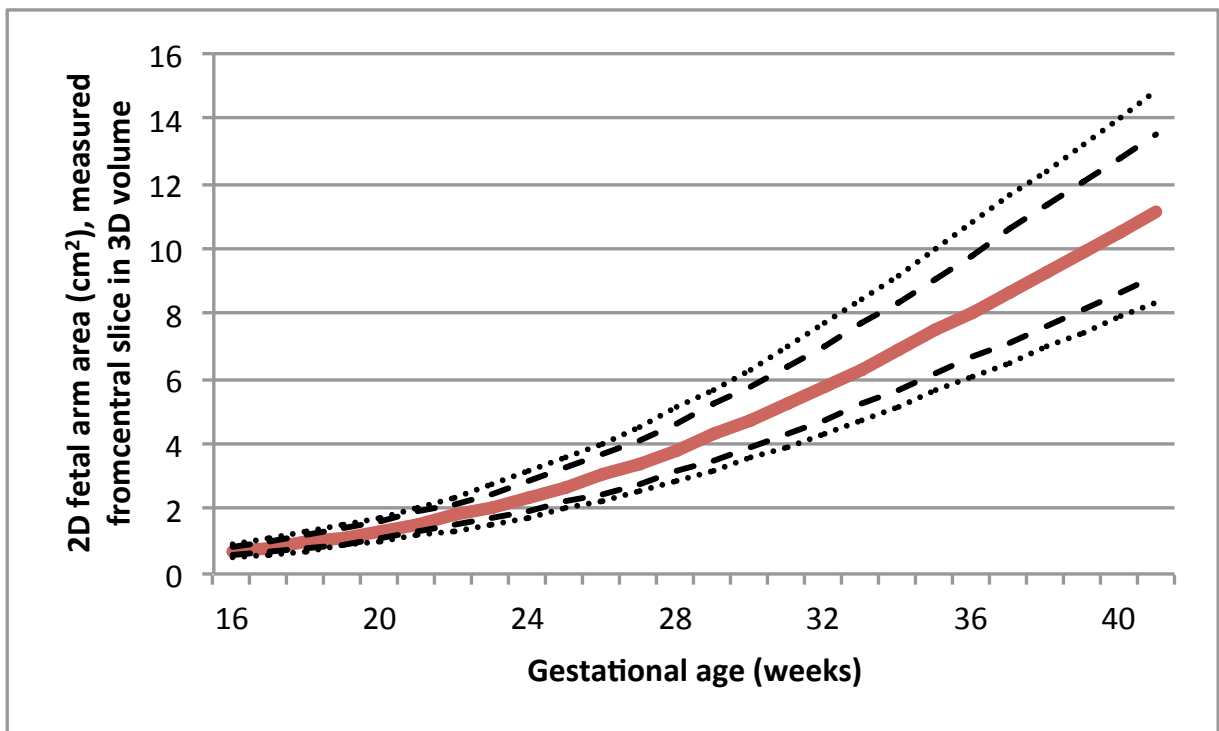


Figure 112: Fetal arm area, from central slice in 3D volume (3rd, 10th, 50th, 90th and 97th centiles).

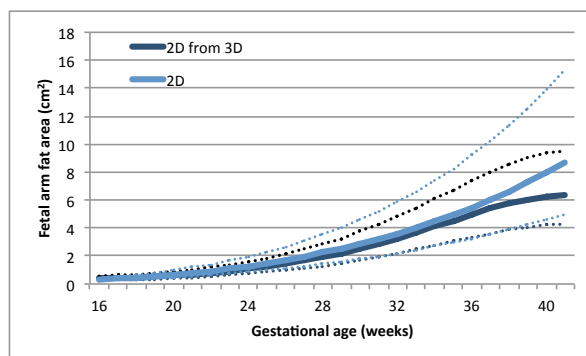
Table 29: Centile values for arm areas for each week of gestation, from central slice in 3D volume.

GA (weeks)	Fetal arm area (cm ²) centiles (from 3D)						
	3 rd (mean -1.88 SD)	5 th (mean - 1.64 SD)	10 th (mean - 1.28 SD)	50 th (mean)	90 th (mean + 1.28 SD)	95 th (mean + 1.64 SD)	97 th (mean +1.88 SD)
16	0.51	0.53	0.56	0.68	0.83	0.88	0.91
17	0.61	0.63	0.67	0.81	0.99	1.05	1.09
18	0.72	0.75	0.79	0.96	1.17	1.24	1.29
19	0.85	0.88	0.93	1.14	1.38	1.46	1.52
20	0.99	1.03	1.09	1.33	1.62	1.71	1.77
21	1.16	1.20	1.27	1.54	1.88	1.99	2.06
22	1.34	1.39	1.47	1.78	2.17	2.30	2.38
23	1.54	1.60	1.69	2.05	2.50	2.64	2.74
24	1.76	1.82	1.93	2.35	2.86	3.02	3.13
25	2.00	2.08	2.19	2.67	3.25	3.44	3.57
26	2.27	2.35	2.49	3.03	3.68	3.89	4.04
27	2.55	2.65	2.80	3.41	4.15	4.38	4.55
28	2.86	2.97	3.14	3.82	4.65	4.92	5.10
29	3.20	3.32	3.51	4.27	5.19	5.49	5.69
30	3.55	3.69	3.89	4.74	5.77	6.10	6.33
31	3.93	4.07	4.31	5.24	6.38	6.74	6.99
32	4.32	4.48	4.74	5.77	7.02	7.42	7.70
33	4.74	4.91	5.19	6.32	7.69	8.13	8.43
34	5.16	5.36	5.66	6.89	8.39	8.87	9.20
35	5.61	5.82	6.15	7.48	9.11	9.62	9.99
36	6.06	6.29	6.64	8.09	9.84	10.40	10.79
37	6.52	6.76	7.15	8.70	10.58	11.19	11.61
38	6.98	7.24	7.65	9.31	11.33	11.98	12.43
39	7.44	7.72	8.15	9.92	12.08	12.77	13.24
40	7.89	8.19	8.65	10.53	12.81	13.54	14.05
41	8.33	8.64	9.14	11.12	13.53	14.30	14.84

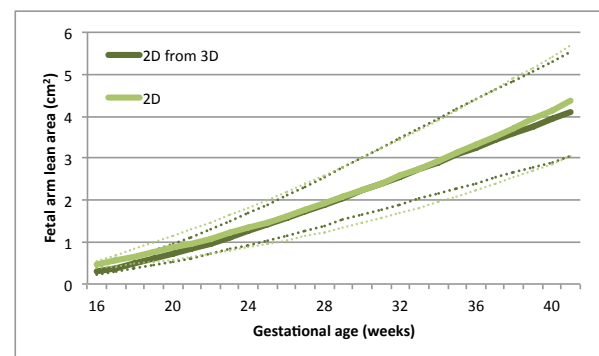
Discussion

This work provides reference ranges for 2D fetal whole arm, fat, lean, and fat as a percentage of arm areas. The comparison work in section 5e provided evidence that the use of the central slice of a 3D volume gave comparable results to 2D acquired images, but with the benefit of the 3D-acquired images being based around perpendicular measurements.

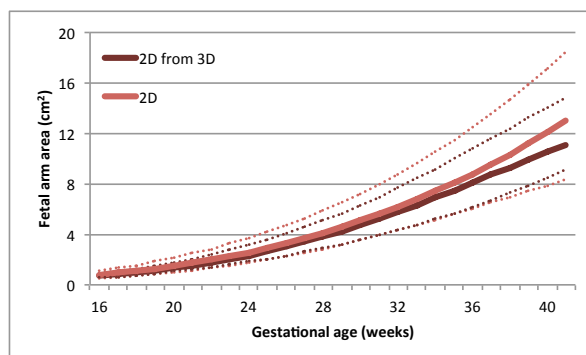
The reference centiles presented in this section and in section 4c are based on differing numbers of data points – 180 for the 2D and 501 for the 2D-from-3D – with the fetuses from the first study also being represented in the second. The two sets of centiles were superimposed as shown below [Figure 113].



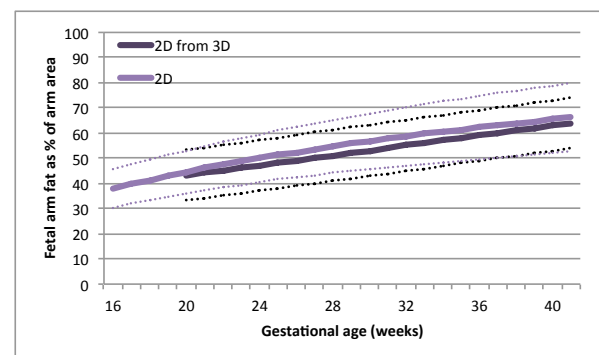
(a) Fat



(b) Lean



(c) Arm.



(d) Fat as percentage of arm.

Figure 113: Comparison of centiles from 2D images (paler line) (n=180) and from 2D extracted from central slice of 3D volumes (darker line) (n=501) (3rd, 50th and 97th centiles shown).

The lean area centiles are extremely similar for both data sets although the 2D results give a slightly higher 50th centile, with a slightly wider 3rd to 97th centile range. Not all of the 2D images will have been entirely perpendicular, which will produce larger areas. The 50th centiles for fat expressed as a percentage are also very similar (with the 2D again being the greater). There is some variation in the 3rd and 97th centiles which may have arisen due to 2D centiles using 16-41 week data whereas the 2D-from-3D centiles used 20-41 week data.

The fat area centiles are similar until 36 weeks but show a different pattern after 37 weeks. The 2D 50th centile shows an upwards exponential-type trend with diverging 3rd and 97th centiles, whilst the 2D-from-3D centiles show a reduced rate of growth. (This is mimicked in the arm area centiles as these represent combined fat and lean areas.) Measurement differences or errors at this later gestation may have contributed to this difference, as might the smaller number of data points at 40 and 41 weeks. The mathematical modelling would thus fit a less exponential curve to this part of the dataset. The current 2D arm fat area literature (section 1g) would suggest that an ongoing upwards course is to be expected, and it is likely that the 'true' 50th centile lies somewhere between the two lines shown on the graph: the 2D may have an element of over-estimation due to non-perpendicularity, whilst the 2D-from-3D may have an element of under-estimation.

Conclusion

Reference centiles are presented for fetal arm, fat and lean areas, and for fat as a percentage of arm area, acquired from the central 2D slice of 3D volumes. The results are similar to original 2D datasets, but the fat measurements acquired from the two techniques show some small differences (likely methodological or due to curve fitting) from 37 weeks.

5f. Fetal Fat Index (arm)

i. Fetal Fat Index (arm): reference ranges

Aim

To assess the correlation of the Fetal Fat Index (FFI) of the arm with fractional arm volume, and to produce reference centiles for future research.

Method

This work built upon the manual FFI work presented in section 4g. Manually segmented 3D volumes (section 5c) were used in this study. M.A. extracted the central 2D slice from the saved .mat file for each volume. A tool (developed by S.R.) then automatically measured the thickness between the inner and outer segmentation of the fat at three fixed points, at the 9, 12 and 3 o'clock positions [Figure 114]. These were added together to generate the arm FFI for each fetus, which was then correlated with its arm fat volume.



Figure 114: Example of fat tracing acquired from manual segmentation, showing three positions of automatic measurement of fat thickness to calculate FFI.

Results and discussion

FFI was measured in 499 scans from 207 women (from section 5c), ranging from 16⁺⁶ to 41⁺⁰ weeks of gestation. 2 scans had not been saved in the correct file type to allow automated FFI calculation.

The difference between the smallest and largest of the three measurements in each FFI ranged from 0-9.7mm (mean 1.8mm), demonstrating the variation in the arm fat.

There was a good correlation between FFI and fractional arm fat volume ($r=0.94$, $r^2=0.89$) [Figure 115]; this was similar to the correlation shown between FFI and fat area (section 4g).

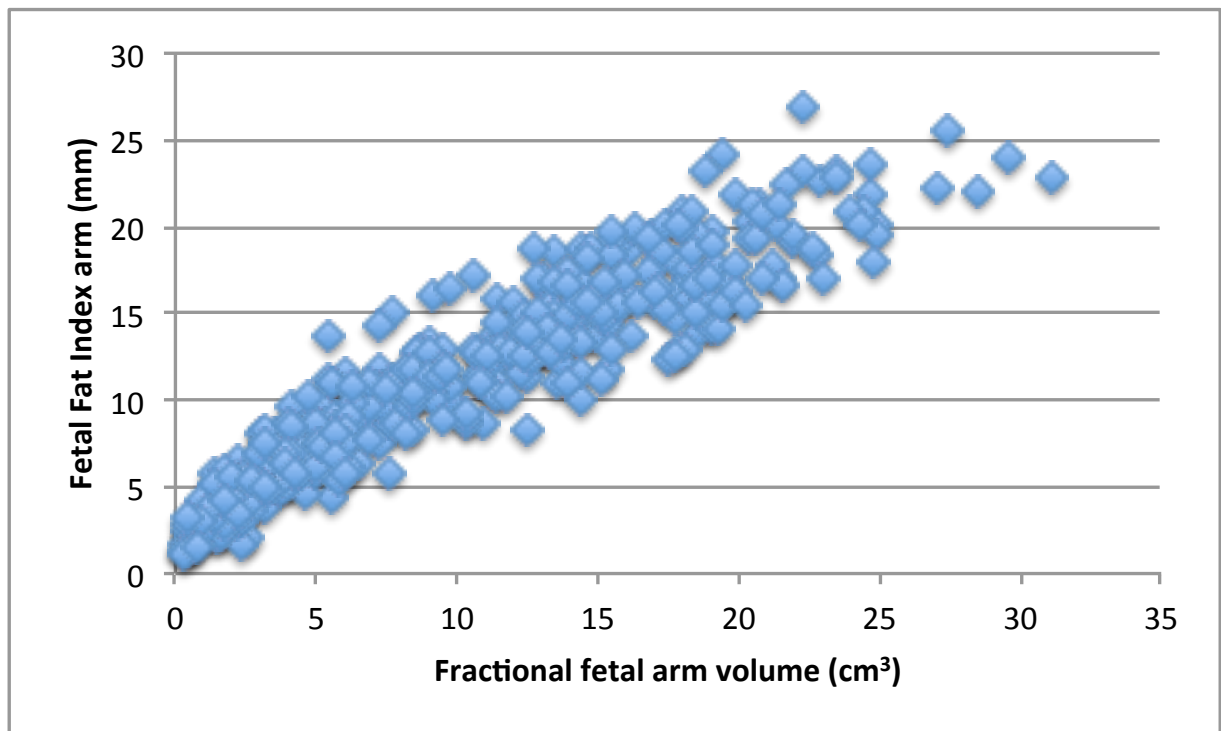
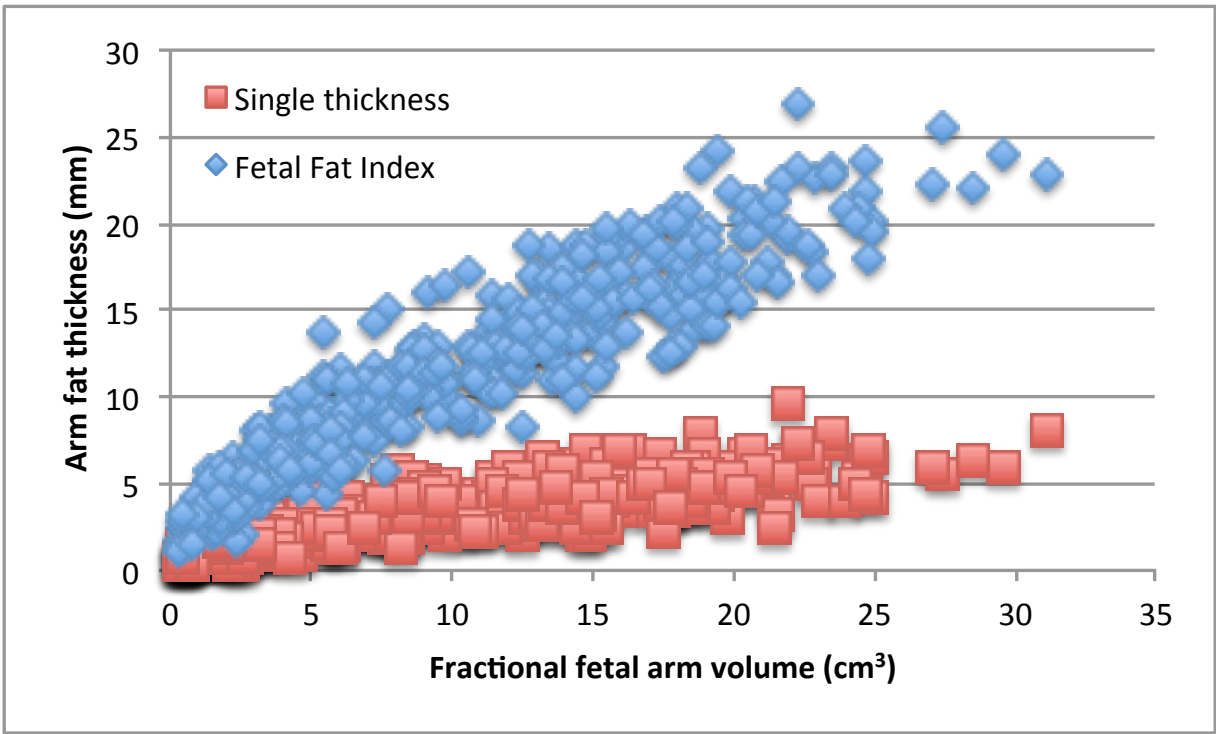
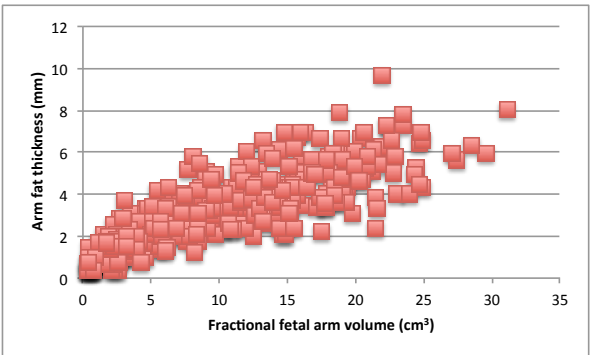


Figure 115: FFI measurements plotted against fractional fetal arm fat volume (n=499).

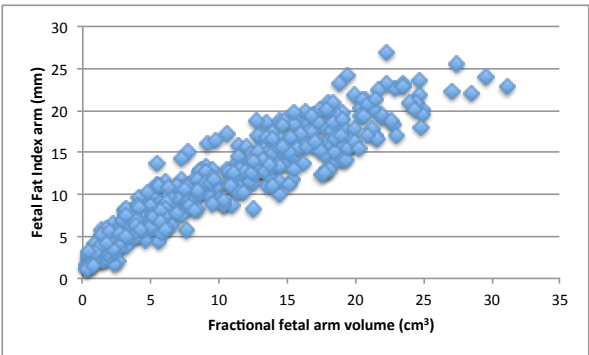
Correlation between single thickness and fat volume was less strong ($r=0.85$, $r^2=0.72$) than for FFI and fat volume [Figure 116], as might have been anticipated by the variation in fat around the arm, and by the single thickness results compared with fetal arm fat area (section 4g). This may also be partly due to the limited range of values available for a single measurement of fat thickness.



(a) Superimposed FFI and single fat thickness vs. fractional fetal arm fat volume (n=499).



(b) Single thickness vs. fractional fetal arm fat volume.



(c) FFI vs. fractional fetal arm fat volume.

Figure 116: Comparison of arm FFI and single thickness measurements, with fractional fetal arm fat volume.

FFI values were then plotted against gestational age [Figure 117], demonstrating a similar curve to the fractional fetal arm fat volumes, with increasing variation in the third trimester.

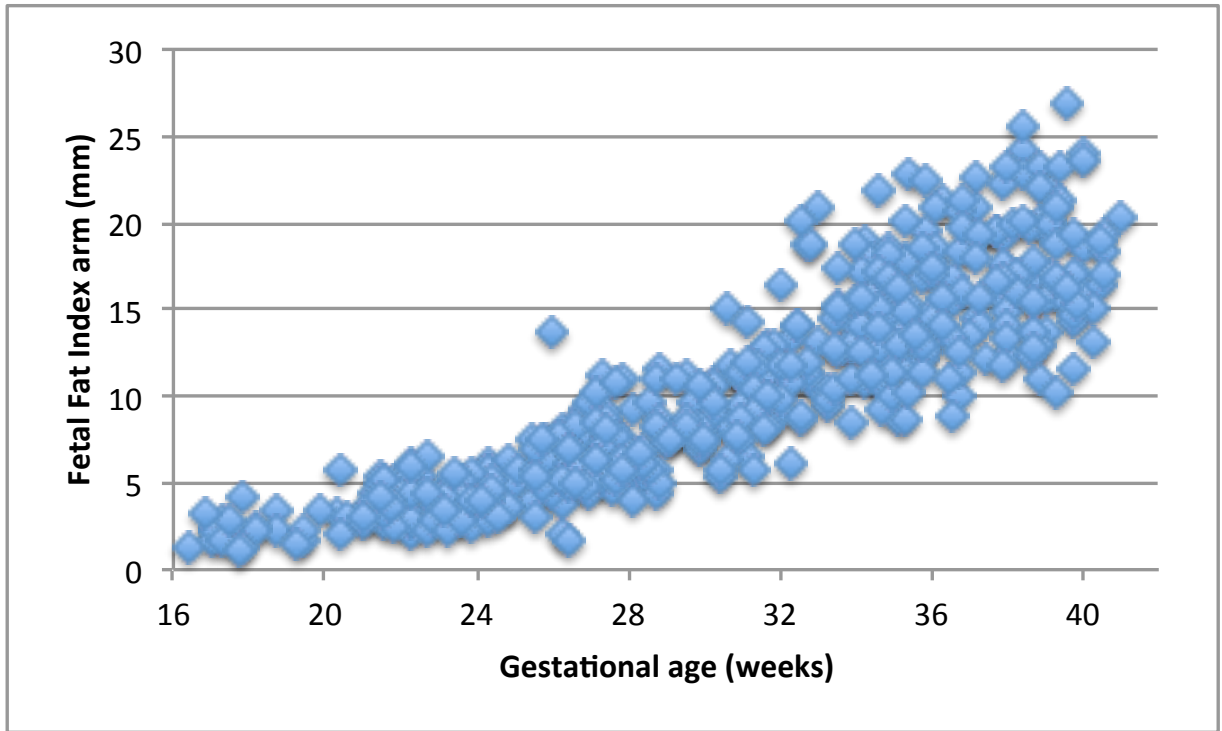


Figure 117: FFI plotted against gestational age (n=499).

Mean / 50th centile (C50) and SD were calculated using fractional polynomial regression, and 3rd, 5th, 10th, 90th, 95th and 97th centiles as previously stated [Figure 117, Table 29]. As with some other measurements, values below 20 weeks were excluded due to tool limitations, measurement challenges, and low likelihood of clinical relevance:

$$\begin{aligned} \text{Fetal Fat Index (arm) C50} &= ((\text{GA}/10)^{-2} \times 184.9485) + \\ &\quad ((\text{GA}/10)^{-0.5} \times -239.8859) + 127.2778 \end{aligned}$$

$$\text{SD of Fetal Fat Index (arm) C50} = (\text{GA} \times 0.1713721) - 2.820451$$

Table 30: Centile values for Fetal Fat Index (arm) for each week of gestation, from central slice in 3D volume.

GA (weeks)	Fetal Fat Index – arm (mm) centiles						
	3 rd (mean -1.88 SD)	5 th (mean - 1.64 SD)	10 th (mean - 1.28 SD)	50 th (mean)	90 th (mean + 1.28 SD)	95 th (mean + 1.64 SD)	97 th (mean +1.88 SD)
20	2.7	2.9	3.1	3.9	4.7	4.9	5.0
21	2.2	2.4	2.7	3.7	4.7	5.0	5.1
22	2.0	2.2	2.5	3.8	5.0	5.3	5.5
23	2.0	2.2	2.6	4.1	5.5	5.9	6.2
24	2.1	2.4	2.9	4.5	6.2	6.7	7.0
25	2.4	2.8	3.3	5.2	7.0	7.6	7.9
26	2.8	3.2	3.8	5.9	8.0	8.5	8.9
27	3.3	3.7	4.3	6.7	9.0	9.6	10.1
28	3.8	4.3	5.0	7.5	10.0	10.8	11.2
29	4.4	4.9	5.7	8.4	11.2	11.9	12.4
30	5.0	5.5	6.4	9.3	12.3	13.1	13.7
31	5.6	6.2	7.1	10.3	13.5	14.4	15.0
32	6.2	6.9	7.8	11.2	14.6	15.6	16.2
33	6.9	7.6	8.6	12.2	15.8	16.9	17.5
34	7.5	8.3	9.3	13.2	17.0	18.1	18.8
35	8.2	8.9	10.1	14.2	18.2	19.4	20.1
36	8.8	9.6	10.8	15.1	19.4	20.6	21.4
37	9.5	10.3	11.6	16.1	20.6	21.9	22.7
38	10.1	11.0	12.3	17.0	21.8	23.1	24.0
39	10.7	11.6	13.0	18.0	22.9	24.3	25.2
40	11.3	12.3	13.7	18.9	24.1	25.5	26.5
41	11.9	12.9	14.4	19.8	25.2	26.7	27.7

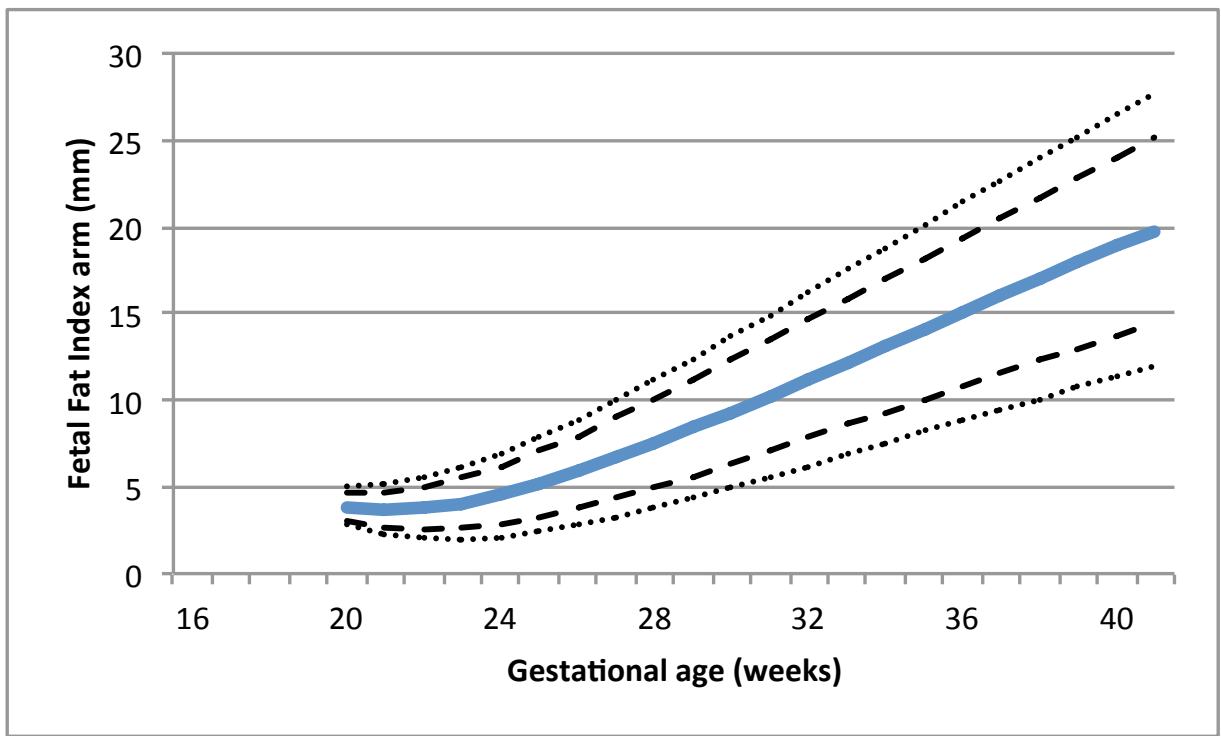


Figure 118: Fetal Fat Index of the arm (3rd, 10th, 50th, 90th and 97th centiles).

Conclusion

This section explores the FFI measurement in more detail and shows that it correlates well with fractional fetal arm fat volume measurements. Reference centiles have been calculated and are demonstrated from 20 to 41 weeks. FFI may prove useful in units with 2D, but not 3D, ultrasound machines, as a simple surrogate for fractional fetal arm fat volume.

ii. Fetal Fat Index (arm): reproducibility

Aim

To assess the intra-observer variability of the FFI technique, both of the overall method but also of its components.

Method

The 23 arm images which were manually measured as a proof-of-concept of FFI in section 4g, ranging from 16⁺³ to 40⁺⁴ weeks, were re-measured by the author. The method used of the four explored in section 4g was that of using the centre of the arm slice as the reference point ("2a" from the list of technique options in that section), and taking measurements at the 9, 12 and 3 o'clock positions, as this was felt to be the most straightforward of the methods. Intra-observer variability was assessed by means of Bland-Altman plots and ICCs, and the correlation between individual measurements was assessed using Pearson correlation coefficients.

Results

The correlation coefficient showed strong correlation between the original and repeat FFI ($r=0.993$, $r^2=0.986$), whilst the Bland-Altman plot gave a mean difference of 0.18% or -0.14mm (limits of agreement -15.1% to 15.4% or -2.2mm to 1.9mm) [Figure 119].

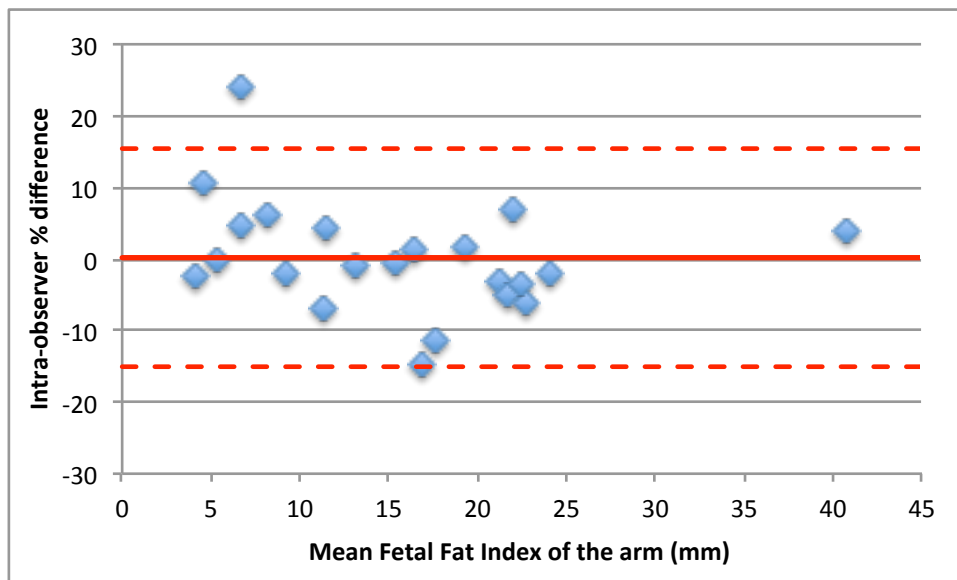


Figure 119: Bland-Altman plot showing intra-observer percentage difference for Fetal Fat Index.

The mean differences for the measurements at the individual positions were: 9 o'clock position mean difference 6.1% or 4.1mm (limits of agreement -2.5 to 14.7%, 0.0 to 8.1mm); 12 o'clock position mean difference -0.5% or 0.2mm (limits of agreement -27.8 to 26.8%, -1.1 to 1.4mm); and 3 o'clock position mean difference 3.2% or 0.1mm (limits of agreement -23.9 to 30.3%, -0.8 to 1.0mm). The correlation coefficients for the measurements at each position were 0.98, 0.97 and 0.98 respectively, and the ICCs similarly strong (0.97-0.98). It is interesting to note that whilst the individual measurements (particularly those at the 9 o'clock position) showed some variation, with broader limits of agreement than the FFI, that the FFI itself showed a small mean difference of 0.18% with narrow limits of agreement.

Conclusion

FFI shows a low mean difference in terms of intra-observer variation, with limits of agreement comparable to most ultrasound techniques.

5g. Publications and presentations arising from this chapter

OP29.05 Fetal arm fat: a better marker than thigh fat for nutritional status?²⁴²
 Sept 2014: listed in full in Chapter 6

P13.20 Fetal limb fat and lean volume reference ranges from an optimally healthy population²⁴³
 Sept 2014: listed in full in Chapter 6

Fetal limb fat and lean tissue assessment using 3D ultrasound and a fractional volume MATLAB tool
 Sept 2014: listed in full in Chapter 6

Fetal Fat Index (FFI) reference ranges: a simple method to assess limb fat²⁴⁴
McKnight, S.Rueda, M.Ahmed, K.Edwards, J.Noble, A.Papageorghiou
J Matern Fetal Neonatal Med, 2014; 27 (s1): 118
 June 2014: XXIV European Congress of Perinatal Medicine, Florence, Italy
 [oral and poster presentation]

OC05.04: Fetal arm volumetry: a novel assessment of fetal body composition in the second and third trimester²⁴⁵
C.L.Knight, S.Rueda, M.Ahmed, K.Edwards, J.A.Noble, A.T.Papageorghiou
Ultrasound Obstet Gynecol 2013; 42 (s1): 10
 Oct 2013: ISUOG 23rd World Congress, Sydney, Australia
 [oral communication; awarded best oral communication in section]

P11.01: Assessing fetal fractional arm volume using varying numbers of cross-sectional slices²⁴⁶
M.Ahmed, **C.L.Knight**, S.Rueda, A.T.Papageorghiou, J.A.Noble
Ultrasound Obstet Gynecol 2013; 42 (s1): 151-152
 Oct 2013: ISUOG 23rd World Congress, Sydney, Australia
 [poster presentation]

Fetal arm fat as an ultrasound marker of nutritional status
 Aug 2013: listed in full in Chapter 7

Ultrasound image segmentation using feature asymmetry and shape guided live wire²⁴⁷
T.M.Rackham, S.Rueda, **C.L.Knight**, J.A.Noble
Feb 2013: SPIE (Society of Photo-Optical Instrumentation Engineers) Medical Imaging
Conference: Image Processing, Florida, USA
[paper and presentation]

P27.07: Assessing fetal fractional arm adipose tissue volume using 3D ultrasound: the
importance of measuring perpendicular to the humerus²⁴⁸
M.Ahmed, S.Rueda, **C.L.Knight**, A.T.Papageorghiou, J.A.Noble
Ultrasound Obstet Gynecol 2012; 40 (s1): 275-276
Sept 2012: ISUOG 22nd World Congress, Copenhagen, Denmark
[poster presentation]

5h. Contributions to the thesis: chapter 5

Other than where indicated below, and acknowledged in appendix A, all the work in this chapter (including concepts and hypotheses, protocols, acquisition and segmentation of scans, analyses, production of graphs and figures) is that of the author.

M.A. developed the MATLAB segmentation tool (3D) under the supervision of S.R., by modifying the 2D tool previously developed by S.R.. S.R. and the author segmented the volumes to assess the effect of the angle of slices; M.A. and the author undertook the analyses on this data, and M.A. provided the figure [Figure 79]. M.A. and the author segmented the volumes to assess the effect of the number of slices. M.A. processed all mat files to extract central slice information for FFI measurement; S.R. designed the tool, and processed the mat files, to generate the FFI values. S.A. retrieved neonatal demographic data from the INTERGROWTH-21st database with the permission of L.C.I. on behalf of the INTERGROWTH-21st collaboration. L.C.I. also oversaw the anthropometry team (with F.P. and S.L.) who undertook the newborn body composition analysis (PEA POD) and anthropometry

Images taken from journal papers and textbooks are individually cited in the figure captions, by name and year, and with a reference to the full bibliographic details.

6. FETAL THIGH FAT ASSESSMENT USING 3D ULTRASOUND

This third results chapter applies previously-developed techniques to the fetal thigh, using 3D ultrasound. It includes the novel measurements of fetal thigh fat and lean volumes, and Fetal Fat Index (FFI). 2D measurements are assessed using the central slice of 3D volumes.

Section 6a introduces the assessment of the fetal thigh, exploring anatomical and segmentation challenges, and demonstrating MRI validation.

Sections 6b and 6c describe the measurement of fetal thigh fat and lean tissue volumes, including scatterplots and centiles describing normal development throughout gestation in an optimally healthy population, and longitudinal studies of thigh fat volumes of individual fetuses with at least 4 scans. Axially-acquired volumes are compared with sagittally-acquired volumes from the same fetus, in preparation for fetal and neonatal assessments (chapter 7).

Section 6d explores the thigh, fat, lean, and fat as percentage of thigh areas for the central slice of all 3D scans originally measured in section 6b, producing scatterplots and reference ranges. This is similar to the assessment of the fetal arm in section 5e.

Section 6e examines FFI through central slice extractions from all thigh volumes, comparing them with thigh fat volume measurements in the same fetuses. This is similar to that assessed in the arm in section 5f. Reference centiles for FFI are also presented.

Section 6f lists the publications and presentations arising from the work in this chapter, and **section 6g** lists the contributions to this chapter.

6a. Measuring fetal thigh fat using 3D ultrasound: challenges

Having explored, in detail, 2D and 3D assessment and measurement techniques of arm fat and lean tissues, the same tools and protocols were used to assess the fetal thigh. The 3D volumes, enabling both 3D and 2D analyses, were utilised, rather than also analysing the separate 2D images, as volumes had been more useful. The volumes were acquired using the same protocol (see section 4a and appendix B), assessed using a pragmatic subjective approach, and segmented using the same 5 perpendicular slice technique (see section 5a and appendix D) [Figure 120] as those used for the arm. However, there were challenges associated with acquisition of images and volumes, and measurement of, the fetal thigh.

Minor modifications were made to the arm tool – for example, to include the amount of X-Z rotation in the results files, and use generic terms so the tool could be used for arm or thigh.

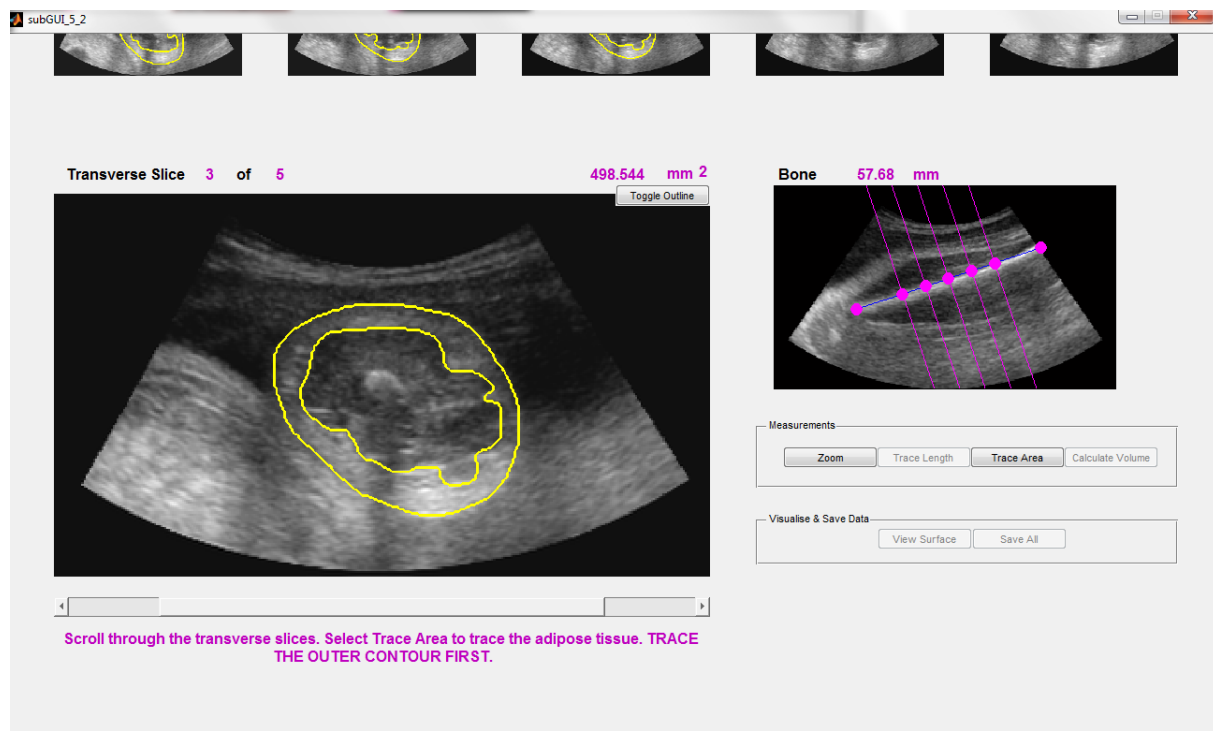


Figure 120: Sample segmentation of 32 week fetal thigh. The slight modifications to the tool caused cropping of the five upper images; however, these could still be reviewed in the main screen using the horizontal slider.

i Anatomical challenges and MRI validation

The more complicated anatomy of the fetal thigh, compared to the arm, has been shown in fetal anatomy specimens [Figure 12], and was a factor in beginning the exploration of limb fat measurement using the simpler structure of the arm. The correlation with anatomical specimens is particularly notable in the late third trimester of pregnancy [Figure 121]. This complex anatomy made segmenting the thigh fat rather more challenging than in the arm.

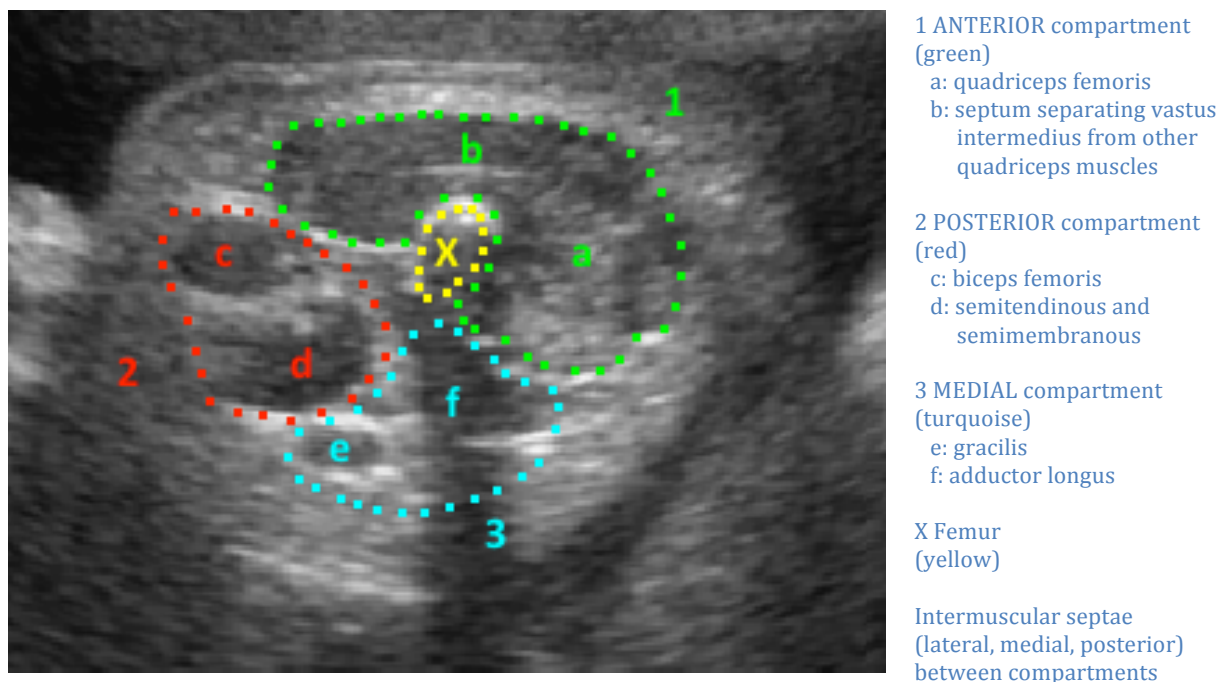
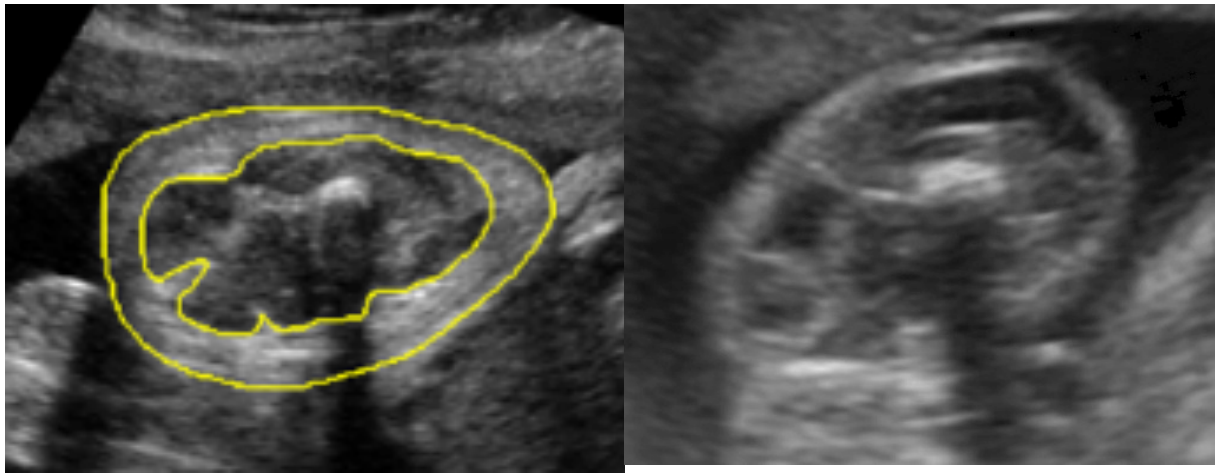


Figure 121: Ultrasound image of 37 week fetal thigh showing musculoskeletal anatomy.

The MRI validation of the arm images [Figure 62] had already suggested that there was some fat between the muscles, which then led into the intermuscular septae. The same approach was therefore taken when segmenting thigh fat, i.e. incorporating such indentations on the inner fat border [Figure 122a]. This was used throughout the range of gestational ages – some details of the thigh, for example, the anterior septum within the quadriceps femoris muscle groups, could be identified in scans even at early gestational ages [Figure 122b].

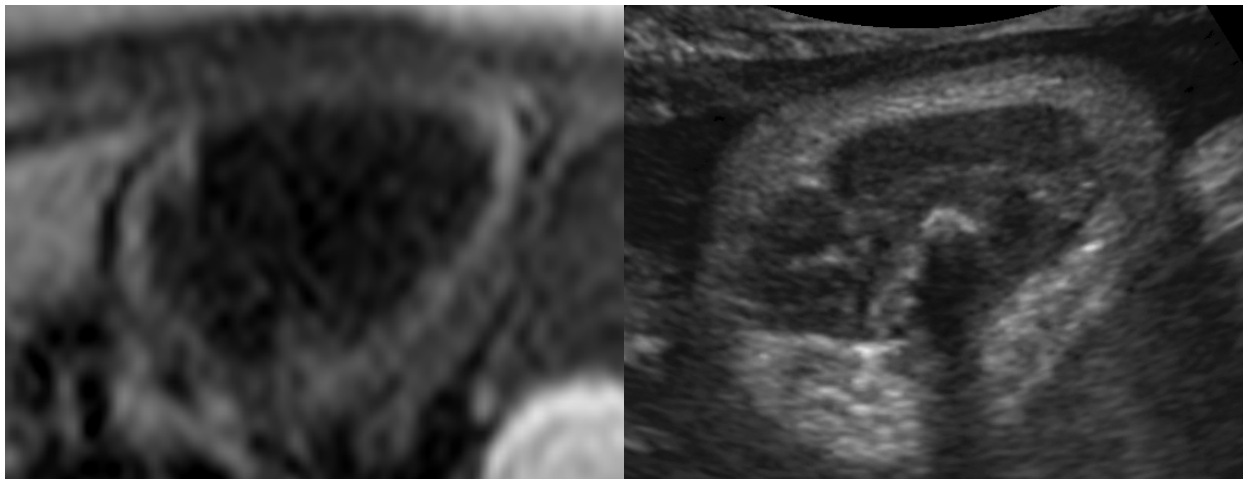


(a)

(b)

Figure 122: Challenges of outlining fetal thigh fat (a) 37 week fetus, as shown in Figure 121 (b) 23 week fetus showing anterior compartment with septum, and prominent posterior compartment muscles (left in image).

MRI was also used to validate the more complex thigh anatomy. Visual comparison of thigh images acquired as part of the MRI validation study (see section 4e) confirmed the presence of fat within the ‘base’ of the intermuscular septae, suggesting our segmentation approach was correct [Figure 123].



(a) MRI

(b) ultrasound

Figure 123: MRI validation of ultrasound images – (a) MRI and (b) ultrasound axial images of 37 week fetal thigh, taken less than an hour apart. Note the continuation of fat within the base of the lateral and medial intermuscular septae.

ii Imaging and segmentation challenges

The thigh images and volumes provided some specific challenges, mainly due to the increased size of the thigh when compared with the arm.

At later gestational ages, it could be difficult to capture the full length of the femur in the maximum 70° angle sweep [Figure 124]. This resulted in incomplete volumes which had to be rejected at the segmentation stage. This also happened at earlier gestational ages, if the sagittal image (upper right) was not checked prior to saving the volume – however, in these cases it would have physically been possible to capture the full FL within the volume.

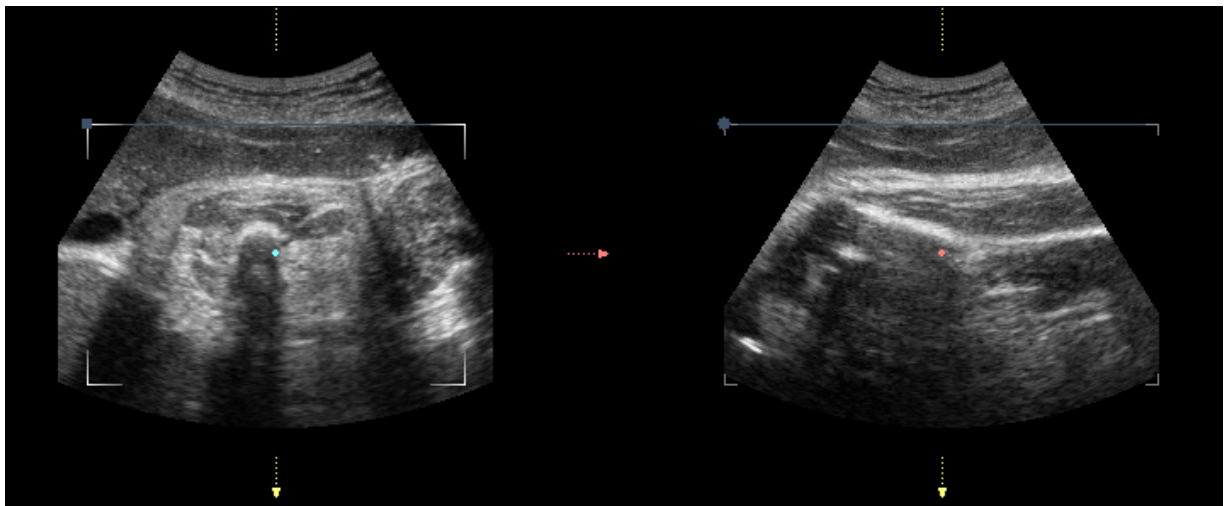


Figure 124: Axially-acquired thigh volume from 39 week fetus showing incomplete capture of the femur length in the sagittal plane (right).

Increased acoustic shadowing [Figure 125] was also noted, particularly at later gestational ages, as the fetal tissues became increasingly dense and echoreflective. Shadowing could also occur due to the fetal thigh being under the maternal umbilicus (which may cause shadows), or due to overlying fetal arms or crossed legs [Figure 126]; the latter is far less of a problem for arm imaging than for thigh.

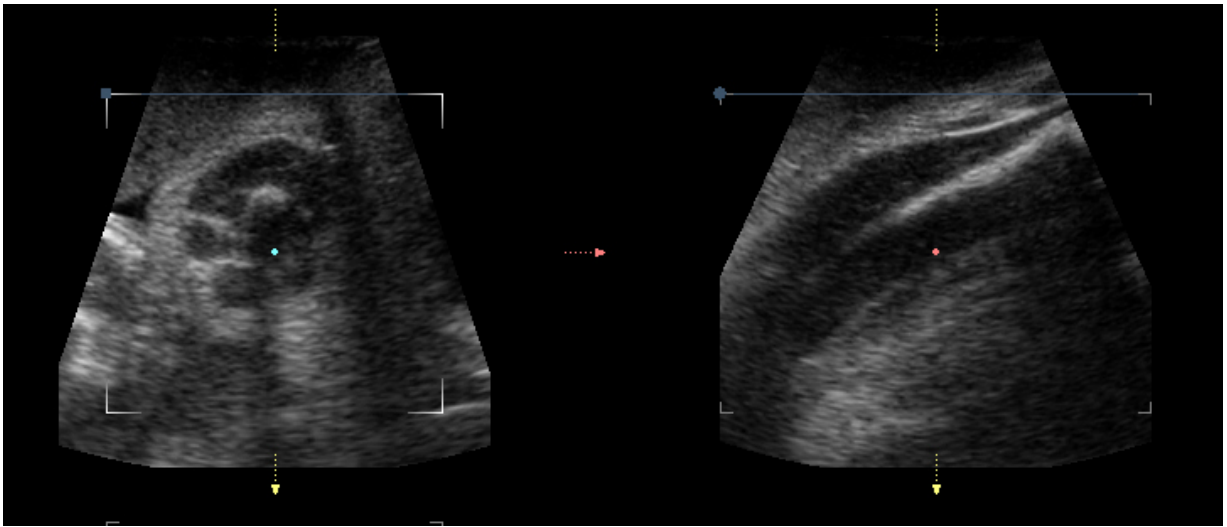


Figure 125: Fetal thigh at 34 weeks showing shadowing, rendering accurate fat segmentation impossible.

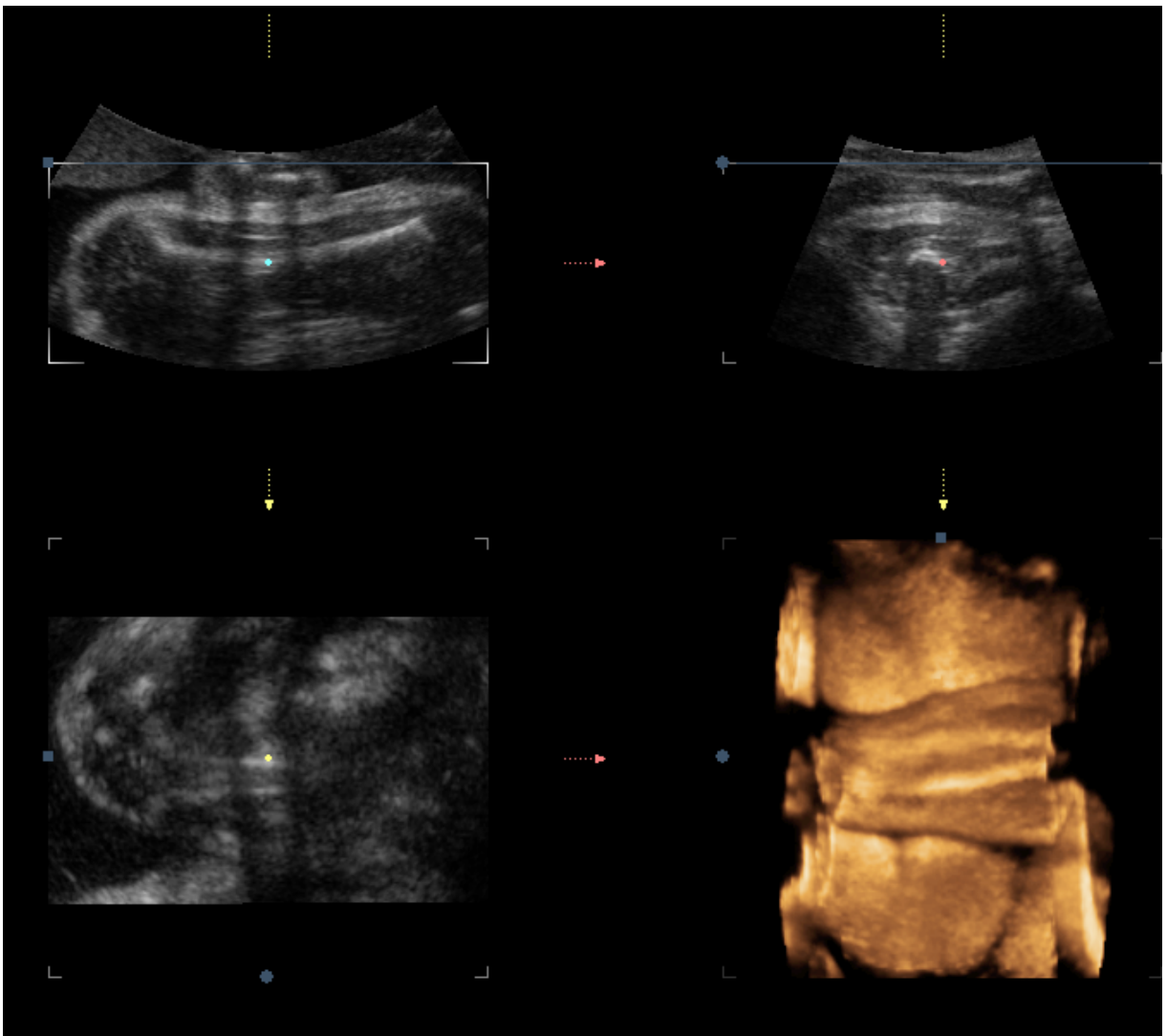
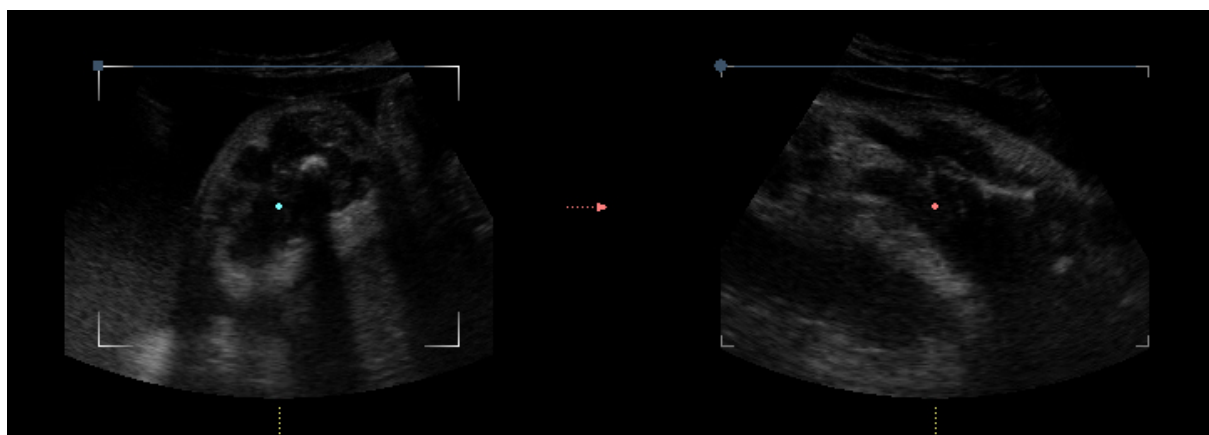
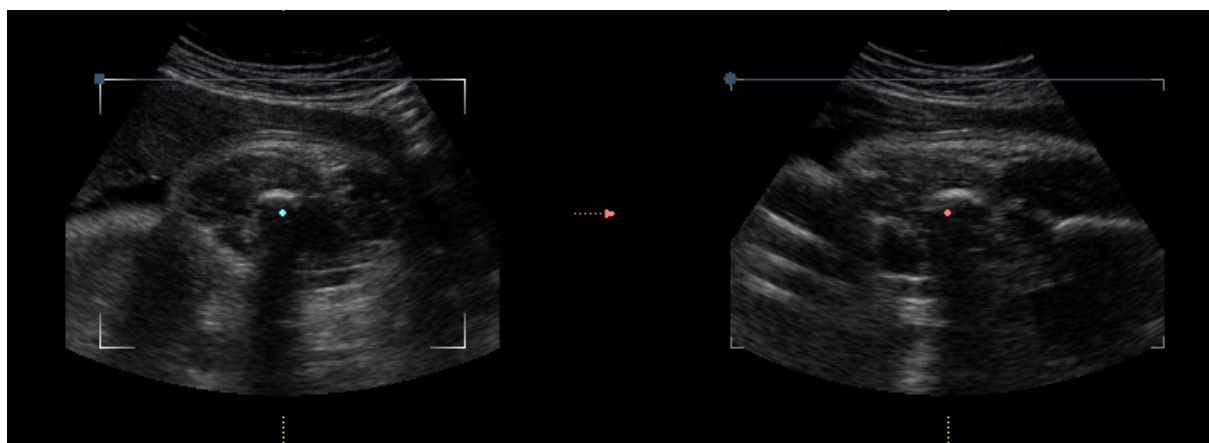


Figure 126: Sagittally-acquired thigh volume from 32 week fetus showing acoustic shadows from overlying bones from the other lower leg, and resulting disruption to the thigh images in the sagittal and coronal planes. Disruption to the axial segmentation slices also occurred, where they fell within a shadowed section.

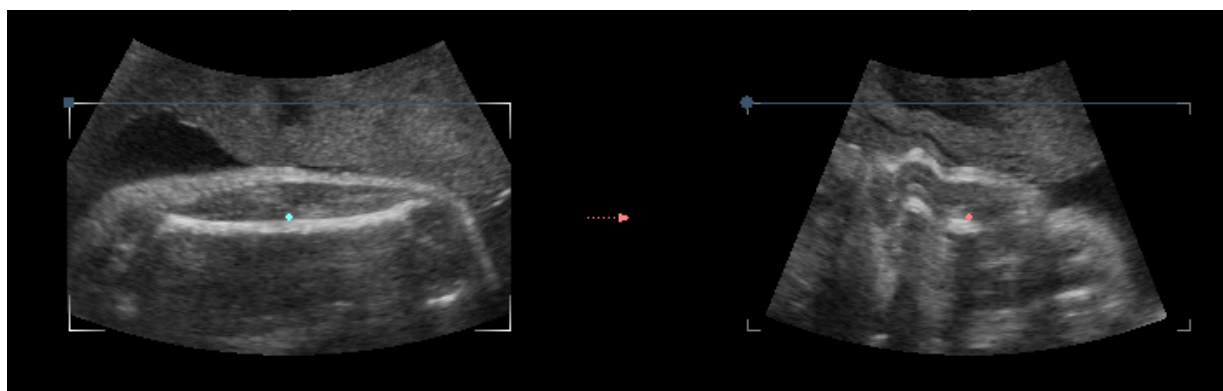
Other acquisition and segmentation issues provide challenges for both the arm and thigh [Figure 127a-h].



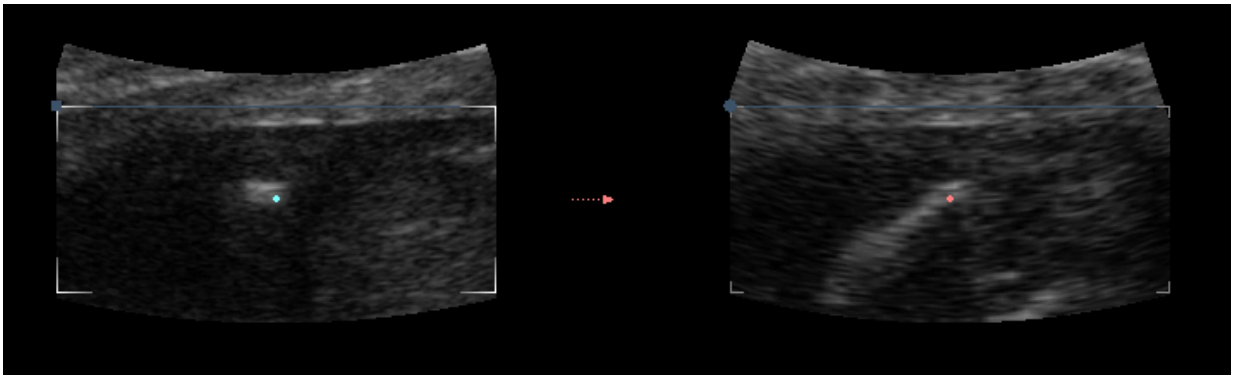
(a) dark image (32 weeks).



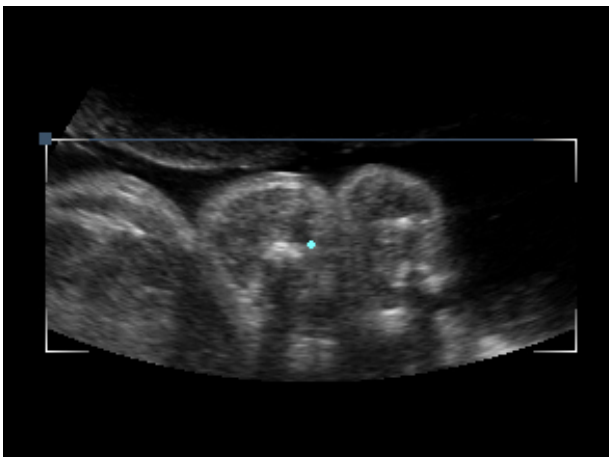
(b) rotated image (36 weeks).



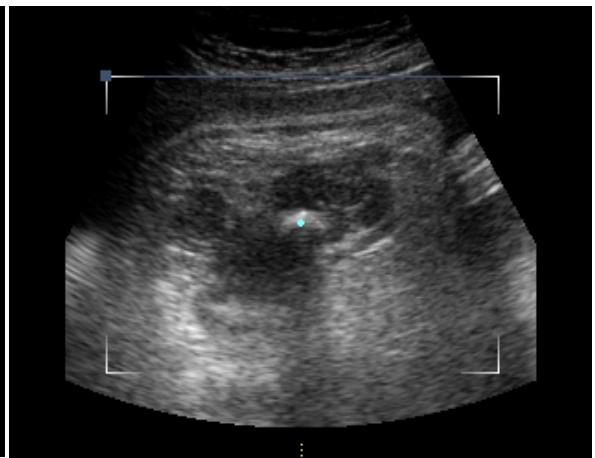
(c) movement artefact (32 weeks, sagittally acquired).



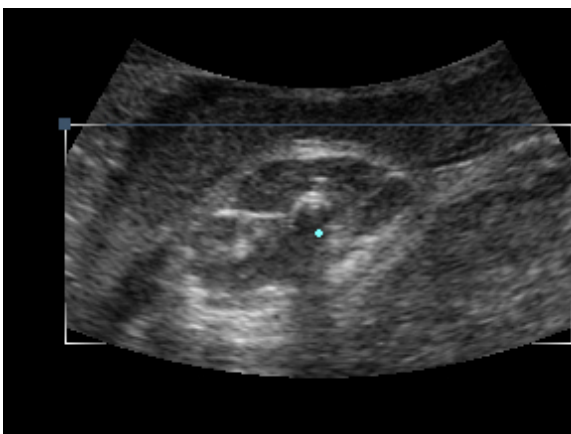
(d) early gestation (16 weeks).



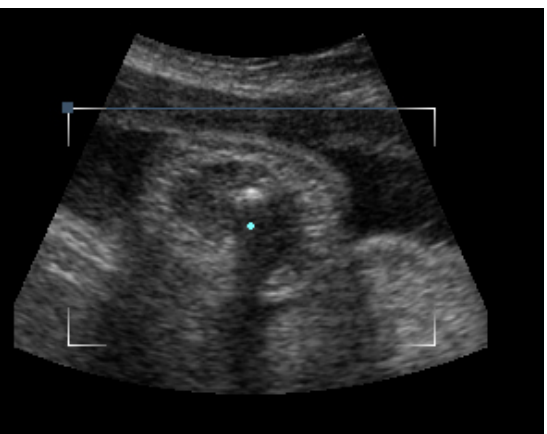
(e) disruption by other structures (33 weeks, flexed leg with calf on left of axial image).



(f) fat lower inner and outer border identification (40 weeks).



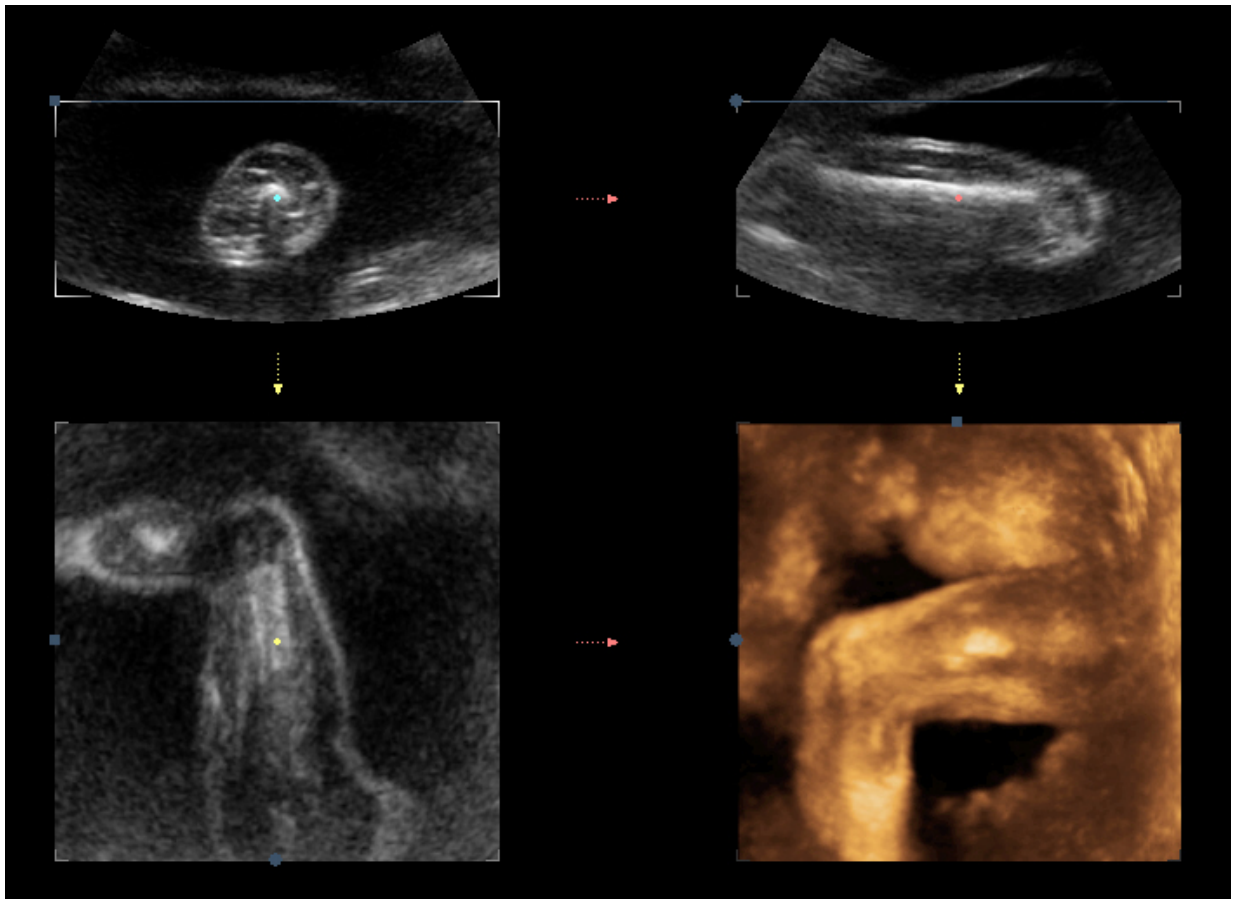
(g) fat lower outer border identification (32 weeks).



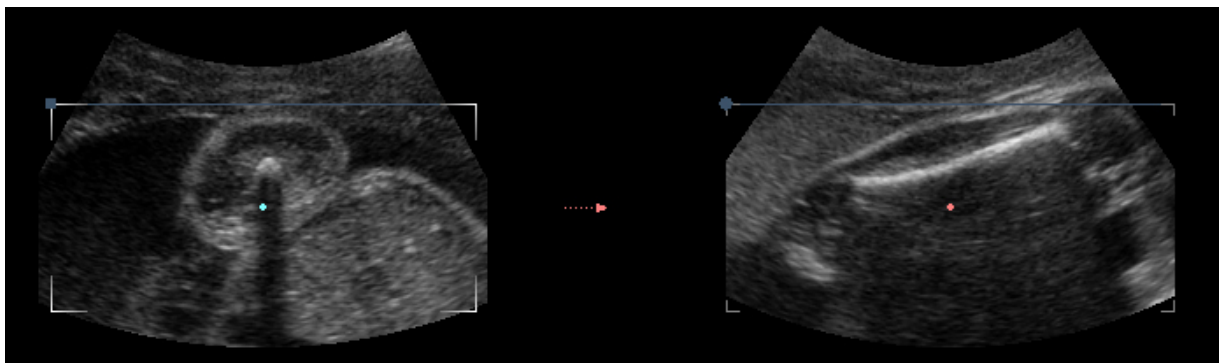
(h) fat/skin upper border identification (37 weeks).

Figure 127: Challenges in limb volume acquisition and segmentation.

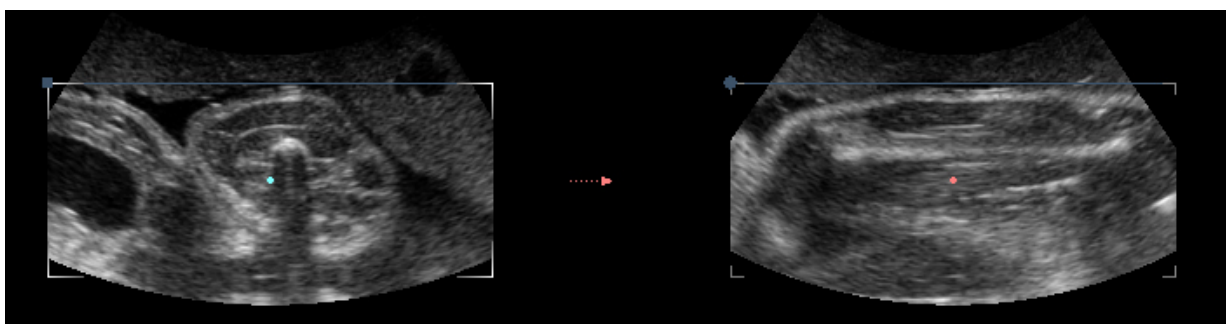
Despite the challenges, excellent quality thigh volumes were also acquired [Figure 128].



(a) 22 weeks.



(b) 27 weeks.



(c) 35 weeks.

Figure 128: Excellent quality fetal thigh volumes showing clear axial images and full sagittal femur length.

6b. Measuring thigh fat and lean tissue volumes, and establishing reference ranges

i. Normal development of fetal thigh fat and lean volumes

Aim

To assess the fetal thigh using 3D ultrasound, and produce reference ranges for fat and lean volumes, from an optimally healthy population.

Method

Axial mid-femoral volumes from 15-41 weeks' gestation were acquired from women participating in the INTERGROWTH-21st study between January 2011 and May 2012. The best volume was selected from each scan based on visual assessment. These were measured (segmented) using the specifically-designed MATLAB tool (section 5a) by five people: the author, M.A. (biomedical engineer), K.E. (research midwife), V.D. (research fellow), and G.P. (teaching fellow, University of Auckland). All underwent training in the segmentation tool and techniques by the author. The same approach was used for the thigh as for the arm (see section 5c), namely a perpendicular-slice, 5-slice, 50% fractional volume approach¹⁴³ with careful division of patients and scans between segmenters to minimise bias.

Lean volume (muscle and bone) was subtracted from thigh volume to generate fat volume. Fat was also expressed as a percentage of thigh volume. The volumes (in cm³) were plotted against gestational age. Statistical analyses were carried out using STATA to calculate centiles (3rd, 5th, 10th, 50th, 90th, 95th and 97th), using fractional polynomial regression, with log transformation where necessary.

Results

714 thigh volumes were obtained from 242 women taking part in the INTERGROWTH-21st study; each woman was scanned 1-6 times (median 4, mean 3 scans). The gestational ages ranged from 15⁺² weeks to 41⁺⁶ weeks. Fat and lean volumes could be measured in 557 (78%) [Figure 129].

The ease of segmentation was recorded in 334/557 cases, of which only 44% were stated to be “easily segmentable”. This is considerably lower than the 71% easily segmented arm volumes, and may reflect the more difficult anatomy of the thigh, or the other factors discussed above (section 6a).

In those thigh volumes which could not be segmented, the majority (86 volumes, or 55%) were not acquired according to the protocol (60 did not acquire the full sagittal length of the femur; 23 were too rotated to be corrected and then measured; and 3 did not capture all of the thigh fat within the volume). In 66 cases it was not possible to segment the fat due to problems of visualisation. Similar to the arm, this was evident at lower gestational ages (22/66 were <21 weeks). Of the 44 scans which could not be segmented at gestational ages of 21 weeks or later (i.e. after the usual timing of the anomaly scan): 20 were due to difficulties with the outer border, and 24 were due to difficulties with both the outer and inner fat borders. This is twice as many, in percentage terms, as the arm volumes, although the reasons were similar, divided between difficulties with the outer border or with both borders. Again, this higher number in the thigh volumes may reflect the more complex anatomy which makes the segmentation more challenging; or it may reflect the shadows and other imaging artefacts which are particularly evident at later gestations.

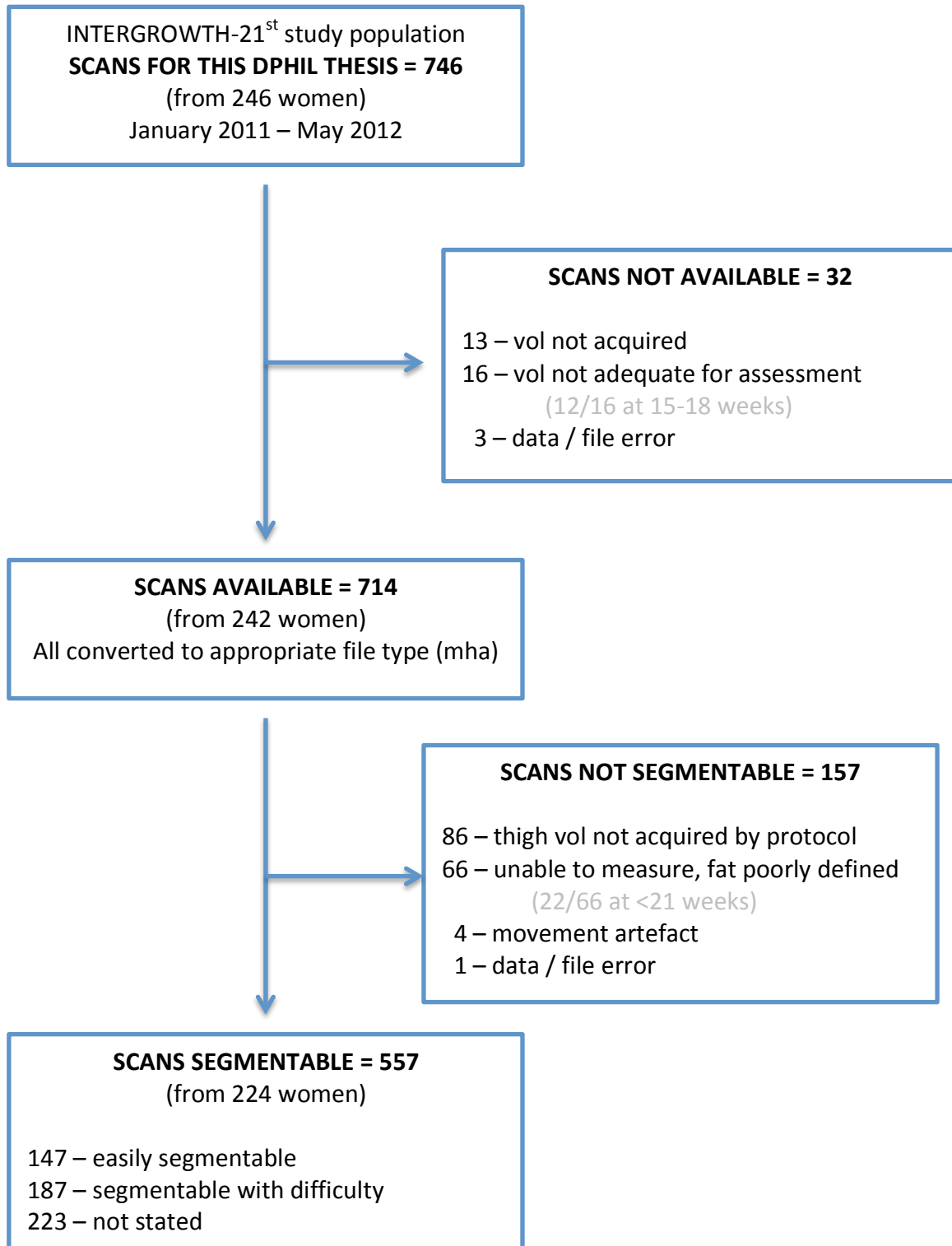


Figure 129: Flow diagram showing generation of fetal thigh volume results.

An assessment of thigh volumes was undertaken using femur length (FL), similar to that undertaken for arm volumes using humeral length (section 5c). The measured volumes were assessed visually, and inadequately rotated volumes excluded from the analysis. To aid with the identification of such cases, the FL measurements were compared with Chitty's reference centiles for FL,²³² using the mean $\pm$ 3SD.

Overall, this resulted in the exclusion of 53 (9.5%) cases (49 below 3SD and 4 above 3SD), leaving 504 cases for analysis from 221 women. This was a very similar exclusion rate to the arm volumes (55/556, or 9.9%). The excluded cases ranged from 20⁺³ weeks to 40⁺⁶ weeks, with more at later gestational ages (median 34⁺⁵ weeks, inter-quartile range 26⁺⁵ to 37⁺⁴ weeks). The final segmentation rates after excluding inadequately rotated volumes, was an average of 71% of thigh volumes being able to be segmented. This was lower than the 79% equivalent value for the arm volume segmentation rate, but better than might have been anticipated from the lower "ease of segmentation" assessment.

The calculated fat, lean and thigh 50% fractional volumes (in cm³) were plotted against gestational age [Figure 130 - Figure 133].

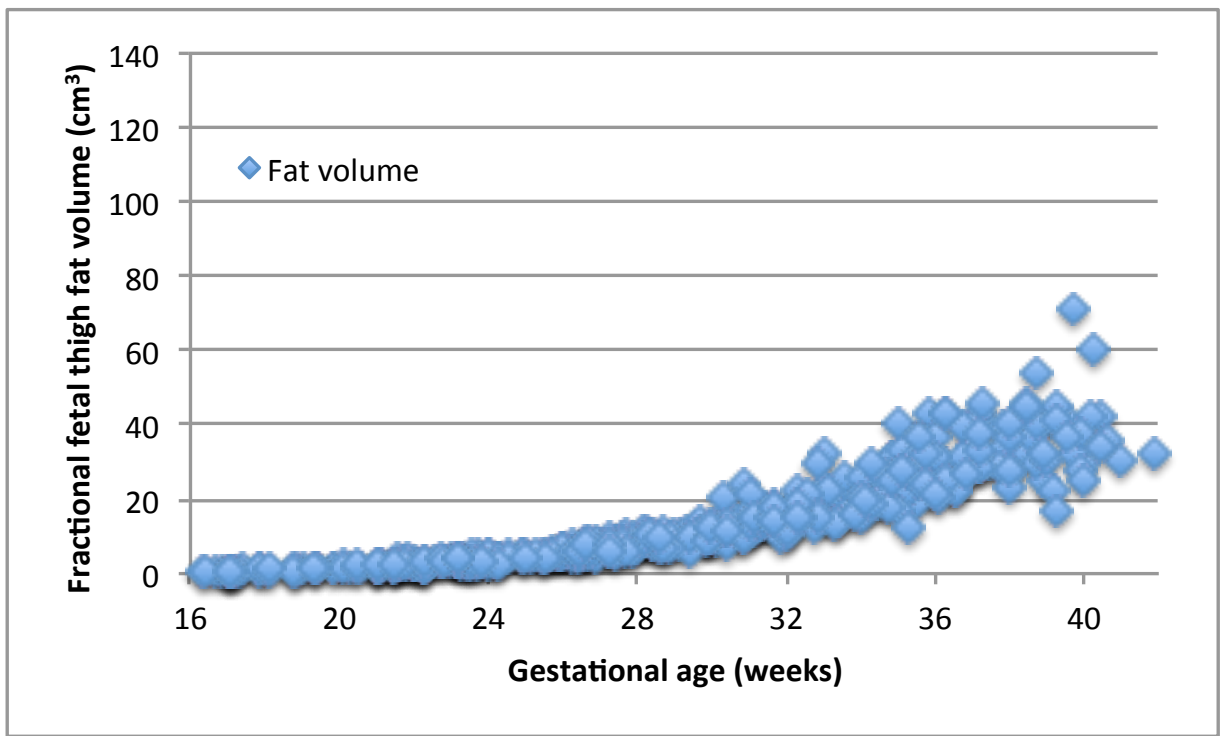


Figure 130: Fractional fetal thigh fat volume (50% fractional volumes, acquired axially) (n=504).

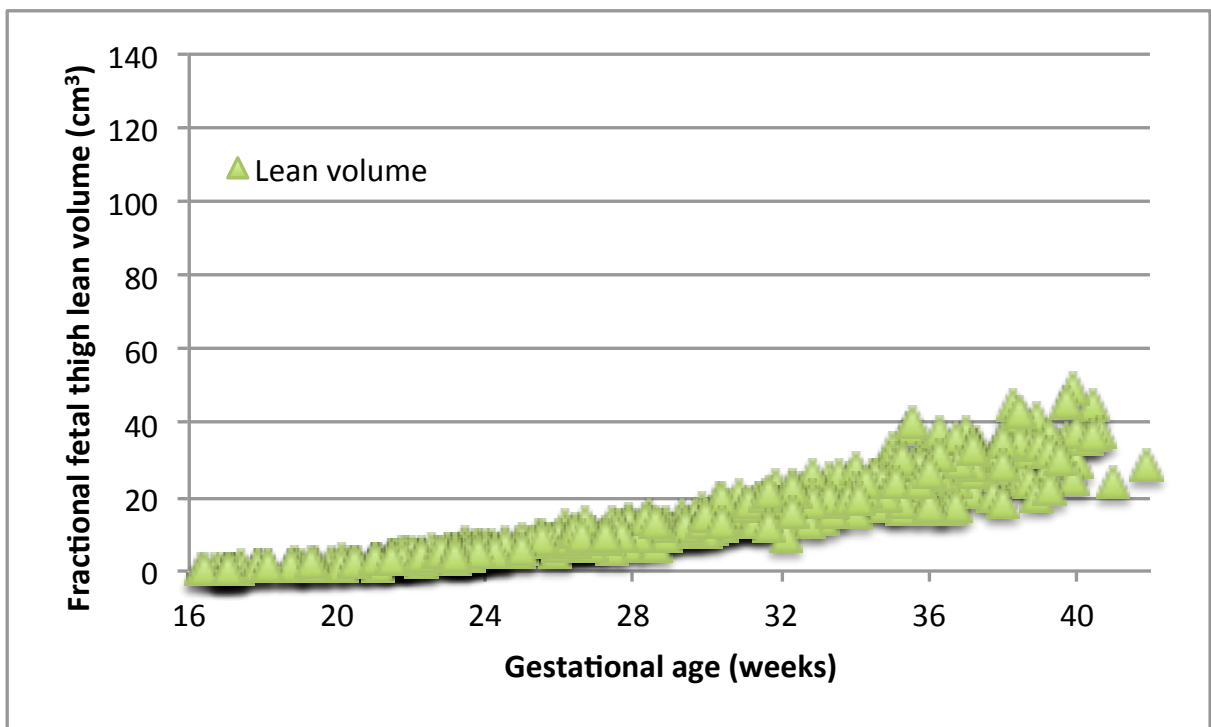


Figure 131: Fractional fetal thigh lean volume (50% fractional volumes, acquired axially) (n=504).

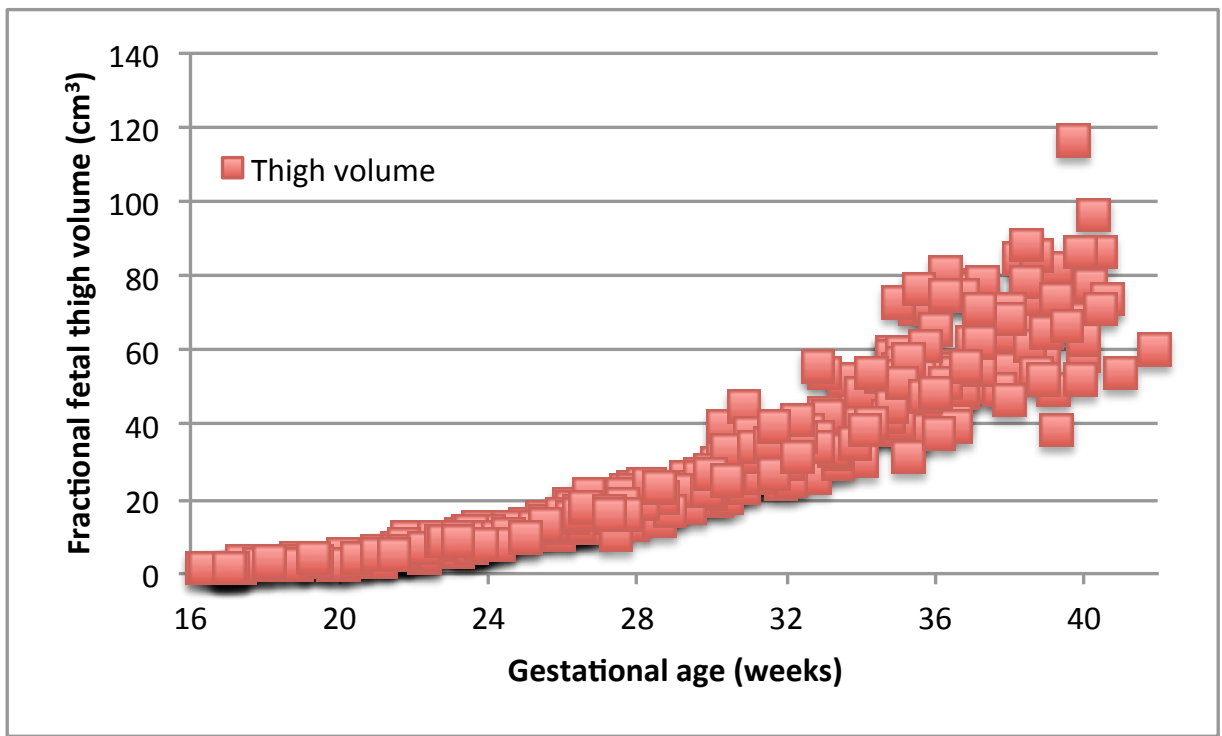


Figure 132: Fractional fetal thigh volume (50% fractional volumes, acquired axially) (n=504).

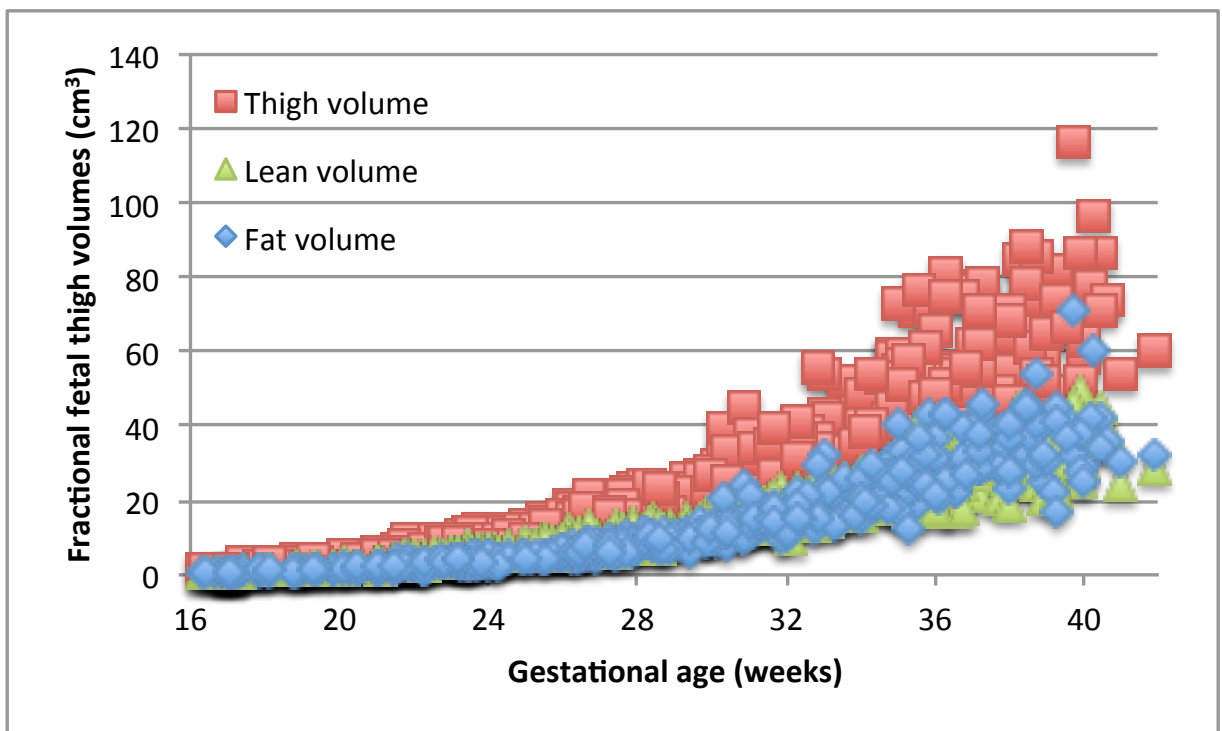


Figure 133: Fractional fetal thigh, fat and lean volumes (50% fractional volumes, acquired axially) superimposed (n=504).

Fractional polynomial regression^{206,207} was used to generate equations to calculate the mean / 50th centile (C50) values for thigh fat volume and lean volume (50% fractional volumes) for a given gestational age (GA):

$$\begin{aligned} \text{Log (thigh fat volume) C50} &= ((\text{GA}/10)^{0.5} \times 7.807605) + ((\text{GA}/10)^3 \times -0.0241565) \\ &\quad -10.40706 \end{aligned}$$

$$\text{Log (thigh lean volume) C50} = ((\text{GA}/10)^{-1} \times -10.84032) + 6.264174$$

The relationship with standard deviation (SD) was also generated using the same approach.

$$\text{SD of log (thigh fat vol) C50} = 0.20$$

$$\text{SD of log (thigh lean vol) C50} = 0.17$$

This enabled calculation of the centiles for both sets of data:

$$3^{\text{rd}} / 97^{\text{th}} \text{ centiles} = \text{mean} \text{ } -/+ 1.88 \text{ SD}$$

$$5^{\text{th}} / 95^{\text{th}} \text{ centiles} = \text{mean} \text{ } -/+ 1.64 \text{ SD}$$

$$10^{\text{th}} / 90^{\text{th}} \text{ centiles} = \text{mean} \text{ } -/+ 1.28 \text{ SD}$$

For both the fractional thigh fat volume and the fractional thigh lean volume, SD was used to calculate the log-transformed centiles, and the results then transformed back to generate results in cm³ [Table 31, Table 32].

Table 31: Centile values for fractional fetal thigh fat volume for each week of gestation.

GA (weeks)	Fractional fetal thigh fat volume (cm ³) centiles						
	3 rd (mean -1.88 SD)	5 th (mean - 1.64 SD)	10 th (mean - 1.28 SD)	50 th (mean)	90 th (mean + 1.28 SD)	95 th (mean + 1.64 SD)	97 th (mean +1.88 SD)
16	0.36	0.38	0.41	0.53	0.69	0.74	0.78
17	0.48	0.51	0.55	0.71	0.92	0.99	1.04
18	0.64	0.67	0.72	0.93	1.20	1.30	1.36
19	0.83	0.87	0.93	1.21	1.57	1.68	1.77
20	1.06	1.12	1.20	1.55	2.02	2.17	2.28
21	1.35	1.42	1.53	1.98	2.57	2.76	2.90
22	1.71	1.79	1.93	2.50	3.24	3.49	3.66
23	2.14	2.24	2.41	3.13	4.05	4.36	4.58
24	2.65	2.78	2.99	3.87	5.02	5.40	5.67
25	3.25	3.41	3.67	4.76	6.17	6.64	6.97
26	3.96	4.16	4.47	5.80	7.52	8.09	8.49
27	4.79	5.02	5.40	7.01	9.08	9.77	10.25
28	5.73	6.02	6.47	8.39	10.88	11.70	12.28
29	6.81	7.15	7.69	9.97	12.92	13.90	14.59
30	8.03	8.43	9.07	11.75	15.23	16.38	17.20
31	9.38	9.85	10.60	13.74	17.80	19.15	20.10
32	10.88	11.42	12.29	15.93	20.64	22.21	23.31
33	12.52	13.14	14.14	18.32	23.75	25.54	26.82
34	14.28	14.99	16.13	20.91	27.10	29.15	30.60
35	16.17	16.97	18.26	23.66	30.67	32.99	34.64
36	18.15	19.06	20.50	26.57	34.44	37.04	38.89
37	20.22	21.23	22.83	29.59	38.36	41.26	43.32
38	22.34	23.45	25.23	32.70	42.38	45.58	47.86
39	24.48	25.70	27.64	35.83	46.44	49.95	52.44
40	26.61	27.93	30.05	38.94	50.48	54.30	57.00
41	28.68	30.11	32.39	41.98	54.41	58.53	61.45

Table 32: Centile values for fractional fetal thigh lean volume for each week of gestation.

GA (weeks)	Fractional fetal thigh lean volume (cm ³) centiles						
	3 rd (mean -1.88 SD)	5 th (mean - 1.64 SD)	10 th (mean - 1.28 SD)	50 th (mean)	90 th (mean + 1.28 SD)	95 th (mean + 1.64 SD)	97 th (mean +1.88 SD)
16	0.43	0.45	0.48	0.60	0.75	0.80	0.84
17	0.65	0.67	0.72	0.89	1.12	1.19	1.24
18	0.92	0.96	1.02	1.27	1.60	1.70	1.77
19	1.26	1.32	1.40	1.75	2.19	2.33	2.43
20	1.68	1.75	1.87	2.33	2.91	3.10	3.23
21	2.17	2.27	2.41	3.01	3.76	4.01	4.18
22	2.75	2.87	3.05	3.81	4.76	5.07	5.28
23	3.40	3.55	3.78	4.72	5.89	6.27	6.54
24	4.14	4.32	4.60	5.74	7.17	7.63	7.96
25	4.96	5.17	5.50	6.88	8.59	9.14	9.53
26	5.86	6.11	6.50	8.12	10.14	10.79	11.25
27	6.83	7.12	7.58	9.48	11.83	12.59	13.13
28	7.88	8.22	8.75	10.94	13.65	14.53	15.15
29	9.01	9.39	10.00	12.51	15.60	16.60	17.31
30	10.20	10.63	11.32	14.16	17.66	18.80	19.60
31	11.46	11.95	12.72	15.92	19.84	21.12	22.02
32	12.78	13.32	14.18	17.75	22.13	23.55	24.56
33	14.16	14.76	15.71	19.67	24.51	26.10	27.21
34	15.59	16.25	17.30	21.67	26.99	28.74	29.96
35	17.07	17.80	18.95	23.73	29.56	31.47	32.81
36	18.61	19.40	20.65	25.87	32.21	34.29	35.75
37	20.18	21.04	22.40	28.06	34.94	37.20	38.78
38	21.79	22.72	24.19	30.31	37.74	40.17	41.88
39	23.44	24.44	26.02	32.61	40.59	43.21	45.05
40	25.13	26.20	27.89	34.96	43.51	46.32	48.29
41	26.84	27.98	29.79	37.34	46.48	49.47	51.58

Table 31 and Table 32 are shown in graph form below for fractional thigh fat [Figure 134] and lean volumes [Figure 135] (50th centile coloured line; 10th / 90th centiles dashed lines; 3rd / 97th centiles dotted lines):

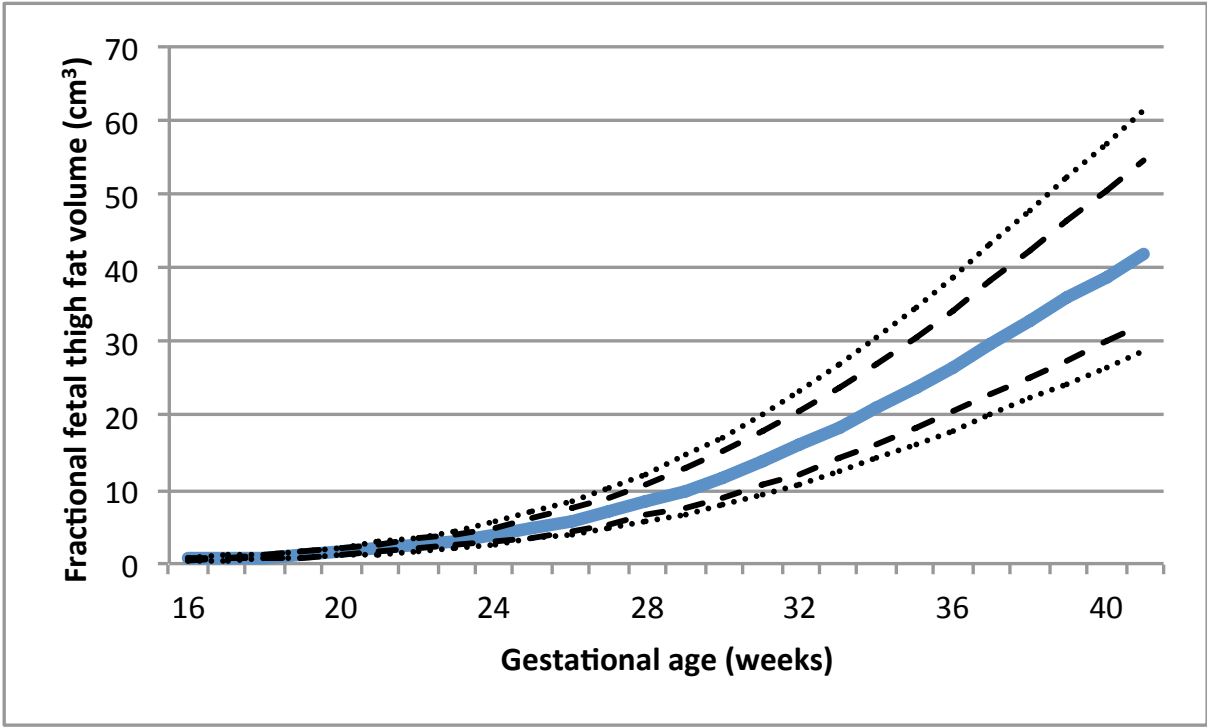


Figure 134: Fractional fetal thigh fat volume (3rd, 10th, 50th, 90th and 97th centiles).



Figure 135: Fractional fetal thigh lean volume (3rd, 10th, 50th, 90th and 97th centiles).

Thigh fat was also assessed in the form of its percentage of the fractional thigh volume, using fractional polynomial regression (without logarithmic transformation):

$$\text{Fat as \% thigh volume C50} = ((\text{GA}/10)^{-2} \times 101.8806) +$$

$$(((\text{GA}/10)^{-2} \times \ln(\text{GA}/10)) \times -384.0338) + 80.78733$$

$$\text{SD of fat as \% thigh vol C50} = 4.99$$

Table 33: Centile values for fractional fetal thigh fat as a percentage of thigh volume for each week of gestation.

GA (weeks)	Fractional fetal thigh fat (as % of thigh volume) centiles						
	3 rd	5 th	10 th	50 th	90 th	95 th	97 th
	(mean -1.88 SD)	(mean - 1.64 SD)	(mean - 1.28 SD)	(mean)	(mean + 1.28 SD)	(mean + 1.64 SD)	(mean +1.88 SD)
20	30.3	31.5	33.3	39.7	46.1	47.9	49.1
21	29.9	31.1	32.9	39.3	45.7	47.5	48.7
22	29.9	31.1	32.9	39.3	45.7	47.5	48.7
23	30.2	31.4	33.2	39.6	46.0	47.8	49.0
24	30.7	31.9	33.7	40.1	46.5	48.3	49.5
25	31.4	32.6	34.4	40.8	47.2	49.0	50.2
26	32.2	33.4	35.2	41.6	48.0	49.8	51.0
27	33.1	34.3	36.0	42.4	48.8	50.6	51.8
28	34.0	35.2	37.0	43.3	49.7	51.5	52.7
29	34.9	36.1	37.9	44.3	50.7	52.5	53.7
30	35.8	37.0	38.8	45.2	51.6	53.4	54.6
31	36.8	38.0	39.8	46.2	52.6	54.4	55.6
32	37.7	38.9	40.7	47.1	53.5	55.3	56.5
33	38.7	39.9	41.6	48.0	54.4	56.2	57.4
34	39.6	40.8	42.6	48.9	55.3	57.1	58.3
35	40.4	41.6	43.4	49.8	56.2	58.0	59.2
36	41.3	42.5	44.3	50.7	57.1	58.9	60.1
37	42.1	43.3	45.1	51.5	57.9	59.7	60.9
38	43.0	44.2	45.9	52.3	58.7	60.5	61.7
39	43.7	44.9	46.7	53.1	59.5	61.3	62.5
40	44.5	45.7	47.5	53.9	60.3	62.1	63.3
41	45.2	46.4	48.2	54.6	61.0	62.8	64.0

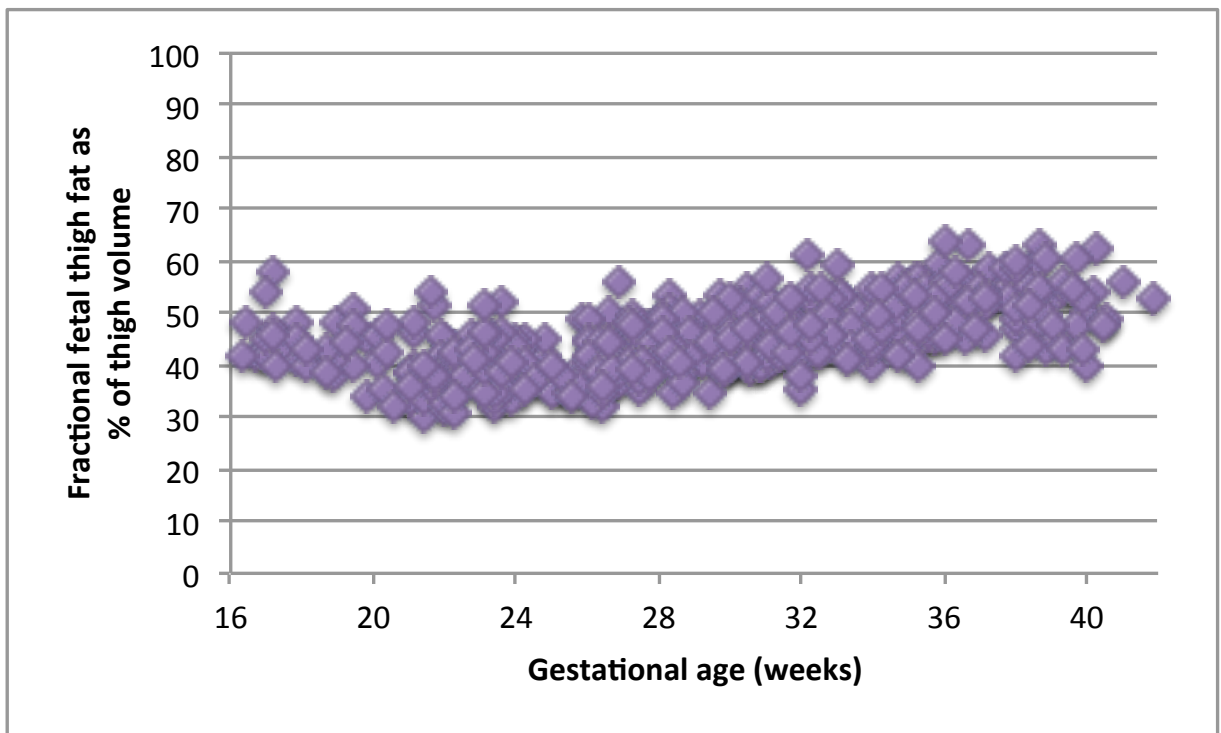


Figure 136: Fractional fetal thigh fat as percentage of thigh volume (50% fractional volume, axial) (n=504).

The percentage of thigh fat centiles were calculated from 20 weeks due to an anomalous amount of fat below this gestation, for reasons explored previously (section 5c).

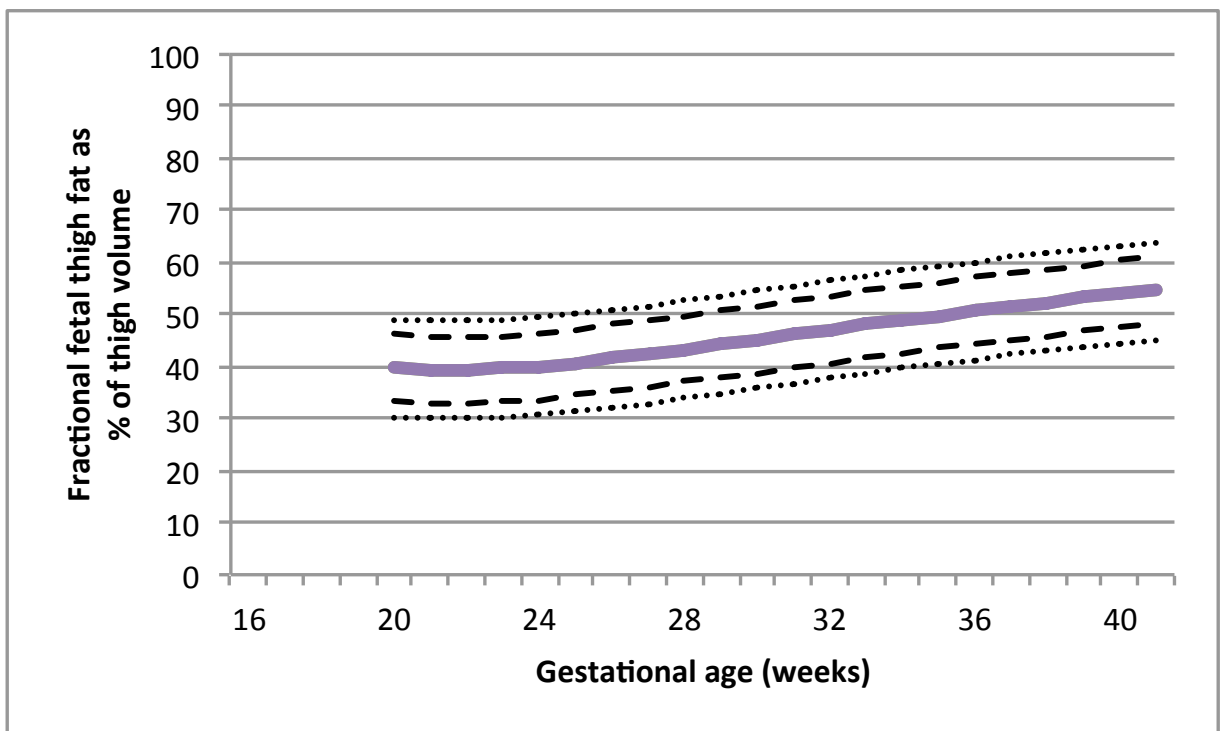


Figure 137: Fractional fetal thigh fat as percentage of thigh volume (3rd, 10th, 50th, 90th and 97th centiles).

Finally, mean and centile values were calculated for fractional thigh volume, using polynomial regression and standard deviation as before [Table 34, Figure 138]:

$$\begin{aligned} \text{Log (thigh volume) C50} &= ((GA/10)^{-2} \times -4.86213) + ((\ln(GA/10)) \times 2.965361) \\ &\quad + 0.5340931 \end{aligned}$$

$$\text{SD of log (thigh vol) C50} = ((GA/10)^3 \times 0.0010586) + 0.1289684$$

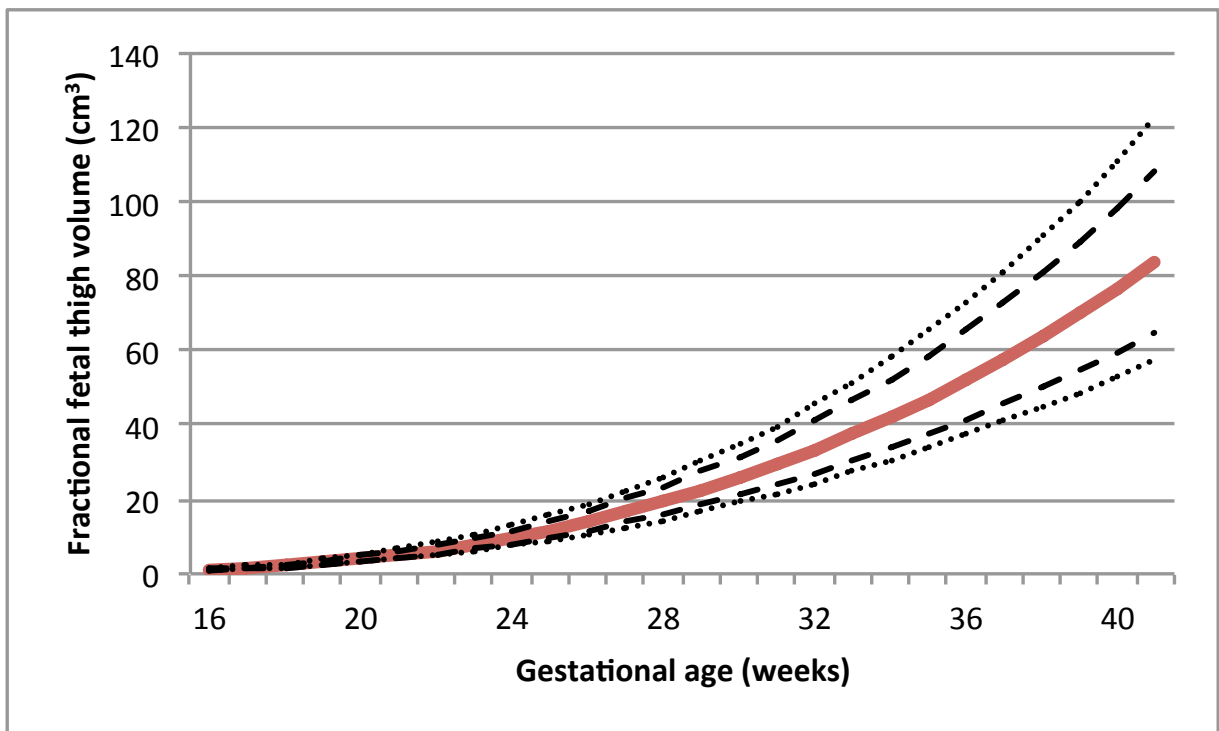


Figure 138: Fractional fetal thigh volume (3rd, 10th, 50th, 90th and 97th centiles).

Table 34: Centile values for fractional fetal thigh volume for each week of gestation.

GA (weeks)	Fractional fetal thigh volume (cm ³) centiles						
	3 rd (mean -1.88 SD)	5 th (mean - 1.64 SD)	10 th (mean - 1.28 SD)	50 th (mean)	90 th (mean + 1.28 SD)	95 th (mean + 1.64 SD)	97 th (mean +1.88 SD)
16	0.80	0.83	0.87	1.03	1.22	1.28	1.32
17	1.19	1.23	1.29	1.53	1.82	1.91	1.97
18	1.69	1.74	1.83	2.17	2.58	2.71	2.80
19	2.30	2.38	2.50	2.98	3.54	3.72	3.84
20	3.05	3.15	3.31	3.95	4.71	4.95	5.12
21	3.94	4.07	4.28	5.11	6.11	6.42	6.64
22	4.97	5.14	5.41	6.47	7.75	8.15	8.43
23	6.16	6.37	6.71	8.04	9.64	10.15	10.50
24	7.51	7.77	8.18	9.84	11.82	12.45	12.88
25	9.02	9.34	9.85	11.86	14.29	15.06	15.59
26	10.71	11.09	11.70	14.13	17.07	18.00	18.65
27	12.56	13.02	13.75	16.65	20.17	21.29	22.07
28	14.60	15.14	16.00	19.44	23.62	24.95	25.87
29	16.81	17.45	18.45	22.49	27.42	28.99	30.09
30	19.21	19.95	21.11	25.83	31.60	33.45	34.74
31	21.79	22.64	23.99	29.46	36.18	38.34	39.84
32	24.55	25.53	27.08	33.40	41.18	43.68	45.43
33	27.50	28.62	30.39	37.64	46.61	49.50	51.52
34	30.62	31.90	33.92	42.20	52.50	55.82	58.15
35	33.93	35.38	37.67	47.09	58.87	62.68	65.36
36	37.41	39.05	41.64	52.32	65.74	70.10	73.16
37	41.07	42.91	45.83	57.89	73.14	78.11	81.60
38	44.90	46.96	50.23	63.82	81.09	86.74	90.72
39	48.90	51.20	54.86	70.12	89.63	96.03	100.56
40	53.05	55.62	59.70	76.79	98.78	106.03	111.15
41	57.36	60.21	64.75	83.84	108.57	116.76	122.56

Discussion

Fetal thigh fat volumes showed a 79-fold increase from 16 to 41 weeks (50th centile: 0.53cm³ to 41.98cm³); this is more than double the 30-fold increase in fetal whole body fat suggested by chemical composition studies of fat.⁸⁸ The third trimester fetal thigh fat volumes, however, showed a 5-fold increase (8.39cm³ to 41.98cm³), similar to the 4.5-fold increase in chemical composition studies.⁸⁵ The third trimester results are similar to those seen for arm fat volumes (4.6-fold), although the increase from 16 to 41 weeks is larger for the thigh than for the arm (59-fold).

Fetal thigh lean volumes showed a similarly dramatic 62-fold increase from 16 to 41 weeks (50th centile: 0.60cm³ to 37.34cm³). The more modest third trimester results (3.4-fold increase, from 10.94cm³ to 37.34cm³) are similar to the arm lean volumes (2.9-fold); again, the 16 to 41 week increase is greater for the thigh than the arm (38-fold).

These differences between limb and whole body composition from 16 to 41 weeks might be explained by differential laying down of central and peripheral fat, and/or errors associated with the measurement of volumes at low gestational ages, as previously discussed related to the fetal arm (section 5c). It is possible that at the earlier gestations there is only a small amount of fat deposition on the thighs, but this increases during the second trimester. The increase in both compartments is, like the arm, much more aligned with other modalities in the third trimester (28 to 41 weeks).

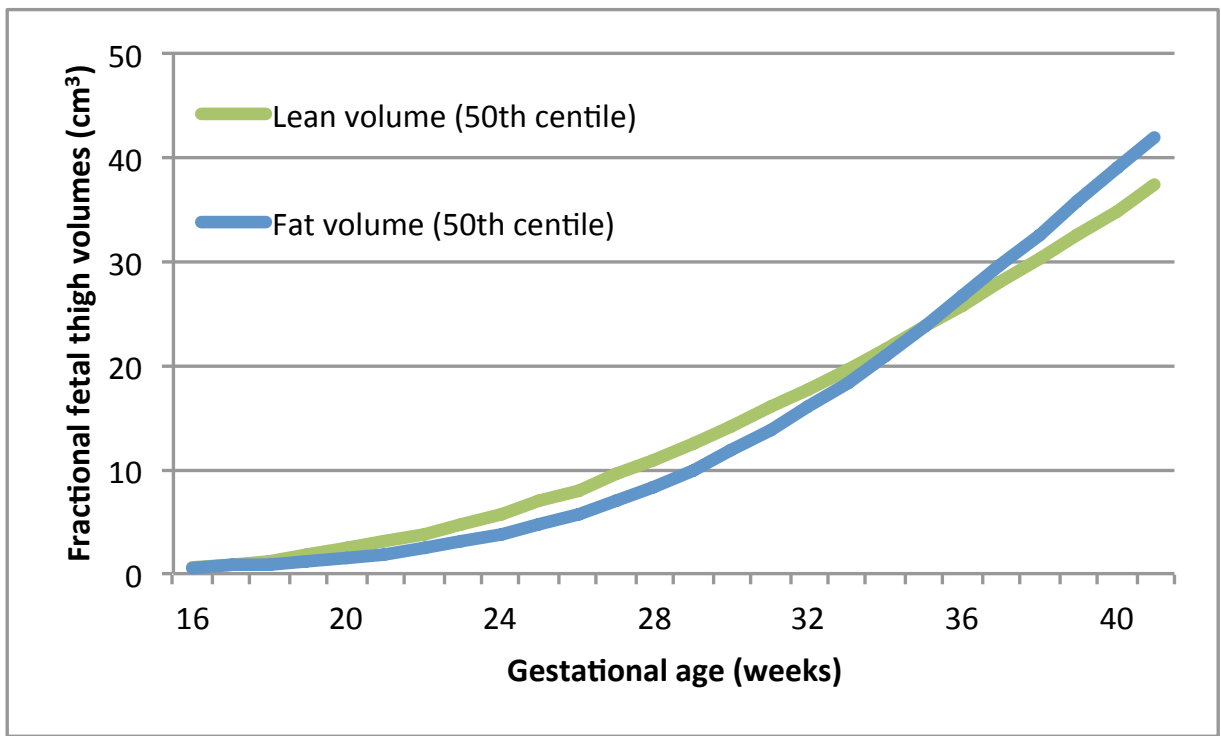
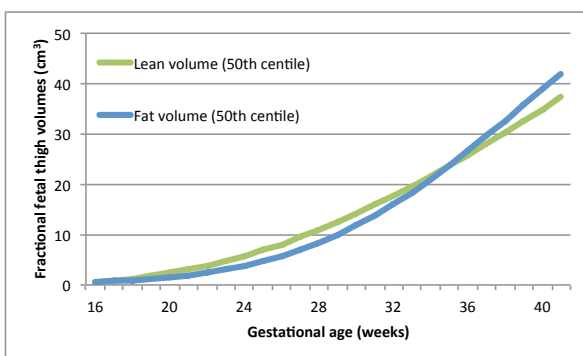
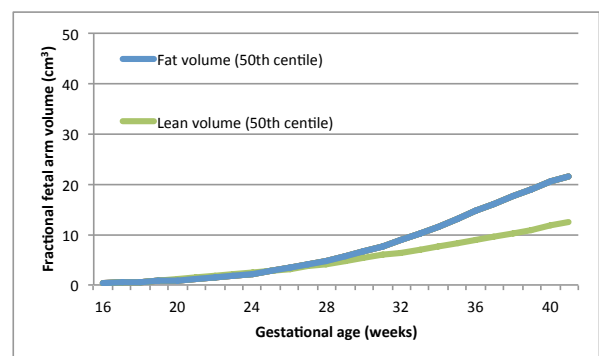


Figure 139: 50th centile lines for fractional fetal thigh fat and lean volumes.

However, the most obvious difference between these and the equivalent arm graphs [Figure 87-Figure 88] is that the relative proportions of the two thigh tissue compartments are very similar [Figure 139]. This is markedly different to the arm, which is a smaller structure in absolute size terms, but shows an increasing amount of fat compared with lean volume from 26 weeks of gestation, and almost double the amount of fat compared with lean volume at 41 weeks [Figure 140].



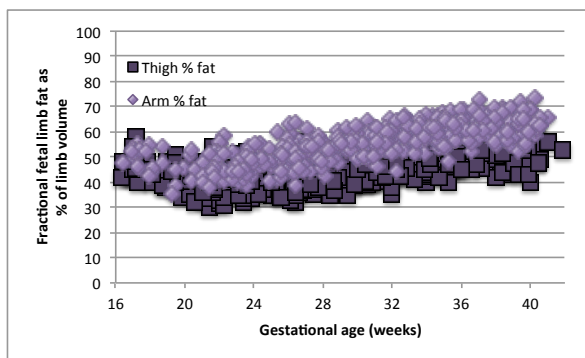
(a)



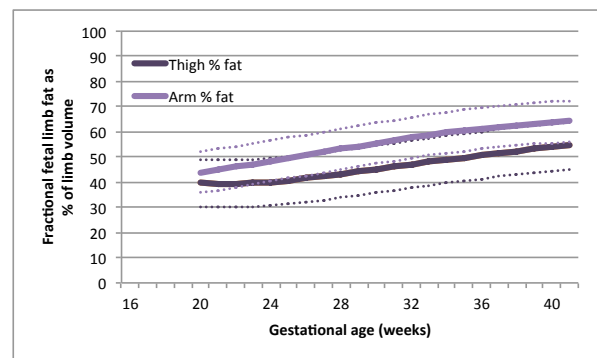
(b)

Figure 140: Graphs showing 50th centile lines for fat and lean in (a) thigh and (b) arm [same y axis scale].

Again, when compared with the fetal arm, thigh fat occupies a smaller percentage of the limb volume across all gestational ages [Figure 141a]. Whilst the rate of increase is very similar, as illustrated by the near-parallel 50th centile lines on the superimposed graph, the difference between the two lines, in absolute numbers, is very marked: the 50th centile line of the percentage arm fat is very similar to the 97th centile of the percentage thigh fat, whilst the 50th percentile line of the percentage thigh fat is very similar to the 3rd centile of the percentage arm fat [Figure 141b].



(a)



(b)

Figure 141: Comparison of fetal arm and thigh fat volumes, expressed as percentage of fractional limb volume using (a) scatterplots of raw data and (b) calculated centiles (3rd, 50th and 97th shown for each).

When the 50th centile values are compared, the thigh fat occupies an average of 9.3 percentage values less of the limb volume, with the smallest percentage difference at 20 weeks (4.2 percentage values difference: 43.9% arm vs. 39.7% thigh) and the greatest at 35 weeks (10.7 percentage values difference: 60.6% arm vs. 49.8% thigh) [Figure 141b].

This analysis, and the comparison of relative fat and lean compartments, show that the fetal arm has a greater percentage of fat compared to the fetal thigh. It is possible that differences in fat deposition would be more noticeable in the arm, making it the better choice of limb for assessment of fat and thus hopefully of fetal nutrition.

The difference in the fat proportions or percentages may be due to the arm receiving better-nourished and oxygenated blood compared with the thigh. The brachial arteries, which supply the arm, arise from the subclavian arteries, which arise from the arch of the aorta, either directly (left) or indirectly via the brachiocephalic trunk (right). The right common carotid artery also arises from the brachiocephalic trunk, whilst the left common carotid arises directly from the aortic arch, prior to the left subclavian artery. The blood from the placenta, rich in nutrients and oxygen, travels in the umbilical vein via the fetal liver (ductus venosus) and the inferior vena cava to the fetal heart. The fetal circulation prioritises the blood stream from the ductus venosus (with higher oxygenation) via the crista terminalis in the right atrium, across the foramen ovale, and up to the aortic arch. This blood has an oxygen saturation of approximately 65% [Figure 142].²⁴⁹ The fetal arm therefore receives well-nourished and well-oxygenated blood, which presumably enables not only growth but also the deposition of fat.

The fetal thigh, however, is supplied by the femoral artery, a continuation of the external iliac artery which arises from the terminal branch of the aorta. The majority of the deoxygenated blood from the inferior and superior vena cavae gets directed via the right ventricle and ductus arteriosus to the descending aorta, where it mixes with the oxygenated blood which has traversed the arch of the aorta but not been directed to the upper body. Studies on fetal circulation in sheep suggest that the oxygen saturation of the blood supplying the abdomen and lower limbs is thus 5-10% lower than that being directed to the aortic arch, brain and upper limbs.²⁴⁹ In addition, the umbilical arteries arise as a branch of each internal iliac artery – hence the thigh is being supplied by blood of a similar quality to that being transported to the placenta for waste removal and replenishment of nutrients and oxygen.

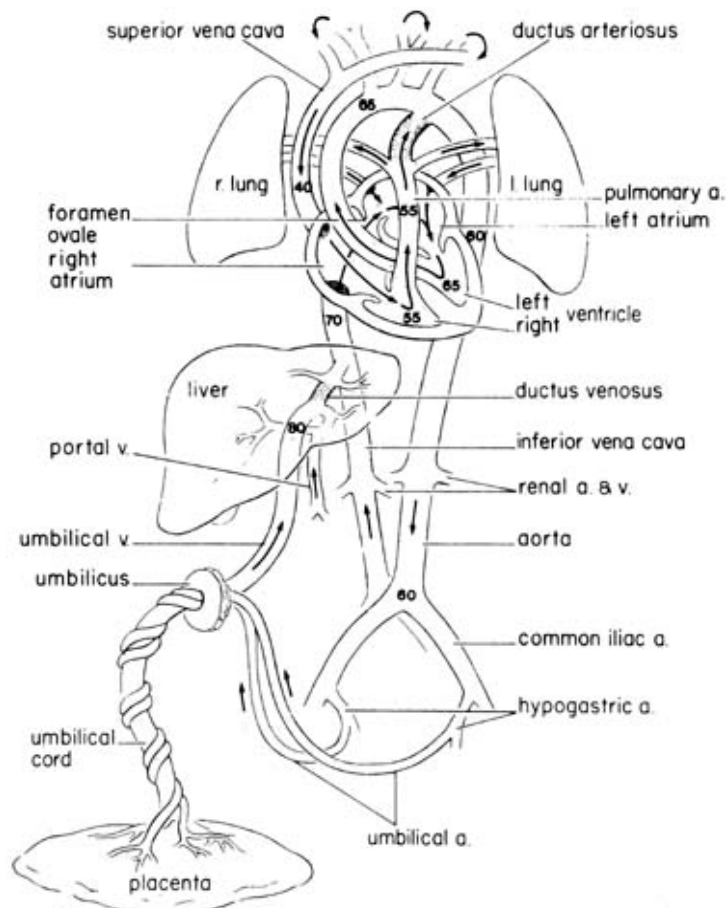
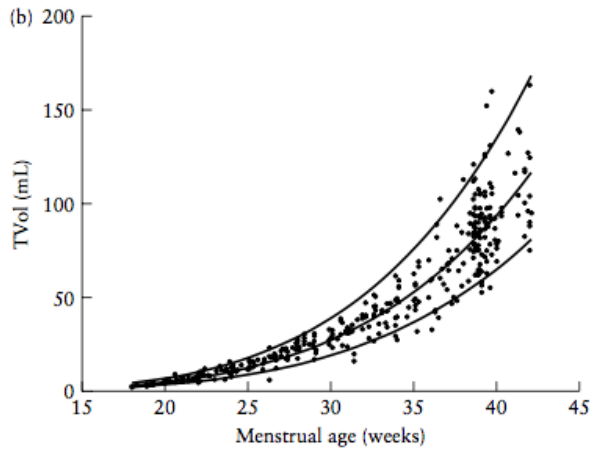


Figure 142: The fetal circulation. Numbers represent approximate values of percentage saturation of blood with oxygen *in utero* (taken from Parer 2008²⁴⁹).

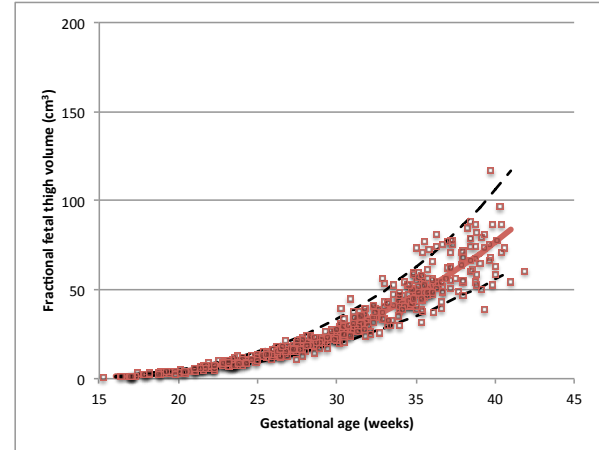
The saturation differences can further be demonstrated by fetal blood gas values in animal work. The partial pressure of oxygen (PO_2) in healthy fetuses is 28-32mmHg in the umbilical vein, 21-25mmHg in the ascending aorta, and 20-23mmHg in the descending aorta.²⁵⁰

In addition, in growth-restricted fetuses, the fetal circulation preferentially supplies the fetal brain at the expense of the abdomen/liver (the concept of asymmetric, or head-sparing growth). Such babies [Figure 7] have thin abdomens with thin, wasted limbs. The differences in limb fat between FGR and appropriately-grown fetuses might be anticipated to be seen in the arms, with a greater percentage of fat than the thighs, particularly if local mechanism(s) reduce brachial blood flow in an FGR fetus; alternatively, the thighs, supplied with poorer blood and more affected by head-sparing, may show an earlier change.

In a similar manner to the fractional arm volume (AVol) measurements, it is possible to compare my fractional thigh volume (TVol) data with the reference range of a previous study.³ The scatterplots, with 5th, 50th and 95th centiles, are shown below [Figure 143]:



(a) Scatterplot of TVol vs. menstrual age, with 5th, 50th and 95th centiles, taken from Lee et al 2009.³



(b) Scatterplot of TVol vs. gestational age, with 5th, 50th and 95th centiles, from this thesis (axes as per Lee et al).

Figure 143: Comparison of TVol scatterplots and reference ranges.

Lee's work uses 379 data points from gestational ages 18.0 to 42.1 weeks;³ mine has 487 data points for the same gestational age range (18.1 to 41.9 weeks). I took the same approach as with the arm data, namely calculating the centile in the middle of each week of gestational age (18⁺³ etc) to enable comparison [Figure 144]. The "log SD" was generated through an equation for this dataset, rather than being a constant as it had been for the arm volumes; the back-transformed values ranged from 1.1 to 1.3.

Table 2 Fitted percentiles for fractional thigh volume during second and third trimesters of pregnancy

<i>Menstrual age (weeks)</i>																	
n	5 th	10 th	25 th	50 th	75 th	90 th	95 th	1SD		GA	n	5th	10th	50th	90th	95th	log SD
18–18.9	16	2.4	2.6	2.9	3.4	3.9	4.4	4.7	1.23	18+3	6	2.0	2.1	2.5	3.0	3.1	0.14
19–19.9	12	2.9	3.2	3.6	4.1	4.7	5.4	5.8	1.23	19+3	7	2.7	2.8	3.4	4.0	4.2	0.14
20–20.9	11	3.7	4.0	4.6	5.3	6.1	6.9	7.4	1.23	20+3	6	3.5	3.7	4.4	5.3	5.6	0.14
21–21.9	17	4.5	4.9	5.6	6.4	7.4	8.4	9.1	1.23	21+3	24	4.5	4.7	5.7	6.8	7.1	0.14
22–22.9	15	5.3	5.7	6.5	7.5	8.6	9.8	10.6	1.24	22+3	19	5.7	5.9	7.1	8.5	9.0	0.14
23–23.9	12	6.9	7.4	8.4	9.7	11.2	12.8	13.8	1.24	23+3	32	7.0	7.3	8.8	10.5	11.1	0.14
24–24.9	10	7.6	8.2	9.4	10.8	12.5	14.2	15.4	1.24	24+3	16	8.4	8.9	10.7	12.8	13.5	0.14
25–25.9	14	9.3	10.1	11.5	13.3	15.3	17.5	18.9	1.24	25+3	14	10.1	10.6	12.8	15.4	16.3	0.15
26–26.9	13	11.1	12.0	13.7	15.8	18.3	20.8	22.5	1.24	26+3	35	11.9	12.6	15.2	18.4	19.4	0.15
27–27.9	12	13.0	14.0	16.0	18.5	21.3	24.3	26.3	1.24	27+3	37	13.9	14.7	17.8	21.6	22.8	0.15
28–28.9	14	14.9	16.1	18.3	21.2	24.5	27.9	30.2	1.24	28+3	26	16.1	17.0	20.7	25.2	26.6	0.15
29–29.9	11	17.2	18.6	21.2	24.5	28.4	32.3	35.0	1.24	29+3	20	18.5	19.6	23.9	29.2	30.9	0.16
30–30.9	14	20.1	21.8	24.8	28.7	33.2	37.9	41.0	1.24	30+3	28	21.1	22.3	27.3	33.5	35.5	0.16
31–31.9	13	23.1	25.0	28.6	33.1	38.3	43.7	47.2	1.24	31+3	34	23.9	25.3	31.1	38.3	40.6	0.16
32–32.9	15	26.9	29.2	33.3	38.5	44.6	50.9	55.1	1.24	32+3	20	26.8	28.5	35.1	43.5	46.1	0.17
33–33.9	14	31.0	33.5	38.3	44.3	51.4	58.7	63.5	1.24	33+3	19	30.0	31.9	39.5	49.1	52.2	0.17
34–34.9	9	33.3	36.1	41.2	47.8	55.4	63.2	68.4	1.24	34+3	34	33.4	35.5	44.2	55.2	58.7	0.17
35–35.9	13	38.0	41.2	47.0	54.5	63.2	72.2	78.2	1.25	35+3	33	36.9	39.4	49.2	61.8	65.8	0.18
36–36.9	13	43.6	47.2	54.0	62.6	72.6	83.0	89.9	1.25	36+3	21	40.7	43.4	54.6	68.9	73.5	0.18
37–37.9	15	49.1	53.1	60.8	70.5	81.8	93.5	101.3	1.25	37+3	14	44.6	47.7	60.2	76.5	81.7	0.18
38–38.9	45	56.2	61.0	69.7	80.9	93.9	107.4	116.4	1.25	38+3	21	48.8	52.2	66.3	84.7	90.7	0.19
39–39.9	49	60.6	65.7	75.1	87.2	101.3	115.9	125.6	1.25	39+3	12	53.1	56.9	72.7	93.5	100.2	0.19
40–40.9	9	66.6	72.2	82.6	95.9	111.4	127.5	138.2	1.25	40+3	7	57.6	61.8	79.5	102.9	110.5	0.20
41–41.9	13	76.7	83.2	95.2	110.7	128.6	147.2	159.6	1.25	41+3	2	62.2	67.0	86.7	113.0	121.6	0.20
42–42.9	8	80.2	87.0	99.6	115.8	134.5	154.0	167.0	1.25								

(a)

(b)

Figure 144: TVol centiles from (a) Lee et al 2009³ and (b) this thesis. (The number of observations in (b) relate to the number of scans at that completed week eg 18+3 = 18+0 to 18+6.)

The 5th, 50th and 95th centiles were then plotted to enable visual comparison [Figure 144]:

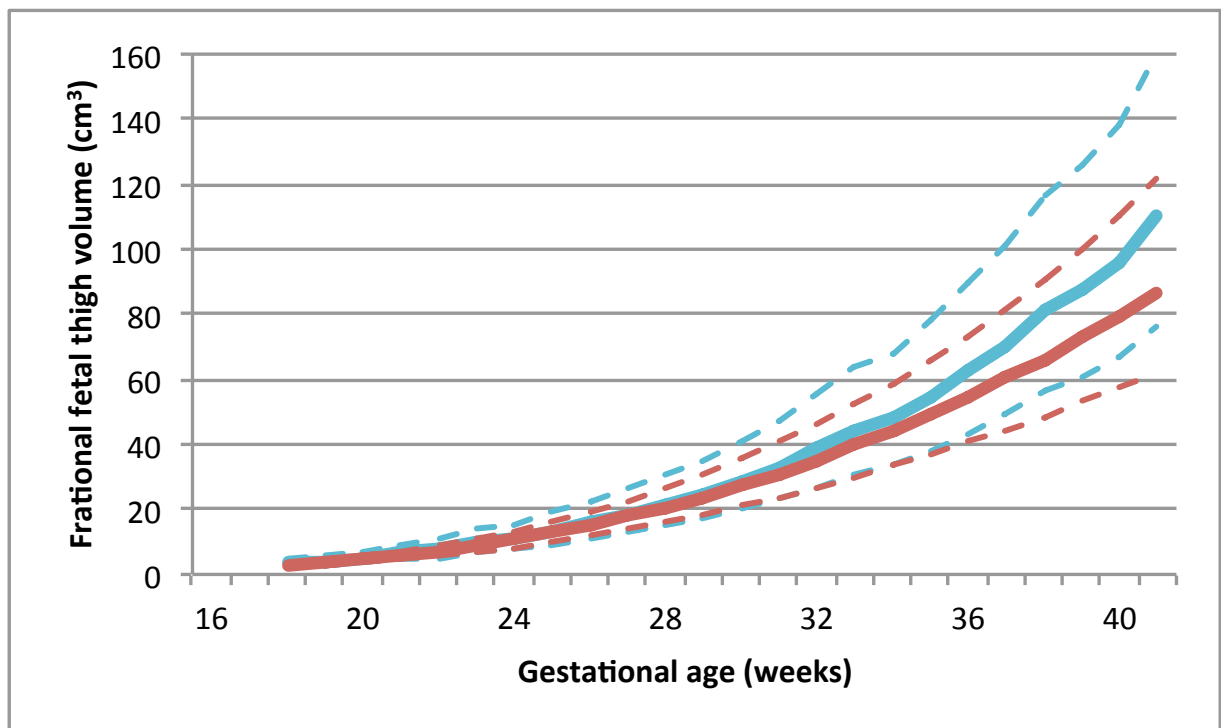


Figure 145: Comparison of TVol centiles: 50th centile solid line, 5th and 95th dashed lines (author's work in red, compared with that of Lee et al 2009³ in turquoise).

It is immediately evident, as with the AVol data, that Lee's TVol values are higher than those calculated in this thesis. The TVol 50th centile lines are comparable until 31 weeks; Lee's centile continues at a higher, and increasing, value, (32% greater at 40 weeks, 95.9 vs. 72.7cm³). The 5th centiles are similar until 35 weeks, after which Lee's increases (16% greater at 40 weeks (66.6 vs. 57.6cm³). Lee's 95th centile is higher than mine throughout the gestational age range, with a 25% difference at 40 weeks (138.2 vs. 110.5cm³).

The centile lines generally show a similar pattern to that demonstrated by the AVol centiles [Figure 96], although the difference between the 50th centile values at 40 weeks is greater for the thigh (32%) than for the arm (19%). The 5th and 95th centile differences between arm and thigh are almost identical (13% and 16%; 25% and 25%).

A direct comparison of the raw thesis data, by completed weeks of gestation, with Lee's 50th centile shows notable increases in the difference between the two at 29, 32-33, and 36-37 weeks with a much larger increase from 38-41 weeks. This accelerated difference in the last 4 weeks would explain the larger difference between the 50th centiles of AVol (19%) and TVol (32%).

The same possible methodological and population factors, as explored in section 5c for AVol, are also valid in trying to explain the differences which are seen in TVol.

Table 35: Comparison of thesis TVol raw data (mean) with fitted centiles (Lee 2009³).

GA (weeks)	Lee n	TVol 50th centile (cm3)	Knight n	TVol mean (cm3)	Difference (Lee- Knight)
18	16	3.4	6	2.5	0.5
19	12	4.1	7	3.4	0.7
20	11	5.3	6	4.4	1.5
21	17	6.4	24	5.7	0.4
22	15	7.5	19	7.1	0.4
23	12	9.7	32	8.8	0.8
24	10	10.8	16	10.7	0.5
25	14	13.3	14	12.8	0.2
26	13	15.8	35	15.2	0.2
27	12	18.5	37	17.8	0.3
28	14	21.2	26	20.7	0.9
29	11	24.5	20	23.9	1.4
30	14	28.7	28	27.3	1.1
31	13	33.1	34	31.1	1.5
32	15	38.5	20	35.1	3.2
33	14	44.3	19	39.5	4.1
34	9	47.8	34	44.2	2.2
35	13	54.5	33	49.2	2.2
36	13	62.6	21	54.6	7.8
37	15	70.5	14	60.2	5.4
38	45	80.9	21	66.3	12.8
39	49	87.2	12	72.7	16.1
40	9	95.9	7	79.5	24.1
41	13	110.7	2	86.7	24.0

Conclusion

The novel measurements of fractional fetal thigh fat and lean volumes are presented, with reference ranges from 16 to 41 weeks of gestation. This study also adds to the body of work on fractional thigh volumes. Reference centiles for fractional thigh fat volume expressed as a percentage of fractional thigh volume are presented; this is also a novel measurement. The thigh has a lower percentage of fat than the arm, and does not show the same increase in fat in the late third trimester, so the arm may be better for fetal nutritional assessment.

ii. Comparing axial and sagittal 3D thigh fat volumes

Aim

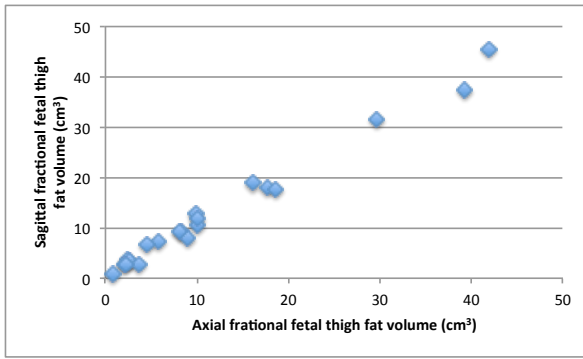
To compare fractional thigh volumes acquired with an axial (previously used in this thesis) and a sagittal (standard INTERGROWTH-21st) plane from the same fetuses.

Method

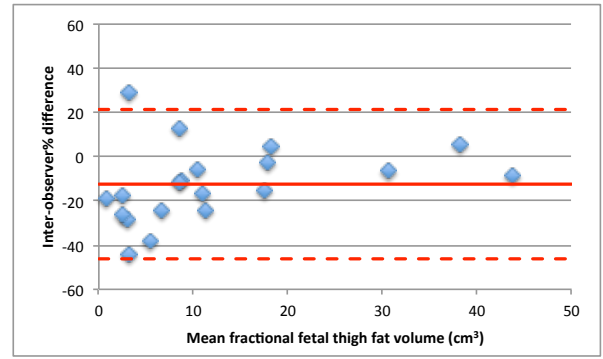
A random number generator (www.random.org) was used to select 20 fetuses from those whose axial thigh volumes were originally segmented by the author. The sagittally-acquired thigh volumes from these fetuses were then segmented using the MATLAB tool and the results compared using Bland-Altman plots and correlation coefficients.

Results

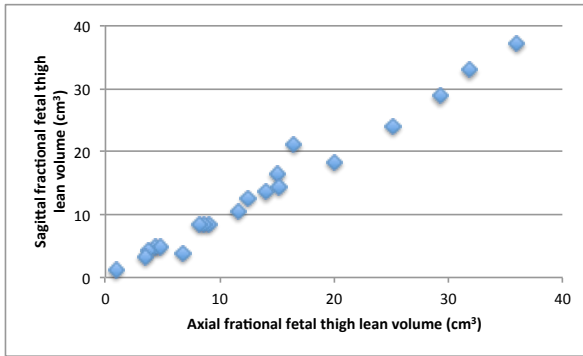
The 20 fetuses were randomly selected and showed a representative sampling across a range of gestational ages, from 17⁺¹ to 40⁺⁰ weeks. The scatterplots and Bland-Altman plots are shown below [Figure 146], together with the correlation, mean percentage difference, and limits of agreement [Table 36].



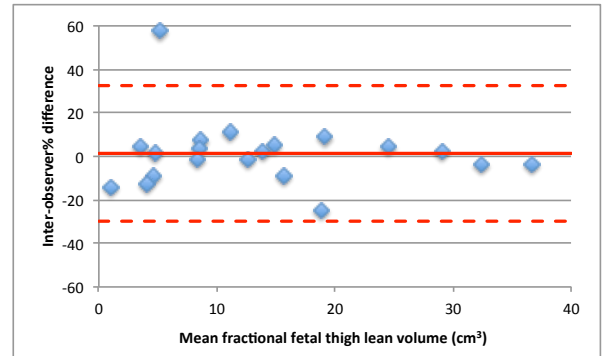
(a) thigh fat.



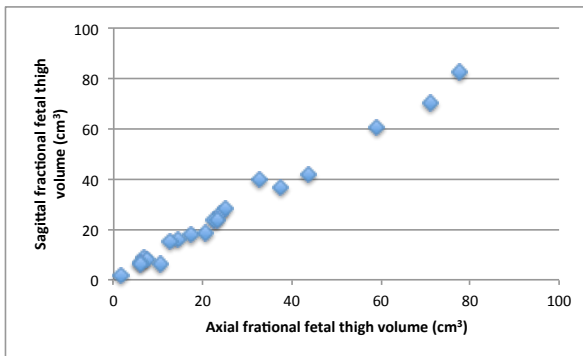
(b) thigh fat.



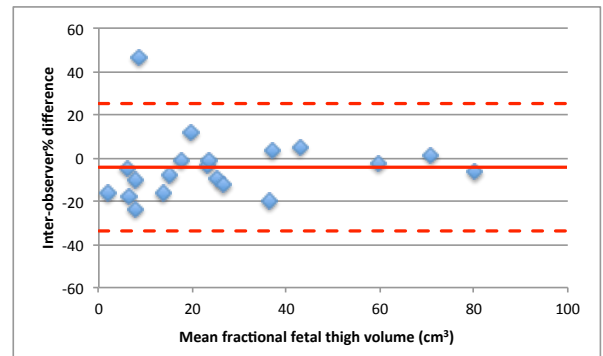
(c) thigh lean.



(d) thigh lean.



(e) thigh.



(f) thigh.

Figure 146: Scatterplots showing fractional measurements from axially- and sagittally-acquired fetal thigh volumes, and comparisons between the volumes using Bland-Altman plots for fat [(a) and (b)], lean [(c) and (d)] and thigh [(e) and (f)] (n=20).

The fractional thigh volumes showed good correlations of 0.990-0.994, and the inter-observer limits of agreement were similar to those of axial arm volumes (section 5c). The mean differences in thigh and lean volume were also similar to those of the fetal arm; there was, however a larger difference in the fat volumes, with the measurements of the axially-acquired volumes being larger than the sagittally-acquired volumes (12.4% difference). This may partly reflect the challenges of measuring limb fat, the error generated by the calculation from two different volume measurements (thigh minus lean), and possibly also some variation due to the sagittally-acquired volumes having poorer resolution (although this does not seem to have affected the other measurements unduly).

Table 36: Correlation and inter-observer differences for axial and sagittal fetal thigh volume measurements.

Volume	Correlation (<i>r</i>)	Mean difference	Limits of agreement
Thigh fat	0.993	-12.4%	+/-33.8%
Thigh lean	0.990	1.4%	+/-31.2%
Thigh	0.994	-4.3%	+/-29.5%

The 2D areas extracted from the central slice of the 3D volumes were also compared, and showed strong correlations of 0.983 (fat area), 0.983 (lean area), and 0.990 (thigh area).

Conclusion

The different volume acquisition methods produced comparable results. This is important as it enables sagittally acquired volumes from the standard INTERGROWTH-21st protocol to be assessed for case-control studies such as SGA vs. AGA (section 7h).

6c. Longitudinal scans (3D) in individual fetuses

Aim

To assess the development of fat in individual fetuses by measuring thigh fat volume.

Method

A similar method was used to that for longitudinal assessment of fetal arm fat volume. The same cohort was used as in section 6b, but only fetuses which had been scanned four, five or six times, and who had then undergone body composition analysis with the PEA POD, were included in this analysis. Of these, those who had had their first scan before 26 weeks gestation, and their last scan at 36 weeks or later, were included in the analysis (the original <25 to $\geq$ 37 week range, as used for arm fat, would have only included 14 fetuses, so was expanded). Individual fat volume curves were plotted and assessed visually. The fetuses were divided into those with a continuous upward trajectory, and those with an apparent slowing of the rate of fat volume gain at the end of the third trimester. Birthweight, gender, and whole body fat percentage data were then collated, and the groups compared.

Results

28 fetuses had at least four scans and had also undergone newborn PEA POD body composition analysis. Of these, 19 met the gestational age criteria. The curves were plotted: 6 showed a slowing of rate of fat volume accrual at the last (late third trimester) scan [Figure 148], whilst the remaining 13 showed a continuous upward trend [Figure 147].

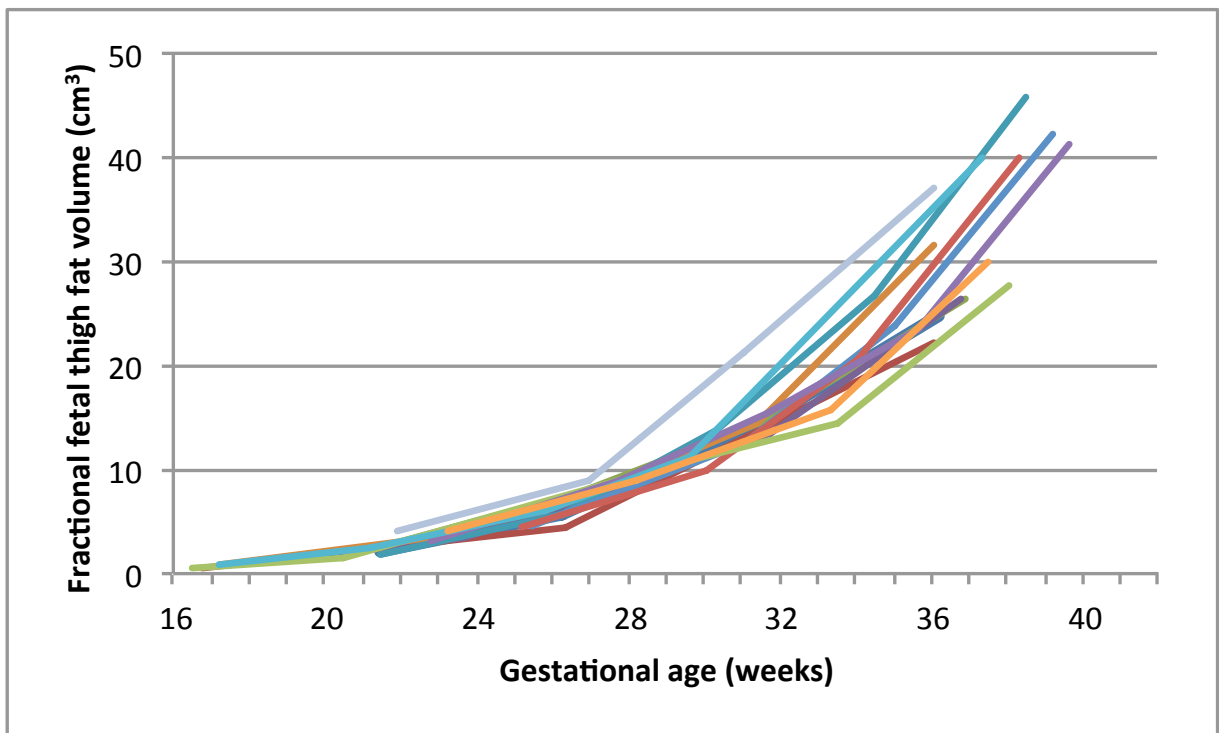


Figure 147: Longitudinal fractional fetal thigh fat volumes in individual fetuses, demonstrating continuous upward trend.

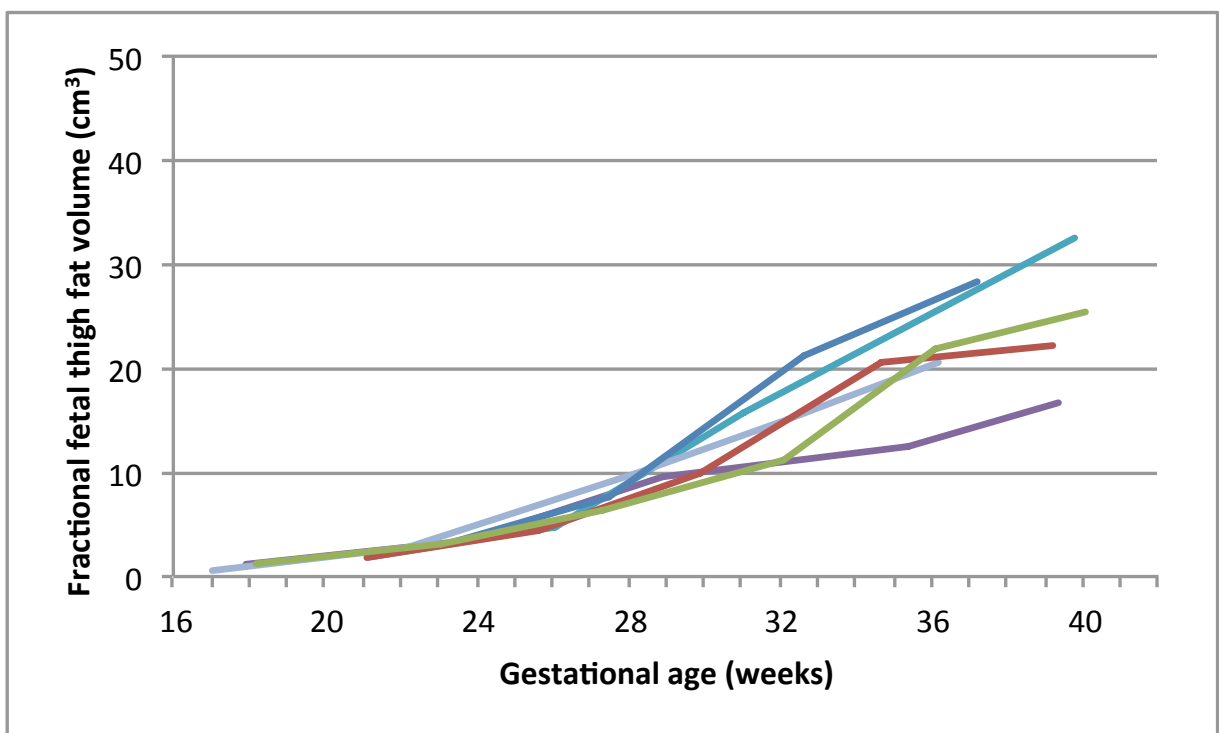


Figure 148: Longitudinal fractional fetal thigh fat volumes in individual fetuses, demonstrating slowing of rate of fat volume accrual in the late third trimester.

The gestational age at birth, birthweight (and centile, using RCPCH/WHO year 0-4 data²²⁰), percentage body fat using neonatal PEA POD body composition analysis, and body fat percentile were recorded for each of these infants [Table 37].

Table 37: Neonatal birthweight and body fat, for fetuses showing different patterns of antenatal thigh fat volume growth.

	ID	GA at birth	Sex	Birthweight	Weight centile ²²⁰	PEA POD %body fat	PEA POD centile ²²¹
Normal thigh fat growth 3 rd trimester (n=13)	6458	39 ⁺⁶	f	3736g	69 th	15.6%	75-90 th
	6506	40 ⁺⁶	m	4178g	84 th	17.3%	95-97.5 th
	6546	40 ⁺⁰	f	3183g	27 th	11.2%	25-50 th
	6558	40 ⁺³	f	2810g	12 th	7.7%	10-25 th
	6580	40 ⁺⁶	f	3635g	72 nd	17.1%	75-90 th
	6582	41 ⁺¹	m	3262g	32 nd	10.0%	50-75 th
	6615	42 ⁺¹	f	4354g	95 th	16.0%	75-90 th
	6616	40 ⁺⁰	f	3219g	29 th	10.9%	25-50 th
	6626	38 ⁺³	m	3507g	51 st	13.5%	75-90 th
	6638	40 ⁺⁵	f	3219g	29 th	9.5%	25-50 th
	6654	39 ⁺⁰	m	3600g	46 th	12.9%	75-90 th
	6668	41 ⁺⁰	m	3266g	22 nd	8.9%	25-50 th
	6676	40 ⁺¹	m	3374g	29 th	5.4%	10-25 th
	<i>Mean</i>	<i>40⁺³</i>	<i>---</i>	<i>3488g</i>	<i>46th</i>	<i>12.0%</i>	<i>50-75th (median)</i>
Slow thigh fat growth 3 rd trimester (n=6)	6554	39 ⁺³	f	2760g	6 th	5.7%	5-10 th
	6589	41 ⁺⁶	m	3407g	43 rd	9.7%	25-50 th
	6606	42 ⁺¹	m	3880g	78 th	7.0%	25-50 th
	6636	39 ⁺¹	f	3070g	19 th	6.8%	10-25 th
	6644	40 ⁺³	f	3045g	18 th	7.5%	10-25 th
	6686	40 ⁺¹	f	3458g	48 th	14.8%	50-75 th
	<i>Mean</i>	<i>40⁺⁴</i>	<i>---</i>	<i>3270g</i>	<i>35th</i>	<i>8.6%</i>	<i>25th (median)</i>

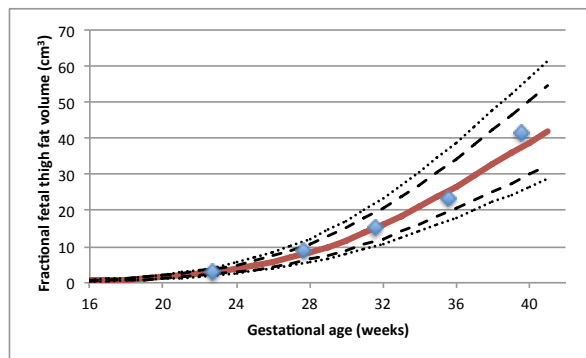
These results showed, similar to the arm volume results in section 5d, a variety of patterns. Of the 13 fetuses with an upwards fat trajectory graphs: 6 showed synchronous results (same, or within 3 centiles of the same, centile bracket eg birthweight 72nd, PEA POD 75-90th); 5 showed a higher PEA POD than birthweight centile; but 2 showed a higher birthweight than PEA POD centile. The hypothesis was that these would show the first two categories of growth (the majority, 11/13, are), but not the last. Meanwhile, for the fetuses with slow fat trajectory graphs: 5 showed synchronous or near-synchronous results, and 1 showed a birthweight centile higher than PEA POD centile (the latter being as anticipated).

The mean and median results may be more in line with predictions than those for the arm: the mean gestational ages are almost identical, but the upwards trajectory group had a higher mean birthweight and birthweight centile, with a higher percentage of fat and higher median neonatal whole body fat centile when compared with the slower trajectory group.

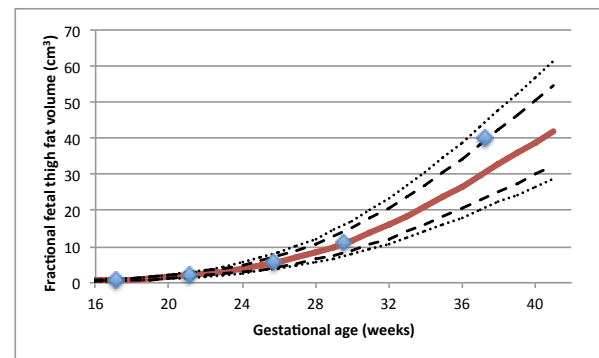
Comparison of birthweight and PEA POD centiles also provides preliminary evidence of the latter's additional information on neonatal growth: the upwards trajectory group had a mean birthweight on the 46th centile, with a median PEA POD neonatal whole body fat centile group of 50-75th i.e. 2 to 27 centiles above that which might have been predicted by the birthweight. Meanwhile, the slow trajectory group had a mean birthweight on the 35th centile, but the median PEA POD neonatal whole body fat centile group in this case was 25th i.e. 10 centiles below that which might have been predicted by the birthweight.

The inconsistencies in these data are partly due to the small numbers involved, which make it hard to identify clear patterns but again, may suggest that thigh (or arm) fat alone may not be a reliable marker of whole body fat. The correlation will be explored further in Chapter 7.

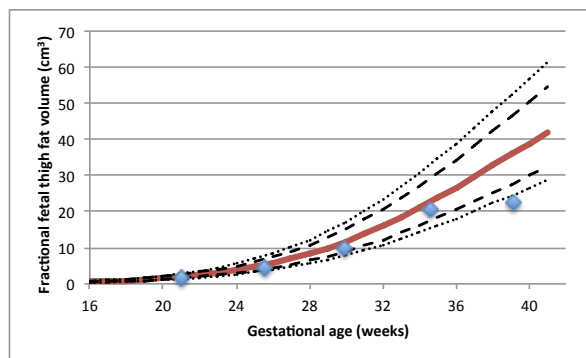
It was possible to identify different patterns of growth when assessing the individual fetuses. These included appropriate growth (following 50th centile), accelerated growth (crossing increasing centiles), late-onset FGR (static growth, falling below 3rd centile), and failure to meet growth potential (falling centiles, remaining within 10th-90th range) [Figure 149].



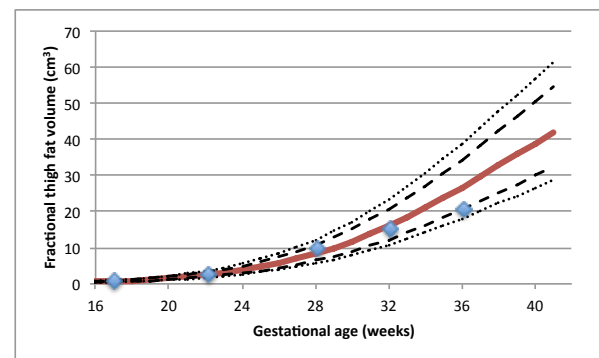
(a) appropriate growth (50th centile).



(b) accelerated growth (50th to >90th centile).



(c) late-onset FGR (<3rd centile, 39 weeks).



(d) failure to meet growth potential, within 10-90th centile.

Figure 149: Patterns of fractional fetal thigh fat volume graphs in individual fetuses.

Conclusion

This section demonstrates longitudinal changes in thigh fat volume in individual fetuses, including different patterns of growth across the second and third trimesters. However the differing shape of the graphs does not, similar to the arm volume results, seem to correspond well with variations in neonatal whole body fat. This may be due to the small numbers involved, or due to whole body fat not being accurately reflected by limb fat.

6d. 2D fat, lean and thigh areas: reference ranges (extracted from 3D volumes)

Aim

To describe the normal development of fetal thigh fat and lean tissues, using optimal 2D images extracted from 3D volumes.

Background

This work is similar to that on the arm volumes in section 5e, using optimal slices acquired from 3D volumes.

Methods

This data was acquired from the same group of INTERGROWTH-21st women as those assessed in section 6b, after correction for FL. The volumes were segmented in the 5-slice fractional limb volume as described in section 6b and the central slice extracted to provide an optimal 2D image. Fractional polynomials were used to produce centiles, as before.

Results

The data were analysed from 504 scans from 221 women (as per section 6b). Fractional fetal arm, fat and lean volumes were plotted against gestational age [Figure 150 - Figure 153].

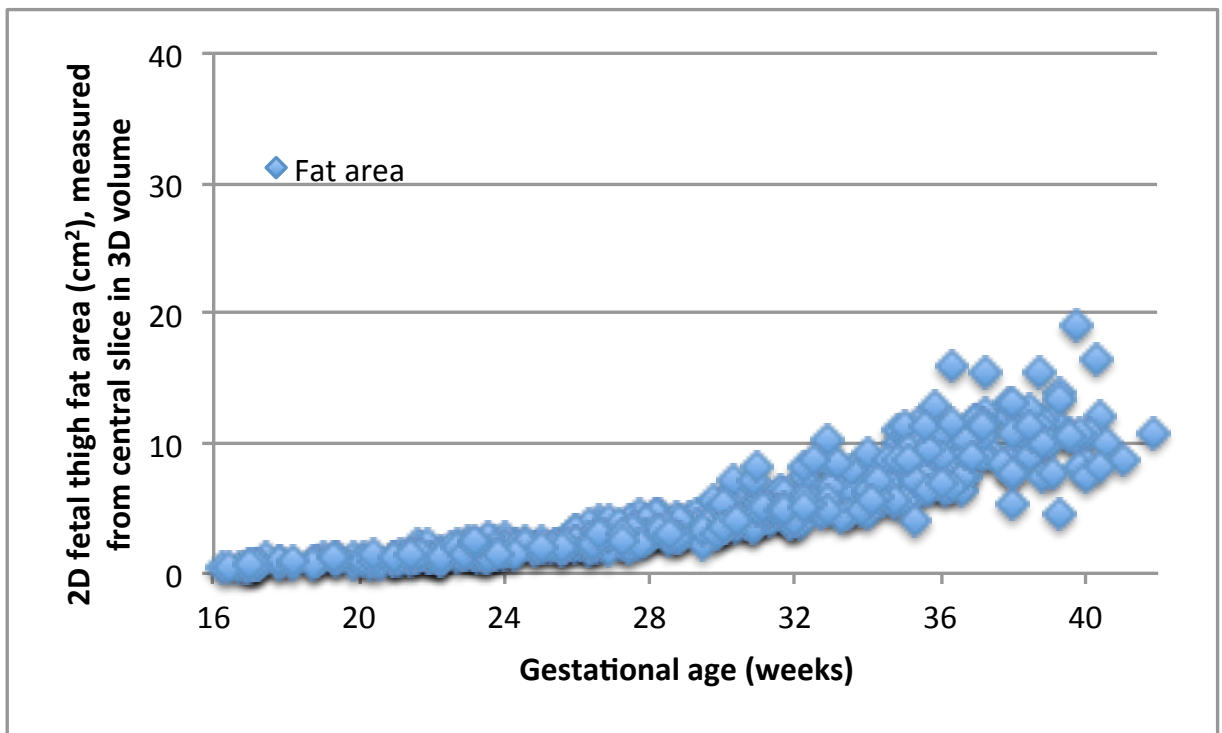


Figure 150: 2D fetal thigh fat area, measured from central slice in 3D volume (n=504).

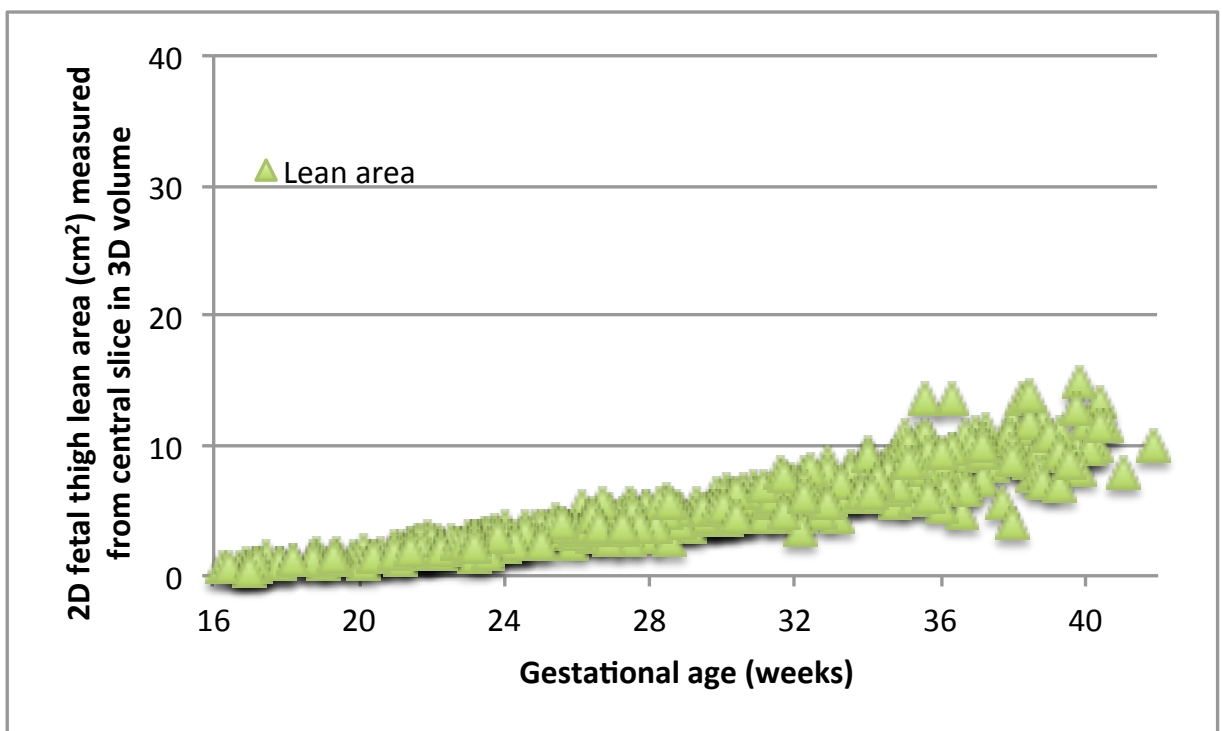


Figure 151: 2D fetal thigh lean area, measured from central slice in 3D volume (n=504).

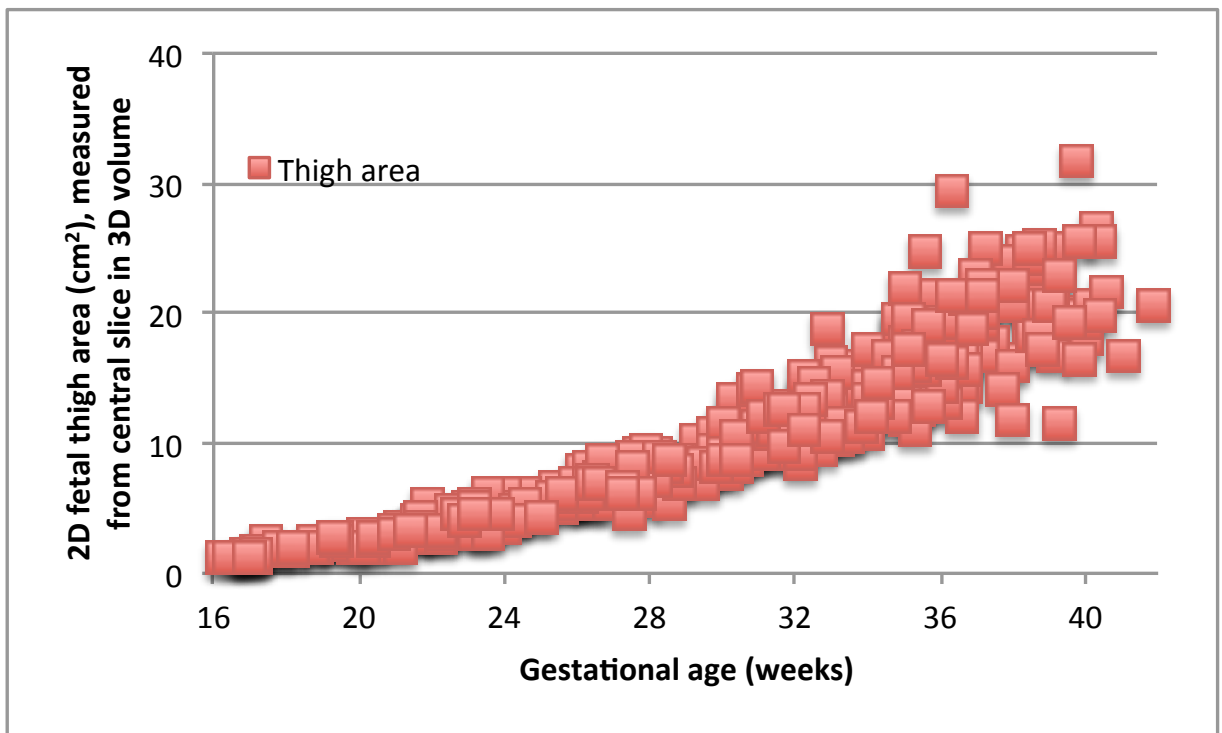


Figure 152: 2D fetal thigh area, measured from central slice in 3D volume (n=504).

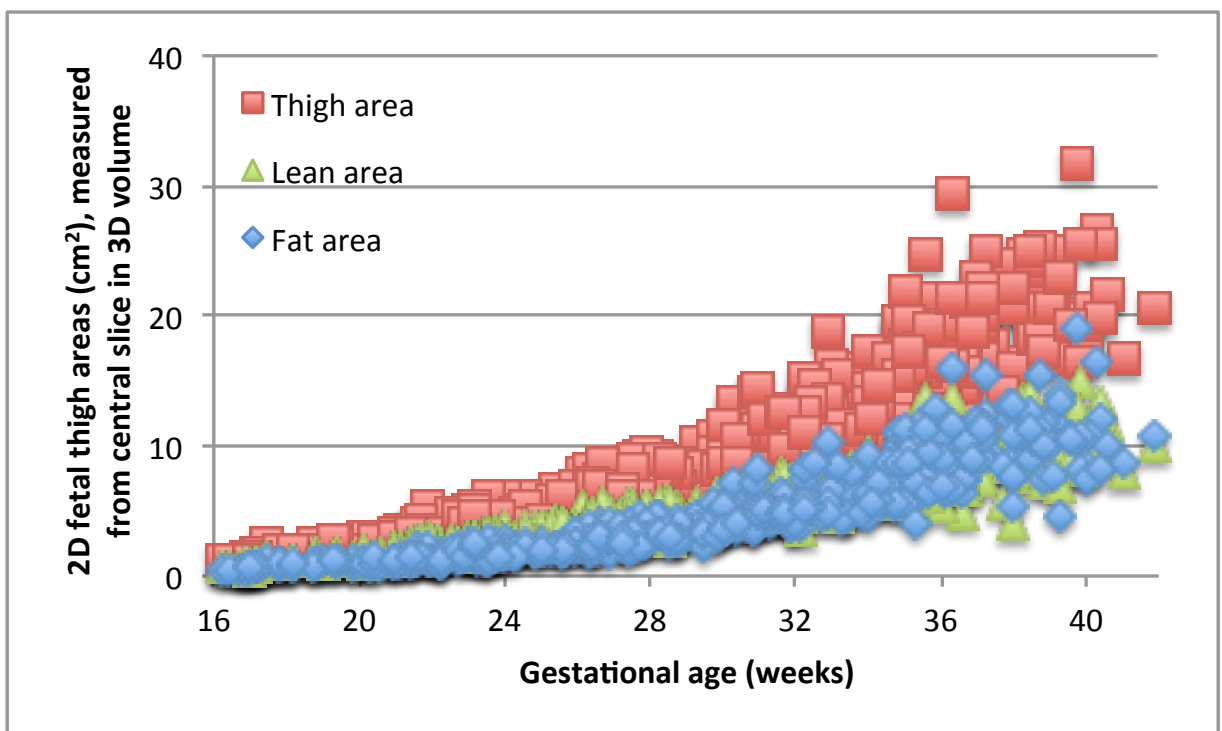


Figure 153: 2D fetal thigh, fat and lean areas superimposed, measured from central slice in 3D volume (n=504).

Fractional polynomial regression^{206,207} was used to generate equations to calculate the mean / 50th centile (C50) values for fat area and lean area for a given gestational age (GA):

$$\begin{aligned} \text{Log (thigh fat area) C50} &= ((\text{GA}/10)^2 \times 0.9139514) + \\ &((\text{GA}/10)^2 \times (\ln(\text{GA}/10))) \times -0.4418934 + 2.199917 \end{aligned}$$

$$\text{Log (thigh lean area) C50} = ((\text{GA}/10)^{-1} \times -7.591939) + 8.824307$$

The relationship with standard deviation (SD) was also generated using the same approach.

$$\text{SD of log (thigh fat area) C50} = 0.21$$

$$\text{SD of log (thigh lean area) C50} = 0.16$$

This enabled calculation of the centiles for both sets of data:

$$3^{\text{rd}} / 97^{\text{th}} \text{ centiles} = \text{mean} \pm 1.88 \text{ SD}$$

$$5^{\text{th}} / 95^{\text{th}} \text{ centiles} = \text{mean} \pm 1.64 \text{ SD}$$

$$10^{\text{th}} / 90^{\text{th}} \text{ centiles} = \text{mean} \pm 1.28 \text{ SD}$$

For the thigh fat and lean areas, SD was used to calculate the log-transformed centiles, and the results then transformed back to mm², and divided by 100 to generate results in cm² [Table 38, Table 39].

Table 38: Centile values for thigh fat areas for each week of gestation, from central slice in 3D volume.

GA (weeks)	Fetal thigh fat area (cm ²) centiles (from 3D)						
	3 rd (mean -1.88 SD)	5 th (mean - 1.64 SD)	10 th (mean - 1.28 SD)	50 th (mean)	90 th (mean + 1.28 SD)	95 th (mean + 1.64 SD)	97 th (mean +1.88 SD)
16	0.37	0.35	0.42	0.55	0.72	0.86	0.82
17	0.43	0.41	0.49	0.64	0.84	1.00	0.96
18	0.51	0.48	0.57	0.75	0.98	1.17	1.12
19	0.59	0.56	0.67	0.88	1.15	1.37	1.31
20	0.69	0.66	0.78	1.03	1.34	1.60	1.53
21	0.80	0.77	0.91	1.20	1.57	1.86	1.78
22	0.94	0.89	1.06	1.39	1.83	2.17	2.07
23	1.09	1.04	1.24	1.62	2.12	2.52	2.41
24	1.26	1.21	1.43	1.88	2.46	2.93	2.79
25	1.46	1.40	1.66	2.17	2.85	3.39	3.23
26	1.69	1.61	1.91	2.51	3.28	3.90	3.73
27	1.94	1.85	2.20	2.88	3.77	4.49	4.28
28	2.22	2.12	2.52	3.30	4.32	5.14	4.90
29	2.53	2.41	2.87	3.76	4.93	5.86	5.59
30	2.87	2.74	3.26	4.27	5.59	6.65	6.35
31	3.24	3.10	3.68	4.82	6.32	7.51	7.17
32	3.65	3.48	4.14	5.42	7.10	8.45	8.06
33	4.08	3.89	4.63	6.06	7.94	9.44	9.02
34	4.53	4.33	5.15	6.74	8.84	10.50	10.03
35	5.01	4.79	5.69	7.46	9.77	11.62	11.09
36	5.51	5.26	6.26	8.20	10.74	12.77	12.19
37	6.02	5.75	6.83	8.95	11.73	13.95	13.32
38	6.53	6.24	7.41	9.71	12.73	15.13	14.45
39	7.04	6.72	7.99	10.47	13.72	16.31	15.57
40	7.53	7.19	8.55	11.21	14.68	17.46	16.67
41	8.01	7.64	9.09	11.91	15.60	18.55	17.71

Table 39: Centile values for thigh lean areas for each week of gestation, from central slice in 3D volume.

GA (weeks)	Fetal thigh lean area (cm ²) centiles (from 3D)						
	3 rd (mean -1.88 SD)	5 th (mean - 1.64 SD)	10 th (mean - 1.28 SD)	50 th (mean)	90 th (mean + 1.28 SD)	95 th (mean + 1.64 SD)	97 th (mean +1.88 SD)
16	0.43	0.45	0.48	0.59	0.73	0.77	0.81
17	0.57	0.60	0.63	0.78	0.96	1.02	1.07
18	0.73	0.76	0.81	1.00	1.24	1.31	1.37
19	0.92	0.95	1.01	1.25	1.54	1.64	1.70
20	1.12	1.17	1.24	1.53	1.89	2.00	2.08
21	1.34	1.40	1.48	1.83	2.26	2.40	2.49
22	1.58	1.65	1.75	2.16	2.66	2.83	2.94
23	1.84	1.91	2.03	2.51	3.09	3.28	3.42
24	2.11	2.19	2.33	2.87	3.55	3.77	3.92
25	2.39	2.49	2.64	3.26	4.03	4.28	4.45
26	2.69	2.80	2.97	3.67	4.53	4.80	5.00
27	3.00	3.12	3.31	4.08	5.04	5.35	5.57
28	3.31	3.45	3.66	4.52	5.58	5.92	6.16
29	3.64	3.78	4.02	4.96	6.12	6.50	6.76
30	3.97	4.13	4.38	5.41	6.68	7.09	7.38
31	4.31	4.48	4.75	5.87	7.25	7.69	8.01
32	4.65	4.84	5.13	6.34	7.83	8.31	8.64
33	5.00	5.20	5.52	6.81	8.41	8.93	9.29
34	5.35	5.56	5.90	7.29	9.00	9.55	9.94
35	5.70	5.93	6.29	7.77	9.59	10.18	10.59
36	6.05	6.30	6.68	8.25	10.19	10.81	11.25
37	6.41	6.66	7.07	8.73	10.79	11.45	11.91
38	6.76	7.03	7.46	9.22	11.39	12.08	12.57
39	7.12	7.40	7.86	9.70	11.98	12.72	13.23
40	7.47	7.77	8.25	10.19	12.58	13.35	13.89
41	7.83	8.14	8.64	10.67	13.18	13.98	14.55

The tables above are shown as graphs for fat [Figure 154] and lean areas [Figure 155] (50th centile coloured line; 10th / 90th centiles dashed lines; 3rd / 97th centiles dotted lines):

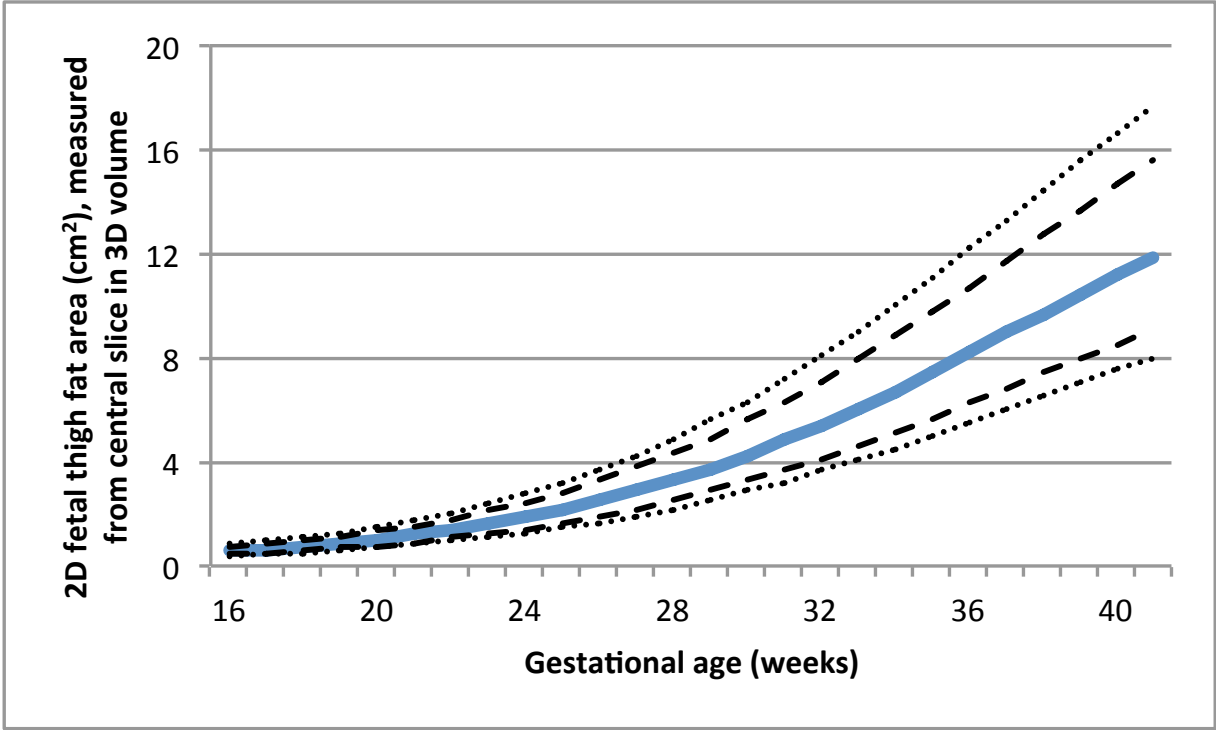


Figure 154: Fetal thigh fat area, from central slice in 3D volume (3rd, 10th, 50th, 90th and 97th centiles).

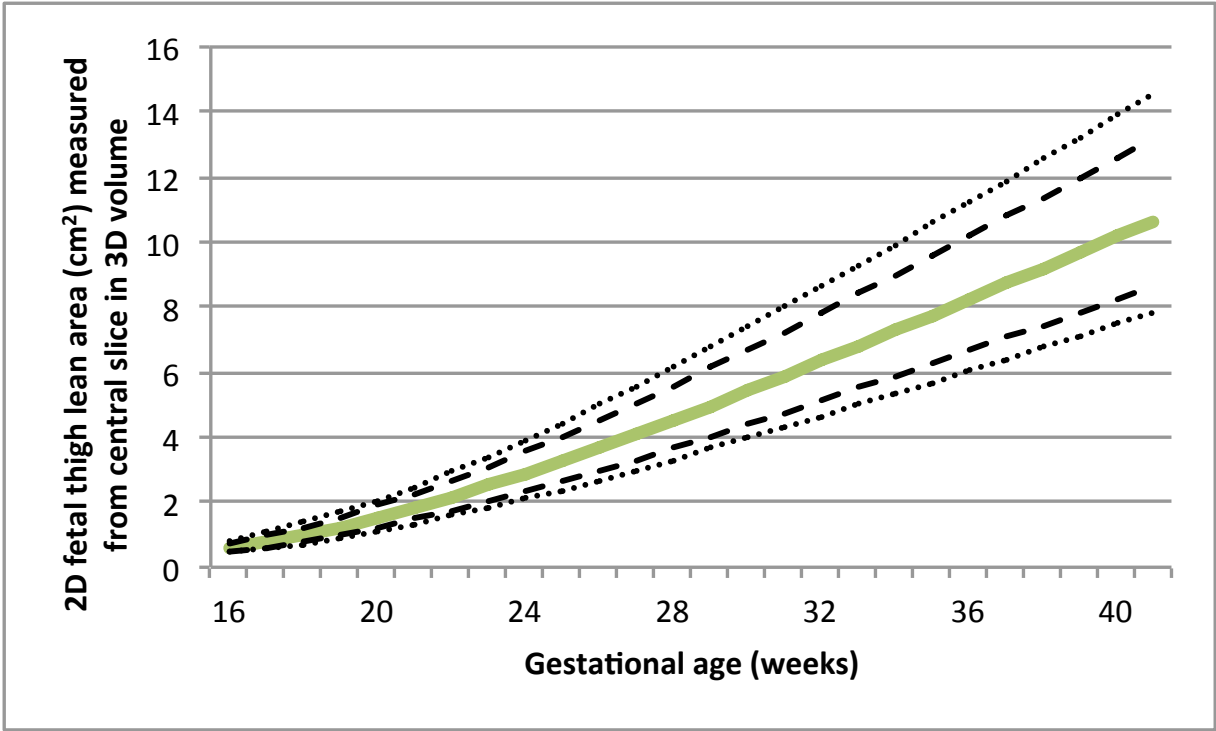


Figure 155: Fetal thigh lean area, from central slice in 3D volume (3rd, 10th, 50th, 90th and 97th centiles).

Thigh fat was also assessed in the form of the percentage of the whole thigh area it occupied, with mean, SD and centiles calculated using fractional polynomial regression:

$$\text{Fat area as \% thigh area C50} = (\text{GA} \times 0.7604302) + 21.83206$$

$$\text{SD of fat area as \% thigh area C50} = 5.63$$

Table 40: Centile values for 2D fetal thigh fat area as a percentage of central slice thigh area in 3D volume, for each week of gestation.

GA (weeks)	Fetal thigh fat area (as a percentage of central slice thigh area) centiles (from 3D)						
	3 rd (mean -1.88 SD)	5 th (mean - 1.64 SD)	10 th (mean - 1.28 SD)	50 th (mean)	90 th (mean + 1.28 SD)	95 th (mean + 1.64 SD)	97 th (mean +1.88 SD)
20	26.5	27.8	29.8	37.0	44.2	46.3	47.6
21	27.2	28.6	30.6	37.8	45.0	47.0	48.4
22	28.0	29.3	31.4	38.6	45.8	47.8	49.1
23	28.7	30.1	32.1	39.3	46.5	48.6	49.9
24	29.5	30.9	32.9	40.1	47.3	49.3	50.7
25	30.3	31.6	33.6	40.8	48.0	50.1	51.4
26	31.0	32.4	34.4	41.6	48.8	50.8	52.2
27	31.8	33.1	35.2	42.4	49.6	51.6	52.9
28	32.5	33.9	35.9	43.1	50.3	52.4	53.7
29	33.3	34.7	36.7	43.9	51.1	53.1	54.5
30	34.1	35.4	37.4	44.6	51.8	53.9	55.2
31	34.8	36.2	38.2	45.4	52.6	54.6	56.0
32	35.6	36.9	39.0	46.2	53.4	55.4	56.7
33	36.3	37.7	39.7	46.9	54.1	56.2	57.5
34	37.1	38.5	40.5	47.7	54.9	56.9	58.3
35	37.9	39.2	41.2	48.4	55.7	57.7	59.0
36	38.6	40.0	42.0	49.2	56.4	58.4	59.8
37	39.4	40.7	42.8	50.0	57.2	59.2	60.5
38	40.1	41.5	43.5	50.7	57.9	60.0	61.3
39	40.9	42.3	44.3	51.5	58.7	60.7	62.1
40	41.7	43.0	45.0	52.2	59.5	61.5	62.8
41	42.4	43.8	45.8	53.0	60.2	62.2	63.6

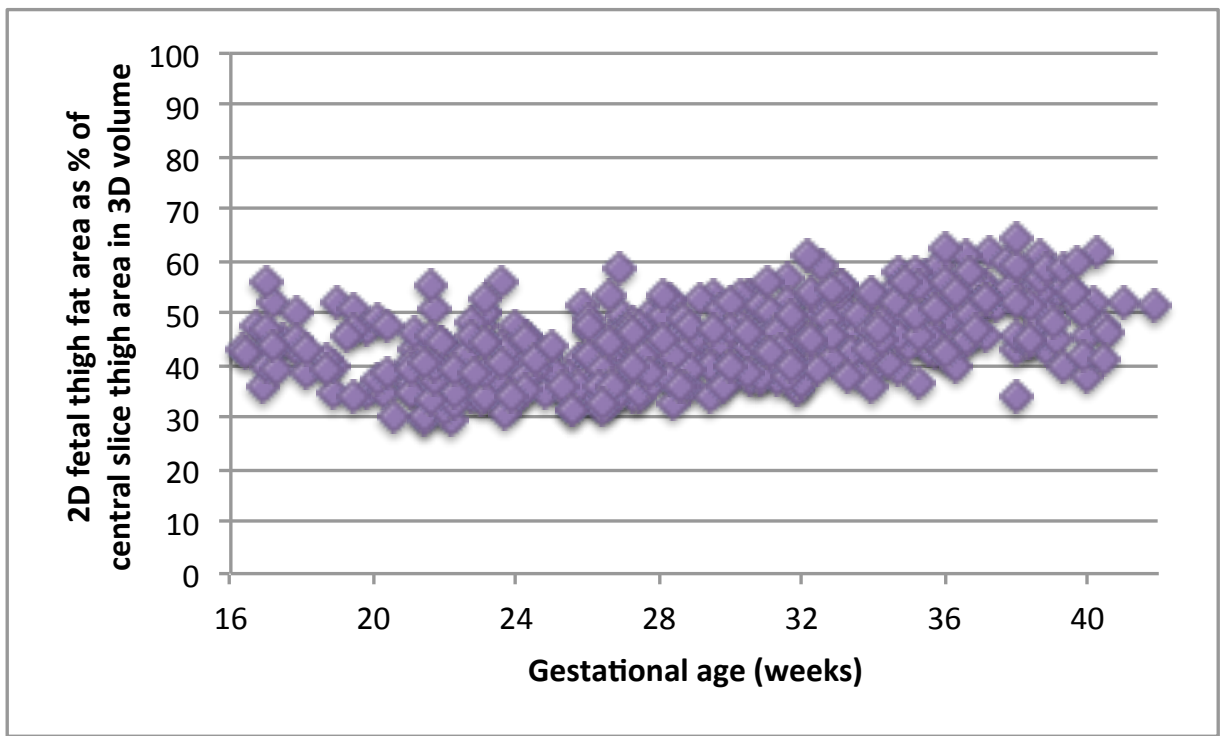


Figure 156: Fetal thigh fat as percentage of thigh area, from central slice in 3D volume (n=504).

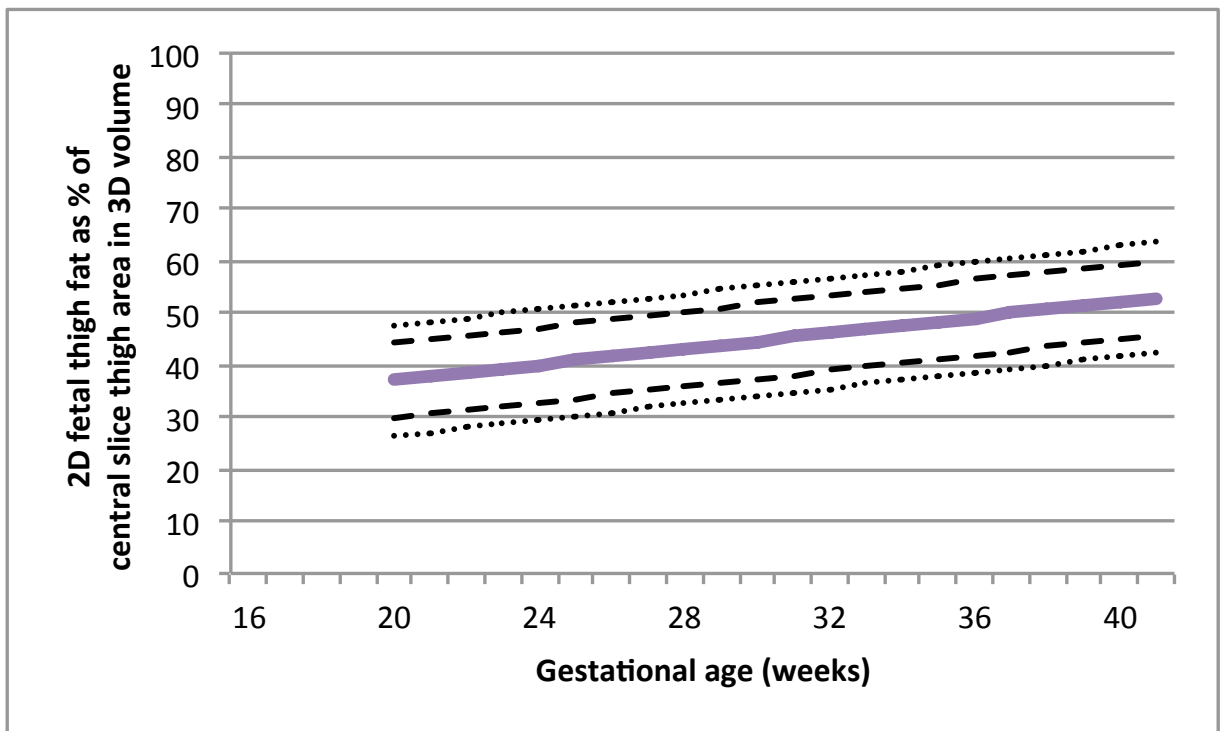


Figure 157: Fetal thigh fat as percentage of thigh area, from central slice in 3D volume (3rd, 10th, 50th, 90th and 97th centiles) (n=504).

Finally, thigh area centiles were calculated using fractional polynomial regression, with centiles calculated from SD:

$$\text{Log (thigh area) C50} = ((\text{GA}/10)^{-0.5} \times -10.26813) + 12.81058$$

$$\text{SD of log (thigh area) C50} = ((\text{GA}/10)^3 \times 0.0026418) + 0.0711358$$

These are shown below [Figure 158, Table 41].

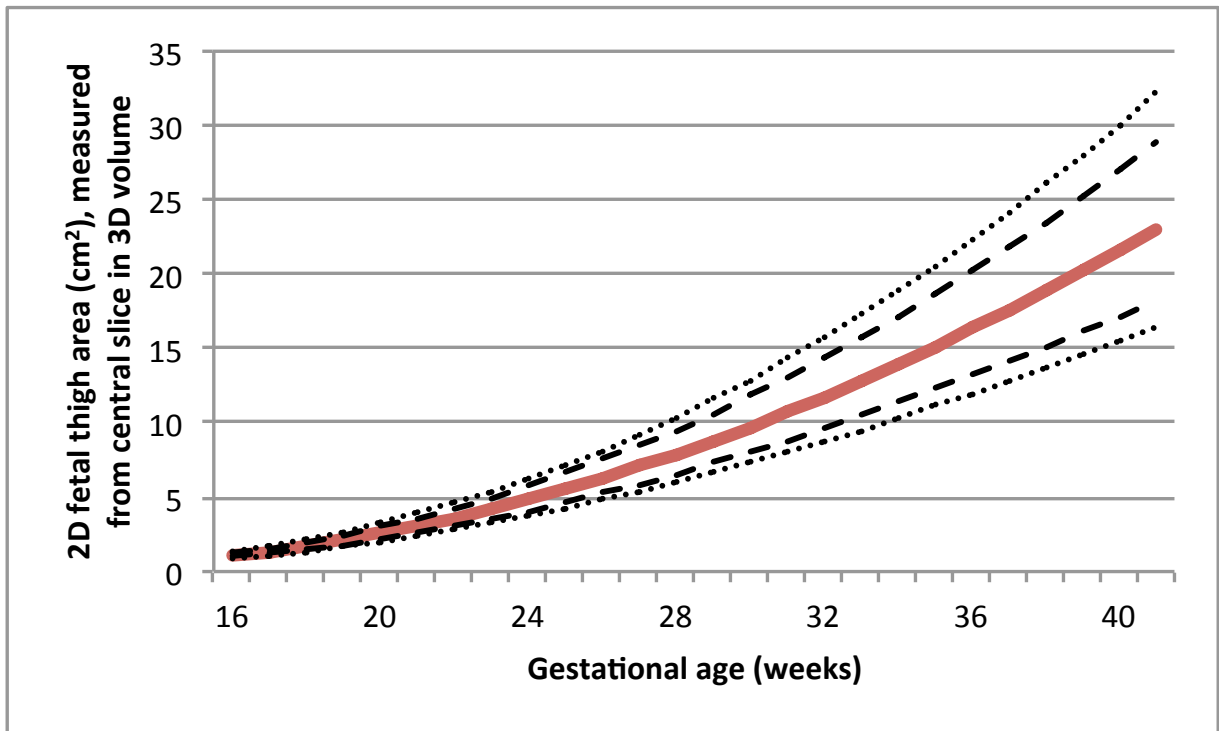


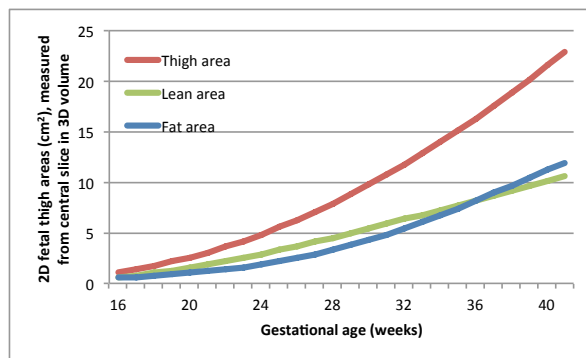
Figure 158: Fetal thigh area, from central slice in 3D volume (3rd, 10th, 50th, 90th and 97th centiles) (n=504).

Table 41: Centile values for thigh areas for each week of gestation, from central slice in 3D volume.

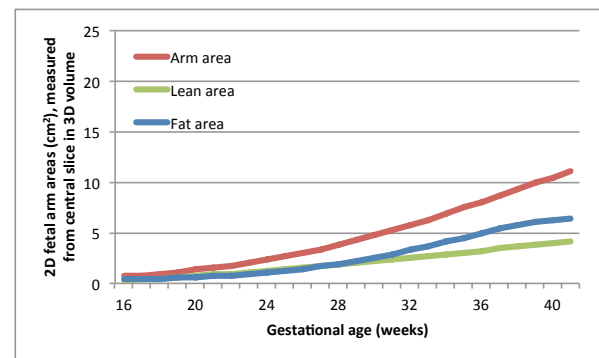
GA (weeks)	Fetal thigh area (cm ²) centiles (from 3D)						
	3 rd (mean -1.88 SD)	5 th (mean - 1.64 SD)	10 th (mean - 1.28 SD)	50 th (mean)	90 th (mean + 1.28 SD)	95 th (mean + 1.64 SD)	97 th (mean +1.88 SD)
16	0.88	0.91	0.94	1.09	1.26	1.31	1.35
17	1.12	1.15	1.20	1.39	1.61	1.68	1.73
18	1.39	1.43	1.49	1.74	2.02	2.11	2.17
19	1.70	1.75	1.82	2.13	2.49	2.60	2.68
20	2.04	2.10	2.19	2.57	3.01	3.15	3.25
21	2.41	2.49	2.61	3.06	3.60	3.77	3.89
22	2.83	2.92	3.06	3.61	4.25	4.46	4.60
23	3.28	3.38	3.55	4.20	4.97	5.21	5.38
24	3.76	3.88	4.08	4.84	5.75	6.04	6.24
25	4.28	4.42	4.64	5.54	6.60	6.93	7.16
26	4.83	4.99	5.25	6.28	7.51	7.90	8.17
27	5.41	5.60	5.89	7.07	8.49	8.94	9.25
28	6.03	6.24	6.58	7.92	9.53	10.04	10.40
29	6.67	6.91	7.29	8.81	10.64	11.22	11.63
30	7.35	7.62	8.04	9.75	11.82	12.48	12.93
31	8.05	8.35	8.83	10.73	13.06	13.80	14.31
32	8.78	9.12	9.64	11.77	14.36	15.19	15.77
33	9.54	9.91	10.49	12.85	15.73	16.65	17.30
34	10.32	10.73	11.37	13.97	17.16	18.19	18.90
35	11.13	11.57	12.27	15.13	18.66	19.79	20.58
36	11.95	12.44	13.21	16.34	20.21	21.46	22.34
37	12.80	13.33	14.17	17.59	21.83	23.20	24.16
38	13.67	14.25	15.16	18.88	23.51	25.01	26.06
39	14.56	15.18	16.17	20.21	25.25	26.89	28.03
40	15.47	16.14	17.20	21.57	27.05	28.83	30.08
41	16.40	17.12	18.26	22.97	28.91	30.83	32.19

Discussion

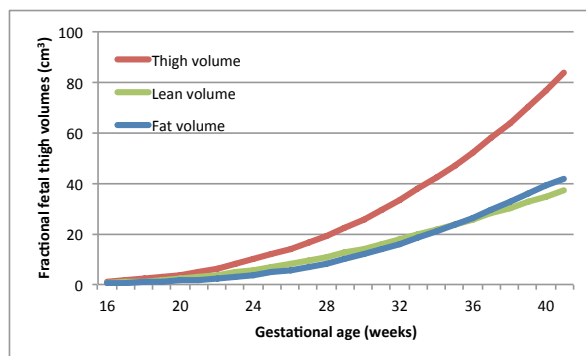
The 2D thigh area results show a similar pattern, in terms of 50th centile graphs, to the volumes, with similar fat and lean areas across all gestational ages. In comparison, the arm 2D and 3D show an increasing fat deposition from mid-third trimester [Figure 159].



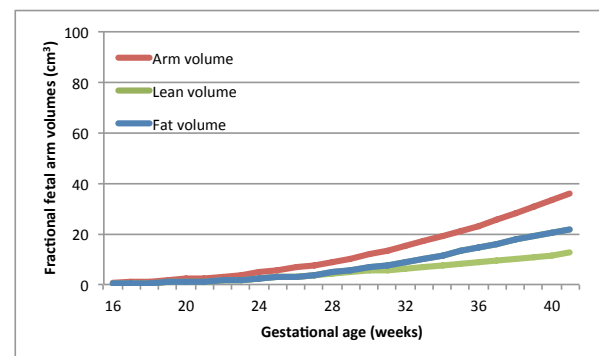
(a) thigh 2D areas (central slice 3D volume).



(b) arm 2D areas (central slice 3D volume).



(c) thigh 3D volumes.



(d) arm 3D volumes.

Figure 159: Comparison of 2D area and 3D volume 50th centiles, from fetal thigh and arm.

Conclusion

Reference centiles are presented for fetal thigh, fat and lean areas, and for fat as a percentage of thigh area, acquired from 3D volumes. The results show a similar pattern of growth to the equivalent 3D volumes, suggesting that 2D areas may provide a surrogate measure of 3D volumes. Thigh fat and lean tissue areas are very similar throughout the second and third trimesters, unlike the arm where the fat increases more towards term.

6e. Fetal Fat Index (thigh)

Aim

To assess the correlation of the Fetal Fat Index (FFI) of the thigh with fractional thigh volume, and to produce reference centiles for future research.

Method

This work built upon the FFI work presented in sections 4g and 5f. The tool devised by S.R. was used to assess the central 2D slice of each manually segmented 3D volume. M.A. prepared the volume segmentation mat files for the tool which then automatically measured the thickness between the inner and outer segmentation of the fat at three fixed points, equivalent to the FFI's 9, 12 and 3 o'clock positions. These were added together to generate the FFIs, which were then correlated with the fat volume.

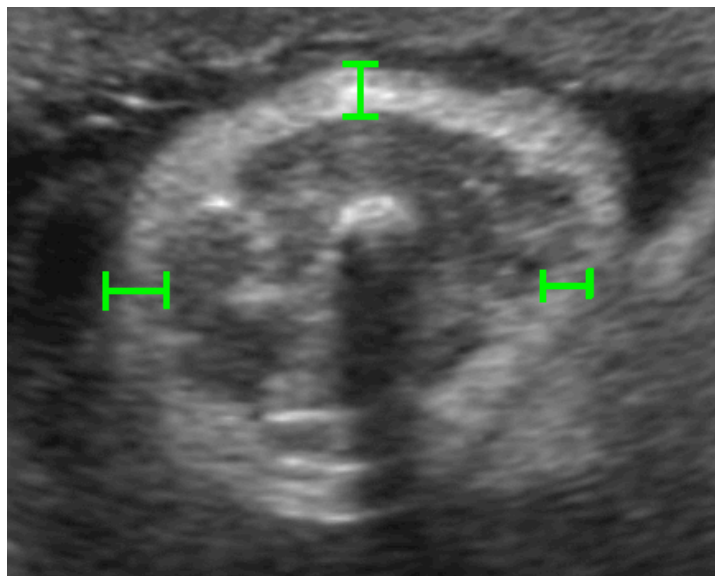


Figure 160: Axial image of fetal thigh showing Fetal Fat Index measurements at 9, 12 and 3 o'clock positions.

Results and discussion

FFI was measured in 491 scans from 217 women (from section 6b), ranging from 15⁺³ to 41⁺⁶ weeks of gestation. 13 scans could not be measured as the mat file had been misplaced after the original segmentation process.

The difference between the smallest and largest of the three measurements in each FFI ranged from 0.1-10.1mm (mean 1.4mm), demonstrating the variation in the thigh fat. This was a similar range of differences to those found in the arm, although the mean difference was greater in the arm (1.9mm) when compared with the thigh.

There was a good correlation between FFI and fractional thigh fat volume ($r=0.88$, $r^2=0.77$), although this was not as strong as the correlation seen for the arm ($r=0.93$, $r^2=0.87$).

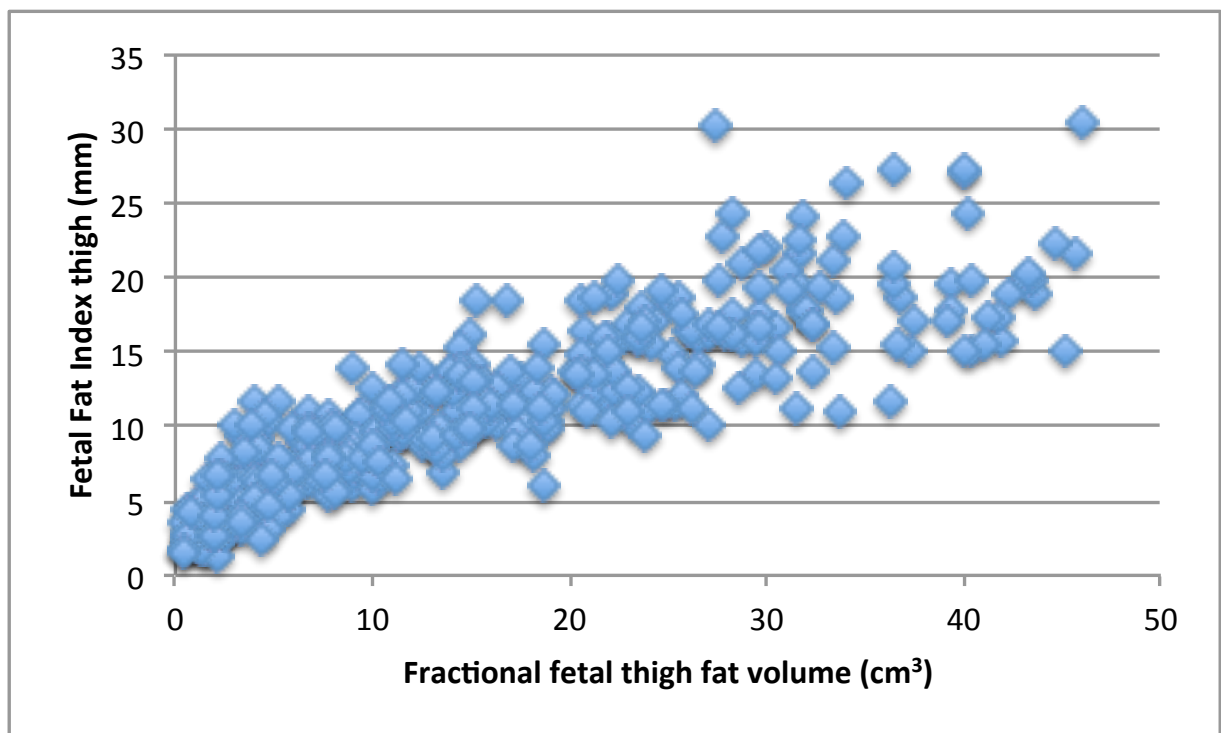
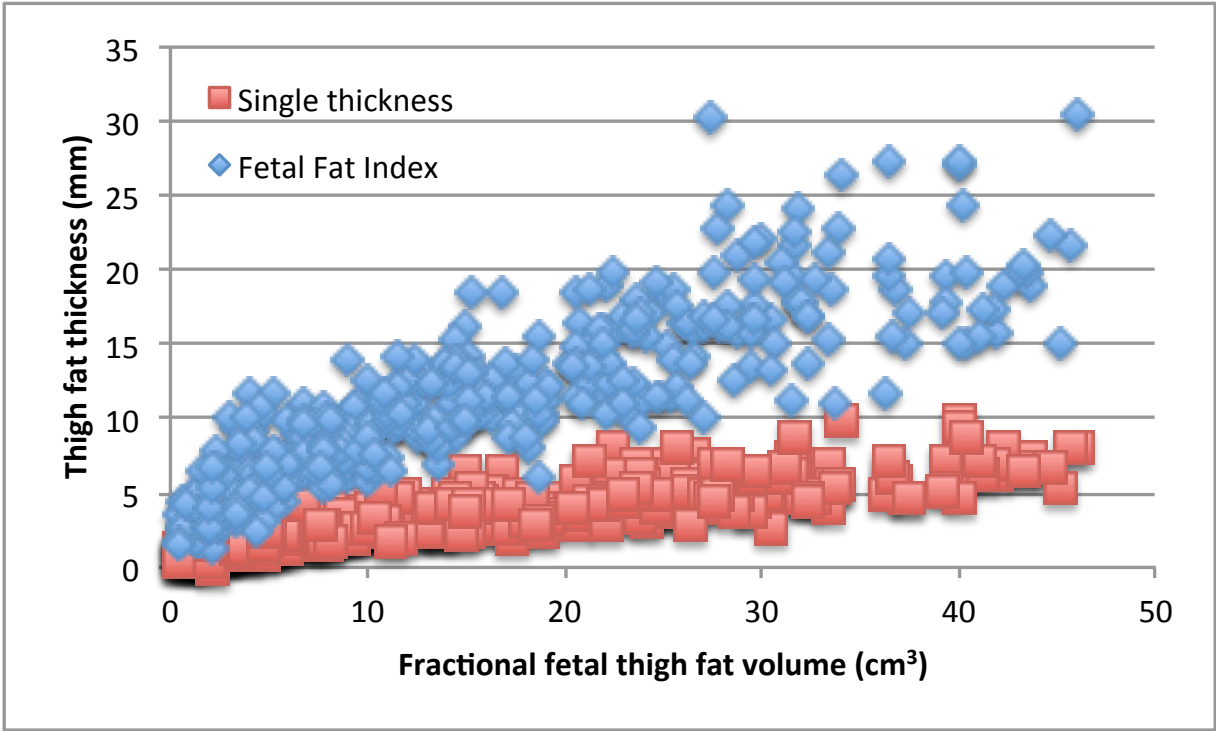
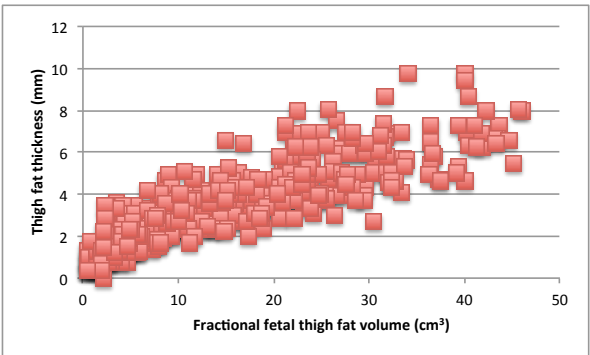


Figure 161: FFI measurements plotted against fractional fetal thigh fat volume (n=491).

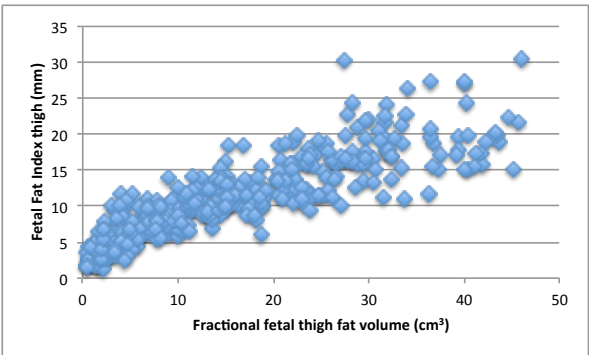
Correlation between single thickness and fat volume was not as strong ($r=0.86$, $r^2=0.73$) as that of FFI and fat volume [Figure 162]. Although there is still variation in fat around the thigh, there is also the more complicated anatomy which may contribute to a less strong correlation. As previously discussed for arm FFI, the limited range of values available for a single measurement of fat thickness may also contribute to this less strong correlation.



(a) Superimposed FFI and single fat thickness vs. fractional fetal thigh fat volume.



(b) Single thickness vs. fractional fetal thigh fat volume.



(c) FFI vs. fractional fetal thigh fat volume.

Figure 162: Comparison of thigh FFI and single thickness measurements, with fractional fetal thigh fat volume.

FFI values were then plotted against gestational age [Figure 163], demonstrating a similar curve to the fractional fetal thigh fat volumes, with increasing variation in the third trimester.

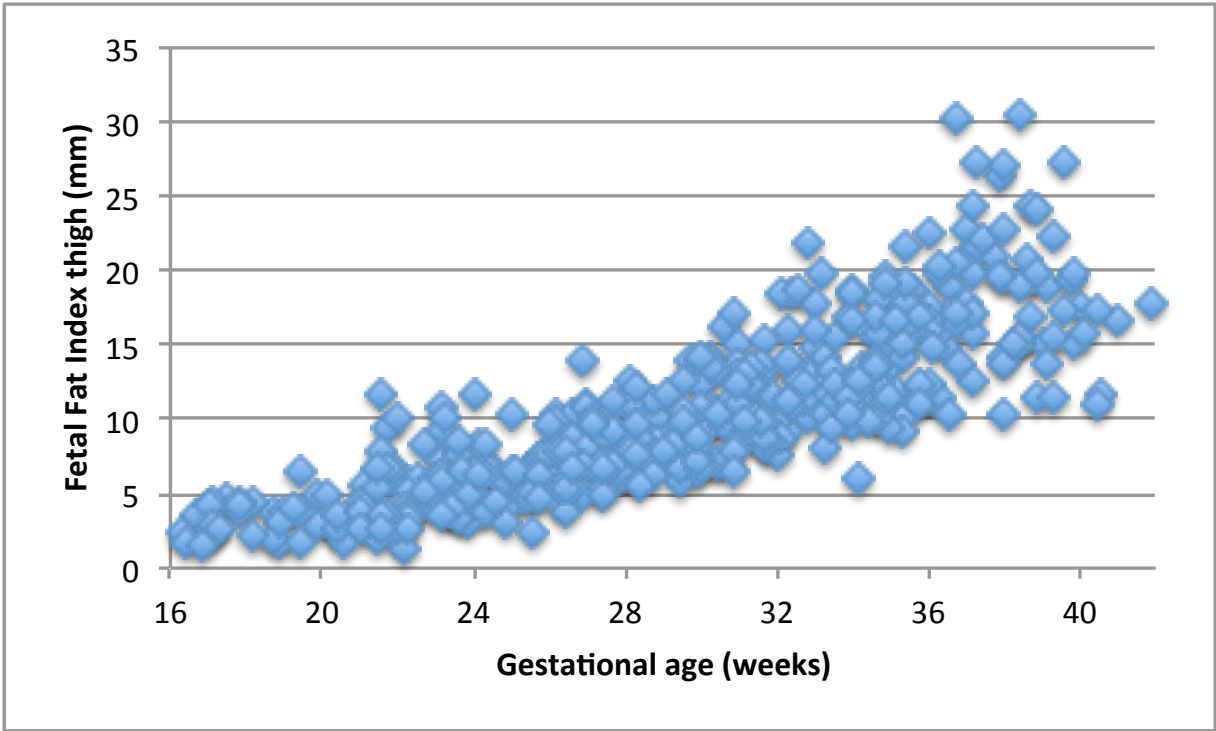


Figure 163: FFI plotted against gestational age (n=491).

Mean / 50th centile (C50) and SD were calculated using fractional polynomial regression, and 3rd, 5th, 10th, 90th, 95th and 97th centiles as previously stated [Table 42, Figure 164]. As with some other measurements, values below 20 weeks were excluded due to tool limitations, measurement challenges, and low likelihood of clinical relevance:

Fetal Fat Index (thigh) C50	=	$((GA/10)^2 \times 1.337324) - 1.789101$
SD of Fetal Fat Index (thigh) C50	=	$((GA/10)^3 \times 0.0678584) + 0.8347644$

Table 42: Centile values for Fetal Fat Index (thigh) for each week of gestation, from central slice in 3D volume.

GA (weeks)	Fetal Fat Index – thigh (mm) centiles						
	3 rd (mean -1.88 SD)	5 th (mean - 1.64 SD)	10 th (mean - 1.28 SD)	50 th (mean)	90 th (mean + 1.28 SD)	95 th (mean + 1.64 SD)	97 th (mean +1.88 SD)
20	1.0	1.3	1.8	3.6	5.3	5.8	6.2
21	1.4	1.7	2.2	4.1	6.0	6.5	6.9
22	1.8	2.1	2.7	4.7	6.7	7.2	7.6
23	2.2	2.6	3.2	5.3	7.4	8.0	8.4
24	2.6	3.0	3.6	5.9	8.2	8.8	9.2
25	3.0	3.5	4.1	6.6	9.0	9.7	10.1
26	3.4	3.9	4.7	7.3	9.8	10.6	11.1
27	3.9	4.4	5.2	8.0	10.7	11.5	12.0
28	4.3	4.9	5.7	8.7	11.7	12.5	13.1
29	4.8	5.4	6.3	9.5	12.6	13.5	14.1
30	5.2	5.9	6.8	10.2	13.7	14.6	15.3
31	5.7	6.4	7.4	11.1	14.7	15.7	16.4
32	6.2	6.9	8.0	11.9	15.8	16.9	17.7
33	6.6	7.4	8.6	12.8	17.0	18.1	18.9
34	7.1	7.9	9.2	13.7	18.2	19.4	20.3
35	7.6	8.5	9.8	14.6	19.4	20.7	21.6
36	8.0	9.0	10.4	15.5	20.7	22.1	23.1
37	8.5	9.5	11.1	16.5	22.0	23.5	24.6
38	9.0	10.0	11.7	17.5	23.4	25.0	26.1
39	9.4	10.6	12.3	18.6	24.8	26.5	27.7
40	9.9	11.1	13.0	19.6	26.2	28.1	29.3
41	10.3	11.7	13.6	20.7	27.7	29.7	31.1

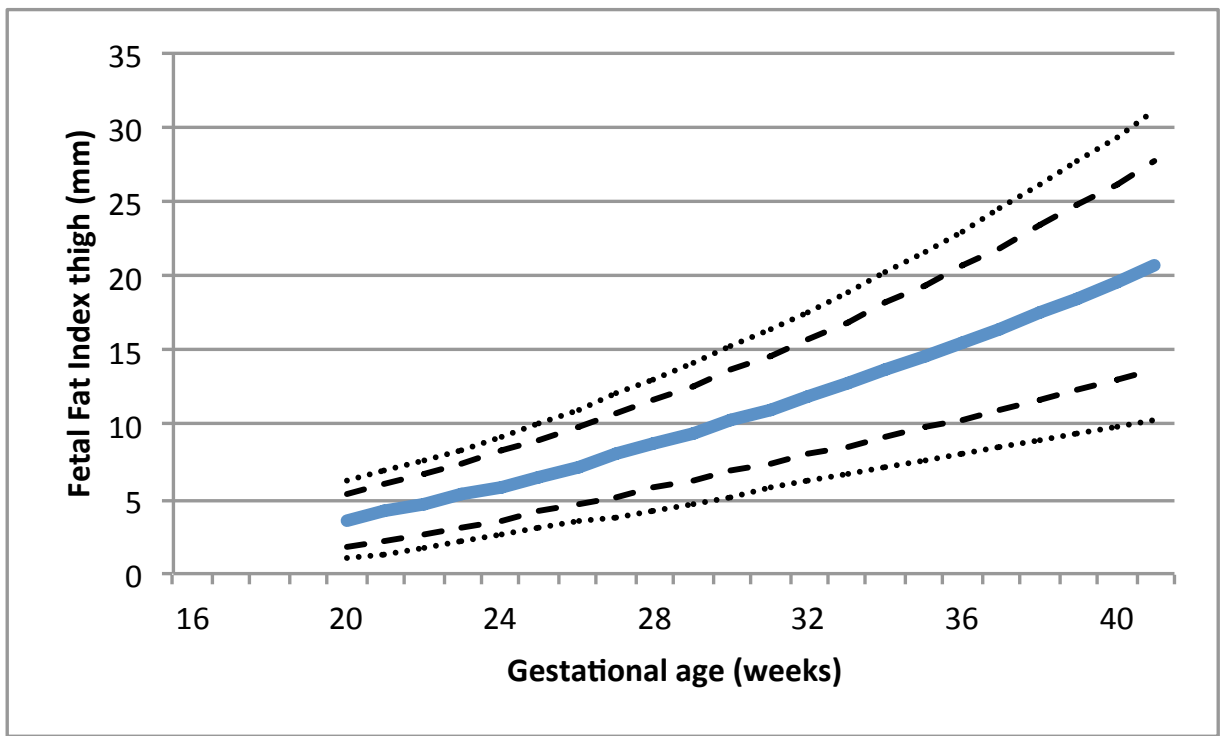


Figure 164: Fetal Fat Index of the thigh (3rd, 10th, 50th, 90th and 97th centiles).

Conclusion

This section explores the FFI measurement as applied to the fetal thigh, and shows that it correlates well with fractional fetal thigh fat volume measurements. Reference centiles have been calculated and are demonstrated from 20 to 41 weeks. FFI may prove useful in units with 2D, but not 3D, ultrasound machines, as a simple surrogate for fractional fetal thigh fat volume.

6f. Publications and presentations arising from this chapter

Fetal Fat Index (FFI): references ranges for fetal thigh fat

C.L.Knight, M.Ahmed, K.Edwards, V.Donadono, G.Parry, S.Rueda, J.A.Noble,
A.T.Papageorghiou

Oct 2014: Third International Conference on Fetal Growth, Oxford, UK

[oral presentation]

OP29.05 Fetal arm fat: a better marker than thigh fat for nutritional status?²⁴²

C.L.Knight, M.Ahmed, K.Edwards, V.Donadono, G.Parry, S.Rueda, J.A.Noble,
A.T.Papageorghiou

Ultrasound Obstet Gynaecol, 2014; 44 (s1): 156

Sept 2014: ISUOG 24th World Congress, Barcelona, Spain

[oral presentation] (see also abbreviated listing in Chapter 5)

P13.20 Fetal limb fat and lean volume reference ranges from an optimally healthy population²⁴³

C.L.Knight, M.Ahmed, K.Edwards, V.Donadono, G.Parry, S.Rueda, J.A.Noble,
A.T.Papageorghiou

Ultrasound Obstet Gynaecol, 2014; 44 (s1): 259

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[poster presentation] (see also abbreviated listing in Chapter 5)

Fetal limb fat and lean tissue assessment using 3D ultrasound and a fractional volume
MATLAB tool

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Sept 2014: Medical Engineering Centres Annual Meeting, London, UK

[poster presentation] (see also abbreviated listing in Chapter 5)

6g. Contributions to the thesis: chapter 6

Other than where indicated below, and acknowledged in appendix A, all the work in this chapter (including concepts and hypotheses, protocols, acquisition and segmentation of scans, analyses, production of graphs and figures) is that of the author.

The 3D tools, and process for producing FFI values, used in this chapter were developed and utilised by biomedical engineering colleagues, as previously stated. Data was retrieved from the INTERGROWTH-21st database, and neonatal body composition and anthropometry undertaken, by NDOG colleagues, as previously stated. MRI study scans acquired by G.Q. with radiologists and radiographers at the John Radcliffe Hospital, Oxford.

Images taken from journal papers and textbooks are individually cited in the figure captions, by name and year, and with a reference to the full bibliographic details. The sagittally-acquired volumes demonstrating anatomical and segmentation challenges were selected from the whole INTERGROWTH-21st scanning database (i.e. from all centres).

7. CORRELATING FETAL AND NEONATAL MEASUREMENTS: “ANTENATAL BODY COMPOSITION”

This final results chapter considers the correlation of fetal limb fat assessment with neonatal anthropometry and body composition measurements, and potential application of the methods within these contexts. The work presented includes analyses on fetuses and infants from all study populations described in section 3b.

Sections 7a and 7b discuss the INTERGROWTH-21st and MAVIDOS neonatal measurements. Protocols are described for neonatal body composition analysis (PEA POD, ADP) and anthropometry (INTERGROWTH-21st) and neonatal body composition (DXA) (MAVIDOS).

Section 7c compares whole body and limb-regional analyses of neonatal fat and body composition. This provides evidence for the biological plausibility of using fetal limb fat as a marker of fetal whole body fat *in utero*.

Section 7d, 7e and 7f compare fetal limb ultrasound measurements with neonatal body composition and anthropometry. Analyses include the frequency distribution of neonatal body fat, comparison of late third trimester fetal limb fat in babies with high and low body fat, the use of raw data and Z-scores to compare antenatal and neonatal measurements (18-41 weeks), and fat assessment at 34 weeks compared with neonatal measurements.

Section 7g assesses the effect of sex upon fractional arm and thigh volumes (fat and lean tissue) across the second and third trimesters, as some papers have suggested sex differences in neonatal body fat.

Section 7h considers the use of limb fat in the assessment of SGA fetuses. A proof-of-concept analysis was undertaken on sagittally-acquired fetal thigh volumes obtained on the Thai-Burmese border; thigh fat and lean tissues (2D and 3D) were then compared in SGA and AGA fetuses at 30-34 weeks, using a subset of the global INTERGROWTH-21st population.

Section 7i lists the publications and presentations arising from the work in this chapter, and **section 7j** lists the contributions to this chapter.

7a. INTERGROWTH-21st neonatal measurement protocols

Participants in the INTERGROWTH-21st study in Oxford were given verbal and written information about neonatal body composition and anthropometry studies. The subset of women who agreed to additional measurements for their babies then signed a consent form. These extra measurements (with associated paperwork) formed part of a substantial amendment to the original INTERGROWTH-21st ethics application (REC reference 08/H0606/139). I wrote the information leaflet and consent form (see appendix E).

The INTERGROWTH-21st anthropometry team were informed when study participants were in labour. After delivery, the babies underwent anthropometry and body composition analysis, ideally within 24 hours of birth.

i. INTERGROWTH-21st neonatal body composition analysis (PEA POD) protocol.

The PEA POD machine calculates neonatal body composition using air displacement plethysmography (ADP, see section 1i). The machine was set up in a carefully adapted room, so as to provide the necessary stable environment (in terms of temperature, vibration, and being on a stable, horizontal surface) for its optimal operation. The quality control and calibration of the machine was carried out daily as per the manufacturer's instructions. A protocol was developed through collaboration between the author, INTERGROWTH-21st anthropometry team (led by L.C.I, F.P. and S.L.) and neonatal colleagues who had some experience of the techniques. Members of the anthropometry team then underwent training in the use of the machine.

The volume and scale calibrations were carried out prior to each neonatal measurement, with appropriate allowances for any objects attached to the infant, such as umbilical cord clamps and ankle identification bands. Infants were fully undressed and their hair flattened against their scalp using baby oil; those with lots of hair or very curly hair had a lightweight, close-fitting cap applied [Figure 165b]. These procedures were to minimise volumetric errors caused by hair standing out from the head. The infants were then weighed on the PEA POD scales before being laid on the PEA POD test chamber tray and being slid into the warmed test chamber [Figure 165a]. The volumetric testing within the test chamber lasted 2-3 minutes with most infants tolerating it very well – the measurement has been set up so that varying infant behaviours, including movement and crying, do not affect the test result. The chamber had a window so that if the infant became distressed, the test could be stopped immediately. To minimise the chance of this being necessary, the infants were measured, whenever possible, when calm. The full PEA POD test took about 7 minutes.

The infant's volume was calculated using Boyle's law, by measuring pressure changes, which are inversely proportional to mass. Density was then calculated (mass divided by volume) and known densitometric equations allowed the calculation of the percentage of fat- and fat-free mass (see section 1i).

Results generated included fat mass and fat-free mass (in grams, and as a percentage of total mass), and total mass (i.e. weight) [Figure 166]. Additional data recorded included study ID, time and date of birth, gender, time and date of PEA POD test, additional behaviour (eg crying, urinating), mode of feeding, and time of most recent feed. Results were saved on the machine and in a spreadsheet for future analyses.

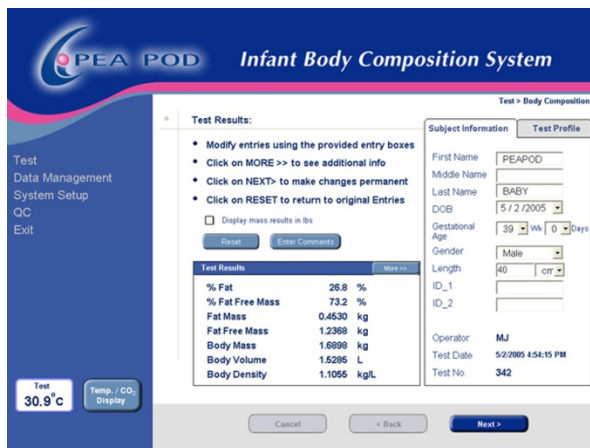


(a) PEA POD machine showing scales, screen and infant within measurement chamber.



(b) PEA POD machine showing infant wearing cap, prior to being measured.

Figure 165: PEA POD infant body composition machine.

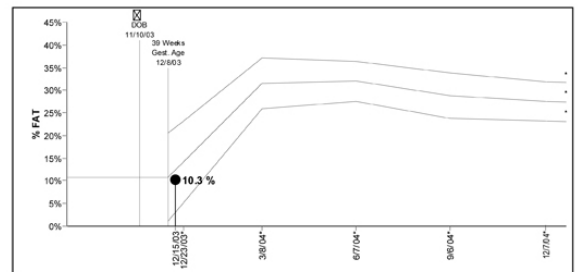


(a) Screenshot from PEA POD computer

(b) Print out from PEA POD showing neonatal whole body fat percentage

TEST RESULTS		SUBJECT INFORMATION	
% FAT	10.3 %	FIRST NAME	PEAPOD
% FAT FREE MASS	89.7 %	MIDDLE NAME	
FAT MASS	0.2910 kg	LAST NAME	BABY
FAT FREE MASS	2.5218 kg	GESTATIONAL AGE	35.0 Weeks
BODY MASS	2.8128 kg	DATE OF BIRTH	11/15/2003
BODY VOLUME	2.6029 L	AGE	5.1 Weeks
BODY DENSITY	1.0445 kg/L	GENDER	Female
OPERATOR COMMENTS		LENGTH	44 cm
		ID_1	555-55-5555
		ID_2	AA 55 21
		GROUP	None
		OPERATOR	Alex
		TEST TIME/DATE	11:15 AM 12/15/2003
		TEST NUMBER	317

% FAT TEST RESULTS		TEST DATE	AGE	% FAT	NORMATIVE* % FAT	NORMATIVE* % FAT SD	NORMATIVE* % FAT + 1 SD	NORMATIVE* % FAT - 1 SD
		12/15/03	5.1 Weeks	10.3 %	12.5 %	9.3 %	21.8 %	3.1 %



*Normative data are calculated based on results by Butte NF, Hopkins JM, Wong WW, Smith EO, Ellis KJ. Body composition during the first 2 years of life: an updated reference. *Pediatr Res* 2000;47:579-85. Data provided are for informational purposes only and are not intended as a substitute for advice provided by physician or other medical professional.

PEA POD

COSMED USA, Inc. • www.cosmed.com

Figure 166: PEA POD sample results (provided by COSMED).

ii. INTERGROWTH-21st neonatal anthropometry protocol.

All infants had birthweight, length, and head circumference recorded within the first 24 hours of life as part of the Fetal Growth Longitudinal Study (FLGS) within the INTERGROWTH-21st study.¹⁹⁸ If the parents consented (as above) to additional anthropometry as well as the body composition measurements, these were taken following the PEA POD body composition assessment. The infants who underwent the additional anthropometry are a smaller cohort; these were started after the ADP measurements as a protocol had to be developed in line with WHO reports¹⁷⁷ and approved by the INTERGROWTH-21st team. The INTERGROWTH-21st anthropometry team also underwent training by expert anthropometrists before undertaking the measurements.

Additional anthropometric measurements acquired were: mid-upper arm circumference (MUAC), abdominal circumference (AC), and mid-thigh circumference (MTC) [Figure 168]. MUAC proved to be the most important of the measurements for this thesis, so is outlined in more detail below [Figure 167].

MUAC is measured between the acromion process of the scapula and the olecranon.

1. Locate the landmarks:

- Looking at a child's back, locate the acromion process as the most lateral bony protuberance of the shoulder and mark it.
- Bend the child's arm at a 90° angle at the elbow, palm up, the olecranon stands out as the elbow bone.

2. Measure the distance between the 2 landmarks and mark the mid-point.

3. Measure the arm circumference at the level of the mid-arm with the tape; this should be kept flat against the skin, without compressing the skin or tissue.

4. Record the measure at the last completed 1mm.

Figure 167: Neonatal mid upper arm circumference (MUAC) measurement protocol.



(a) MUAC: measuring acromion process to olecranon.



(b) MUAC: marking the midpoint of the arm.



(c) MUAC: measurement being taken.



(d) AC: measurement being taken.



(e) MTC: measuring abdomen-thigh flexion crease to knee.



(f) MTC: measurement being taken.

Figure 168: Neonatal anthropometry.

7b. MAVIDOS neonatal body composition analysis (DXA) protocol

Within 14 days of delivery, babies within the MAVIDOS cohort underwent DXA body composition scanning at the Nuffield Orthopaedic Centre, Oxford, by trained MAVIDOS radiographers, overseen by K.J.. Initially the DXA scans were done using a Discovery A machine, but this was changed to a Lunar iDXA machine specific to the research centre, which allowed more detailed scans including sub-analyses of limb fat [Figure 169].

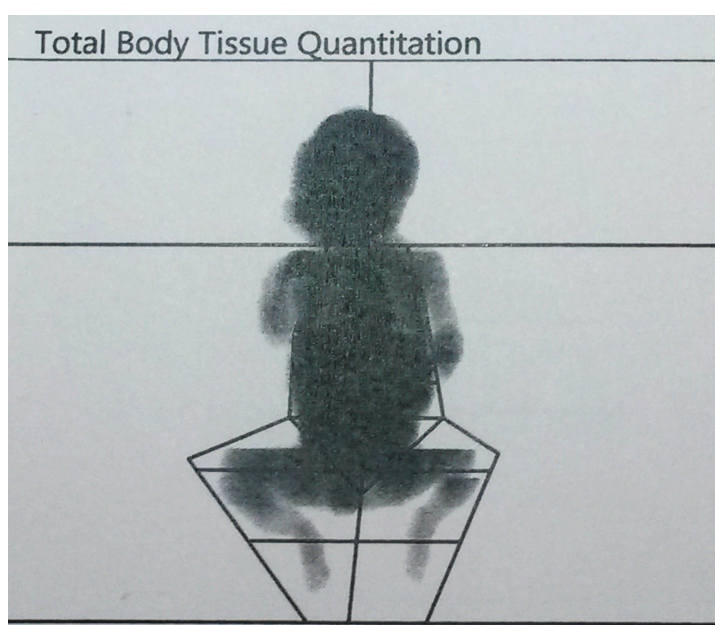


Figure 169: DXA print-out showing body composition analysis of a MAVIDOS infant including limb fat.

The DXA scans were acquired as per the MAVIDOS protocol.²⁰⁰ The baby was pacified, fed, fully undressed and then swaddled in a towel before being placed on the densitometer. The scanner was calibrated against a spine phantom every day together with daily quality assurance and calibration all performed as per manufacturer's instructions. Whole body and lumbar spine bone area, bone mineral content, bone mineral density and body composition were measured. The total radiation dose (0.04 mSv) is equivalent to around 2 day's exposure to background radiation in Cornwall (UK) or 7 days' exposure in other parts of the UK.²⁰⁰

7c. Neonatal body composition using DXA: limb fat as a surrogate for whole body fat?

Aim

To compare limb fat against whole body fat in infants, measured using DXA, to see whether they show good correlation.

Background and method

The GE Lunar iDXA machine allowed for more detailed sub-analyses of neonatal body composition than the original Hologic Discovery A machine, which only provided whole body analysis. Using the GE Lunar iDXA machine, it was possible in some cases (depending on the baby's position and behaviour) to analyse the fat within their arms, legs, and whole body. The results were given in absolute (fat in grams) and percentage values (percentage of limb, or whole body, occupied by fat). Comparison and correlation of these neonatal values produced some evidence for the use of fetal limb fat as an approximate for whole body fat.

Results and discussion

19 babies within the MAVIDOS cohort with my limb scans had undergone DXA body composition with sub-analyses of limb composition. Two babies were excluded as they were scanned 25 and 48 days after birth; in the remainder, the mean age was 11 days.

The percentage of fat in the arm and thigh confirmed the differences shown in the fetal ultrasound findings: the neonatal arm had a greater amount of fat, with a mean of 35.1% (range 27.2-41.0%) compared with 31.6% in the leg (23.8-38.4%). The difference in the limb sizes was obviously reflected in the absolute amount of fat, with the arm having a mean of 143g (range 72-206g) and the leg 253g (113-445g). The mean whole body fat percentage was 20.2% (range 16.2-24.4%), or 782g (418-1149g). The mean whole body weight at the time of the DXA was 3.735kg (range 2.935-4.739kg).

Limb fat showed very strong ($r>0.80$)²⁵¹ correlation with whole body fat [Figure 170]:

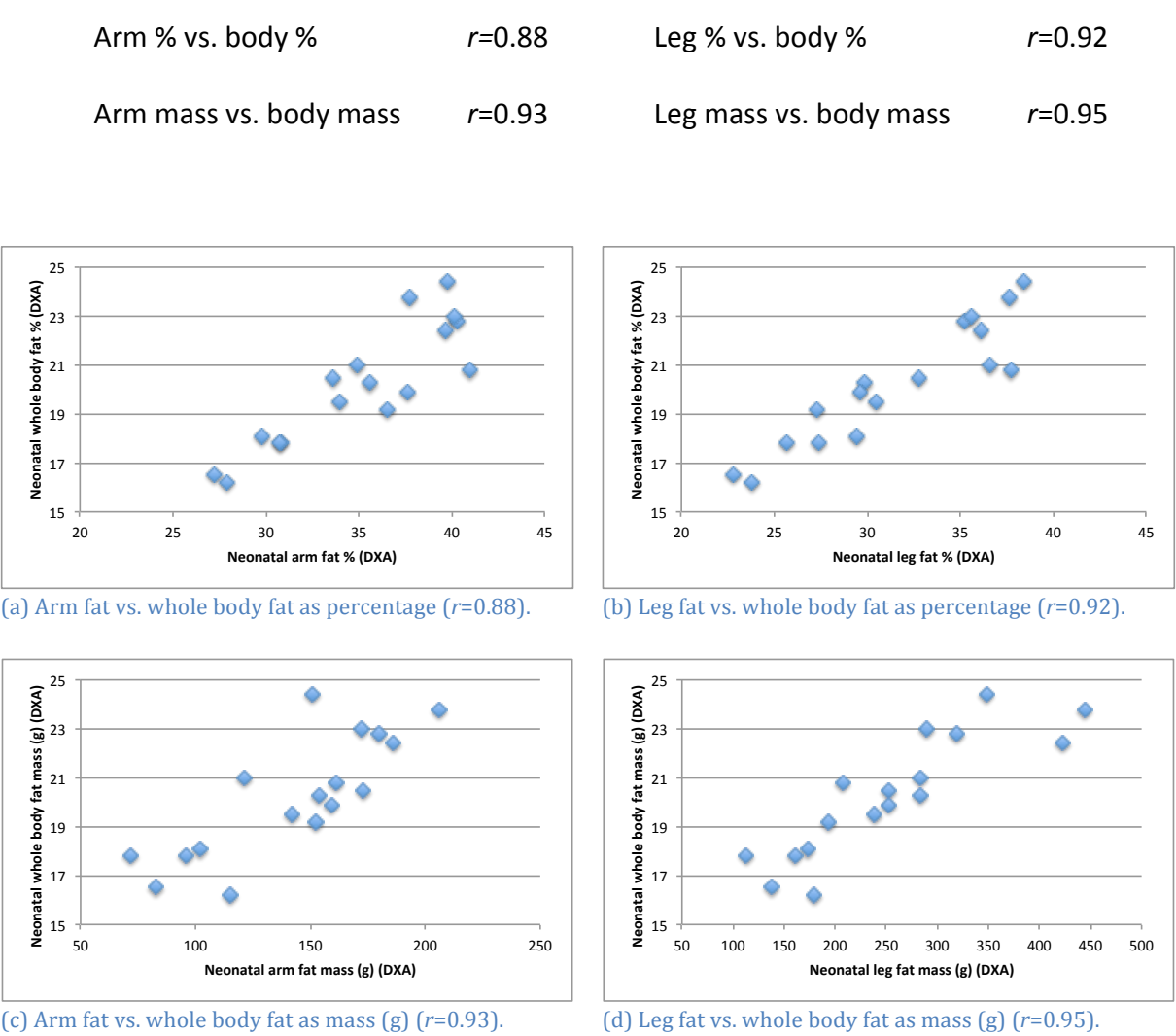


Figure 170: Correlation of neonatal limb fat and whole body fat using DXA (MAVIDOS).

When different measurement methods were compared, for example arm and leg mass with whole body percentage (mimicking the comparison of fetal fat areas or volumes with neonatal whole body fat percentage for the INTERGROWTH-21st babies), the correlation coefficients were, unsurprisingly lower, but still showed a very strong correlation:

Arm mass (g) vs. body %	$r=0.81$	Leg mass (g) vs. body %	$r=0.87$
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It is notable that the percentage fat values from DXA are significantly higher than those obtained from the PEA POD measurements: DXA mean whole body fat 20.2% (16.2%-24.4% range) compared with PEA POD 10.9% (2.9-20.1%) This is not solely due to differences in the infants' age (mean 11 days vs. 20 hours) or weight (mean 3.735kg vs. 3.413kg) at the time of the test. It has been described on several occasions in the literature that body composition analysis methods are not interchangeable¹⁸⁷ and that it is clinically inappropriate to compare the percentage of body fat across different methods.²⁵² Papers have also shown that, whilst there is a good correlation between methods, DXA tends to give higher percentage of fat and absolute fat mass values than ADP in both infants²⁵³ and adults.²⁵² The mean whole body fat percentage seen in the MAVIDOS cohort does align with the results of a DXA body composition study of 64 AGA babies by Koo et al,²⁵⁴ who calculated that 20% is a 50th centile whole body fat percentage for babies weighing 4kg.

Conclusion

The comparison of limb sub-analyses with whole body fat using DXA in neonates has shown a good correlation between limb and whole body measurements, suggesting it is plausible to use fetal limb measurements as an approximation marker for whole body composition.

7d. Correlation of fetal limb fat with neonatal body composition: PEA POD

i. Proof of concept: comparison of fetal limb measurements ≥ 36 weeks in neonates with high and low whole body fat (PEA POD)

Aim

To assess whether fetal arm fat area and volume measurements in the last 5 weeks of pregnancy can be used as a surrogate marker of body composition, by distinguishing infants with high and low whole body fat percentages (measured with PEA POD).

Methods

A subset of the Oxford INTERGROWTH-21st neonates underwent body composition analysis using the PEA POD machine (ADP) to measure their whole body fat and fat-free mass. The results were translated into sex- and gestational age-specific centiles as per the paper by Hawkes et al²²¹ and the distribution assessed. The five infants born at 42⁺⁰ or 42⁺¹ weeks were assessed against centiles for 40⁺⁰-41⁺⁶ weeks.

Fetal arm and thigh volumes were compared with the neonatal whole body fat percentage centiles. The numbers were too small to compare <10th with >90th centile babies (i.e. SGA and LGA), so instead the <25th and >75th groups were compared. As this was a proof of concept study, the last scan (≥ 36 weeks) prior to delivery was assessed: we hypothesised this would have the strongest correlation with the neonatal measurement, being the closest in terms of time of measurement. The fractional volume and 2D slice area measurements were used for the arm and thigh fat and for the arm and thigh as a whole.

Results

110 infants underwent body composition analysis as well as having had fetal limb fat scans. Within this cohort, 78 had body composition measured within the first 24 hours of life (the earliest at 3 hours of age); 29 within the first 24-48 hours; and 3 within the first 48-72 hours (the latest at 71 hours).

Neonatal whole body fat showed a normal distribution, both overall [Figure 171] and when male and female infants were analysed separately [Figure 172]. Even within this optimally healthy pregnant population, there were still 8 babies (8/110, or 7%) with <10th centile whole body fat. Whilst a range of whole body fat values would have been anticipated, it might have been expected that there would be fewer with fat <10th centile given the selection of optimally healthy women. This finding reflects that of Schmelze et al who found that appropriate birthweight did not guarantee appropriate body fat (in their case, 10% of babies of normal birthweight had <10% body fat).⁹²

It is also interesting to note that the range was wider for female than for male infants, with only 1 male falling either <5th or >95th centile, compared with 5 female. The median values also differed: 25-50th centile for female infants, and 50-75th for male. The absolute values for these centiles were 9.2-12.7% fat (50-75th) for male, mean 10.9%, and 5.7-12.5% fat (25-50th) for female, mean 9.2%. This finding thus differs from the Hawkes paper, where male infants generally had a lower percentage of fat than females.²²¹ The influence of sex upon body fat varies within the scientific literature, with some papers showing female infants to have more fat²⁵⁵⁻²⁵⁷, whilst others show a non-significant increase²⁵⁸, or no difference.^{259,260} Male infants having a greater percentage of fat has not previously been demonstrated.

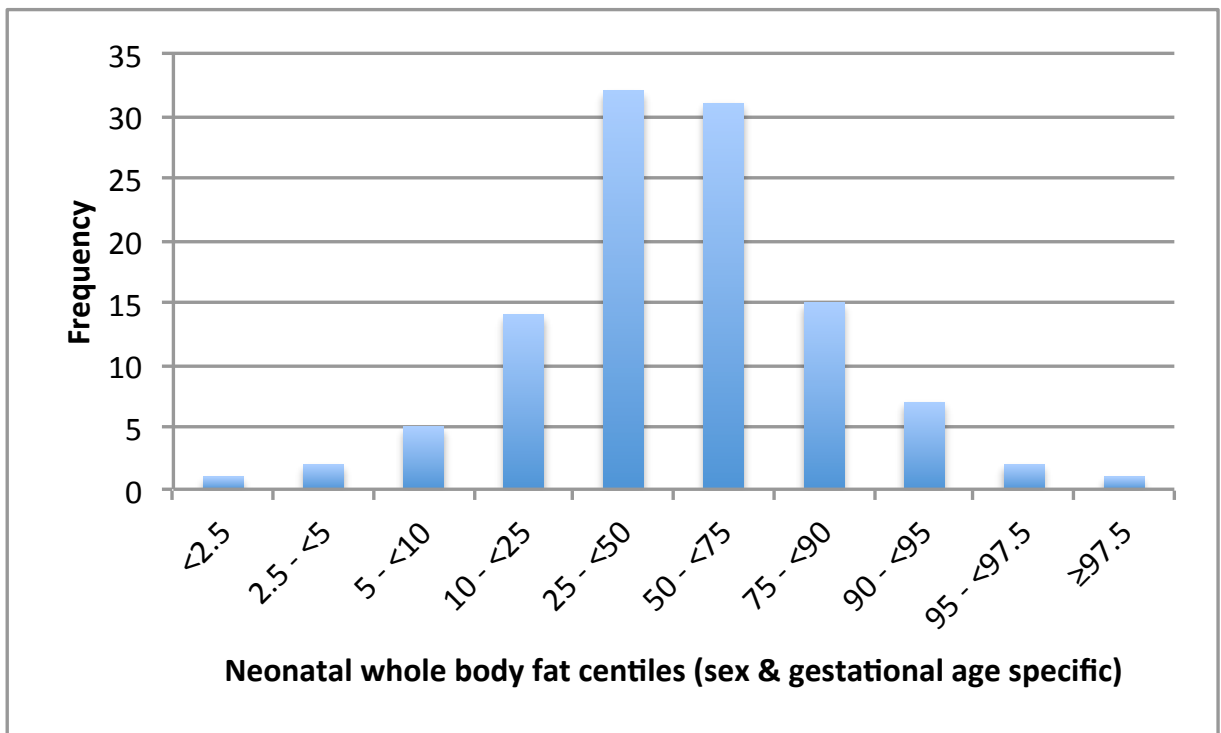


Figure 171: Neonatal whole body fat centiles (n=110).

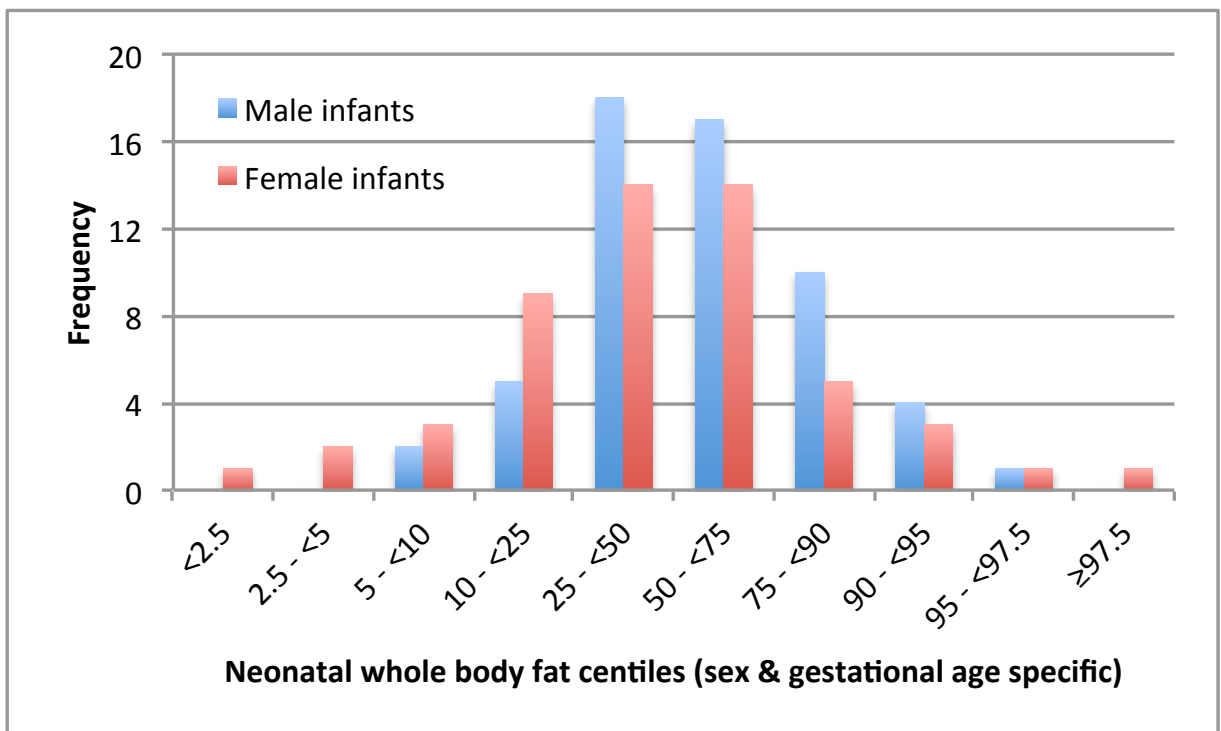


Figure 172: Neonatal whole body fat centiles, by infant sex (n=110: 53 female, 57 male).

The Hawkes paper had the largest sample size (n=786) and provided sex- and gestational age-specific centiles, so was used as the reference for the neonatal body fat in this thesis.

Having confirmed the normal distribution of neonatal whole body fat, the analysis of those infants with <25th centile (n=14) compared with those >75th centile (n=15) was undertaken [Table 43], with evaluation of fetal arm parameters. There was no statistically significant difference between the groups in terms of gestational age at birth, interval from last scan to PEA POD assessment, or age at PEA POD assessment. There was a statistically significant difference ($p=0.01$) in the sex distribution of the babies within the <25th and >75th body fat centile groups, when thigh measurements were assessed [Table 44]; this was not the case for the arms. The numbers in each centile bracket are too small to draw any definitive conclusions in terms of sex and whole body fat but when neonatal whole body fat quartiles and neonatal sex were assessed, there were no statistically significant differences (chi square, $p=0.15$).

Infants with the lower amount of whole body fat had a lower birthweight than those with the higher amount of whole body fat (mean 2985g vs. 3913g, $p<0.001$). However it is worth noting that, probably due to the INTERGROWTH-21st selection of optimally healthy women, all of the infants except one were of healthy weight; (>2500g) the one categorised as low birth weight (<2500g)¹⁷⁵ weighed 2421g.

Birthweight centiles, specific to sex and gestational age (WHO/RCPCH²²⁰), also generally reflected the body fat centiles: the lower fat group had a mean 15th centile for birthweight, whilst the higher fat group had a mean 71st centile for birthweight. There were, however, discrepant results, such as the infant whose birthweight was on the 39th centile but who was in the higher fat group (>75th centile for whole body fat). This illustrates the additional information provided by body composition results, and some of the limitations of using birthweight alone.

Table 43: Comparison of fetal arm measurements in infants either <25th or >75th centile for neonatal whole body fat, as assessed by PEA POD.

	<25 th centile	>75 th centile	t-test <i>p</i> value (*chi square)
<i>n</i>	14	15	n/a
Sex distribution (male:female)	4 : 10	9 : 6	0.09*
Mean gestational age at birth (weeks)	40 ⁺⁰ (38 ⁺¹ – 42 ⁺⁰)	40 ⁺⁴ (38 ⁺³ – 41 ⁺⁶)	0.16
Mean birthweight (g)	2985 (2421 – 3374)	3913 (3416 – 4460)	<0.001
Mean birthweight centile²²⁰	15 th (1 st – 29 th)	71 st (39 th – 93 rd)	<0.001
Mean interval from last scan to PEA POD (days)	14 (0 – 32)	14 (0 – 30)	0.89
Mean age at PEA POD (hours)	19 (3 – 30)	22 (4 – 47)	0.45
Mean fetal fractional arm fat volume (cm³)	15.4 (10.3 – 20.5)	23.8 (15.5 – 34.2)	<0.001
Mean fetal fractional arm volume (cm³)	25.5 (19.9 – 31.0)	34.5 (20.9 – 46.4)	<0.001
Mean fetal arm fat area (cm²)	5.1 (3.8 – 7.5)	7.6 (5.3 – 12.2)	<0.001
Mean fetal arm area (cm²)	8.7 (7.1 – 11.2)	11.1 (7.8 – 15.5)	<0.001

The differences in the arm volumes and area measurements between the two groups were highly statistically significant ($p < 0.001$). This shows the potential for limb, and limb fat, measurements to be able to distinguish antenatally, in the late third trimester, those fetuses that will have low or high body fat at birth.

A similar evaluation was undertaken for fetal thigh parameters. The group sizes were slightly smaller and involved some fetuses from the arm analyses, together with some additional scans not previously assessed (not all arm and thigh volumes could be segmented for all fetuses) [Table 44].

In this case, there was a statistically significant difference in the sex distribution of the groups with, again, a similar trend to the arm groups, with more females in the $<25^{\text{th}}$ whole body fat group, and more males in the $>75^{\text{th}}$ group. There were no differences in any of the variables related to gestational age or timing of the measurements. The birthweights showed a statistically significant difference, with a similar range of weights and birthweight centiles to the arm data.

Table 44: Comparison of fetal thigh measurements in infants either <25th or >75th centile for neonatal whole body fat, as assessed by PEA POD.

	<25 th centile	>75 th centile	t-test <i>p</i> value (*chi square)
<i>n</i>	11	13	n/a
Sex distribution (male:female)	2 : 9	9 : 4	0.01*
Mean gestational age at birth (weeks)	40 ⁺² (38 ⁺⁴ – 42 ⁺⁰)	40 ⁺³ (38 ⁺³ – 41 ⁺⁶)	0.67
Mean birthweight (g)	3037 (2760 – 3374)	3908 (3416 – 4354)	<0.001
Mean birthweight centile ²²⁰	17 th (6 th – 29 th)	69 th (39 th – 95 th)	<0.001
Mean interval from last scan to PEA POD (days)	15 (2 – 32)	17 (2 – 34)	0.86
Mean age at PEA POD (hours)	17 (3 – 29)	20 (3 – 34)	0.34
Mean fetal fractional thigh fat volume (cm ³)	25.3 (16.8 – 33.8)	39.6 (25.7 – 60.3)	<0.001
Mean fetal fractional thigh volume (cm ³)	51.3 (37.3 – 71.0)	72.5 (48.5 – 96.5)	<0.001
Mean fetal thigh fat area (cm ²)	7.5 (4.6 – 9.1)	11.3 (8.2 – 16.5)	<0.001
Mean fetal thigh area (cm ²)	16.1 (11.6 – 19.9)	21.3 (14.4 – 26.5)	<0.001

Again, the results for thigh fractional volume and fractional fat volume, and 2D area and fat area, were all highly statistically significantly different between the two groups ($p < 0.001$). The lean volume also showed a statistically significant difference with a p value of 0.025, but the lean area showed a non-significant difference with a p value of 0.13. Although it would be expected that generally larger and heavier babies would have larger amounts of muscle than smaller babies, this provides evidence in support of the previous work by Galan et al⁹⁵ which suggested that the major differences in body composition between infants lie within the fat, rather than the fat-free compartments.

Conclusion

Neonatal whole body fat was normally distributed amongst infants from the optimally healthy INTERGROWTH-21st cohort. There was some suggestion that, contrary to some published data, male infants may have more fat compared with female infants, however the numbers were too small to draw definitive conclusions.

Fetal arm and thigh, and their equivalent fat measurements, were statistically significantly different (in both 2D and 3D) at 36 weeks or later in fetuses who went on, as newborn infants, to have low ($< 25^{\text{th}}$ centile) and high ($> 75^{\text{th}}$ centile) amounts of whole body fat. These data provide the first, preliminary evidence that such ultrasound measurements may allow *in utero* assessment of body composition measurements.

ii. Comparison of fetal limb measurements with neonatal whole body fat (PEA POD)

Aim

To assess the correlation between fetal arm and leg measurements (fractional volume and fractional fat volume; 2D central slice area and fat area, FFI) and neonatal whole body fat, including how the correlation varies across different fetal gestational age ranges.

Method

These analyses used the same dataset as section 7b.i, namely the subset of the Oxford INTERGROWTH-21st neonates who underwent body composition analysis using the PEA POD machine to measure their whole body fat and fat-free mass. This time the complete dataset was used; the fat was analysed as percentage fat mass rather than by centile.

Fetal arm and thigh volumes, previously measured using the MATLAB tool (see section 5c), were compared with the neonatal whole body fat percentage centiles. The proof of concept study had shown that late third trimester scans had the potential to distinguish babies of low and high whole body fat. This was extended to all infants and scans of all gestational ages.

The raw data were initially compared using correlation coefficients: moderate correlation 0.40-0.59, strong 0.60-0.79, very strong 0.80-1.00.²⁵¹ Z-scores were then calculated ((observed-mean)/standard deviation) for both the PEA POD neonatal whole body fat and for the fetal limb measurements, as it was felt this SD-based assessment might enable a more accurate comparison of data from two different measurement methods.

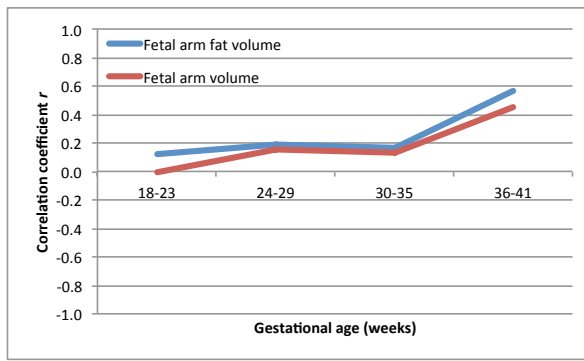
Results and discussion: comparison of raw data

Having compared the late third trimester scans at 36-41 weeks with the PEA POD results in the section above, the same gestational age ranges were used but moving to earlier gestational ages. This gave four gestational age 'windows' of 18-23, 24-29, 30-35 and 36-41 weeks. Arm and thigh measurements from each individual fetus were correlated with its neonatal PEA POD whole body fat measurement for each scan [Table 45, Figure 173].

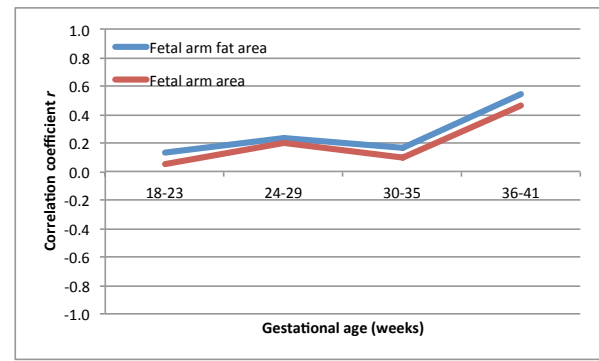
Table 45: Correlation between fetal limb measurement (6-week gestational age brackets) and neonatal whole body fat percentage. (Strong correlation coefficients (≥ 0.60) shown in red.)

Gestational age	<i>n</i>	Correlation between fetal arm measurement and neonatal whole body fat percentage (coefficient <i>r</i>)			
		Arm fat volume	Arm volume	Arm fat area	Arm area
18-23 weeks	49	0.12	-0.01	0.13	0.05
24-29 weeks	79	0.19	0.16	0.24	0.20
30-35 weeks	93	0.17	0.13	0.16	0.10
36-41 weeks	61	0.56	0.45	0.54	0.46

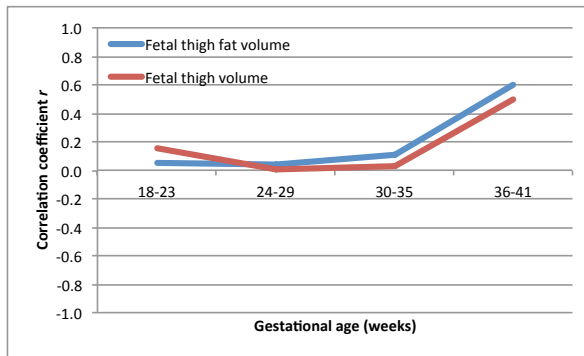
Gestational age	<i>n</i>	Correlation between fetal thigh measurement and neonatal whole body fat percentage (coefficient <i>r</i>)			
		Thigh fat volume	Thigh volume	Thigh fat area	Thigh area
18-23 weeks	53	0.05	0.15	0.02	0.14
24-29 weeks	83	0.04	0.00	0.09	0.05
30-35 weeks	91	0.11	0.03	0.16	0.08
36-41 weeks	61	0.60	0.50	0.58	0.47



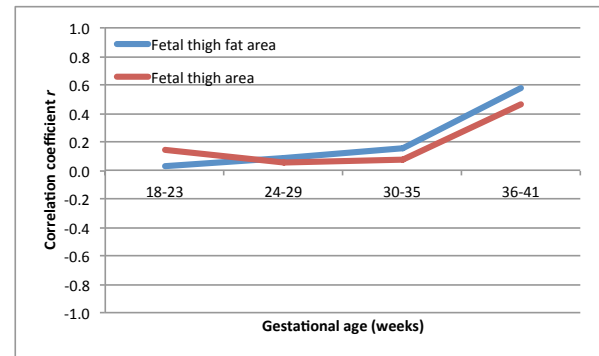
(a) Fetal arm fat volume and arm volume.



(b) Fetal arm fat area and arm area.



(c) Fetal thigh fat volume and thigh volume.

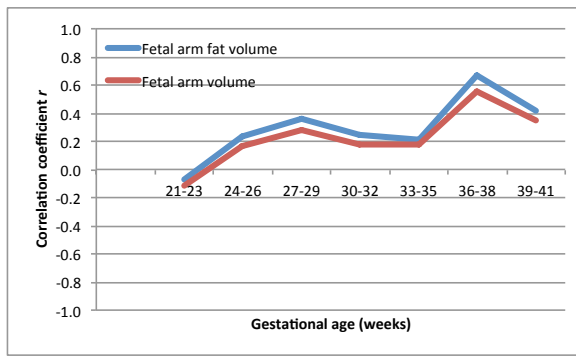


(d) Fetal thigh fat area and thigh area.

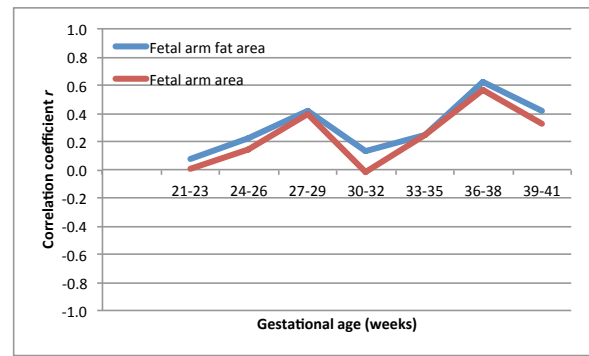
Figure 173: Correlation, expressed as correlation coefficient r , between fetal limb measurements (specified above) at differing 6-week gestational age ranges and neonatal whole body fat percentage.

The most immediate similarity between all four panels above [Figure 173] is that the corresponding fat and limb measurements (blue and red, respectively) show very similar results. However, limb fat gives a correlation coefficient that is, on average, 0.1 higher than the overall limb measurement. This may reflect the fact that the limb measurement is used to calculate the fat measurement (the lean measurement is subtracted from the limb measurement to generate the fat measurement).

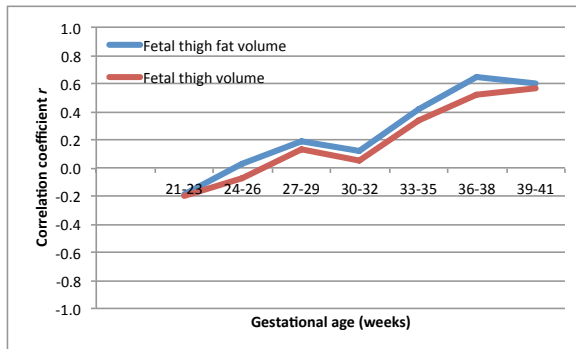
There was also a striking increase in the strength of correlation from earlier gestational age windows to the last, 36-41 week, window. This differed from the expected gradual increase. The gestational age brackets were reduced to 3-weekly blocks to see if this clarified the pattern [Figure 174]. The 18-20 week bracket was not used due to small numbers ($n=7$).



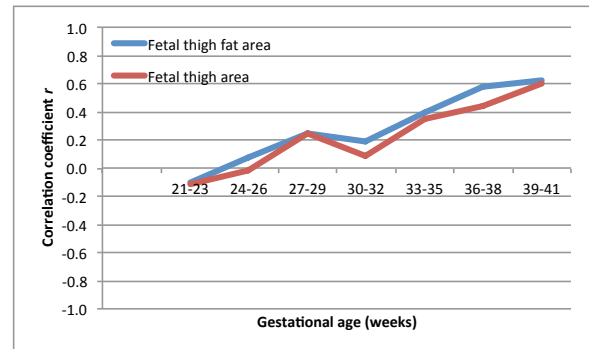
(a) Fetal arm fat volume and arm volume.



(b) Fetal arm fat area and arm area.



(c) Fetal thigh fat volume and thigh volume.



(d) Fetal thigh fat area and thigh area.

Figure 174: Correlation, expressed as correlation coefficient r , between fetal limb measurements (specified above) at differing 3-week gestational age ranges and neonatal whole body fat percentage.

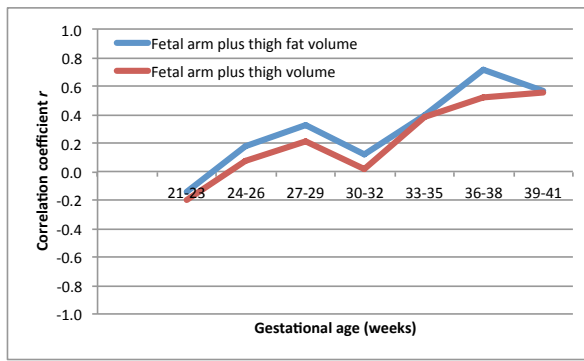
These graphs showed the same correspondence between the fat and limb measurement plots. Again, fat measurement showed a slightly higher correlation in all cases. It seems that the best correlation occurs at 36-38 weeks of gestation. This could be of clinical relevance in the detection of late FGR, for example, at a 36 week scan. The slightly reduced correlation which follows, at 38-41 weeks, may partly be due to the smaller sample size in this group: less than half the size of all preceding groups, for the arm measurements, and less than a third of the size for the thigh groups. It may also partly reflect the difficulties of measuring fat at this late gestation, due to the challenges of late third trimester ultrasound imaging. It would otherwise be expected that, the nearer the scan measurement to the neonatal measurement (i.e. the later the gestational age), the stronger the correlation.

It is also evident that the 2D results are generally reflective of the 3D results. Whilst this might have been anticipated to a certain extent, as the 2D areas are extracted from the 3D volumes, our hypothesis was that 3D volumes would provide additional information and a better correlation to the whole body fat of the neonate. The 3D volume measurements, both of the limb overall and the fat specifically, do tend to show a slightly higher correlation when compared with the 2D areas, but (in a similar way to the fat compared with the limb measurements) this is only a small difference and may not be clinically significant.

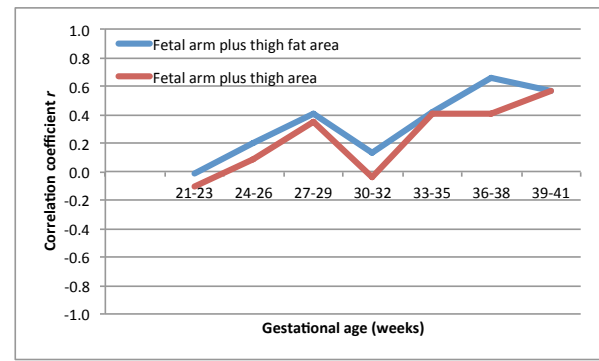
It seemed biologically plausible that combining the arm plus thigh measurements, in 2D and 3D, might better reflect neonatal whole body fat and lead to improved correlation. Scans with results for both arm and thigh were thus assessed [Table 46, Figure 175].

Table 46: Correlation between fetal arm plus thigh measurement (3-week gestational age brackets) and neonatal whole body fat percentage. (Strong correlation coefficients (≥ 0.60) shown in red.)

Gestational age	<i>n</i>	Correlation between fetal arm plus thigh measurement and neonatal whole body fat percentage (coefficient <i>r</i>)			
		Arm plus thigh fat volume	Arm plus thigh volume	Arm plus thigh fat area	Arm plus thigh area
21-23 weeks	37	-0.14	-0.21	-0.01	-0.11
24-26 weeks	34	0.18	0.07	0.20	0.09
27-29 weeks	39	0.32	0.22	0.40	0.35
30-32 weeks	36	0.12	0.02	0.13	-0.05
33-35 weeks	30	0.40	0.38	0.42	0.41
36-38 weeks	25	0.71	0.52	0.65	0.40
39-41 weeks	11	0.57	0.55	0.57	0.56



(a) Fetal arm plus thigh fat volume and volume.



(b) Fetal arm plus thigh fat area and area.

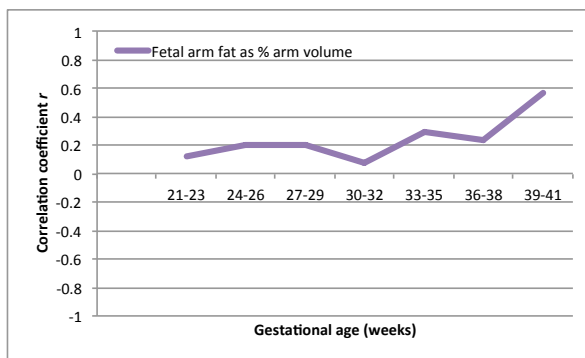
Figure 175: Correlation, expressed as correlation coefficient r , between fetal arm plus thigh measurements (specified above) at differing 3-week gestational age ranges and neonatal whole body fat percentage.

The combination of arm plus thigh data improved the 3D correlations slightly (maximum $r=0.71$ for arm fat volume at 36-38 weeks) but did not improve the 2D correlations.

The analyses were repeated with the percentage fat values [Table 47, Figure 176]. These had been useful for comparing the relative amounts of fat in fetal arms and thighs [Figure 141, section 6b] but otherwise seemed less likely to be clinically relevant as there were relatively small changes in the percentage of fat, within a comparatively narrow range of values, on a week-by-week basis. However, it was possible that the fetal limb fat expressed as a percentage might show an improved correlation with the neonatal whole body fat expressed as a percentage.

Table 47: Correlation between fetal limb fat measurement expressed as a percentage (3-week gestational age brackets) and neonatal whole body fat percentage. (Strong correlation coefficients (≥ 0.60) shown in red.)

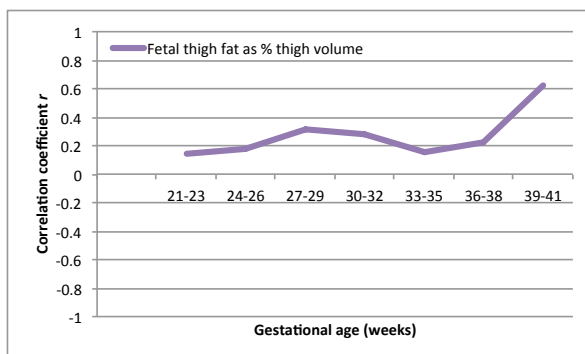
Gestational age	Correlation between fetal limb fat measurements expressed as a percentage and neonatal whole body fat percentage (coefficient r)					
	n	Arm fat % volume	Arm fat % area	n	Thigh fat % volume	Thigh fat % area
21-23 weeks	42	0.12	0.11	43	0.14	-0.31
24-26 weeks	39	0.20	0.16	37	0.18	-0.11
27-29 weeks	40	0.20	0.11	46	0.32	-0.26
30-32 weeks	44	0.07	0.05	45	0.28	0.02
33-35 weeks	49	0.29	0.24	46	0.16	0.02
36-38 weeks	45	0.23	0.20	38	0.22	0.26
39-41 weeks	23	0.56	0.54	13	0.63	-0.18



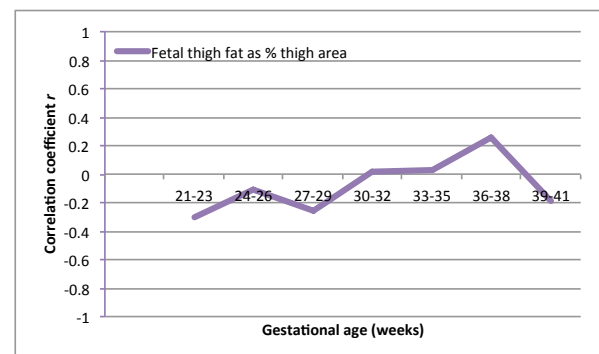
(a) Fetal arm fat as % of arm volume.



(b) Fetal arm fat as % of arm area.



(c) Fetal thigh fat as % of thigh volume.



(d) Fetal thigh fat as % of thigh area.

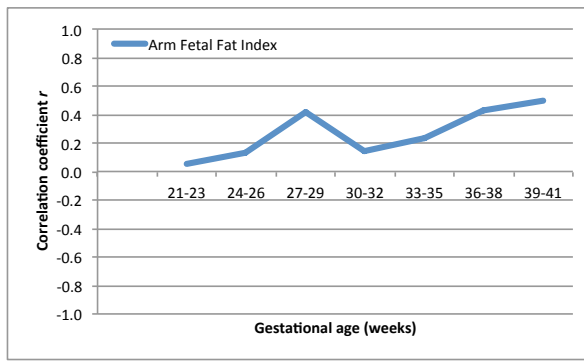
Figure 176: Correlation, expressed as correlation coefficient r , between fetal limb fat expressed as a percentage at differing 3-week gestational age ranges and neonatal whole body fat percentage.

Using the fat expressed as a percentage seemed to “smooth” the graphs for the arm and thigh volume when compared with the raw volume data [Figure 174]. It also resulted in an increasing (maximum) correlation coefficient at 39-41 weeks, whereas the raw data had a reduced correlation. The arm fat area as a percentage had the same smoothing effect, but the thigh fat area as a percentage showed poor correlation across the range of gestational ages, with a negative correlation coefficient at 39-41 weeks. It is not clear why the thigh area percentage should give this result. In all cases, the fat as percentage did not overall improve the correlation (with the exception of 39-41 weeks) when compared with the raw data.

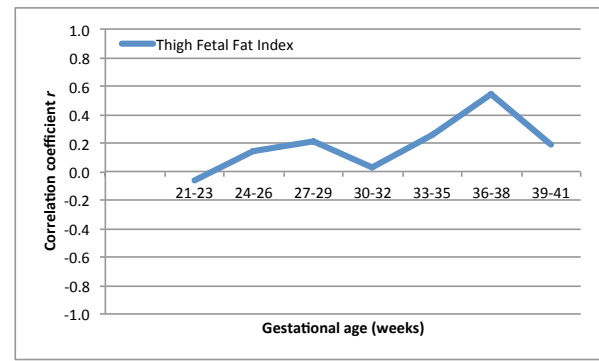
Finally, the Fetal Fat Index of the arm and thigh measurements were correlated with the neonatal whole body fat results [Table 48, Figure 177].

Table 48: Correlation between fetal limb Fetal Fat Index (3-week gestational age brackets) and neonatal whole body fat percentage.

Gestational age	Correlation between fetal limb Fetal Fat Index and neonatal whole body fat percentage (coefficient <i>r</i>)			
	<i>n</i>	Arm FFI (mm)	<i>n</i>	Thigh FFI (mm)
21-23 weeks	41	0.16	44	-0.06
24-26 weeks	39	0.19	34	0.11
27-29 weeks	40	0.39	46	0.21
30-32 weeks	44	0.15	43	0.03
33-35 weeks	49	0.24	47	0.23
36-38 weeks	47	0.44	38	0.56
39-41 weeks	22	0.50	11	0.19



(a) Arm Fetal Fat Index.



(b) Thigh Fetal Fat Index.

Figure 177: Correlation, expressed as correlation coefficient r , between Fetal Fat Index measurements at differing 3-week gestational age ranges and neonatal whole body fat percentage.

The arm FFI showed similar correlation to the arm volume and area (although lower at 36-38 weeks) and slightly better than the limb measurements. The thigh FFI, meanwhile, showed a slightly stronger correlation than fat (2D and 3D) until 27-29 weeks, but weaker than the limb measurements thereafter. Overall, results were generally comparable.

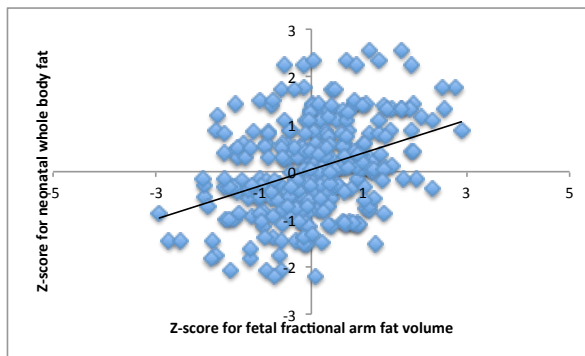
Results and discussion: Z-scores

There is a promising correlation between fat measurement in fetal limbs and neonatal body composition. Nevertheless, it should be noted that although they both involve body fat, the former measures fat area or volume in the periphery (and in only one limb) whilst the latter measures whole body fat as a percentage of whole body mass. It seemed it might be helpful to compare these two types of measurements using Z-scores. These were calculated specific to this study population, using the mean (expected measurement) and standard deviation for each set of measurements. For the antenatal measurements, these were gestational age-specific mean and SD (by day of gestation), as previously calculated.

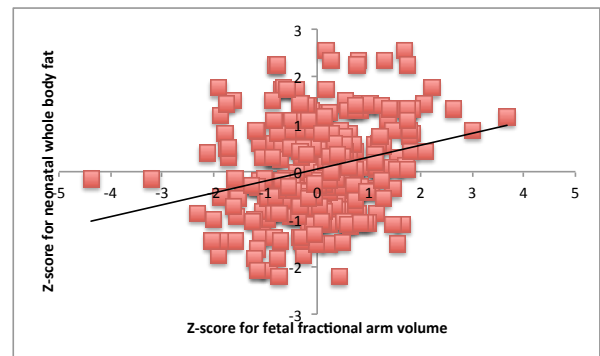
$$\text{Z-score} = (\text{measurement}_{\text{observed}} - \text{measurement}_{\text{expected}}) / \text{SD}$$

As the 3D volume measurements had shown slightly better correlation with the neonatal body fat, when compared with the performance of the 2D measurements, these were converted to Z-scores and used to explore the method.

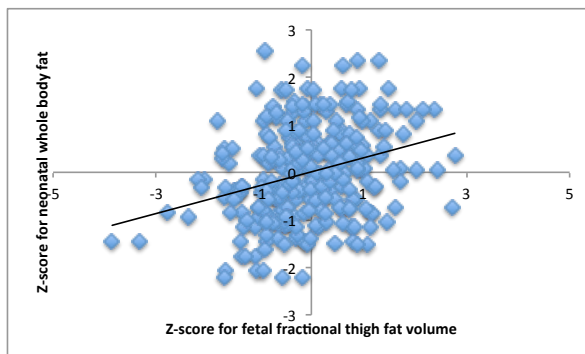
Scatterplots were initially used to visualise the correlations. These were plotted for neonatal whole body fat compared with fetal fractional arm fat volume; arm volume; thigh fat volume; and thigh volume [Figure 178].



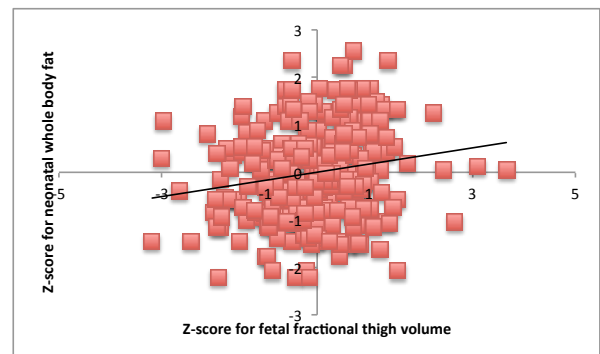
(a) Fetal arm fat volume ($r=0.36$).



(b) Fetal arm volume ($r=0.26$).



(c) Fetal thigh fat volume ($r=0.30$).



(d) Fetal thigh volume ($r=0.17$).

Figure 178: Scatterplots comparing Z-scores of fetal limb measurements with neonatal whole body fat.

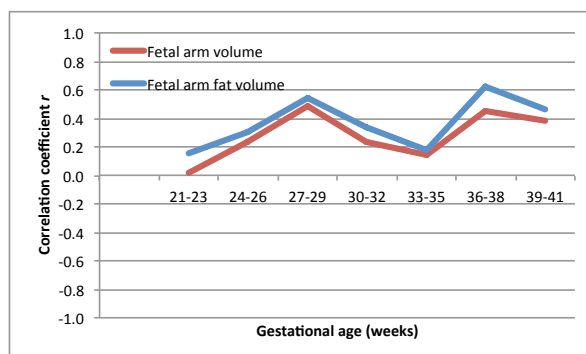
The scatterplots showed a generally positive correlation with overall correlation coefficients, $r=0.36$ (arm fat volume); 0.26 (arm volume); 0.30 (thigh fat volume); 0.17 (thigh volume).

The arm correlation coefficients were thus stronger than the thigh equivalents, but both the fat volume coefficients were stronger than the limb volume coefficients.

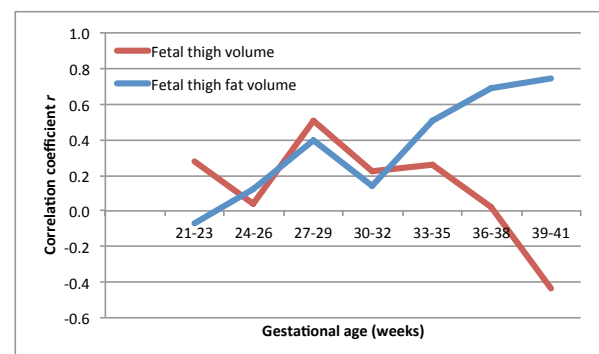
Analyses were then undertaken in a similar way to those for the raw data, above. The same 3-weekly gestational age brackets were used for the correlation [Table 49, Figure 179] so as to enable comparison with the raw data tables and graphs.

Table 49: Correlation between fetal arm and fetal thigh measurements, and neonatal whole body fat percentage. (Strong correlation coefficients (≥ 0.60) shown in red.)

Gestational age	Correlation between fetal arm and thigh measurements and neonatal whole body fat percentage (coefficient r), with measurements expressed as Z-scores					
	n	Arm fat volume	Arm volume	n	Thigh fat volume	Thigh volume
21-23 weeks	37	0.15	0.01	43	-0.07	0.27
24-26 weeks	34	0.30	0.23	37	0.12	0.04
27-29 weeks	39	0.54	0.49	46	0.40	0.50
30-32 weeks	36	0.33	0.24	45	0.14	0.22
33-35 weeks	30	0.18	0.14	46	0.50	0.26
36-38 weeks	25	0.62	0.45	38	0.69	0.02
39-41 weeks	11	0.46	0.38	13	0.74	-0.44



(a) Fetal arm fat volume and arm volume.



(b) Fetal thigh fat volume and thigh volume.

Figure 179: Correlation, expressed as correlation coefficient r , between Z-scores of fetal limb volumes (specified above) at differing 3-week gestational age ranges and Z-scores of neonatal whole body fat %.

The correlation between Z-scores of fetal arm fat and neonatal fat showed a similar pattern to the raw 3D data, although with stronger correlation at 27-29 weeks and a correspondingly steeper 'fall' in correlation to 33-35 weeks. The peak correlation coefficient, at 36-38 weeks, was similar to that of the 3D data ($r=0.62$ Z-score; 0.67 raw data).

The correlation between Z-scores of fetal thigh fat and neonatal fat showed a similar pattern to the raw 3D data for the thigh fat volume, with a peak correlation coefficient, at 39-41 weeks, similar to but, unlike the arm data, slightly stronger than that of the 3D data ($r=0.74$ Z-score; 0.60 raw data). The coefficient at 36-38 week also showed a stronger correlation ($r=0.69$ Z-score; 0.58 raw data).

However, the correlation with fetal thigh volume and neonatal fat Z-scores showed an inconsistent pattern between 21 to 32 weeks, including a drop in correlation at 24-26 weeks which was not seen with the raw data, and followed by a falling strength of correlation at the end of the third trimester, ending with a negative correlation $r=-0.44$. It is not clear why Z-score analyses of the data should have revealed this pattern; it was not seen with the raw data and does not make biological sense. Review of the Z-scores and calculations did not reveal any anomalous values which might explain the result. The 39-41 week group was smaller than the other groups, but this was also the case for the arm data which showed continuing parallel correlations between the arm volume-neonatal and arm fat volume-neonatal Z-score results.

Z-score analyses did not markedly improve the correlation between the fetal limb ultrasound measurements and the neonatal whole body fat measurements, so were not explored further (for example, for the 2D measurements).

Conclusions

Measurements of fat in the fetal arm and thigh – including fractional fat volume and fractional limb volume, fat area and limb area, and Fetal Fat Index – show varying strengths of correlation with neonatal whole body fat. This was explored in various gestational age brackets, although the numbers were too small to enable weekly correlations, which would have been the most clinically useful. The strength of correlation increases with increasing gestational age, with the strongest correlation seen in the late third trimester ($r=0.67$, arm fat volume at 36-38 weeks). The 3D volumes generally showed a more reliable increase in correlation, whereas the 2D areas had more variable measurements.

Alternative approaches were explored, however combining arm and thigh fat measurements together (in 3D and 2D); using the fat expressed as a percentage of the limb measurement (in 2D and 3D); and translating both the volume data and the body composition data into Z-scores (3D only) brought slight increases in correlation of individual measurements at late gestation but did not markedly change the correlations or the patterns with gestational age.

7e. Comparison of antenatal limb fat volume and area with neonatal anthropometry

i Comparison of fetal arm measurements and neonatal mid-upper arm circumference

Aim

To compare antenatal ultrasound findings with neonatal anthropometry, to see whether antenatal limb fat could be used to distinguish between babies with above or below mean limb circumferences.

Background

Neonatal anthropometric measurements, including mid-upper arm circumference (MUAC) and mid-thigh circumference (MTC), have been shown to be helpful measurements for identifying low birthweight babies in developing countries, where scales may not be available (see section 1i). Such studies, however, tend to be in developing countries and thus their values are unlikely to be applicable to an optimally healthy population in a developed country.

A paper from the WHO¹⁶⁷ was used as a reference. This listed mean MUAC at birth for 2091 infants in four European cities (Istanbul, Turkey; St Petersburg, Russia; Szeged, Hungary; and Yerevan, Armenia), within a total 7924 infants from 22 cities around the world (also including Asia, Africa, Middle East and Latin America). The paper did not assess MTC. The mean MUAC for infants in the European centres was 10.95cm. This resulted in 29/58 (50%) of the INTERGROWTH-21st infants being below the mean, and 29/58 above, which suggested this fitted our study population well.

Method

Infants from the INTERGROWTH-21st study population who had undergone additional neonatal anthropometric measurements at birth were divided into two groups by their MUAC: above and below mean (10.95cm). Within each group, antenatal arm ultrasound segmentation results were then identified within three 6-week gestational age ranges: 36-41 weeks (late third trimester / last scan), 30-35 weeks (likely period for a third trimester growth scan), and 24-29 weeks (late second / early third trimester). Only one scan per fetus was used within each gestational age group; if two scans were available then the later was used. The arm volumes outlined in section 5c were used for these analyses, i.e. after exclusion of inadequately rotated volumes.

Results

58 INTERGROWTH-21st infants had anthropometry, including MUAC, measurements taken shortly after birth. Of these, 55 had arm volume segmentation results within one, two or three of the gestational age ranges for analysis, whilst 3 did not have any arm volume results available within those ranges. A total of 121 scans were included in the analysis: 63 in the above mean MUAC, and 58 in the below mean MUAC. The number of available results varied within each gestational age group; all of the gestational age groups involved at least 50% of all possible (55) fetuses.

An initial comparison of the groups was done in terms of gestational age at scan, to ensure that the groups were comparable and that any difference shown could not be attributed to gestational age differences. T-tests showed no statistically significant differences between the groups [Table 50].

Table 50: Gestational age range groups for comparison by neonatal MUAC.

	Below mean MUAC: gestational age of fetuses	Above mean MUAC: gestational age of fetuses	t-test to compare gestational ages of fetuses in groups
24-29 weeks	mean 27^{+0} ($24^{+2} - 29^{+4}$) n=19	mean 27^{+2} ($24^{+0} - 29^{+6}$) n=26	$p=0.595$
30-35 weeks	mean 32^{+6} ($30^{+0} - 35^{+6}$) n=22	mean 33^{+4} ($30^{+6} - 35^{+6}$) n=23	$p=0.142$
36-41 weeks	mean 38^{+5} ($36^{+1} - 40^{+4}$) n=17	mean 38^{+2} ($36^{+0} - 40^{+4}$) n=14	$p=0.371$

The mean value for each parameter (arm and fat volume; arm and fat area) was calculated within each gestational age group. These values were compared for the fetuses above and below the mean MUAC, using a t-test [Table 51, Table 52].

Table 51: Comparison of fetal fat and arm volumes at different gestational age ranges from infants with above or below mean MUAC (significant t-test p values <0.05 shown in red).

	Mean antenatal arm fat volume (cm ³)		Mean antenatal arm volume (cm ³)	
	Below mean MUAC	Above mean MUAC	Below mean MUAC	Above mean MUAC
24-29 weeks	4.0cm ³	4.6cm ³	7.6cm ³	8.7cm ³
	<i>p</i> =0.145		<i>p</i> =0.078	
30-35 weeks	9.9cm ³	12.2cm ³	16.7cm ³	20.2cm ³
	<i>p</i> =0.038		<i>p</i> =0.021	
36-41 weeks	17.1cm ³	19.3cm ³	27.9cm ³	30.8cm ³
	<i>p</i> =0.072		<i>p</i> =0.089	

Table 52: Comparison of fetal fat and arm areas at different gestational age ranges from infants with above or below mean MUAC (significant t-test p values <0.05 shown in red).

	Mean antenatal arm fat area (cm ²)		Mean antenatal arm area (cm ²)	
	Below mean MUAC	Above mean MUAC	Below mean MUAC	Above mean MUAC
24-29 weeks	0.17cm ²	0.18cm ²	0.34cm ²	0.37cm ²
	<i>p</i> =0.641		<i>p</i> =0.251	
30-35 weeks	3.46cm ²	4.16cm ²	6.18cm ²	7.19cm ²
	<i>p</i> =0.049		<i>p</i> =0.023	
36-41 weeks	5.39cm ²	6.26cm ²	9.16cm ²	10.21cm ²
	<i>p</i> =0.009		<i>p</i> =0.029	

At 24-29 weeks there is no statistically significant difference between any of the measurements. Even at this early gestation, though, each of the arm parameters is smaller in the below mean MUAC group.

At 30-35 weeks there is a statistically significant difference between all of the measurements, suggesting that either 2D or 3D measurements of the arm or its fat may be able to distinguish babies which may be of high or low birthweight.

At 36-41 weeks, the mean measurements in below average MUAC were still smaller than those with above average MUAC, but this was no longer statistically significant for fetal arm volume or fetal arm fat volume, most likely due to the smaller sample size. Measurement difficulties in the third trimester, where scans can be harder to segment with increasing artefacts, may have also been a factor.

Statistically significant differences are retained in the fetal arm area and fetal arm fat area measurements; it may thus be the case that, rather than making the differences more pronounced by using more detailed measurements, as we had hoped, the volumes are actually making the measurements less precise (possibly through the contributory and additive effects of measurement errors in each slice).

Conclusion

This work provides preliminary evidence that arm, and arm fat, areas and volumes may be able to distinguish fetuses who will ultimately have large or small MUAC. It also suggests that 2D fetal measurements may be more useful than 3D.

ii. Comparison of fetal thigh measurements and neonatal mid thigh circumference

Aim and Method

There was no published material to provide a reference for developed country neonatal mid-thigh circumferences (MTC) at birth; however, as the WHO¹⁶⁷ reference had divided this thesis' arm circumferences into two even groups, it seemed plausible to use the mean MTC of this thesis' results (15.23cm) to generate two groups for comparison. The aims and method were as described above, but with fetal thigh measurements and neonatal MTC.

Results

There were no differences in the gestational ages of the groups [Table 53].

Table 53: Gestational age range groups for comparison by neonatal MUAC.

	Below mean MTC: gestational age of fetuses	Above mean MTC: gestational age of fetuses	t-test to compare gestational ages of fetuses in groups
24-29 weeks	mean 27^{+1} ($24^{+2} - 29^{+4}$) n=20	mean 27^{+1} ($25^{+0} - 29^{+6}$) n=22	$p=0.928$
30-35 weeks	mean 33^{+3} ($30^{+3} - 35^{+4}$) n=18	mean 33^{+1} ($30^{+3} - 35^{+5}$) n=20	$p=0.565$
36-41 weeks	mean 37^{+5} ($36^{+0} - 40^{+3}$) n=13	mean 38^{+2} ($36^{+5} - 39^{+5}$) n=10	$p=0.438$

There were no significant differences between any of the thigh area or volume measurements, apart from the fetal thigh fat area at 36-41 weeks [Table 54, Table 55].

Table 54: Comparison of fetal fat and thigh volumes at different gestational age ranges from infants with above or below mean MTC .

	Mean antenatal thigh fat volume (cm ³)		Mean antenatal thigh volume (cm ³)	
	Below mean MTC	Above mean MTC	Below mean MTC	Above mean MTC
24-29 weeks	7.3cm ³	7.2cm ³	16.9cm ³	17.2cm ³
	<i>p</i> =0.941		<i>p</i> =0.796	
30-35 weeks	18.5cm ³	19.5cm ³	39.3cm ³	39.7cm ³
	<i>p</i> =0.590		<i>p</i> =0.881	
36-41 weeks	29.3cm ³	33.3cm ³	57.7cm ³	63.5cm ³
	<i>p</i> =0.136		<i>p</i> =0.262	

Table 55: Comparison of fetal fat and thigh areas at different gestational age ranges from infants with above or below mean MTC (significant t-test p values <0.05 shown in red).

	Mean antenatal thigh fat area (cm ²)		Mean antenatal thigh area (cm ²)	
	Below mean MTC	Above mean MTC	Below mean MTC	Above mean MTC
24-29 weeks	2.92cm ²	2.96cm ²	6.84cm ²	7.04cm ²
	<i>p</i> =0.902		<i>p</i> =0.619	
30-35 weeks	5.97cm ²	6.45cm ²	13.10cm ²	13.34cm ²
	<i>p</i> =0.380		<i>p</i> =0.785	
36-41 weeks	8.68cm ²	10.22cm ²	17.77cm ²	19.47cm ²
	<i>p</i> =0.042		<i>p</i> =0.242	

Conclusion

Fetal thigh, and thigh fat, measurements are larger in the above average neonatal MTC group at all gestational ages (except fat volume, 24-29 weeks), but the differences are small and not statistically significant. (One borderline result at 36-41 weeks is likely to have arisen by chance.) Therefore, thigh measurements do not distinguish fetuses who will have large or small neonatal MTC; the arm assessments (section 7d.i) seem more useful.

7f. Comparison of fetal limb fat at 34 weeks with neonatal body and limb fat (DXA)

Aim

To compare and correlate fetal arm and thigh volumes and areas (fat and limb), measured at the 34-week MAVIDOS scan, with neonatal body composition data (whole body, arm and leg) measured using DXA.

Methods

Women taking part in the MAVIDOS trial underwent an ultrasound scan at 34 weeks; in addition to the standard growth scan, we captured arm and thigh volumes so as to be able to measure fetal limb fat. These were segmented by the author and K.E..

After the babies were born, they underwent DXA body composition analysis (see section 7b); those more than 3 weeks old at measurement were excluded from this analysis.

Correlations were undertaken between the neonatal whole body and limb fat, and the fetal limb fat measurements. The DXA machine, and the measurement approach, was changed half way through the study, which unfortunately resulted in small sample sizes (different DXA machine results are not interchangeable¹⁹¹).

Results

A total of 33 babies had whole body composition measurements with DXA and a 34-week scan with limb volume acquisition using 3D ultrasound [Table 56-Table 58].

In 11 babies these measurements were done using the Hologic Discovery A DXA machine. In 1 scan an arm volume had been captured but not a thigh volume; the remaining 10 had both. Segmentation, and one exclusion, gave a final dataset of 8 arm volumes and 6 thigh volumes.

The other 22 babies had whole body composition measurements using the GE Lunar iDXA machine. Of these, 18 fetal thigh volumes and 15 fetal arm volumes were available for assessment – the remainder were missing scans (either due to the scans not being obtained, being obtained incorrectly, or being saved in an incorrect file format). Segmentation, and three exclusions, gave a final dataset of 11 arm volumes and 11 thigh volumes. Within these, there was a subset where DXA subanalysis was possible: 7 arm volumes and 8 thigh volumes.

Table 56: Correlation between fetal limb measurements at 34 weeks and neonatal whole body fat, measured by Hologic Discovery A DXA machine.

Hologic Discovery A DXA (whole body fat)	Correlation coefficient, <i>r</i>	
	Arm (<i>n</i> =8)	Leg (<i>n</i> =6)
Fetal limb fat volume (cm ³) vs. neonatal whole body fat (%)	-0.06	-0.24
Fetal limb fat volume (%) vs. neonatal whole body fat (%)	0.58	-0.36
Fetal limb volume (cm ³) vs. neonatal whole body fat (%)	-0.20	-0.14

Table 57: Correlation between fetal limb measurements at 34 weeks and neonatal whole body fat, measured by GE iDXA machine.

GE Lunar iDXA (whole body fat)	Correlation coefficient, <i>r</i>	
	Arm (<i>n</i> =11)	Leg (<i>n</i> =11)
Fetal limb fat volume (cm ³) vs. neonatal whole body fat (%)	0.13	0.28
Fetal limb fat volume (%) vs. neonatal whole body fat (%)	0.37	0.10
Fetal limb volume (cm ³) vs. neonatal whole body fat (%)	-0.01	0.37

Table 58: Correlation between fetal limb measurements at 34 weeks and neonatal limb fat, measured by GE iDXA machine.

GE Lunar iDXA (limb fat)	Correlation coefficient, <i>r</i>	
	Arm (<i>n</i> =7)	Leg (<i>n</i> =8)
Fetal limb fat volume (cm ³) vs. neonatal limb fat (%)	-0.02	0.32
Fetal limb fat volume (%) vs. neonatal limb fat (%)	0.41	0.23
Fetal limb volume (cm ³) vs. neonatal limb fat (%)	-0.20	0.52

Discussion and conclusion

Overall it is not possible to discern a pattern of correlation between fetal fat measurements by ultrasound at 34 weeks and neonatal fat measurements by DXA; this is probably due to the small sample sizes. These arose through a combination of factors, but were primarily due to the change in DXA machines during the MAVIDOS study.

7g. The effect of fetal sex upon fractional arm and thigh fat and lean volumes

Aim

To assess whether being of male or female sex results in different fetal arm and/or thigh fat or lean volumes.

Background

PEA POD body composition data has shown varying results as to whether there are sex differences in infant whole body fat percentages. Some studies have shown that female infants have a significantly higher percentage fat mass compared to male infants at birth,²²¹ at a month of age,²⁵⁵ and at 4.5 months of age;²⁵⁷ whilst others have shown a tendency for female infants to have more fat, but not statistically significant, within the first 72 hours,²⁶¹ and at 1 week.²⁵⁸ Others have shown no difference between male and female infant fat in the first four months of life,²⁶⁰ and no difference at 6 months of age.²⁵⁵ Fields et al produced sex-specific mean +/- 2SD curves for PEA POD fat mass from birth to 4 months, with female infants having higher values throughout.²⁵⁶ Meanwhile, a study on Ethiopian infants by Andersen et al showed that girls had a lower birthweight and fat-free mass, but a similar fat mass, to boys.²⁵⁹

Based on this varying data, I thought it would be interesting to assess whether possible sex differences in whole body fat might be due to differences in fetal limb fat, whilst central fat (not assessed in this thesis), which includes brown fat (see section 1g.i) might be relatively constant between the sexes.

Methods

The data used presented in section 5c (arms) and 6b (thighs) were divided into two groups based on the sex of the fetuses, documented at birth. The data points were displayed as scatterplots, and compared using t-tests to ascertain whether there was a difference in fat volume between male and female fetuses. The data was acquired and measured in a blinded fashion i.e. fetal sex was not known at the time of image acquisition or segmentation.

Results

There were a small number of data points excluded from this analysis due to women withdrawing from the study: 3 results from 3 women (arms) and 4 results from 3 women (thighs). In these cases, the infant's sex was unknown.

The fractional arm fat volumes from female fetuses had 240 data points from 101 women; male infants had 258 data points from 101 women [Figure 180]. There was no difference between the fractional arm fat volume between male and female fetuses (t-test: $p=0.46$).

The fractional thigh fat volumes from female fetuses had 245 data points from 111 women; male fetuses had 255 data points from 106 women [Figure 181]. There seemed to be a statistically significant difference between the fractional thigh fat volume between male and female fetuses (t-test: $p=0.04$), but on looking at the graph it was evident that the two scatterplots looked very similar apart from two (male) outliers at very late gestational ages which, given the smaller sample size, might have skewed the results. When these were removed, there was no significant difference between the two groups (t-test: $p=0.07$).

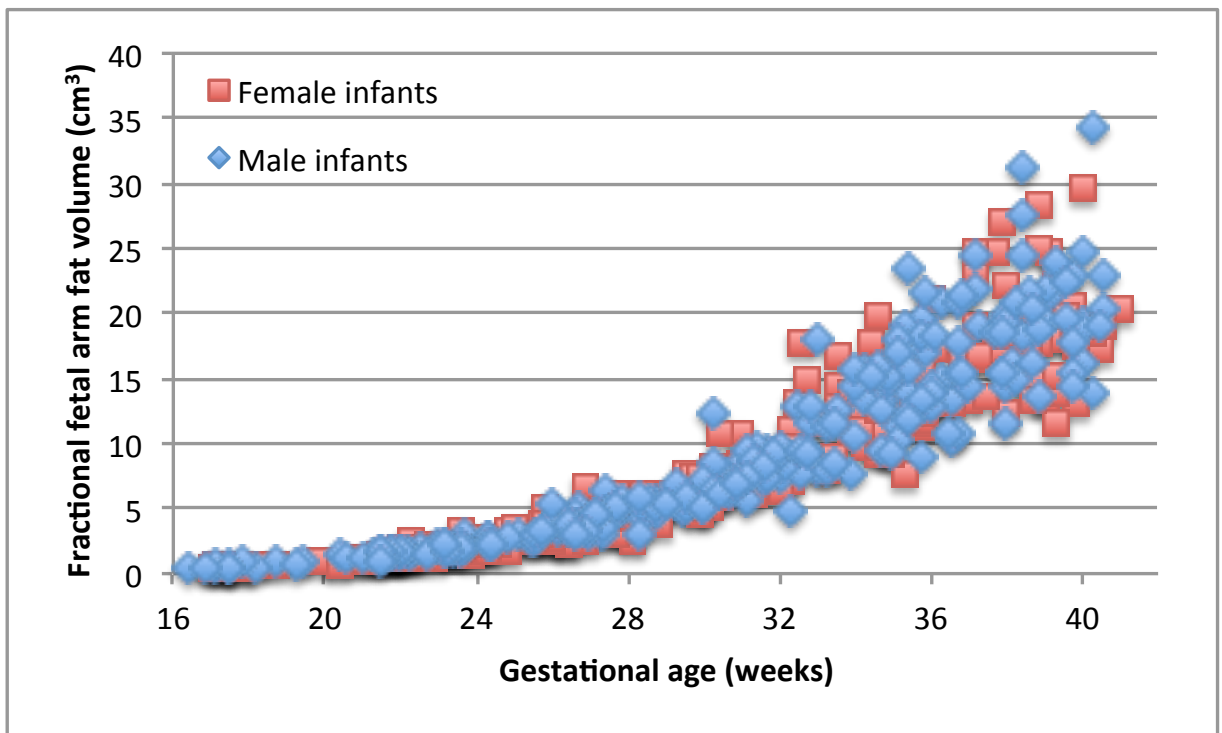


Figure 180: Fractional fetal arm fat volumes of male and female fetuses.

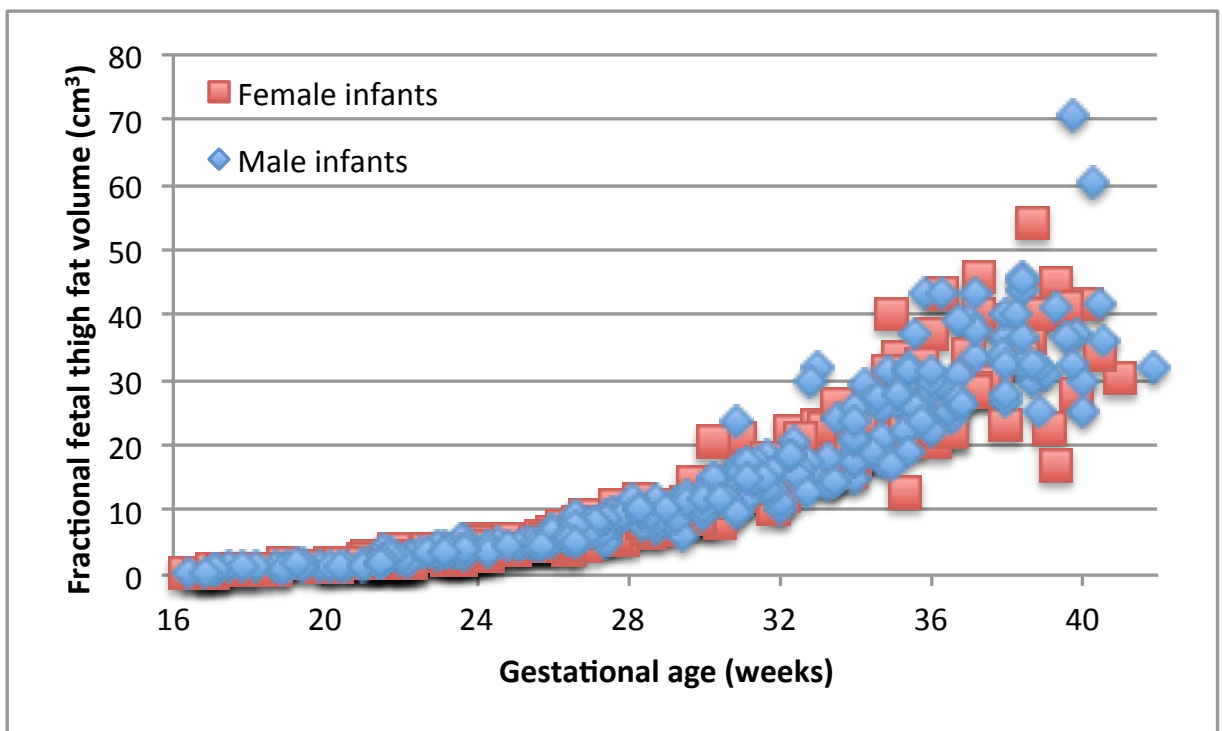


Figure 181: Fractional fetal thigh fat volumes of male and female fetuses.

Andersen et al²⁵⁹ had suggested there might be differences in the fat free, rather than fat, mass between male and female infants, so I also analysed fetal arm and thigh lean volumes:

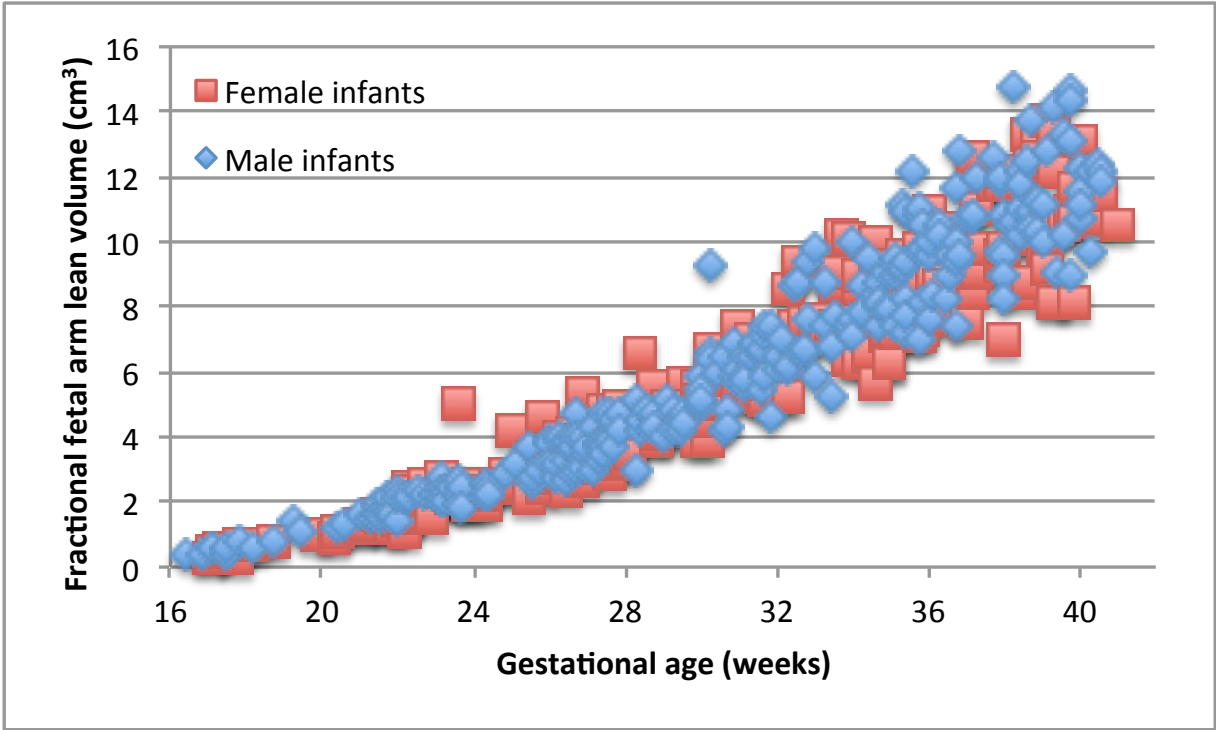


Figure 182: Fractional fetal arm lean volumes of male and female fetuses.

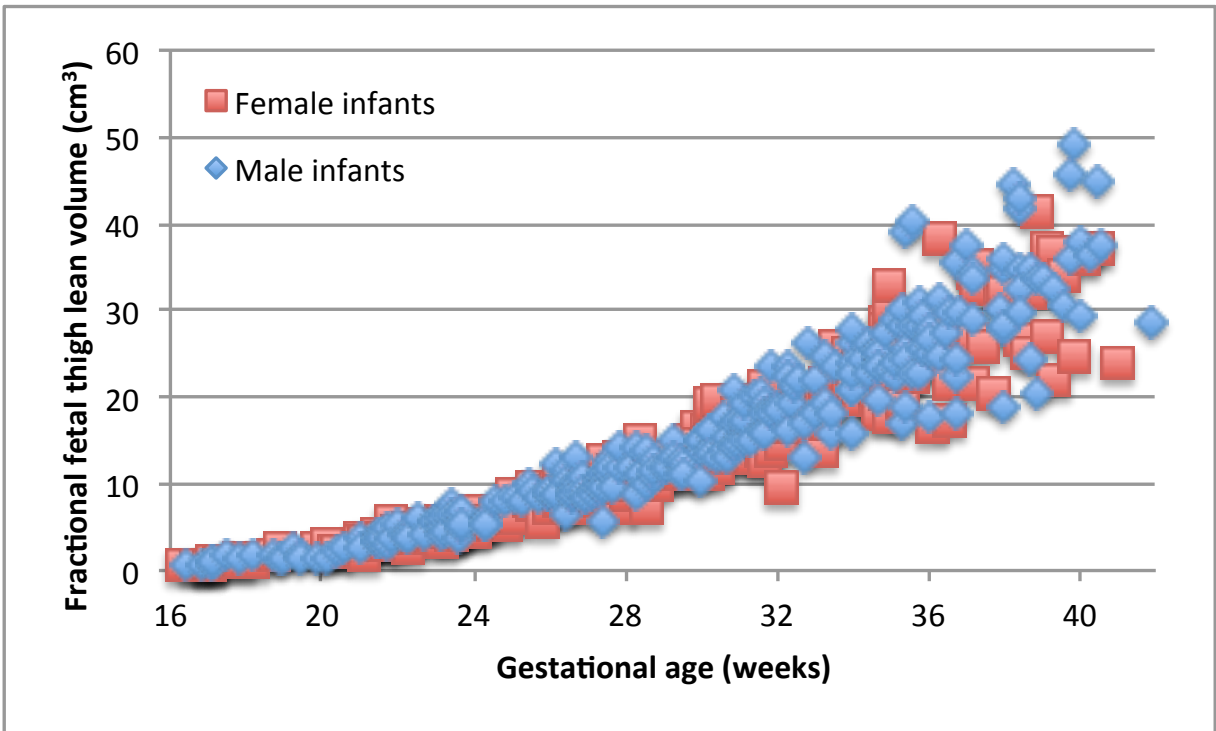


Figure 183: Fractional fetal thigh lean volumes of male and female fetuses.

The scatterplots for fractional fetal arm lean volume [Figure 182] and thigh lean volume [Figure 183] did not show any obvious visual differences.

There were no statistically significant differences between the arm lean volumes in male and female fetuses (t-test: $p=0.197$).

Comparison of the thigh lean volumes, however, produced a statistically significant difference (t-test: $p=0.005$). This was present irrespective of the presence or exclusion of the two male fetuses with outlier thigh fat volumes, whose volumes were likely to be inaccurate. This significant difference is not evident from the scatterplot. All of the plots seem to show a wider range for the data points from the female fetuses compared with those from the male fetuses, but the thigh lean plot does not seem to be notably different to any of the others. It may be a chance finding which has occurred within multiple comparisons within the dataset.

Contrary to my initial supposition, the antenatal limb fat measurements do not show any differences between male and female fetuses. It is thus possible that variations in neonatal body fat percentages between male and female infants (PEA POD results, section 7d) are due to differences in central (including brown) fat.

Conclusion

There were no significant differences in the antenatal limb fat volumes between male and female fetuses in the study population.

7h. Assessing fetal thigh fat in SGA and AGA fetuses

i. Feasibility study: sagittally-acquired thigh volumes

Aim

To assess the feasibility of fat measurement in sagittally-acquired thigh volumes which were not obtained specifically for fat volume assessment.

Background and Method

A Dutch medical student, E.G., who visited the Nuffield Department of Obstetrics and Gynaecology in Oxford, was measuring fractional thigh volumes (TVol) as part of a research component of her medical degree, supervised by M.R., a Dutch postdoctoral research fellow. The thigh volumes had been obtained on the Thai-Burmese border, using INTERBIO-21st scanning protocols.

I provided quality assurance, and enabled assessment of inter-observer variability, by assessing, and where possible, segmenting, every tenth scan from the study. TVol was measured using the commercially-available 4Dview tool (GE Healthcare, Amersham, UK).

During these measurements, I also visually assessed the likelihood of being able to segment the thigh fat within the volumes. The volumes had all been acquired sagittally, as per the INTERGROWTH-21st and INTERBIO-21st ultrasound protocols, and this orientation could have had an impact upon the quality of the reconstructed axial slices.

Results and discussion

There were 1141 thigh volume scans in the original cohort; I assessed 129 for quality assurance purposes.

Of the 129 scans, I felt it was only possible to measure TVol in 87 (67%), with the majority of those rejected due to a poorly defined thigh outline, and a minority due to an incomplete volume. Of the 87, I felt that it would probably have been possible to segment the fat in 43 of the 87 (49% of the 87 TVol-segmentable, but only 33% of the 129 original) [Figure 184], and that a further 6 might have been possible (taking the potential total to 49/87 or 56%; or 38% of the 129 original).

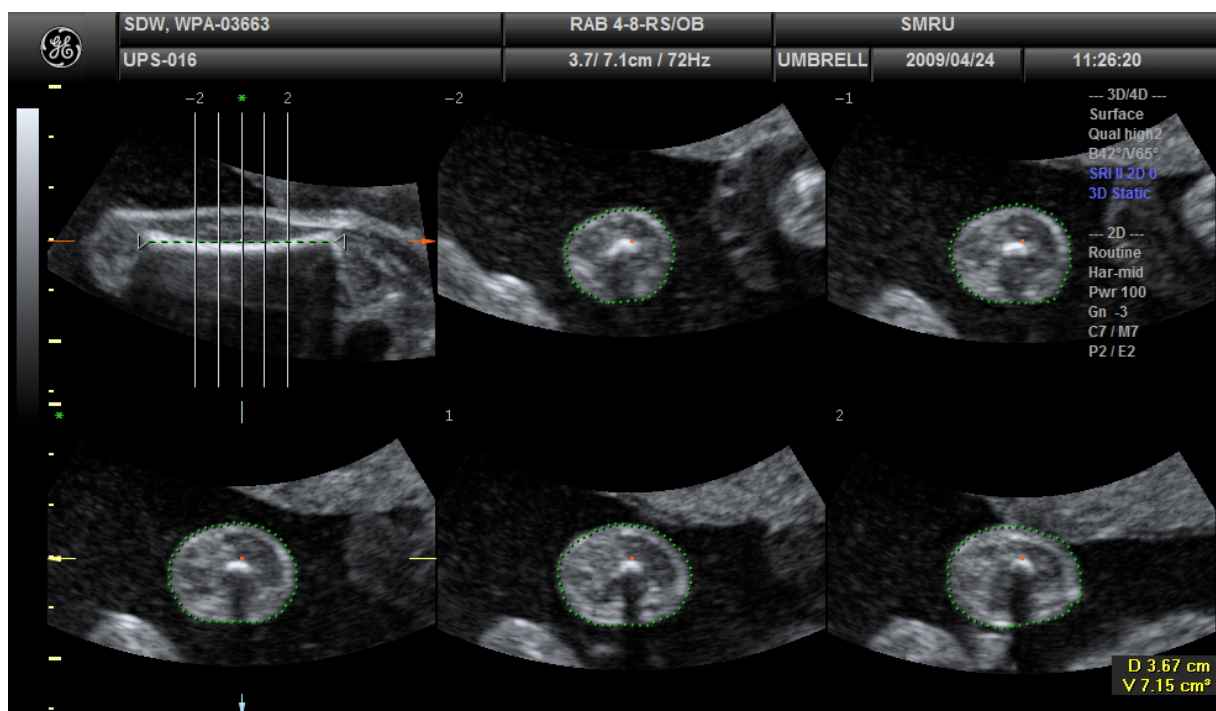


Figure 184: Sample fractional thigh volume (sagittally-acquired) where it would have been possible to segment the fat.

The main reasons why scans would have been rejected was that the fat was indistinct or not clearly visible [Figure 185]

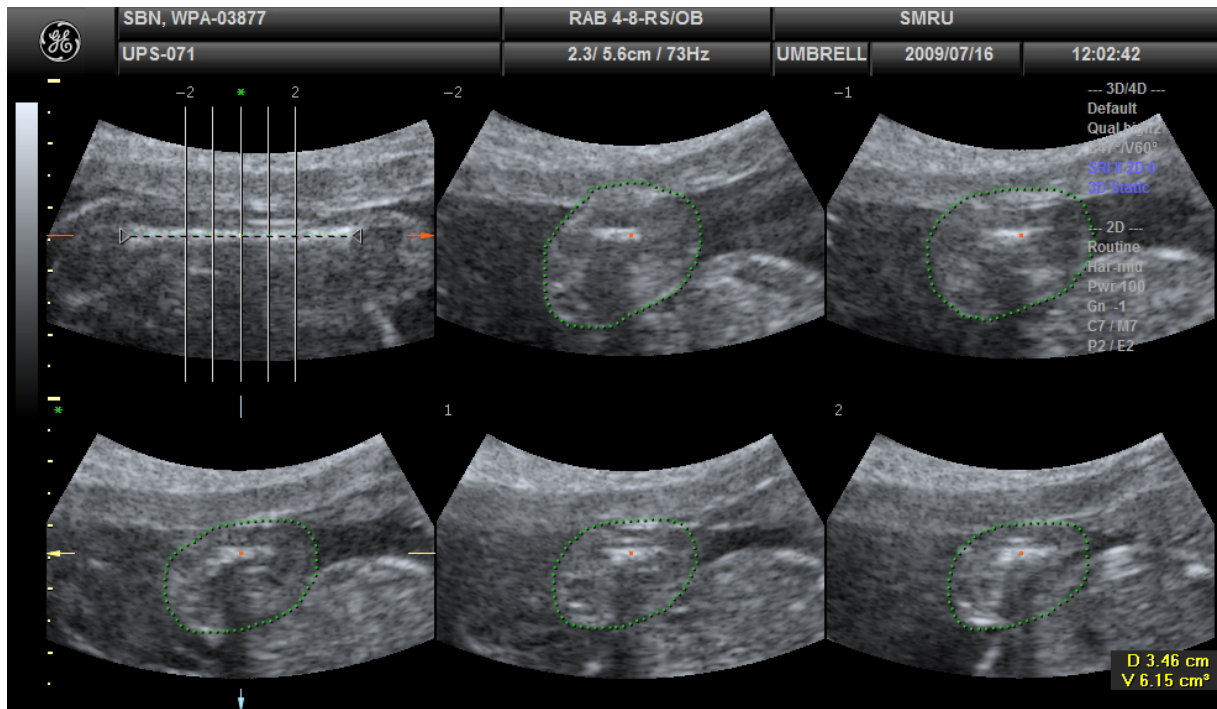


Figure 185: Sample fractional thigh volume (sagittally-acquired) where it would not have been possible to segment the fat.

Conclusion

This work showed that it was feasible to aim to measure thigh fat in sagittally-acquired thigh volumes, and in those which had not been acquired for the primary purpose of fat measurement – for example, volumes from the worldwide INTERGROWTH-21st participants. The likely low segmentation rate, however, demonstrated the importance of having a reasonable number of subjects for any future research, so as to ensure an adequate number of results once those which were unable to be segmented had been excluded.

ii INTERGROWTH-21st: SGA vs. AGA

Aim

To assess the performance of thigh fat volume measurements, acquired as part of the standard INTERGROWTH-21st protocol, in distinguishing SGA and AGA fetuses. (Arm volumes, though acquired for work specific to this thesis, were not routinely obtained within the INTERGROWTH-21st project, so were not available for analysis.)

Method

The INTERGROWTH-21st database was assessed for pairs of “cases” and “controls”. Cases were babies who were delivered at 36⁺⁰ to 41⁺⁶ weeks of gestation and whose birthweight was below the 5th centile, as calculated using the entire FGLS cohort within INTERGROWTH-21st. Controls were the next baby born at the same gestational age (completed weeks) but whose birthweight was within the 10th-90th centiles.

The gestational age at each INTERGROWTH-21st scan for each participant was assessed. A target gestational age range was identified, based upon the likely gestational age of a third trimester growth scan [Table 59]. Pairs of cases and controls, who both had a scan within this gestational age range, were identified, and they were included if the two scans fell within 14 days of each other. This was chosen as a limit as growth is not re-measured in an individual fetus at less than 14 days; thus differences between the pairs of fetuses should be able to be ascribed to the actual difference in their size, rather than the differences due to their gestational ages.

Scan volumes were then retrieved from the INTERGROWTH-21st database for both cases and controls, and the subjectively best thigh volume (sagittally acquired) chosen. These volumes were converted to enable assessment by the MATLAB tool, and the fat was segmented by the author, M.A., K.E. and V.D., blinded as to which were cases and which were controls.

Results and Discussion

Information was gathered on ultrasound scanning practice in developed countries. Seven countries were identified as routinely offering a third trimester growth scan [Table 59]. Based upon this, a gestational age range of 30-34 completed weeks was chosen for analysis.

Table 59: Countries offering a routine third trimester ultrasound scan.

Country	Gestation (weeks)	Reference
Austria	30	EUROCAT 2009 ²⁶²
Belgium	30-32	EUROCAT 2009, ²⁶² FMF Belgium ²⁶³
Croatia	28-32	EUROCAT 2009 ²⁶²
France	32	EUROCAT 2009 ²⁶²
Germany	29-32	BZgA (Federal Centre for Health Education) 2010 ²⁶⁴
Italy	30-34	EUROCAT 2009 ²⁶²
Spain (Catalonia)	30-35	EUROCAT 2009, ²⁶² Sokol 2009 ²⁶⁵
Australia, Canada, Denmark, Finland, Ireland, Malta, Netherlands, New Zealand, Norway, Sweden, Switzerland, UK and USA do not recommend routine third trimester ultrasound scan although these may be carried out in private practice ^{262, 266-268}		

Analysis of the INTERGROWTH-21st database identified 179 pairs of SGA-AGA babies. Of the 179 pairs, 156 had scans within the 30-34 week range; of these, 122 had scans within 14 days of each other.

Unfortunately, due to issues with the INTERGROWTH-21st database, and despite repeated attempts, only some of the scans could be retrieved: ultimately, only 73 pairs. A 7 further pairs of scans were retrieved from the local Oxford database, resulting in a cohort of 80 pairs of scans [Table 60]. These volumes were then segmented by the author, M.A., K.E. and V.D.

Table 60: INTERGROWTH-21st countries of origin of scans used for SGA-AGA study.

Country	SGA-AGA pairs, 30-34 weeks, scans originally taken ≤14 days apart	Of these SGA-AGA pairs, those where scans were available for segmentation
01 – Brazil	0	0
02 – China	7	6
04 – India	33	20
05 – Kenya	27	14
06 – Oman	31	27
07 – UK	12	7
08 – USA	4	0
09 - Italy	8	6
Total	122	80

Of the 80 pairs where scans were available to measure: 45 pairs were able to be segmented; 20 of the pairs had one scan being able to be measured but the other not; and in 15 pairs neither scan was able to be measured. This was an overall segmentable rate of 56%, which was higher than anticipated, given these thigh volumes were not acquired specifically with the aim of measuring fat. This rate was also better than had been anticipated from the feasibility experiment (section 7h.i), which had suggested that, at best, 38% might be able to be measured.

The majority of the scans which could not be measured were due to an incomplete volume capture, whereby the thigh fat was missing from the edge of the images (31/50). The next most common issue was with difficulties visualising the outer +/- inner border of the fat (10/50), with the remaining images due to varying reasons (incomplete capture of sagittal femur length; blurry image; or reason not recorded).

The gestational age range was 30⁺¹ to 34⁺⁴, with a median of 32⁺³ weeks. The mean difference between the pairs of scans was 0.34 weeks, or 2.4 days (range 0-14 days).

Table 61: Differences in thigh measurements between SGA and AGA fetuses, with scans within 14 days of each other (n=45 pairs). Statistically significant results ($p < 0.05$) are shown in red.

2D	<i>t-test p value</i>	3D	<i>t-test p value</i>
Thigh fat area	0.008	Thigh fat volume	0.002
Thigh lean area	0.172	Thigh lean volume	0.016
Thigh area	0.026	Thigh volume	0.003
Fat as % area	0.100	Fat as % volume	0.142
Femur length	0.010	-----	

These results showed a statistically significant difference between the cases and controls for all parameters apart from thigh lean area and the percentage fat parameters.

As previous data have suggested that it is the fat component that varies, rather than the lean component,⁹⁵ it did not seem surprising that there was no significant difference in lean areas between the two groups. It is more surprising that the lean volume did show a difference between the groups. It may reflect the ability of volumetric measurements to distinguish finer details between subjects' biometry. The femur length, as might have been expected, was shorter in the SGA fetuses when compared with the AGA fetuses (mean 57.6mm vs. 59.4mm).

These data may suggest that the fat component has greater changes in SGA fetuses when compared with AGA controls, but is not definitive. It is also important to note that these were SGA, but not necessarily FGR, fetuses. The latter may have shown different results, being more compromised than SGA fetuses.

Conclusion

This work demonstrates the potential use of thigh fat volume and area measurements to be able to distinguish SGA fetuses from AGA fetuses at a third trimester scan.

7i. Publications and presentations arising from this chapter

Effect of malaria during pregnancy on fetal thigh volume: a longitudinal observational study.

Marcus J Rijken, Eloise Groenveld, Aris T Papageorghiou, Suporn Kiricharoen, Noaeni

Karunkonkowitz, **Caroline Knight**, Ellen Tromp, Els de Jong, Sasithon Pukrittayakamee,

Stephen Kennedy, Rose McGready, Francois Nosten

Submitted to *Malaria* 2014

Fetal arm fat as an ultrasound marker of nutritional status

Knight, C. L., Rueda, S., Ahmed, M., Noble, J. A., and Papageorghiou, A. T.

Aug 2013: Medical Engineering Centres Annual Meeting, Ascot, UK

[oral presentation] (see also abbreviated listing in Chapters 4 and 5)

Fetal arm ultrasound as a marker of fetal body composition

Knight, C. L., Rueda, S., Noble, J. A., and Papageorghiou, A. T.

Dec 2012: Blair Bell / RCOG Annual Scientific Meeting, London, UK

[poster presentation] (see also abbreviated listing in Chapter 4)

OP17.05: Fetal arm fat: an *in utero* marker of body composition?²³¹

Knight, C. L., Rueda, S., Noble, J. A., and Papageorghiou, A. T.

Ultrasound Obstet Gynecol 2012; 40 (s1): 106

Sept 2012: ISUOG 22nd World Congress, Copenhagen, Denmark

[oral presentation] (see also abbreviated listing in Chapter 4)

7j. Contributions to the thesis: chapter 7

Other than where indicated below, and acknowledged in appendix A, all the work in this chapter (including concepts and hypotheses, protocols, acquisition and segmentation of scans, analyses, production of graphs and figures) is that of the author.

The INTERGROWTH-21st infant growth study information leaflet and consent form were written by the author, and reviewed by A.P.. The leaflets were given to the women by F.R. or the author, and consent was obtained by the author or by trained members of the INTERGROWTH-21st anthropometry team. Initial training in the use of the PEA POD machine for the author, F.P and S.L. was provided by Zedong Jiang (research fellow, Department of Paediatrics, University of Oxford). The PEA POD and anthropometry protocols were developed by F.P. in collaboration with the author, and overseen by L.C.I., who also arranged training for the anthropometrists. The DXA measurements were undertaken by trained MAVIDOS radiographers, with the study overseen by Kassim Javaid (lecturer, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford) at the Oxford study site. The DXA results were stored and coordinated by K.E.. Thigh volumes used in this thesis for sagittal fat feasibility were acquired by researchers supervised by M.R. at the Shoklo Malaria Research Unit, Maesot, Thailand. TVol segmentation undertaken by E.G., supervised by M.R.

The 3D tools, and process for producing FFI values, used in this chapter were developed and utilised by biomedical engineering colleagues, as previously stated. Data was retrieved from the INTERGROWTH-21st database, and neonatal body composition and anthropometry undertaken, by NDOG colleagues, as previously stated. Some additional data were retrieved by Sana Fathima (DPhil student, IBME/NDOG).

Images taken from journal papers and textbooks are individually cited in the figure captions, by name and year, and with a reference to the full bibliographic details. Figure 165b courtesy of Michael Schmidt, University of Minnesota, USA. Figure 168 photos courtesy of F.P.; taken during the INTERGROWTH-21st anthropometry training sessions arranged by L.C.I. in Turin, Italy, in September 2011

8. DISCUSSION, FUTURE WORK, AND CONCLUSIONS

8a. Principal achievements and findings

Ultrasound scanning protocols were developed to enable accurate acquisition of 2D images and 3D volumes of fetal arms and thighs. These were refined over time, with evident improvements in image quality and consistency. The final protocol was distributed amongst, and taught to, other members of the Oxford INTERGROWTH-21st and INTERBIO-21st scanning teams. The importance of acquiring scans by such protocols was demonstrated as, when segmentation of images was not possible, this was often due to scans not being acquired in line with protocols – for example, not checking for a full-length sagittal reconstructed image in 3D volumes. One advantage of the 3D methods over the 2D is that it is easier to identify inadequate volumes, whereas 2D images, due to lack of landmarks, are harder to identify.

Methods of measuring fat in ultrasound images were also developed. The principal methods used throughout this thesis for both arm and leg fat analysis were the MATLAB manual segmentation tools developed by collaborators at the Institute of Biomedical Engineering. These are suitable as a research tool and have certain benefits, including the generation of multiple datasets (fat, lean and limb; 2D and 3D data), and the generation of results into excel spreadsheets for ease of analysis; but would be too time-consuming for day-to-day clinical use. Our original hope was that an automated, or semi-automated, method would be developed; this is still in progress but has proved an extremely complicated engineering challenge. Other, simpler, methods were also assessed. The two-ellipse approach proved potentially useful at lower gestational ages but less so towards term, so was abandoned. The three-thickness Fetal Fat Index, however, shows promise, and has the significant advantage of being simple and rapid, and thus of potential clinical use.

Reproducibility was assessed throughout the thesis. Systematic and random errors were assessed using Bland-Altman plots. All methods were assessed: 2D and 3D manual segmentations, ellipses, and Fetal Fat Index. Intra- and inter-observer variation was assessed for the 2D and 3D manual segmentation methods, including analyses between different pairs of segmenters. Results were compared with published data; and also assessed over time to allow for experiential learning with these novel methods. The methods showed acceptable reproducibility, whilst accepting that the limits of agreement for the 3D fat method in particular will always be broad, as it is a technique which involves multiple levels of potential error: each slice is calculated using two freehand traced areas, with interpolation of the volume between each of 5 slices to generate the final volume result.

Validity was assessed by comparing 2D ultrasound with MRI images of the same fetus, obtained within an hour of each other. The arm anatomy was much clearer with MRI. Interestingly, however, lean and arm areas were slightly larger when measured on the MRI compared with the ultrasound, whilst fat areas were slightly smaller. This was the opposite of what might have been expected; it might be thought that ultrasound, with its acoustic “fuzziness” would yield larger values. Images from the same study were also used to confirm the anatomy of the fetal thigh, to ensure accuracy of the thigh fat measurement method.

In addition, fat area results, estimated using the MATLAB tool, were compared with measurements of the same images obtained with a commercially-available tool. Validation was also undertaken between ultrasound methods: 2D area measurements were compared with those obtained from the central slice of 3D volumes from the same fetuses; and sagittally-acquired thigh volumes were validated by comparing them with axially-acquired thigh volumes, also from the same fetuses.

Reference ranges were calculated for all measurements within this thesis:

- 2D area reference ranges (based on 180 measurements): mid-humeral axial slice arm area, arm fat area, arm lean area, and arm fat as percentage of arm area.
- 2D areas acquired from central slice of fractional limb volume references ranges (based on approximately 500 measurements): central slice arm area, arm fat area, arm lean area, and arm fat as percentage of arm area; and central slice thigh area, thigh fat area, thigh lean area, and thigh fat as percentage of thigh area; Fetal Fat Index of the arm and of the thigh.
- 3D fractional (50%, 5-slice) limb volume reference ranges (based on approximately 500 measurements): arm volume, arm fat volume, arm lean volume, and arm fat as percentage of arm volume; thigh volume, thigh fat volume, thigh lean volume, and thigh fat as percentage of thigh volume.

These reference ranges were all calculated using fractional polynomial regression, with log-transformation of data where required, and using standard deviations to calculate centiles. In addition, the volume data were scrutinised so as to optimise the accuracy of the reference centiles.

The reference ranges were then used elsewhere in the thesis, including correlation with MRI measurements, comparison with neonatal arm area data, and comparison with published data.³

This last comparison produced some surprising results, with reference ranges for both fractional arm and thigh volume in this thesis being considerably lower than those published by Lee et al³ (19% (arm) and 32% (thigh) lower at 40 weeks), although they were very similar at lower gestational ages. The possible reasons behind these differences have been explored in some detail.

In contrast, the 2D data from this thesis (from a subset of the fetuses used to generate the 3D data) generally showed a greater amount of fat than previously published results. This suggests that the data in this thesis are somewhere between those from previous studies; again, some of the possible reasons behind these differences are explored in the appropriate chapter.

A key finding of this thesis has been the potential usefulness of arm fat measurements, rather than thigh fat, as a marker of fetal nutrition. I have shown that the fetal arm, whilst being a smaller structure than the thigh, has a greater percentage of its area or volume occupied by fat than the thigh. Arm fat also shows a greater rate of increase compared with lean tissue in the third trimester, whereas thigh fat and lean tissue increase by similar rates throughout pregnancy. This may be due to better-oxygenated and nourished blood supplying the arms (due to their blood supply sharing similar origins to that for the brain) compared with the lower levels of oxygenations in the blood supplying the legs. The fetal thigh has previously been explored in greater detail, in part through its familiarity from the use of femur length as a standard biometric measurement; however my work suggests that we should focus on the arm as well. Further work is needed to elucidate which limb is the better biomarker to assess fetal nutritional status.

Correlation with neonatal body composition and anthropometry was undertaken. Initially, using DXA data, I was able to demonstrate a very strong correlation between neonatal limb fat measurements and whole body fat measurements in the same babies. This provided biological plausibility for the use of fetal limb fat as a surrogate for whole body composition.

Correlations between fetal limb fat measured *in utero*, and neonatal body composition (using PEA POD data) were also demonstrated. These increase across gestational age with the strongest correlations seen, as anticipated, towards the end of the third trimester.

This provides evidence that body composition can be assessed antenatally; until now, body composition, using PEA POD or other methods, could only be measured in the neonate. These tools may therefore become of great interest to researchers studying the development of fetal body composition, as the non-invasive ultrasound-based techniques would allow them to map developments during gestation and, in particular, in the last trimester of pregnancy.

Variations on the correlation were explored, including fat expressed as a percentage, Fetal Fat Index, the combination of arm plus leg volumes, and the use of Z-scores to compare measurements. The differences between 2D and 3D measurements, and between fat and limb measurements were also examined. The combination of arm plus leg showed slightly stronger correlation coefficients than either measurement alone; Z-scores added little; whilst the 3D fetal measurements tended to have stronger correlations with the neonatal measurements than the 2D fetal measurements. Overall, however, these adjustments made only slight differences.

Anthropometry was also correlated with fetal measurements, although this was limited by a lack of newborn data in developed countries to provide a reference range. Mid upper-arm circumferences (MUAC) were compared with fetal arm measurements. Statistically significant differences were identified in gestational age windows from 30 weeks, with areas showing better correlation than volumes. This might have been expected as the MUAC is measured at the level of the axial slice. Mid thigh circumferences (MTC) were also compared with fetal thigh measurements, and provided further evidence that arm fat may be the better marker of body composition and/or size.

SGA and AGA fetuses were compared, using scans from the INTERGROWTH-21st databank, in terms of thigh fat at 30-35 weeks of gestation. Preliminary work, using sagittally-acquired thigh volumes from a population on the Thai-Burmese border, suggested that the fat would be able to be measured in about a third of scans; however just over half of the pairs of scans were able to be measured from the INTERGROWTH-21st cohort. The fat area and volume were significantly different in SGA from AGA fetuses with scans within 14 days of each other, suggesting the potential for limb fat measurement as a marker to distinguish these two groups of fetuses.

Longitudinal scan patterns, and the identification of fetuses who fail to meet their growth potential whilst remaining within the 10th-90th centile range, proved impossible in the time available, although differing longitudinal growth patterns of individual fetuses was explored for 2D arm fat area, 3D arm fat volume, and 3D thigh fat volume.

In addition to the original aims, I also explored fetal arm and thigh fat volumes in males and females, but found no differences; neonatal measurements suggested higher fat in males.

8b. Strengths

One of the main strengths of this thesis is the use of an optimally healthy, carefully-selected population for the reference range calculations. I was fortunate to be able to work with the INTERGROWTH-21st team, and to scan some of the women in Oxford recruited to the study.

Another strength was the multi-disciplinary nature of my research. Without the collaboration with the biomedical engineers, the measurement of the 3D volumes, and the extracted 2D measurements, would have been impossible. The initial 2D work would also have been far more challenging without the MATLAB tool they designed (and which provided the basis for the 3D volume tool). The influence of the engineers can, I believe, also be demonstrated in the extensive checking of reproducibility and validity demonstrated within this thesis; this is an intrinsic part of the engineering approach to research. It has been used increasingly in more recent ultrasound research papers in O&G but some of the earlier papers had very minimal reproducibility studies.

Collaboration, not only with the biomedical engineers, but also with colleagues within the Nuffield Department of O&G, other medical departments, and overseas universities, was a further strength. The training of, and overseeing of, colleagues in collecting and measuring my research scans meant that I was able to obtain data in much larger numbers than would have been possible if I had just been working on the project alone. Collaboration with the INTERGROWTH-21st anthropometry team meant that I was able to obtain neonatal data within 24 hours of birth which, again, might still have been possible had I been working alone, but with much smaller numbers.

Working with the MAVIDOS study team enabled me to access DXA body composition data which, although proving less useful than I had hoped, nevertheless provided useful evidence of the correlation between limb fat and whole body fat in neonates. Close collaboration with radiology colleagues was a necessary part of the successful completion of the MRI validation study, which would have been impossible without them. Within the same study, the involvement of the INTERGROWTH-21st research midwife meant that many more patients received my MRI study information leaflet than would have been possible had I been recruiting patients alone.

Meanwhile, unexpected collaborations arose through networking at international meetings. Working with colleagues from the Netherlands on their work provided a proof-of-concept study for my SGA-AGA work on the INTERGROWTH-21st database, whilst discussions with a colleague from New Zealand has resulted in an ongoing collaboration as well as his contribution to the measurement of some of my limb volumes.

Finally, I think this thesis also shows a structured approach to the problem in question. Whilst the nature of assessing different components of the fetal limb (fat, lean, whole limb; 2D and 3D) necessarily meant that repetition was involved, a logical approach and progression through experiments, from simple to complex (for example, 2D to 3D, and arm to thigh) supported by appropriate exploration around the subject (for example, validation studies, image scoring, number of slices, and axial or sagittal acquisition of volumes) meant that there was ongoing development of the methodology through the first three results chapters, with its application in the fourth results chapter.

8c. Weaknesses and limitations

Every piece of research has its weaknesses and limitations, and this thesis is no exception. Scanning women in the INTERGROWTH-21st study has its negative sides, for example. Against the positive of having access to a large number of optimally healthy women, and the benefit of not having to recruit them myself, was the negative of not obtaining my limb volume scans in all women at all scans, as would have occurred with a dedicated study population. This resulted in large, but incomplete datasets, and varying numbers of patients in different studies. This was seen in the longitudinal studies, the body composition or anthropometry studies, or in studies assessing limb fat within different gestational age brackets, where there were either limited numbers of patients who met the criteria or had all the measurements taken, or else different numbers of patients in different groups.

In addition, against the benefit of others obtaining scans on my behalf, and the resulting increase in data, is the variation in scan quality: it is understandable that the person who is carrying out the research will be more familiar with, more expert at, and care more about, the techniques than another person who is acquiring the scans without direct benefit to themselves. Scan protocols and training did help with this, but it remained noticeable that a higher percentage of scans acquired by others could not be segmented compared with those obtained by the author, usually due to poor quality or non-protocol scans (for example, dark or rotated scans, or fat missing off the edge of the screen). The same point could also be applied to the situation of others undertaking the volume segmentation technique. On the other hand, this is more reflective of real-life scenarios, as clinicians of different skill levels would be involved in any prospective large-scale study, or indeed, in clinical management. It also emphasises the importance of ongoing education or training, and quality control.

Small group sizes were a particular issue with the neonatal measurements. Issues with either the fetal or the neonatal part of the studies could mean that the patient was excluded and the study sample size became smaller. Factors could include incomplete datasets due to patients missing scans, or limb volumes not being obtained; patients withdrawing consent for the neonatal measurements; or the PEA POD machine being out of order.

Although the studies were carefully designed, one methodological criticism is that the 3D volumes were each only measured once (unlike the original 2D areas, which were each measured twice, and the mean measurement used). This decision was taken after the 3D volume measurement tool was developed: measuring each volume twice was very time-consuming. In order to mitigate this, I undertook reproducibility studies to assess potential errors in the measurements, assessing both intra- and inter-observer variability.

An intrinsic weakness of 3D volume calculations is that the additional dimensions add extra amounts of error to the measurements. The errors are even more pronounced in the techniques presented in this thesis, which involve five slices, each of which has two freehand tracings of limb area, which are then interpolated into a volume measurement. This is reflected in the large limits of agreement (random error) seen in the Bland-Altman plots for the 3D values, particularly when compared with the narrower limits of agreement seen in standard 1D biometric measurements such as bi-parietal diameter or femur length.

One result-based criticism is that this research has yielded obvious results: “big babies have lots of fat”. Whilst this is true to some extent, we know from others’ research that this is not the full story, and measuring or estimating fetal weight (or size) does not necessarily reflect body fat (or nutrition). An optimally healthy population such as INTERGROWTH-21st would not be expected to have many babies of suboptimal nutrition, but even here there were some babies with high birthweights but low body fat, or vice versa. The measurements definitely warrant further investigation in less optimal populations.

Another negative result from the study is the suggestion that there may be little clinical benefit in measuring limb fat when compared with the limb itself, whether in 2D or 3D. Whilst this is not a weakness of the study per se, it is potentially disappointing. However, limb fat should still be evaluated in different pathological populations before it is disregarded: for example, it may be more sensitive than the limb measurements in LGA fetuses or those from diabetic pregnancies, where the lean and fat tissues may behave differently when compared with SGA fetuses.

A more technical criticism is that 3D ultrasound is purely a research tool, with little clinical benefit in day-to-day medicine. Whilst it is true that 3D ultrasound has only limited clinical usage in standard obstetrics, that is not to say that it does not hold benefits – for example, in most cases the correlations between fetal and neonatal measurements were stronger for 3D volumes than for 2D areas. The machines are also available in clinical settings, albeit mostly in teaching hospital settings, and in developed countries. However, a recognition of the more common usage of 2D ultrasound, together with the non-availability of 3D machines in more resource-poor settings, have led me to continue to focus on 2D approaches such as the Fetal Fat Index, in addition to the more complex volumetric approaches.

8d. Future work

I had hoped to explore the role of fetal arm and thigh fat in different groups of pregnancies, including those with SGA; FGR; gestational or pre-existing diabetes; and LGA. Having been unable to undertake this work during the course of my DPhil, I hope to be able to continue it in the future, and/or for others to continue working within this area of study.

My original hypothesis was that limb fat measurements would enable the identification of growth restricted fetuses from those that are constitutionally small. Unfortunately I was not able to assess this in the time available to me. It would be interesting to look at a cohort of SGA fetuses with abnormal Doppler measurements (presumed FGR), to ascertain the degree of change from normal limb fat measurements that might be expected in such a population. These fetuses could be compared with another SGA cohort with normal Doppler measurements (presumed constitutionally small) to see whether their fat measurements differ. A further group could include fetuses clinically suspected of being SGA (for example, through reduced symphysial-fundal height measurements): many of these babies will have normal biometry but it would be interesting to see how their limb fat compared with an optimally healthy population.

We had identified 180 pregnancies within the INTERGROWTH-21st study where the women had been optimally healthy at the start of their pregnancies, but had developed gestational diabetes during the course of the pregnancy. I had intended to assess the pattern of fetal thigh fat and lean deposition within these pregnancies (thigh volumes would have been available, as per standard INTERGROWTH-21st protocol; arm volumes would not), to see if there was accelerated growth and, if so, at which stage of the pregnancy.

Volumetric measurements have not previously been undertaken, though others have previously assessed subcutaneous tissue thickness and fat and lean areas in these populations. I wanted to look at both compartments as although previous work has shown greater body fat in fetuses in diabetic compared with non-diabetic pregnancies, there have been contradicting reports about changes in lean measurements.^{96, 116} It would also be interesting to compare fetal limb measurements in pregnancies with gestational and pre-existing diabetes, to see if they demonstrate different patterns of fat and lean tissue growth.

In addition to the fat and lean limb volume measurements within these cohorts, I would like to assess the Fetal Fat Index to see how it performs in populations with growth outside the previously explored healthy ranges. I would also like to compare limb and fat volumes, to see whether the small additional strength of correlation suggested by the neonatal comparison work in this thesis actually translates into a meaningful clinical difference, and/or one which would justify the additional effort required to measure fat.

Potential barriers to fat volume measurements becoming clinically viable include the time needed, and requirement for post-processing, to measure fat volumes, even with specifically-designed tools. An original aim of the work within the Personalised Perinatal Project was for the engineers to develop automated methods of fat area and volume measurement. This proved an extremely challenging task; progress has been made^{203,204,205} but there is still much work to be done (which is continuing) before the vision of an automated, or semi-automated, real-time tool is realised within ultrasound machines. Ongoing collaboration between obstetric and biomedical engineering researchers is thus a necessity for the furthering of this work.

8e. Conclusion

This thesis has explored, in detail, the measurement of fetal arm and thigh fat using both 2D and 3D ultrasound, and has demonstrated that it is correlated to neonatal body composition thus allowing “fetal body composition” to be established as a research tool; the ultimate aim is to be able to distinguish growth-restricted fetuses from those of normal nutritional status.

Novel measurements have been developed, acquisition protocols described, reproducibility assessed, and reference centiles calculated in an optimally healthy population: 2D Fetal Fat Index, 3D fractional limb fat and lean volumes, and limb fat (2D and 3D) as a percentage. The use of MRI to validate fetal fat measurements, described in this thesis, has also not been published in the scientific literature. In addition, this thesis has added to previously published results: 2D fetal fat and lean limb areas, and 3D fractional limb volumes.

Fetal fat measurements correlate better with neonatal whole body fat data than limb measurements, but only by small margins, which may prove clinically insignificant. Similarly, volumetric measurements show only small improvements in correlation above area measurements. The fetal arm has a greater percentage of fat compared with the fetal thigh, and also shows an increased rate of fat deposition compared with lean tissue in the third trimester, so may reflect nutritional status better than the thigh.

Much of the work undertaken has been to develop new methods in a healthy population; preliminary work assessed the measurements in SGA fetuses compared with AGA controls. Whilst the original hypothesis has not been definitely proven, the evidence suggests limb fat measurements are able to distinguish SGA from AGA fetuses, and those with high and low whole body fat, and certainly warrant further assessment as markers of fetal nutrition.

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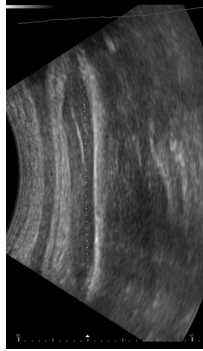
Dr Caroline Knight (523573) Oriel College Antenatal characterisation and postnatal validation of fetal nutritional status using novel fetal imaging methods, neonatal body composition data and anthropometry Additional sheet appended to GSO.3 APPLICATION FOR APPOINTMENT OF EXAMINERS Name: Dr Caroline Knight (523573) College: Oriel Thesis title: Antenatal characterisation and postnatal validation of fetal nutritional status using novel fetal imaging methods, neonatal body composition data and anthropometry (DPHil) Supervisor: Dr Aris Papageorgiou 2.2 Statement of authorship: Details of the parts of my thesis which are not my own work This thesis was written by the author. The majority of the thesis was conceived by, the research carried out by, and the analyses performed by, the author. However, collaborations did form a key part of this research. These are outlined below, by department, and described in full at the end of each thesis chapter. Institute of Biomedical Engineering (IBME), University of Oxford: - The MATLAB tools used to manually measure limb areas and volumes, to measure fat using ellipses, and to obtain the automated Fetal Fat Index measurements, were designed by Dr Sylvia Rueda (postdoctoral researcher, IBME), together with Ms Maryam Ahmed in the case of the 3D tool (masters student then; now DPhil student, IBME) whose work was overseen by Dr Rueda, with clinical and user input from the author; also overseen by Professor Alison Noble (Director of IBME). - Dr Rueda also quality scored the initial 2D arm images, segmented 68 arm areas for inter-observer reproducibility assessment (the remaining 180 arm areas were segmented twice each by the author), segmented volumes to assess the effect of perpendicular vs parallel slices. - Dr Rueda also undertook the analyses and produced graphs assessing the variation in arm fat thickness (min, max, mean etc) around the fetal arm (chapter 4g.i). - Ms Ahmed segmented most volumes to assess the effect of slice number upon resulting volumes (segmentation detail below), undertook the majority of the analyses of perpendicular vs parallel slice volumes, processed the volume mat files to enable extraction of relevant central slice information for automated analysis of fat thickness, and co-wrote (with the author) the segmentation protocol for the 3D MATLAB tool. INTERGROWTH-21st ultrasound scanning team, University of Oxford: - INTERGROWTH-21st scans: the author (73%, 548/746) and Dr Raffaele Napolitano (clinical fellow, Nuffield Department of O&G (NDOG)) (27%, 198/746), to whom I taught the technique and protocols. - The majority of the infant growth (body composition and additional anthropometry) study and fetal MRI study information leaflets were given to study participants by Mrs Fenella Roseman (INTERGROWTH-21st research midwife). - All ultrasound scans (46) with paired MRI images for the MRI study acquired by the author, and patients consented by the author. - MAVIDOS scans: the author (8/33 scans at 33-35 weeks), Dr Napolitano, Dr Sikolia Wanyonwi (clinical fellow, NDOG), Ms Tess Norris (sonographer, NDOG), to whom I taught the technique and protocols.	Dr Caroline Knight (523573) Oriel College Antenatal characterisation and postnatal validation of fetal nutritional status using novel fetal imaging methods, neonatal body composition data and anthropometry INTERGROWTH-21st anthropometry team, University of Oxford: - Team members performed all neonatal PEA POD body composition analyses and additional anthropometry, led by Dr Fabien Puglia (postdoctoral researcher, NDOG) and overseen by Dr Leila Cheikh Ismail (INTERGROWTH-21st anthropometry group leader). - PEA POD and anthropometry protocols written by Dr Puglia with input from the author. Nuffield Department of Obstetrics and Gynaecology (NDOG), University of Oxford: - Neonatal data and Oxford scans retrieved from the INTERGROWTH-21st database by Mr Stephen Ash (INTERGROWTH-21st data manager), with the approval of Dr Cheikh Ismail and Professor José Villar (INTERGROWTH-21st Principal Investigator). - International scans also retrieved from the INTERGROWTH-21st database by Ms Sana Fatima (DPHil student, NDOG/IBME). Oxford University Hospitals, Oxford: - All fetal MRI scans for the MRI-ultrasound study were undertaken by Dr Gerardine Quaghebeur (consultant neuroradiologist) and radiographers at the John Radcliffe Hospital; Dr Quaghebeur also reviewed the MRI study ethics application and developed the MRI scanning protocol in collaboration with the author and Dr Rueda. - All neonatal DXA scans and anthropometry for the MAVIDOS study were undertaken by radiographer team members at the Nuffield Orthopaedic Centre, Oxford overseen by Dr Kassim Javadi (lecturer, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences). Segmentation of fetal limb volumes - Arm areas: all segmented twice by the author (180 x2). - INTERBIO-21st MRI and ultrasound pairs: all segmented by the author. - Arm volumes: the author (40%, 254/638), Ms Ahmed, and Ms Katherine Edwards (research and MAVIDOS research midwife, NDOG). - Thigh volumes: the author (22%, 154/714), Ms Ahmed, Ms Edwards, Dr Vera Donadono (clinical fellow, NDOG), Dr Graham Parry (teaching fellow, University of Western Australia). - MAVIDOS arm and thigh volumes: the author (53%, 183/4) and Ms Edwards. - Malaria in pregnancy thigh volumes: the author (129 volumes for quality control purposes, and proof-of-concept for my research); original segmentations by Ms Eloise Groeneweld (medical student, University of Utrecht, Netherlands) overseen by Dr Marcus Rijken (postdoctoral researchers, University of Utrecht, Netherlands). - SGA-AGA pairs: the author (45%, 72/160), Ms Ahmed, Ms Edwards, Dr Donadono. Ethics applications - The INTERGROWTH-21st, INTERBIO-21st, and MAVIDOS ethics applications undertaken by the respective study teams. - The infant growth study (PEA POD and anthropometry) information leaflet and consent form written by the author – substantial amendment to INTERGROWTH-21st ethics. - The MRI validation of fetal ultrasound study ethics application, and all supporting documents, were written by the author, with supervision by Dr Papageorgiou and input from Dr Quaghebeur, Dr Rueda and Professor Noble. Individual images, whether obtained from others or, rarely, by others, are individually cited within the thesis and at the end of each chapter. All other research, analyses, diagrams, images, and other work not specified above was undertaken by the author.
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Appendix B: Fetal arm, thigh and subscapular ultrasound image acquisition protocol

PROTOCOL FOR ACQUIRING 2D SUBSCAPULAR AND 2D + 3D ARM AND LEG ULTRASOUND IMAGES v.1 – 13.02.2012 All patients in this study are involved in the INTERGROWTH-21st or INTERBIO studies. All scans are performed with the Philips HD-9 machine (Philips Ultrasound, Bothell, WA, USA) Label the first image in each set “SUBSCAP” (subscapular), “HUM” (humerus) or “FEM” (femur) SUBSCAPULAR IMAGES – 2D only (x3 images) - Obtain a sagittal view of the fetal cervical and thoracic spine - Move the probe laterally to whichever shoulder is uppermost, or whichever shoulder is easier for you to scan - Stop when the scapula is seen as a “Y” shaped sagittal cross-section of bone - Zoom the image so that the scapula is in the approximate centre of the screen - Ideally the scapula will - occupy at least 50% of the width of the image - occupy at least 25% of the height of the image - lie horizontally in the image, in relation to its long axis; or at as low an angle to the horizontal as possible (less than 45 degrees) - Rotate or angle the probe slightly, if necessary, to see all ends clearly - Capture 1 still 2D image with harmonics ON, and 2 still 2D images with harmonics OFF - NB at late 3rd trimester gestations a hypoechoic (black) layer may be noted overlying the scapula – this seems to be a normal feature of near-term pregnancies Subscapular view at 32+2 weeks of gestation with harmonics on (above) and off (below) Subscapular view at 36+2 weeks of gestation demonstrating hypoechoic layer	ARM IMAGES – 3D (x2 images) and 2D (x2 images) - These are usually easily obtained straight after the subscapular images - Keep the harmonics off - Move the probe laterally from the scapula until the fetal arm is seen - Rotate or angle the probe if necessary to get a full-length longitudinal view of the humerus, ideally horizontal, in the centre of the screen - Rotate the probe through 90 degrees to get a transverse cross-sectional view of the arm at the mid-point of its length - This should have a ‘target’ appearance - Bone in centre (should be centrally placed within muscle) - Hypoechoic muscle - Echogenic fat around outside - Adjust the zoom as needed to maximise the size of the image on the screen - Move the probe up and down the humerus – the bone should remain in the centre; adjust rotation as needed - Return to the midpoint of the humeral length in the transverse view and perform a 3D sweep at 70 degrees with the speed set to ‘fast’ - Check the image to ensure - a perpendicular transverse view: the longitudinal humerus should be seen top right - no movement artefact - Repeat until ideal image obtained x2 - Exit 3D view to standard 2D, keeping the same (perpendicular / true transverse) image and capture two 2D transverse cross-sectional images of the arm Longitudinal view of humerus at 29+1 weeks 3D ultrasound of arm at 34+1 weeks (above) and 39+6 weeks of gestation (below) 2D transverse view of arm at 39+6 weeks, taken immediately after the 3D image above
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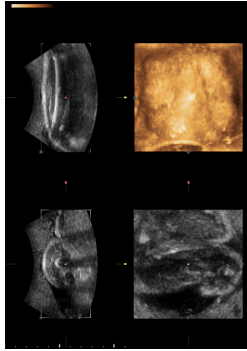
THIGH IMAGES – 3D (x2 images) and 2D (x2 images)

- These are usually easily obtained straight after the femur length images
- Turn the harmonics off
- Rotate or angle the probe if necessary to ensure a full-length longitudinal view of the femur, in the centre of the screen
- Rotate the probe through 90 degrees to get a transverse cross-sectional view of the thigh at the mid-point of its length



Femur length at 38+5 weeks, showing longitudinal echogenic intramuscular septum

- This should have a 'target' appearance
 - Bone in centre (should be centrally placed within muscle)
 - Hypoechoic muscle – more definition than the arm, with compartments and septae
 - Echogenic fat around outside
- Adjust the zoom as needed to maximise the size of the image on the screen
- Move the probe up and down the femur – the bone should remain in the centre; adjust rotation as needed



3D ultrasound of thigh 35+2 weeks – FL view (top R) confirms perpendicular transverse view

- Find the point at which the intramuscular septum in the anterior muscle compartment is visible as an echogenic line in transverse view (this ensures a consistent anatomical level)
- Perform a 3D sweep at 70 degrees with the speed set to 'fast'
- Check the image to ensure
 - a perpendicular transverse view: the longitudinal femur should be seen top right
 - no movement artefact



Transverse view of thigh at 35+2 weeks, taken immediately after the 3D image above; note the presence of the echogenic intramuscular septum above the femur

- Repeat until ideal image obtained x2
- Exit 3D view to standard 2D, keeping the same (perpendicular / true transverse) image and capture two 2D transverse cross-sectional images of the thigh

Appendix C: Fetal MRI study paperwork

 REC reference 12/SC/0141 V. 06.15.02.2012 PROTOCOL: VALIDATING FETAL ULTRASOUND IMAGING USING MRI 1. SUMMARY 2 2. SCIENTIFIC BACKGROUND 3 3. RESEARCH AIMS 6 3.1 Principal research aim 6 3.2 Secondary research aims 6 4. STUDY DESIGN 8 4.1 Overview of study 8 4.2 Recruitment, counselling and consent 8 4.2.1 Inclusion / exclusion 4.2.2 Informed consent 4.2.3 Confidentiality 4.3 Timetable of MRI, ultrasound and PEA POD 10 4.4 Detailed description of fetal MRI scan 11 4.5 Offline analysis and image bank 11 5. SAFETY AND ETHICAL CONSIDERATIONS 13 5.1 Summary of MRI safety data 13 5.2 Detailed account of MRI safety data 13 5.3 Image bank 15 6. DEFINITION OF THE END OF THE STUDY 15 7. ADDITIONAL INFORMATION 16 Publication policy statement Funding Sponsorship Institutional affiliations Conflicts of interest Investigators REFERENCES 17 APPENDIX: TIME LINE FOR THE STUDY 19 This protocol should be read in conjunction with the IRAS application form which contains additional information 1	1. SUMMARY Fetal imaging during pregnancy is usually done by ultrasound as it is safe, cheap and readily available. Three-dimensional ultrasound (3D US) is a recent advance allowing 3D volumetric visualisation and measurement of fetal organs. However, ultrasound has disadvantages: the picture can contain artefacts and shadows. Such image inaccuracies are of particular concern when assessing important aspects of fetal development, for example fetal nutritional status (by measurement of fetal fat tissue) and brain development. We will use Magnetic Resonance Imaging (MRI), a safe and well-established technique, to compare with and validate 3D US. This will aid accurate interpretation of 3D US images, volumes (e.g. limb fat) and organ appearances (e.g. brain, liver, kidneys). We will use a small number of fetal MRI scans as a reference standard to validate 3D US taken on the same fetuses. Engineering techniques (image alignment and segmentation) will be used to compare these images. Such techniques have been used to compare 3D US and MRI in neurology and cardiology in adults (including by our group). The ultimate aim of this validation study is to improve the ability of 3D US to assess fetal growth and nutritional status. In future, this may be used to help predict and manage conditions such as fetal growth restriction, a common cause of stillbirth and premature birth. The study will also provide an “image bank” of paired ultrasound and MRI images for future research. The proposed study will take place at the John Radcliffe Hospital, and is part of the Centre for Excellence in Medical Engineering Theme 1A Personalised Perinatal Monitoring Project funded by the Wellcome Trust and the Engineering and Physical Sciences Research Council (EPSRC). Up to 50 women who are taking part in the INTERGROWTH-21st or INTERBIO studies (REC reference 08/H0606/139) will be recruited over 18 months. They will be invited to have an MRI scan and ultrasound scan on the same day between 20-40 weeks. They will then continue to take part in the INTERGROWTH-21st or INTERBIO studies, which involve serial ultrasound scans during pregnancy and neonatal body composition analysis (to calculate its percentage of body fat) / anthropometry. These measurements will be correlated with the 3D US and MRI findings.
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2. SCIENTIFIC BACKGROUND PURPOSE AND DESIGN OF ULTRASOUND VALIDATION The primary aim of the project is to validate and improve our understanding of 3D US by comparing fetal volume data with fetal MRI. Although standard (two-dimensional) ultrasound has been used for many years in the assessment of pregnancy [1,2], 3D ultrasound is a more recent development [3]. The advantage of 3D US is that it allows capture of volume data; such volumes can then be used for offline image analysis and also for the measurement of volumes of fetal organs or body compartments. The Centre for Excellence in Medical Engineering Theme 1A Personalised Perinatal Monitoring Project, funded by the Wellcome Trust and EPSRC, is investigating how advanced multimodality imaging and analysis can be used to assess the functional state of a fetus and the implications of that on the neonate. The Developmental Origins of Health and Disease (DOHaD) concept [4] suggests that the nine months of gestation are potentially the most consequential period of human life. The time spent in utero, including nutrition received and potential exposure to toxins, may permanently influence future life, for example, the composition of the brain, nutritional status and the functioning of organs such as the heart, liver and pancreas, leading to pathology including cardiovascular disease (hypertension) and metabolic disease (diabetes). A common cause of poor development of fetal organs is intrauterine growth restriction (IUGR). This is an important cause of perinatal morbidity and mortality; improving the detection of this may thus lead to reduced rates of stillbirth and morbidity. The potential for 3D US to both detect key fetal structures and estimate fetal volume measurements may be useful in both detecting growth restriction and also in assessing the severity, by correlating with postnatal body composition which, in turn, may determine the feeding strategy of growth restricted infants. In addition, 3D US may be able to demonstrate some of the associated problems seen in IUGR, such as changes in the brain and other organs (liver, kidneys, adrenals) related to poor nutrient and oxygen delivery.	One of the current questions is how well 3D volumes of the fetus accurately reflect anatomy. For instance, fetal ultrasound images can be “fuzzy”, contain artefacts, be subject to errors due to fetal movement and contain “acoustic shadows” – dark parts of the image where sound waves cannot penetrate (e.g. behind fetal bones). Before 3D US can be applied in large-scale studies of assessment of fetal growth, development and nutritional status it needs to undergo validation. There are different theoretical options to perform such validation: - <u>Validation with Computerised Tomography (CT)</u>: This would be particularly useful for assessing bone development, but is not appropriate for imaging viable fetuses due to the risks of ionising radiation. - <u>Validation of 3D US just before birth with imaging or physical examination just after birth</u>: Due to the inevitable time lag between 3D US, birth, and postnatal imaging, this would only be possible in women at advanced gestations. The major interest of this study, however, is to get validation in the earlier months of pregnancy during key stages of growth. It would also require imaging the newborn by, for example, MRI, which would be difficult to arrange due to the unpredictability of delivery dates. There would also be the challenge of scanning shortly after birth with limited availability of scan appointments, which are usually arranged several weeks in advance. - <u>Validation with fetal MRI</u>: Fetal MRI has been used as a research technique since the 1980s [5] but has become a clinically useful tool more recently due to improved imaging. It is currently used for examination of fetuses with suspected fetal brain and other system abnormalities and is considered a safe technique. It gives very clear images of fetal organs and body compartments. By scanning the fetus using MRI and 3D US within hours of each other one should be able to perform side-by-side comparison of images obtained using these two modalities (image comparison), validation of 3D US measurements and also assessment of images in the “acoustic shadow” of ultrasound (for example, where ultrasound waves are reflected by fetal bone and thus do not penetrate beneath the bone).
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We propose the use of fetal MRI to validate US in this study; such techniques have previously been applied in adults in the fields of neurology [6] and cardiology [7]. By applying similar techniques in pregnancy, 3D US can undergo the required validation which would allow its application as a tool for the assessment of the fetal nutritional status. The ultrasound and MRI will be correlated by manual alignment and delineation of key structures (volumes or linear dimensions) to look at anatomical differences and/or similarities. Using the higher resolution and tissue contrast of an MRI scan as a reference will allow us to better understand how the ultrasonic appearance of key structures changes with gestational age. This project adds to knowledge of fetal imaging (both MRI and ultrasound) and may also allow 3D US to become a tool for more accurate assessment of fetal growth, development and nutritional status.	3. RESEARCH AIMS 3.1 PRINCIPAL RESEARCH AIM <u>To compare fetal three-dimensional ultrasound and fetal MRI with respect to accurate representation of fetal anatomy.</u> Measurements of neonatal organs and body compartments is a useful tool in assessing growth and development, and is used in routine practice, for example clinically (by assessing an infant's weight, ponderal index, height), by measuring body composition (using methods such as plethysmography) or by use of imaging (such as Dual energy X-ray absorptiometry). The relatively recent development of three-dimensional ultrasound has the potential to allow such measurements to take place during fetal life, and this could allow assessment of the functional state of a fetus. It could also improve the detection and assess the severity of fetal growth restriction, and thus afford a "window" into how prenatal nutrition influences postnatal health. However, it is not known how accurately 3D ultrasound volumes reflect anatomy. Therefore, validation of 3D US is of vital importance before it can usefully be applied in the research setting (or indeed used clinically) to assess its value in assessing fetal growth, development and nutritional status. We therefore propose to use fetal MRI to validate 3D US to ensure ultrasound techniques are robust. 3.2 SECONDARY RESEARCH AIMS <u>1. To aid the development of a method of determining the volume of limb fat using three-dimensional obstetric ultrasound.</u> The artefact-free MRI images will be used as a reference standard to aid interpretation and then segmentation (outlining / measuring) of fetal limb fat from the three-dimensional ultrasound images. <u>2. To use the engineering techniques of alignment and registration to compare images arising from fetal ultrasound and fetal MRI.</u> The ultrasound and MRI will be correlated by manual alignment and delineation of key structures (volumes or linear dimensions) to look at anatomical differences and/or similarities. These techniques can be used when comparing different images obtained using one imaging modality, or images of the same structures acquired with different modalities (as in
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this case). Using the higher resolution and tissue contrast of an MRI scan as a reference will allow us to better understand how the ultrasonic appearance of key structures changes with gestational age. 3. To correlate fetal MRI images with 2D and 3D ultrasound images, neonatal measurements and body composition data. MRI images taken as part of this study will be used to categorise fetal fat and non-fat compartments. Findings will then be compared to 2 different methods of assessing fat and non-fat compartments, both of which are already part of the INTERGROWTH-21st and INTERBIO studies (REC 08/H0606/139). The first is 2D and 3D ultrasound, performed on the same day which will measure fat and is a technique we are developing; and the second is postnatal validation of neonatal body composition using whole-body air displacement plethysmography (PEA POD) [8] in the same baby. Correlation between the three ways of fetal / neonatal fat assessment will enhance our understanding of how to measure body composition, as all methods have advantages and limitations. 4. To create an image bank of paired (anonymous) ultrasound and MRI images for use in future research into fetal growth and development. The paired high quality ultrasound volumes and corresponding MRI images will represent a unique resource and will be stored in an anonymised fashion to allow future research on fetal growth and on the development of fetal organs and body compartments. 5. A final purpose of the project is educational: Dr Caroline Knight is currently registered for a DPhil at the University of Oxford on a project entitled "Antenatal characterisation and postnatal validation of fetal nutritional status using novel imaging methods, anthropometry and biomarkers"; this project will form part of her work towards this aim. She has completed GCP training.	4. STUDY DESIGN 4.1 OVERVIEW OF STUDY This is a prospective, observational clinical study. Women who agree to take part will have had a normal routine anomaly scan at 18-22 weeks and have no contraindications to MRI scanning. Women will be recruited from those participating in the INTERGROWTH-21st and INTERBIO studies. In addition to their routine pregnancy care these women will therefore be having 2D and 3D ultrasound scans every 4-6 weeks, and postnatal anthropometry and body composition assessment. The current proposal involves women having a fetal MRI scan on the same day as a scheduled 2D / 3D US scan, at a gestation between 20 and 40 weeks of pregnancy. Recruitment is expected to start in May 2012 and continue for 18 months; we aim to recruit 50 women during this time. 4.2 RECRUITMENT, COUNSELLING AND CONSENT Women will be recruited from those participating in the INTERGROWTH-21st and INTERBIO studies. These women will be having 2D and 3D ultrasound scans every 4-6 weeks' gestation, in addition to their routine pregnancy care. When they attend for a scan, the Clinical Research Fellow and/or Research Midwife will inform them about the additional fetal MRI study and give them a patient information leaflet. The women will have the opportunity to discuss the study and ask the Clinical Research Fellow any questions. Each potential participant will then take the information leaflet away so that they have time to consider whether they wish to take part, and to discuss the study with their partner if they wish. At their next scan appointment (4-6 weeks later) there will be the opportunity to ask any further questions. If they are happy to proceed, they will then sign the consent form and will be given a copy for their records. The consent form will include their name, date of birth and study ID number (to be used on all images and PEA POD data). It will be signed, name printed and dated by the patient and the Clinical Research Fellow and a copy given to the patient. If they decline, they will take no further part in the MRI study but will continue with routine care and the other studies – they will be informed that their decision to not take part will have no influence on the rest of their care.
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4.2.1 INCLUSION / EXCLUSION CRITERIA Women will be eligible for the study if they fulfil all of these criteria: • They are pregnant • They have had a normal routine anomaly ultrasound scan at 18-22 weeks. • They are 18 years of age or older • They weigh less than 150kg or have an abdominal girth of less than 58 inches (the limits of the MRI machine) • They are able to understand the information and are able to give written informed consent • They have no standard contraindications to having an MRI scan (see below) Women will be ineligible for the study if • They have a fetal abnormality at the anomaly scan • Maternal weight exceeds 150kg or abdominal girth exceeds 58 inches (the limits for the MRI scan machine) • They are under 18 years old • There is the presence of objects forming a contraindication to having an MRI: ◦ Permanent pacemaker ◦ Metal in the eye(s) ◦ Any other electronic, mechanical or magnet-sensitive metal implants 4.2.2 INFORMED CONSENT The study will be entirely dependent on volunteer recruitment. A patient information leaflet (enclosed with this application) will be provided to interested mothers detailing the timing of the MRI, and this will be supplemented by personalised counseling by a clinical fellow. It will be emphasised that the study is not being done for the basis of structural or anatomical imaging but only for the purposes of image evaluation and correlation. The images will not be reviewed for clinical detail nor reported. The information leaflet also emphasises that any mother is able to withdraw from the study at any stage.	4.2.3 CONFIDENTIALITY The consent form will include the study participant's full name, participant number, signature and date of signing. A copy will be given to the patient for their records. MRI data will only use the participant number rather than any patient identifiable information. All participants' anonymity will be maintained. They will be identified by their participant number only. All documentation will be stored securely and will only be accessible by study staff and authorised personnel. The study will comply fully with the Data Protection Act. 4.3 TIMETABLE OF MRI, ULTRASOUND AND PEA POD The women will be attending for 2D and 3D ultrasound scans every 4-6 weeks (as part of the INTERGROWTH-21st / INTERBIO studies) which should last for about 30 minutes. We will schedule a fetal MRI on the same day as one of these appointments. The exact date will be based on the gestational age required for the study (see below), convenience for the woman (as she will need to have a longer visit to the hospital on the day when she has both scans), and the availability of research MRI scan appointments. We will be aiming for 4 women to have a fetal MRI scan in each of 5 gestational ranges: 20-23, 24-27, 28-31, 32-35 and 36-40 weeks (ie 20 women total), with another 30 women allocated less prescriptively. For example, based on the preliminary groups it may become evident that scans at the lowest gestational range are less helpful, alternatively a woman may only be able to attend for an MRI on one particular week depending on her other commitments. There will be 50 women in total. Once the baby is born, the INTERGROWTH-21st / INTERBIO measuring team will measure the baby including PEA POD body composition, ideally within 24 hours of birth. One or both parents can attend when these measurements are taken.
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4.4 DETAILED DESCRIPTION OF FETAL MRI SCAN All fetal MRI examinations will be obtained with a Philips 1.5T Achieva machine (system ID 111001.47; Philips, The Netherlands) in the neuroradiology department of the John Radcliffe Hospital, Oxford. A Consultant Neuroradiologist experienced in fetal MRI scanning will obtain the scans working with current hospital protocols and using the manufacturer's predefined imaging sequences; she currently scans patients where there is clinical suspicion of fetal neurological abnormality from antenatal ultrasound imaging. No maternal sedation will be used. Routine checks will be done to ensure the woman does not have any contraindications to MRI scanning. The woman will lie in on her back or in a left lateral tilted position. She will be given ear protection, and a panic button in case she wishes to end the scan at any time. Scans will be taken to assess various organs and fetal structures (eg fetal limb fat, liver, brain) using different standard MRI settings. The total study time should be 30-45 minutes for the fetal MRI. The images will be acquired using a pelvic phased array coil. Rapid T2 weighted sequences using single shot fast spin echo sequences will primarily be utilised. A combination of T1, T2 and T2 fat suppressed sequences will be used and the most appropriate and most rapid sequence will be utilised once this is established. Fetal brain imaging will consist of single shot fast spin echo sequences. Thick slices will be obtained with overlap and generally 2-4 loops (complete sets of overcontiguous slices covering the target volume of the fetal brain) will be obtained in the axial, coronal and sagittal planes with each orientation scanned as a single time series. Motion correction software will then be applied to this data for the purpose of image reconstruction and evaluation. Diffusion Tensor Imaging (DTI) will be attempted when possible. 4.5 OFFLINE ANALYSIS AND IMAGE BANK At the completion of the study it is anticipated that there will be up to 50 sets of MRI data which will be paired with the available ultrasound and PEA POD data, one set for each fetus/baby. The organs or structures of interest will be analysed using an offline technique and the images then saved to form an anonymous image bank for future studies. For validation of ultrasound, common structures will be compared and aligned to ensure the reference planes are the same. The organ or structure of interest will then be segmented throughout its volume by division into several	consecutive slices or planes. The area of interest will then be outlined by manual tracing on each imaging plane and the total area and volume then calculated. This will be done in a blinded manner to avoid bias. The ultrasound and MRI values will then be correlated, with the MRI being used as the reference standard. Where there are difficulties interpreting exact anatomy in ultrasound scans – for example, due to acoustic shadowing under fetal bones, or where fetal tissues of similar density are in close approximation – the MRI scans should show the true anatomical structure free of such artefacts. This should lead to improved interpretation of ultrasound scan images with respect to fetal anatomy. The PEA POD body composition data will be correlated with the areas and volumes acquired from the ultrasound and MRI scans.
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5. SAFETY AND ETHICAL CONSIDERATIONS The study will be conducted in accordance with the principles of the Declaration of Helsinki. There are no increased risks to participating mothers or their fetus: 5.1 SUMMARY OF MRI SAFETY DATA In the United Kingdom, the National Radiation Protection Board and the Medicines and Healthcare Products Regulatory Agency do not recommend MRI in the first trimester and all our scans will be obtained after 20 weeks gestation. [20] The American College of Radiology provide similar guidelines for the use of fetal MRI in the USA. Informed consent will explain that although no risks are known it cannot be demonstrated that absolutely no risk exists. During the preliminary discussions about the design, management and undertaking of the research, women currently involved in the INTERGROWTH-21st studies, and their partners, were unfamiliar with fetal MRI scan as it is an unusual imaging modality to many people. Their main questions were about the safety of their unborn babies. Further safety information and evidence is thus provided in the paragraphs below, to enable a more detailed level of counselling in patients with extended questions or who wish to hear more detail about evidence in this area. 5.2 DETAILED ACCOUNT OF MRI SAFETY DATA Magnetic resonance imaging is considered a safe modality and has been in widespread clinical use since the early 1980s. Intrauterine or fetal MRI has been used on a clinical basis since the mid 1990s and in the United Kingdom since 2000. It has been shown to be a valuable clinical tool for the diagnosis of abnormalities, particularly of the brain and spine, but now increasingly also of the body; and obstetric or intrauterine MRI is used for clinical reasons in many units across the world. [10,11,12,13] Although no large studies have been carried out to judge specifically whether intrauterine MRI is safe, several epidemiological studies have been carried out to assess possible deleterious effects of MRI on the reproductive health of female MRI technicians. [14] They recorded no significant effects of working with MRI on rates of miscarriage, stillbirth or ectopic pregnancy among the 1,915 women responding to the survey. In addition, there was no effect on rates of premature delivery, infertility or low birth weight.	Several studies have also been carried out to try and assess the effects of MRI on human cell development and these have failed to find evidence of abnormalities. Wiskirchen and colleagues 1999 [15] exposed fetal lung fibroblasts to a 1.5T scanner regularly for three weeks and found no changes in proliferation between imaged and controlled cell cultures. This study was repeated by others using time varied magnetic fields such as those produced by gradient coils and again no difference was found. They also found that there was no evidence of teratogenic effects. Poutamo and colleagues [16] assessed cardiotocographic (CTG) patterns of the fetus before and after MRI scan and found no evidence of stress to the unborn fetus during MRI acquisition. They found no evidence of stress to the unborn fetus during MRI acquisition, as there was no increase or decrease in either fetal heart rate or fetal movements. Several longitudinal studies have also been done to assess the postnatal effects of prenatal imaging. The largest study [16] followed 74 women who had undergone imaging during the second and third trimesters of pregnancy and found no effect on imaging on either premature delivery or intrauterine growth restriction. Further studies, including one by Clements [18], found no neurological abnormalities on clinical examination of these infants at nine months of age, and these infants had been imaged four times each in utero. More invasive studies have been performed on animals, showing that a ten week exposure of rats to a 9.4T static magnetic field has no significant effect on memory, incidence of death, changes in heart rate, body weight, food and water consumption, blood and urine composition, and physical pathology. The conclusion has been that there are no adverse biological effects of exposure in male and female rats, nor in their progeny. [19] There are thermal effects of radiofrequency for the body as a result of magnetic resonance imaging. This is as a result of the use of radio frequency electromagnetic fields which are used to produce the image (they do this by temporarily exciting the direction of spin of hydrogen atoms in the body). The excess energy can penetrate the superficial surfaces of the body and could possibly be absorbed by the body's tissues. It is dissipated in the form of heat. In order to ensure that there are no adverse thermal effects the specific absorption rate (SAR) will be limited as per the manufacturer's enforced software routine within the pulse sequence programme.
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Acoustic noise is another potential side effect of magnetic resonance imaging. The risks associated with acoustic noise are a function of both the intensity and the duration of exposure. The FDA has stated that all magnetic resonance diagnostic devices should not produce noise having unweighted sound pressure level higher than 140dB. This is the maximum allowed for impulse noise. Stricter noise standards have been proposed by the IEC and they limit average noise in the MRI scanner to 99dB. These standards are adhered to within the Phillips 1.5T achiever standard and in addition the use of hearing protection devices is mandatory for all patients, including any volunteer mothers. There is a theoretical risk that the noise could have an effect on the fetus. To date, there has been no large study assessing this but no research has been published to show that noise in the scanner causes a problem to unborn babies. Many preterm neonates are imaged on a regular basis and there have been no concerns expressed regarding hearing development in that patient population. 5.3 IMAGE BANK The paired MRI and ultrasound images will be stored in an anonymised fashion in an image bank for 20 years as a resource for future research into fetal growth and development. Women will be asked to give explicit consent for the retention of images in this image bank. If, however, they should change their mind then the images could be withdrawn from the image bank by contacting the team. 5 years after the study finishes any link between the images and the patient will be removed as the master research file is destroyed. It would then be impossible to trace individual images to a particular person. 6. DEFINITION OF THE END OF THE STUDY The end of the study will be the date of the last visit of the last participant. The end of the study will therefore be the date that the neonate undergoes their imaging.	7. ADDITIONAL INFORMATION PUBLICATION POLICY STATEMENT The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Authors will acknowledge that the study was funded by the Wellcome trust and EPSRC. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged. FUNDING Wellcome Trust / EPSRC (grant number WT 088877/Z/09/Z) SPONSORSHIP Heather House Clinical Trials and Research Governance (CTRG) Joint Research Office Block 60, Churchill Hospital Oxford OX3 7LE INSTITUTIONAL AFFILIATIONS These are outlined in the IRAS ethics application CONFLICTS OF INTEREST None INVESTIGATORS <table border="0"> <tr> <td>Aris Papageorgiou</td> <td>Caroline Knight</td> </tr> <tr> <td>Senior Fellow in Fetal Medicine</td> <td>Clinical Research Fellow</td> </tr> <tr> <td>Nuffield Department of Obstetrics and Gynaecology</td> <td>Nuffield Department of Obstetrics and Gynaecology</td> </tr> <tr> <td>John Radcliffe Hospital</td> <td>John Radcliffe Hospital</td> </tr> <tr> <td>Oxford, OX3 9PU</td> <td>Oxford, OX3 9DU</td> </tr> <tr> <td>Tel: 01865 221004</td> <td>Tel: 01865 221004</td> </tr> </table> This protocol should be read in conjunction with the IRAS application form which contains additional relevant information.	Aris Papageorgiou	Caroline Knight	Senior Fellow in Fetal Medicine	Clinical Research Fellow	Nuffield Department of Obstetrics and Gynaecology	Nuffield Department of Obstetrics and Gynaecology	John Radcliffe Hospital	John Radcliffe Hospital	Oxford, OX3 9PU	Oxford, OX3 9DU	Tel: 01865 221004	Tel: 01865 221004
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APPENDIX: TIME LINE FOR STUDY

<12/40	Booking appointment; recruitment to INTERGROWTH-21 st or INTERBIO	R	I	S
10-13/40	Dating scan (CRL); confirms EDD within 7 days of the LMP-calculated EDD	R	I	S
16/40	Starts 2D and 3D ultrasound scans with INTERGROWTH-21 st or INTERBIO		I	
18-22/40	Anomaly scan	R		S
20+/40	4-6 weekly 2D and 3D ultrasound scans (maximum total 6 scans)		I	
	*Study mentioned by research midwife at scan appointment			S
	*Patient information sheet given and patient counselled by clinical research fellow (CRF)			S
	Patient returns 4-6 weeks later for next ultrasound scan		I	S
	Patient consented by CRF * / declines to take part			S
	* Appointment made for study fetal MRI scan, on same date as future ultrasound scan			S
	Patient attends for ultrasound and *MRI scans (one date between 20-40/40)		I	S
	Patient returns to INTERGROWTH-21 st / INTERBIO scan appointments		I	
37+/40	Baby born and weighed	R	I	
	PEA POD measurements and anthropometry taken within 24 hours		I	S

Key:

R = routine care

I = INTERGROWTH-21st / INTERBIO

S = validating fetal ultrasound imaging using MRI study – relevant steps

* = extra steps (in addition to those for INTERGROWTH-21st / INTERBIO) taken if patient consents to take part in validating fetal ultrasound imaging using MRI study

**Magnetic Resonance Imaging Protocol in the Fetal Fat Study
(Validating Fetal Ultrasound Imaging Using MRI)**

REC Ref 12/SC/0141

1. Select the current fetal protocol
2. Do the normal localiser and reference scans
3. Plan a 4mm T2 sequence to cover the whole baby. Coronal or sagittal plane is probably best as this is the sequence that will be given to the parents for demonstrative purposes.
4. 3mm T2 sequence of the humerus. Identify one or both arms and plan this sequence perpendicular to the long axis of the humerus, ideally positioned half way between the shoulder and the elbow. Please obtain a screen-shot of the slice selections.
5. 3mm T2 sequence of the femur. Identify the legs and obtain this through one or both femora. This should again be obtained perpendicular to the long axis of the bones halfway between the hip and the knee, and a screen-shot of the slice selection should be obtained.
6. eTHRIVE breath hold sagittal sequence to demonstrate the entire liver. This is usually the longest sequence of 23 seconds, which allows a larger field of view, and should again be obtained on expiration.
7. A) 3mm sagittal, coronal and axial sequences of the brain, two of each sequence if time allows. (NB for reconstruction and motion correction purposes, all three planes must be acquired, with two sequences for each plane)
B) If short of time, could just acquire 3mm axial and coronal sequences (one in each plane) – these allow anatomical comparison for fetal (axial) and neonatal (coronal) ultrasound images. However these will be insufficient for image reconstruction.
8. Patient is then allowed to leave, either home or, if it is a morning appointment, a porter can be organised (if the patient wishes) to take them to level 4 of the Women's Centre for ultrasound.
9. Please burn a CD of the baby sequence for the family and give this to Amanda Isle who will forward it to the family.
10. Please provide an anonymised CD of all the sequences, give this to Amanda, and she will forward it to Sylvia Rueda in Biomedical Engineering for analysis.
11. If for any reason the scan is not carried out (patient late / DNA etc) then could someone please call Caroline Knight (clinical research fellow. O&G) on 07796 691002 to inform her of the fact so that another MRI can be arranged. Please leave a message if the phone cannot be answered.

V2 08-07-13

REC reference 12/SC/0141 V 05 27/04/12 Institute of Biomedical Engineering University of Oxford Old Road Campus Research Building Oxford OX3 7DQ Nuffield Department of Obstetrics & Gynaecology University of Oxford Level 3, Women's Centre John Radcliffe Hospital Oxford OX3 9DU Supported by wellcome trust NHS Oxford University Hospitals NHS Trust EPSRC Engineering and Physical Sciences Research Council VALIDATING FETAL ULTRASOUND IMAGING USING MRI PATIENT INFORMATION LEAFLET We would like to invite you to take part in our research study. Before you decide whether to take part, we would like you to understand why the research is being done and what it would involve for you. Please read this information sheet carefully. One of our team will go through it with you and answer any questions you have. We suggest this should take about 15 minutes. Talk to others about the study if you wish. Part 1 tells you the purpose of this study and what will happen if you take part. Part 2 gives you more detailed information about the conduct of the study. Please ask us if anything is not clear.	REC reference 12/SC/0141 V 05 27/04/12 PART 1: The purpose of the study and what will happen to you if you take part What is the purpose of the study? Thank you for considering being a participant in this study to assess fetal imaging (ultrasound and magnetic resonance imaging, MRI). This is a PhD student project towards the thesis of Dr Caroline Knight, and is part of the Personalised Perinatal Monitoring Project funded by the Wellcome Trust and Engineering and Physical Sciences Research Council (EPSRC). You are already taking part in the INTERGROWTH-21st or INTERBIO studies (REC reference number 08/H0606/139), and as part of this you are already having regular measurements of your baby using ultrasound and also after birth. This separate study has been set up to improve the information we get about a baby's growth, development and nutritional status when we perform ultrasound scans during pregnancy. We are aiming to recruit 50 women to this study. We are using MRI to look at a number of organs and tissues in the babies, including brain, liver and fat. We will use these high-resolution MRI images to investigate how accurately two and three-dimensional ultrasound represents the baby's organs and tissues. We will also assess how closely they agree with postnatal measurements. This will allow us to develop new methods of assessing fetal growth and hopefully help us to optimise the management of pregnancies with growth-restricted babies. This study will gather paired ultrasound and MRI images of babies. This could be helpful for future research. We will therefore also ask your permission to store these images for future research. This "image bank" will be entirely anonymised, in other words it would not be possible to trace the paired images back to you or your baby. Why have I been invited? You have been invited to take part because you are already taking part in either the INTERGROWTH-21st or INTERBIO studies at the John Radcliffe hospital (REC reference number 08/H0606/139), and thus are already having regular 3D ultrasound scans. Amongst these women, we are inviting those who have had a normal routine antenatal ultrasound scan at 18-22 weeks of pregnancy showing no clinical abnormalities. There are some additional requirements for you to be able to take part in this study: - you must weigh less than 150kg and have a girth measurement of less than 58 inches as these are the limits of the MRI machine - you must have no contra-indications to magnetic resonance image (MRI) scanning: these are discussed in the MRI safety questionnaire you will complete, in line with standard practice, before having the scan, and include - permanent pacemaker - metal in eye(s) - any other electronic, mechanical or magnet-sensitive metal implants
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REC reference 12/SC/0141 V 05.27.04.12 Do I have to take part? It is up to you to decide whether or not you wish to take part. Your decision will not affect the care you receive in any way, either in the NHS or as part of the INTERGROWTH-21st / INTERBIO studies. We will describe the study and go through this information sheet. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time, without giving a reason. If you have any questions or concerns after reading this leaflet, a member of our team will be happy to answer them. You can also call us on the number at the end of this leaflet. What will happen to me if I take part? The study will consist of you having a magnetic resonance (MRI) scan on the same day as one of your INTERGROWTH-21st / INTERBIO ultrasound scans. We ask you to allow a total of 2 hours at the hospital to have both of these scans. You will then continue with the rest of your scans, and we will measure your baby when s/he is born, as per the INTERGROWTH-21st / INTERBIO protocol. MRI has been used in pregnancy for nearly 20 years. It is not used routinely, but is currently used in clinical practice to help diagnose and / or confirm abnormalities suspected from ultrasound scans, such as brain or spine abnormalities. MRI scans use a strong magnetic field and radio waves (not X-rays) to make pictures of the inside of your body. With fetal MRI, we will look specifically at your unborn baby in your womb. The MRI scan will take place in the neuroradiology department on level 1 in the West Wing at the John Radcliffe Hospital. On the day of your MRI scan you can eat and drink as usual. On arrival at the MRI unit a member of staff will go through a one-page MRI safety questionnaire with you – this is standard practice before an MRI scan. You will be asked to remove all metal objects from your person and will be able to put these in a locker. You will not be able to wear clothing that contains metal when having an MRI scan. You can bring suitable clothes to change into, or we can provide a hospital gown for you to wear. You could wear: - a T-shirt or vest top - leggings - tracksuit bottoms - a skirt * - a dress * - trousers * (*elasticated, with no zips, buttons or belts) You should not wear: - a bra - jeans - a belt - hairclips - any clothes with a zip - jewellery / watch / body piercing - make up <i>MRI scanner (NB you will be scanned feet first)</i> 	REC reference 12/SC/0141 V 05.27.04.12 During the scan you will lie on a trolley and go into the scanner feet first. The scanner is open at both ends. Some pregnant women feel faint when lying on their back – if this applies to you then we will tilt your body slightly with a wedge. The scan will last for 30 to 45 minutes. You will need to lie still so we don't get blurry pictures. The scan will not involve any needles or injections. The radiographer will talk to you during the scan and you will be given a buzzer to contact the person doing the scan at any point. During your scan the MRI scanner will make a banging noise as the magnets are turned on and off. We will give you ear protection to make you comfortable and if you would like to bring a music CD with you, this can be played during your scan. We will stop the scan at any point if you want us to. Your partner is welcome to be in the scan room with you during the scan, provided they have no contraindications to MRI. Further information: http://www.nhs.uk/Conditions/MRI-scan/Pages/Introduction.aspx The MRI scans are for research purposes only, and will therefore not be routinely reported. You will already have had a normal routine antenatal ultrasound scan at 18-22 weeks of pregnancy. In the unlikely event of an obvious abnormality being seen on an MRI scan, you will be referred for appropriate follow up with a fetal medicine specialist. Expenses and payments This study will be performed at the same time as your hospital visits for the INTERGROWTH-21st / INTERBIO study, for which you will be able to claim travel expenses. What are the possible disadvantages or risks of taking part? Millions of adults and children across the world have had MRI scans since the 1970s and no problems associated with clinical MRI scanning have been demonstrated, including during pregnancy. There have been no reported harmful effects from fetal MRI, which has been used as a clinical tool for over 10 years, e.g. to diagnose problems with babies' brains. As a precaution, however, current radiological guidelines suggest avoiding MRI in the first trimester of pregnancy. MRI scanners use strong magnets so it is important that no metal objects go into the scan room. If you have any metal implants the radiologist will ensure they are safe for scanning. MRI examinations are noisy. We will give you ear protection to make you comfortable and if you would like to bring a music CD with you, this can be played during your scan. There is the potential for babies to hear the noise from the MRI, but studies have not shown a sustained heart rate increase during fetal MRI examinations, which would be expected had the babies been stressed. Fetal hearing is not yet fully developed and the mother's body and amniotic fluid would markedly reduce the amount of sound that would reach the baby. Babies that have had a fetal MRI have not been shown to have any hearing impairment after they are born.
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REC reference 12/5C/04.11 V 05.27.04.12 The scanner generates heat so patients are scanned with light clothing only and a fan to ensure a comfortable temperature. There is no evidence that any heat from the scanner affects the baby, again because it is protected by the mother's body and amniotic fluid. Some pregnant women may feel unable to undergo a scan because of a feeling of claustrophobia, however this usually affects less than 1% of women. We scan pregnant women feet-first rather than head-first which usually eliminates any claustrophobia as the head is outside the scanner. Occasionally a mother may feel uncomfortable towards the end of an examination. If this occurs simple repositioning is usually all that is required. Rarely she may wish to stop the examination. What are the possible benefits of taking part? Taking part in this study will not benefit you or your baby directly. However, there are indirect benefits in that you will contribute to a greater understanding of how we can use imaging to assess the development of babies during pregnancy. What if there is a problem? Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2. Will my taking part in the study be kept confidential? Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. The details are included in Part 2. This completes Part 1. If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 (next pages) before making any decision.	REC reference 12/5C/04.11 V 05.27.04.12 PART 2: More detailed information about the conduct of the study What will happen if I don't want to carry on with the study? You may withdraw from the study at any time. It would not affect the care you receive in any way. If you withdraw from the study after the MRI has been done, any stored data that identifies you would be destroyed. In addition, any images can be withdrawn in the future by contacting the research team. However, 5 years after the study finishes it will be no longer be possible as any link between the images and who they belong to is removed, meaning there will be no way of linking individual images with a particular person (this is in line with Data Protection guidelines). What if there is a problem? If you have concerns about the way you have been approached or treated during the course of the study, you can contact Dr. Aris Papageorgiou, Senior Fellow in Fetal Medicine, Nuffield Department of Obstetrics & Gynaecology, on 01865 740434 or aris.papageorgiou@obs-gyn.ox.ac.uk, or you may contact the University of Oxford Clinical Trials and Research Governance (CTRG) office on 01865 572224 or the head of CTRG, email heather.house@admin.ox.ac.uk If you remain unhappy and wish to complain formally, you may also follow the normal NHS complaints procedure. Please see: www.nhsdirect.nhs.uk/articles/article.aspx?articleid=569 for more information. The University has arrangements in place to provide for harm arising from participation in a study for which the University is the Research Sponsor. NHS indemnity operates in respect of the clinical treatment with which you are provided. Will my taking part in this study be kept confidential? Our data manager, who has a duty of confidentiality to you and your baby as research participants, is the only person able to identify you and your baby. If you choose to continue with the study, your and your baby's results and pictures will be held anonymously in a secure database ("image bank") to which only the research team will have access. Authorised representatives of the University of Oxford or the Oxford University Hospitals NHS Trust may look at the anonymised results to check that the study is being performed correctly. No individual participants will be identified when the results of the study are published. The data (images and measurements) will be retained in an "image bank" for 20 years after the end of the study in the event that future discussions about fetal/infant growth require the data to be reanalysed, or that the scan images (which are anonymised) could provide useful information for future research into growth and nutrition. After 20 years, the data will be disposed of securely. You have the right to check the accuracy of data held about you and your baby, and to correct any errors.
---	---

Involvement of the General Practitioner/Family doctor (GP)

In clinical research such as this, it is our responsibility to inform your GP that you have agreed to take part in this study. We will ask, on the consent form you will sign, for your agreement to inform your GP.

What will happen to the results of the research study?

The results will be prepared for publication in scientific journals and presentation at local (University of Oxford) and international meetings. We can provide you with a copy of the papers after publication if you wish, and a summary in plain language will also be available upon request. Your name will not appear in any report or publication. Some of the data from the study may be included in the PhD thesis of one or more of the researchers. Your identity will be protected at all times.

Who is organising and funding the research?

The research is being carried out by the Nuffield Department of Obstetrics & Gynaecology at the John Radcliffe Hospital and the Institute of Biomedical Engineering at the University of Oxford. It is being funded by the Wellcome Trust and the Engineering and Physical Sciences Research Council (EPSRC). The doctors and engineers involved in the research are not being paid to include you in the study and have no conflicts of interest with regards to the study.

Who has reviewed the study?

Research is reviewed by an independent group of people called a Research Ethics Committee to protect your interests, safety, rights, well-being and dignity. This study has been reviewed and given favourable opinion by the NRES Committee South Central – Oxford C.

Further information and contact details

If you have any questions, please do not hesitate to contact, Fenella Roseman our Research Midwife. She can also contact the medical and/or engineering teams as needed

Telephone: 07837 846234
Email: fenella.roseman@obs-gyn.ox.ac.uk
Post: NDOG, University of Oxford, John Radcliffe Hospital, Women's Centre,
Level 3, Headington, Oxford OX39DU

You can find our website at www.obs-gyn.ox.ac.uk

This completes Part 2.

You may keep this information leaflet for your records.

If you have any questions, please contact us as above.

If you wish to take part in the study we will ask you to sign a consent form.
We will give you a signed copy to keep for your records

Institute of
Biomedical Engineering
University of Oxford
Old Road Campus
Research Building
Oxford OX3 7DQ



Nuffield Department of
Obstetrics & Gynaecology
University of Oxford
Level 3, Women's Centre
John Radcliffe Hospital
Oxford OX3 9DU

Supported by
wellcome trust

Oxford University Hospitals
NHS
NHS Trust

EPSRC
Engineering and Physical Sciences
Research Council

VALIDATING FETAL ULTRASOUND IMAGING USING MRI

CONSENT FORM

Patient Identification Number for this trial: _____ John Radcliffe Hospital, Oxford

1. I confirm that I have read and understand the information sheet dated 27.04.2012 (version 05) for the above study. I have had the opportunity to consider the information and ask questions, and have had these answered satisfactorily.

☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.

☐

3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the University of Oxford, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

☐

4. I agree to my GP being informed of my participation in the study.

☐

5. I agree to take part in this study ☐ I agree that anonymised images can be stored in an image bank for future use

☐

Name of patient _____ Signature _____ Date _____

Name of person taking consent _____ Signature _____ Date _____

When completed: 1 for participant; 1 for researcher site file; 1 (original) to be kept in medical notes.

NUFFIELD DEPARTMENT OF OBSTETRICS & GYNAECOLOGY

Aris Papageorgiou
Senior Fellow in Fetal Medicine



Date:

Dear Doctor,

Re:

DOB:

Address:

We are writing to advise you that the above named patient has agreed to participate in a research study at The Nuffield Department of Obstetrics & Gynaecology, Level 3, Women's Centre, John Radcliffe Hospital, Headington, Oxford. The patient has given us permission to contact you. The study title and details are as follows:

Validating fetal ultrasound imaging using MRI

The study is part of a project into Personalised Perinatal Care, sponsored by the Wellcome Trust and the EPSRC (Engineering and Physical Science Research Council). We are recruiting women who are already taking part in either the INTERGROWTH-21st or INTERBIO studies (REC reference 08/H0606/139) as they are already having 3D ultrasound scans. The aim is to validate 3D fetal ultrasound with fetal MRI, as well as to provide an image bank of MRI and 3D ultrasound scans for future research. We are aiming to recruit 50 women over 18 months.

Your patient will have a research MRI scan to obtain fetal images on the same day as one of her 3D ultrasound scans. She will otherwise continue with routine care and other aspects already planned as part of the INTERGROWTH-21st or INTERBIO studies.

It should be noted that the MRI images are for research only and will not undergo clinical reporting as MRI is not part of routine care however in the unlikely event of an obvious abnormality being seen on a scan, it will be referred for appropriate follow up to a fetal medicine specialist.

If you have any questions about the research please contact me.

Yours sincerely,

Aris Papageorgiou
Senior Fellow in Fetal Medicine

Women's Centre, John Radcliffe Hospital, Oxford, OX3 9DU. Tel: 01865 221004
aris.papageorgiou@obgyn.ox.ac.uk www.obgyn.ox.ac.uk v 03 27.04.12



Health Research Authority
NRES Committee South Central - Oxford C

Room 002
TEDCO Business Centre
Rolling Mill Road
Didcot
OX11 1JH
NE32 3DT

Telephone: 0191 428 3564
Facsimile: 0191 428 3432

08 May 2012

Dr Aris Papageorgiou
Senior Fellow in Fetal Medicine
Nuffield Department of O&G
Women's Centre
John Radcliffe Hospital
Headington
OX3 9DU

Dear Dr Papageorgiou

Study title: A study in foetal imaging which will use MRI images to validate ultrasound images; to evaluate the correlation between ultrasound and MRI images; and to assess foetal growth, development and nutritional status.

REC reference: 12/SC/0141

Thank you for your letter of 27 April 2012, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see 'Conditions of the favourable opinion' below).

Non-NHS sites

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to

A Research Ethics Committee established by the Health Research Authority

the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at <http://www.rforum.nhs.uk>.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Covering Letter	Dr Caroline Knight	24 February 2012
Evidence of insurance or indemnity	Mrs E Chick (University of Oxford)	23 February 2012
GPI/Consultant Information Sheets	Version 03	27 April 2012
Investigator CV	Aris T. Papageorgiou	01 September 2011
Investigator CV	Dr Caroline Louise Knight	01 December 2011
Other: Letter from Funder	Wellcome Trust	01 June 2009
Participant Consent Form	Version 05	27 April 2012
Participant Information Sheet	Version 06	27 April 2012
Protocol	Version 06	15 February 2012
REC application	IRAS Version 3.4 86994/296626/1/544	27 February 2012
Referees or other scientific critique report	JV Hajnal	11 December 2011
Response to Request for Further Information	Aris Papageorgiou (University of Oxford)	27 April 2012

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators

A Research Ethics Committee established by the Health Research Authority

- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

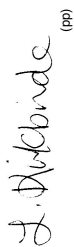
You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

12/SC/0141	Please quote this number on all correspondence
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With the Committee's best wishes for the success of this project

Yours sincerely



(pp)

Professor Nigel Wellman
Chair

Email: laura.kirkbride@sohw.nhs.uk

Enclosures: "After ethical review – guidance for researchers"

Copy to: Ms Heather House

ANNUAL PROGRESS REPORT TO MAIN RESEARCH ETHICS COMMITTEE
(For all studies except clinical trials of investigational medicinal products)

To be completed in typescript and submitted to the main REC by the Chief Investigator. For questions with Yes/No options please indicate answer in bold type.

1. Details of Chief Investigator

Name:	Dr Caroline Knight
Address:	Nuffield Department of Obstetrics & Gynaecology Women's Centre, John Radcliffe Hospital Oxford OX3 9DU
Telephone:	01865 221004
E-mail:	caroline.knight@obs-gyn.ox.ac.uk
Fax:	01865 769141

2. Details of study

Full title of study:	A study in fetal imaging which will use MRI images to validate ultrasound images: to evaluate the correlation between ultrasound and MRI images; and to assess fetal growth, development and nutritional status.
Name of main REC:	NRES Committee South Central – Oxford C
REC reference number:	12/SC/0141
Date of favourable ethical opinion:	08.05.2012
Sponsor:	University of Oxford

3. Commencement and termination dates

Has the study started?	Yes / No
If yes, what was the actual start date?	12.10.2012 (date of Oxford University Hospitals NHS Trust approval)
If no, what are the reasons for the study not commencing?	n/a
What is the expected start date?	
Has the study finished?	Yes / No
If yes, complete and submit "Declaration of end of study" form, available at http://www.nhs.uk/psa/nhs.uk/applications/ate-ethical-review/endstudy	

Annual progress report (non-CTIMP), version 4.2, dated January 2012

If no, what is the expected completion date?	20.05.2014 This is the end of the 18 month period originally proposed – the study may possibly be completed earlier (NB study was suspended from 4 Dec 2013 – 13 Jan 2014 as we were unable to carry out any MRI scans during this period)
If you do not expect the study to be completed, give reason(s)	n/a

4. Registration

Is the study a 'clinical trial'? (Defined as first 4 categories on the IRAS filter page)	Yes / No
(For CTIMP please use CTIMP progress reporting template)	
Is the study registered on a publicly accessible database? (Registration of clinical trials is a condition of approval for studies approved after 30 September 2013)	Yes / No

If yes, please provide the name of the database and the registration number

Database: UKCRN Portfolio Database Registration number: 12783	
If no:	
a. What is the reason for non registration?	
b. What are your intentions for registration?	

5. Site information

Do you plan to increase the total number of sites proposed for the study?	Yes / No
If yes, how many sites do you plan to recruit?	n/a

6. Recruitment of participants

In this section, "participants" includes those who will not be approached but whose samples/data will be studied.

Number of participants recruited: 40	Proposed in original application: 50 Actual number recruited to date: 40
--------------------------------------	---

Annual progress report (non-CTIMP), version 4.3, dated December 2013

Number of participants completing trial: 40 Actual number completed to date: 40	
Number of withdrawals from study to date due to: (a) withdrawal of consent 0 (b) loss to follow-up 0 (c) death (where not the primary outcome) 0 Total study withdrawals: 0 *Number of treatment failures to date (prior to reaching primary outcome) due to: n/a (a) adverse events (b) lack of efficacy Total treatment failures: * Applies to studies involving clinical treatment only	
Have there been any serious difficulties in recruiting participants? If Yes, give details: No serious difficulties; we have had our anticipated recruitment rate from those approached (currently about 1 in 7 accept), and there have been (anticipated) drop-outs prior to the MRI scan and the signing of the consent form eg due to women going into labour, or developing concerns about claustrophobia. There have also been some cancelled appointments due to emergency clinical scans needing to use the scan slots or MRI scanner problems.	Yes / No
Do you plan to increase the planned recruitment of participants into the study? Any increase in planned recruitment should be notified to the main REC as a substantial amendment for ethical review.	Yes / No

6. Safety of participants	
Have there been any related and unexpected serious adverse events (SAEs) in this study?	Yes / No
Have these SAEs been notified to the Committee?	Yes / No / Not applicable
If no, please submit details with this report and give reasons for late notification.	
Have any concerns arisen about the safety of participants in this study?	Yes / No
If yes, give details and say how the concerns have been addressed.	

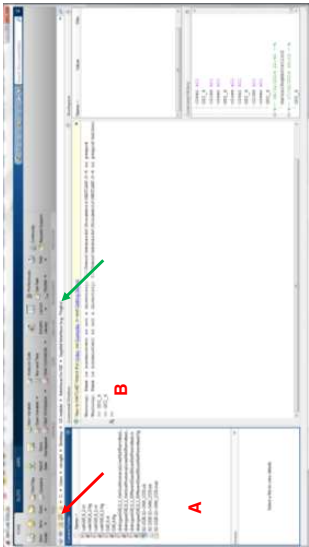
7. Amendments	
Have any substantial amendments been made to the trial during the year?	Yes / No
If yes, please give the date and amendment number for each substantial amendment made.	
8. Serious breaches of the protocol	
Have any serious breaches of the protocol occurred during the year?	Yes / No
If Yes, please enclose a report of any serious breaches not already notified to the REC.	
9. Other issues	
Are there any other developments in the study that you wish to report to the Committee?	Yes / No
Are there any ethical issues on which further advice is required?	Yes / No
If yes to either, please attach separate statement with details.	
10. Declaration	
Signature of Chief Investigator:	Caroline Louise Knight
Print name:	Dr C. L. Knight
Date of submission:	18.02.2014

Appendix D: 3D volume segmentation protocol for MATLAB tool

Instructions for Using Matlab Sagittal and Axial Interfaces to Measure Fetal Limb Adiposity

Before you begin, make sure that all the ultrasound volumes you would like to segment have been moved into the same folder as the files that came with the interface (eg 'Axial Interface' folder). If easier, the volumes can be kept within a folder, which can then be moved into the interface folder.

1. Start Matlab



2. Before typing anything, make sure that the correct folder is selected otherwise the tool will not load.

Use the 'open' button (red arrow), the drop-down menus within the toolbar (green arrow), or click on individual folders within the Current Folder window ('A' above), to select the correct folder. The folder name (eg 'Axial Interface') will then show within the toolbar, and the files it contains within the Current Folder window ('A', above). These files should include those named GUI_6.

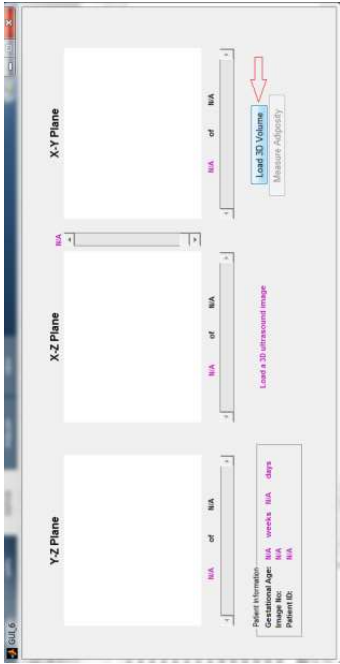
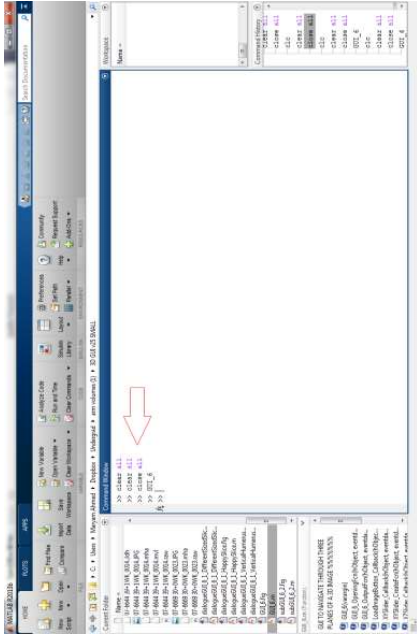
3. in the command window ('B', above), at the '>' prompt, type "close all" and press enter.

4. in the command window, type "clear all" and press enter.

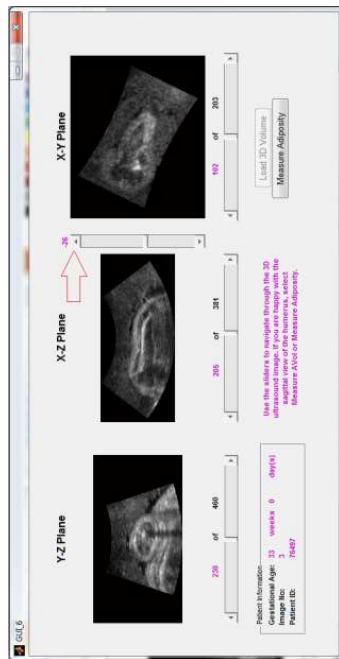
It is very important to repeat steps 3 & 4 every time you are segmenting a new ultrasound volume

5. In the command window, type "GUI_6" and a program window should appear.

This may take a while to appear depending on the speed of your PC. (The arrangement of the three image boxes and buttons may vary slightly from that shown below.)



6. Press 'Load 3D Volume' and select the ultrasound volume you would like to segment from the menu.



9. If the whole length of the bone is not visible in the sagittal view (ie an end is 'cut off', having not been captured in the volume), or if the image is too rotated and cannot be corrected to become wholly visible, close the window and record the reason in the spreadsheet.

10. When you are happy with the view of the bone, select the central sagittal view slice.

Click on the horizontal slider in one direction until the bone is disappearing from view. Click in the other direction (include the 10-click method to save time) to count the number of slices until the bone disappears from view again. Come to the midpoint of the bone (half the number of slices).

11. Press 'Measure Adiposity' and a new program window will appear.

12. Press 'length' and click and HOLD the mouse to draw a line between the endpoints of the bone.

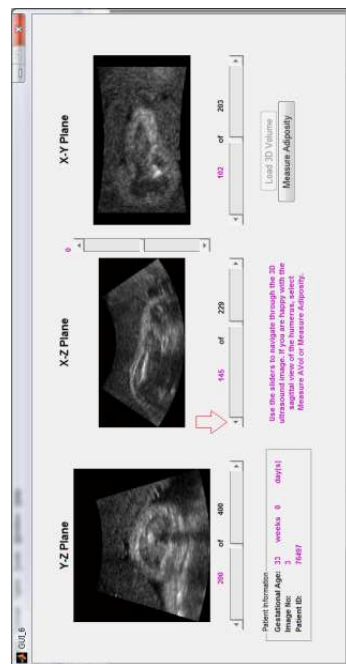
(The image can be zoomed if required, using the 'Zoom' button; this is rarely necessary.)

13. Five cross sectional slices will be automatically extracted and displayed in the small windows above the main window. The first cross sectional slice will appear in the main window.

(The arrangement of the master image box and buttons may vary slightly from that shown below.)

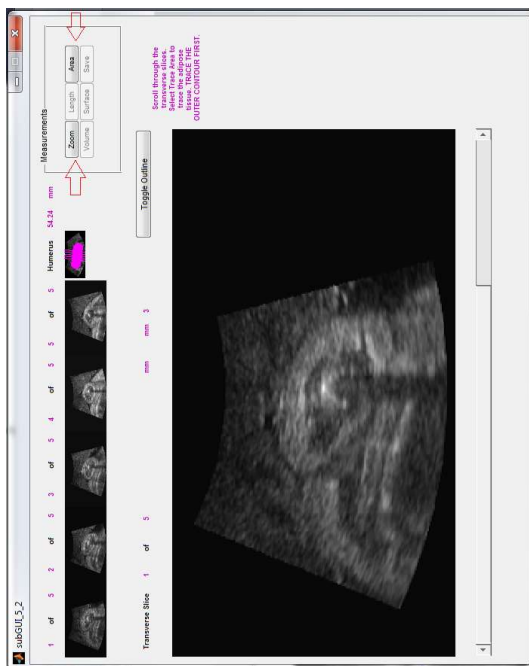
7. Use the horizontal slider to navigate through the XZ plane until the bone (either the humerus or femur, depending on the ultrasound volume) becomes wholly visible.

As you navigate through the bone, both ends should appear and disappear from view at the same time – this means it is parallel to the screen and not rotated, and makes it easier to measure.



8. If the bone is "out of plane" and will not become wholly visible using the horizontal slider, use the vertical slider to rotate the ultrasound volume until a clear view of the bone can be obtained.

Using the vertical slider can be a bit slow depending on the speed of your PC, so please be patient and don't click any other buttons while the volume is being rotated! You will see the images change once the rotation has finished. If you think the image is very rotated, you can save time by clicking within the slider bar. This will move the image by either +10 or -10 (compared with clicking on the slider bar arrows to move it by +1 or -1 per click). Another time-saving tip is to look at the X-Y plane box – if you can see the limb / bone in this box, you are aiming for it to be horizontal. If your initial rotation turns it the 'wrong' way then you know to adjust the vertical slider in the opposite direction.



14. Click through all five slices using the horizontal slider bar below the main image window. This enables previewing of the images to assess whether the volume is able to be measured.

15. If the volume cannot be measured, close the window and record the reason in the volume spreadsheet.

Reasons may include: movement artefact; fat poorly defined (contour edges not clear); fat not visible due to early gestation; image too dark; fat missing off edge of screen. If there is only a small amount of the limb fat missing off one edge of the image, but the outer contour can be accurately judged, then this can be approximated in one or two slices. If there is a large amount of fat missing, or it is missing off more than one edge, or it is missing in more than two slices, then the volume should be recorded as not measurable.

16. The limb cross-section should occupy at least 50% of the width of the image box. Press 'Zoom' if you need to magnify a slice before beginning to segment it.

The cursor will change to a magnifying glass. Click within the image and hold to drag out a box including your area of interest. When you release, the image will resize to this box. This can be repeated if needed. To reset the zoom, double click in the image.

Maryam Ahmed maryam.ahmed@crup.ac.uk (Engineering); Caroline Knight caroline.knight@obs.nyu.ac.uk (Obstetrics) – February 2014

17. The slices can be measured in any order. Press 'Area' and trace the outer contour of the limb first.

Begin at one border of the bone acoustic shadow (6 o'clock position) and trace around the limb to the other border, keeping the mouse / touchpad depressed. When the cursor is released, the gap will auto-complete and the white tracing line will turn yellow. Trace in a smooth fashion. The edge can be difficult to see, particularly towards the bottom of the image; the thickness of the fat should be roughly the same around the limb so it is twice the thickness at one point around the limb then there may have been an error in border identification. NB if you release the mouse by mistake during the tracing of the contour, the outline will auto-complete using the shortest distance between the start and finish point (see point 20: resetting the image, below).

18. WITHOUT pressing 'Area' again, trace the inner contour (fat-muscle border) immediately after the outer contour.

This border can be harder to define; adjusting the screen contrast or partially closing your eyes/squinting can help! Comparison with the previous or following slice may also make the border location more evident.



Maryam Ahmed maryam.ahmed@crup.ac.uk (Engineering); Caroline Knight caroline.knight@obs.nyu.ac.uk (Obstetrics) – February 2014

19. If you are happy with the segmentation, use the slider below the main window to scroll to, and then measure, the next slice.

20. If you would like to repeat the segmentation, press 'Area' again and repeat the process above.

The segmentation can only be reset and repeated if two shapes have been drawn onto the image. If there is a problem with the outer (first) contour and you wish to redo the measurement, trace a second shape (any shape) onto the image and then press 'Area' to start that slice again. If you have already traced two contours, just press 'Area' to start again.

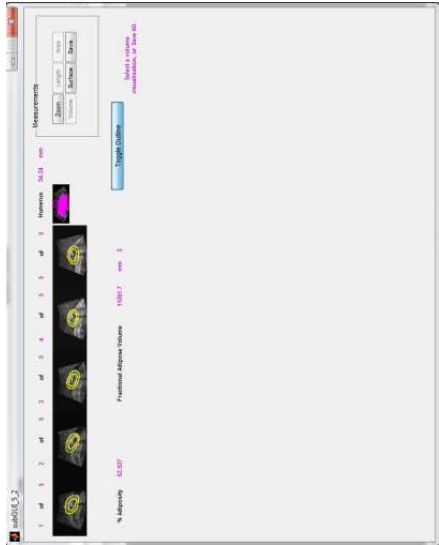
21. The 'Toggle Outline' button can be used to toggle the yellow contours on and off, to view the underlying image and check correct tracing of contours.

22. Once all five slices have been segmented, click through them all quickly using the horizontal slider under the main image box.

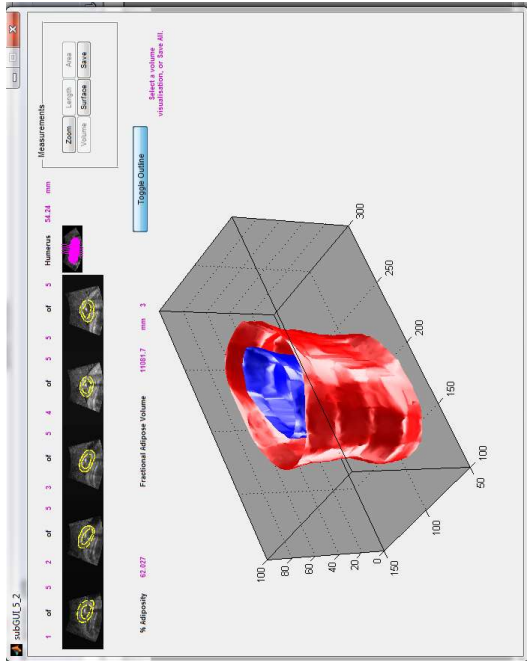
Look for any obvious discrepancies between slices – eg does one suddenly have twice the amount of fat compared with the others? Remeasure any discrepant slices.

23. Press 'Calculate Volume' to calculate the fractional arm volume.

Again, this may take some time so please be patient! You will know the process is complete because the main image box will disappear, and the 'View Surface' and 'Save All' buttons will be activated. (Screen appearance may vary from below.)

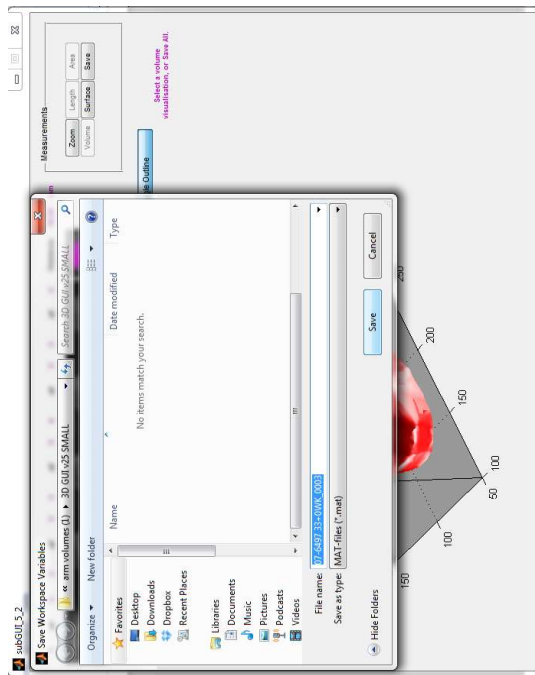


24. If you wish to see the interpolated model of the arm/thigh, press 'View Surface' and click and drag to rotate the model.



25. Otherwise (or after viewing) press 'Save All' to save the segmentation data and fractional arm volumes.

Do not change the automatically generated file name, or change the save location (this will be the folder you selected in step 2, which contains the Matlab code), as this will cause errors. Do not press any buttons or keys while the data is being saved.



26. Once the save is complete, the second window will close and you will see the three image boxes and the buttons in the previous (image select) window have become blank.

27. Close this window manually.

28. Complete the relevant line on the spreadsheet.

Indicate that segmentation was completed successfully. Add whether segmentation was easy or difficult, as well as any additional comments (final column).

29. Type "close all" [enter] "clear all" [enter] in the Matlab Command Window before you start segmenting the next volume.

30. Type "GUI_6" and select your next volume.

The up arrow key on the keyboard can be used to repeat any of the previous "close all", "clear all", or "GUI_6" commands, to save time rather than retyping.

31. Once you have finished a set of volumes, copy the result files out of the Matlab folder, where they will have been saved, and into their original folder (ie containing the image/volumes files).

There will be three files generated for each volume – an excel file (.xls or .xlsx; or .csv from a mac), a mat file (.mat) and a text file (.txt) – all of which should be copied.

32. The folder(s) should then be sent, via dropbox, to Caroline Knight for collation and analysis.

If the folders are too big, just send the .mat and .xls/.xlsx/.csv files.

Appendix E: INTERGROWTH-21st infant growth study paperwork

REC reference 08/H0606/139 V 01 26.04.11  Oxford Radcliffe Hospitals NHS Trust  The International Fetal and Newborn Growth Standards for the 21st Century INTERGROWTH-21st FOLLOW-UP INFANT GROWTH STUDY PATIENT INFORMATION SHEET	REC reference 08/H0606/139 V 01 26.04.11 PART 1: The purpose of the study and what will happen to you if you take part What is the purpose of the study? As you know, the aim of the unique INTERGROWTH-21st study is to chart the growth patterns of unborn babies and newborns all over the world and create an international standard for healthy growth. This is taking place in eight countries on five continents over a five-year period. We hope to recruit around 5,000 volunteers, 650 of whom will come from Oxford. What makes this study unique is its size and the fact that we are assessing healthy mothers and babies all over the world. Our purpose is to set new, international, 'gold' standards for fetal and newborn growth. The success of INTERGROWTH-21st means that we are now able to extend the study beyond birth. The extension involves taking follow-up measurements at 1 and 2 years of age from all infants who were scanned as babies during pregnancy, so as to follow their growth and health. Why have I been invited? You have been invited to take part because you are taking part in the INTERGROWTH-21st study, which uses ultrasound scans to look at the growth of unborn babies. Do I have to take part? It is up to you to decide whether or not you wish to take part. Your decision will not affect the care you receive in any way. We will describe the study and go through this information sheet. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time, without giving a reason. If you have any questions or concerns after reading this leaflet, our research midwife will be happy to answer them. You can also call her on the number at the end of this information leaflet. What will happen to me if I take part? You have already agreed that we can take standard measurements (your baby's weight, length and head circumference) for INTERGROWTH-21st soon after your baby is born. We would like to carry out a few additional measurements at the same time, or shortly after these are done, to help us assess your baby's growth. These are all simple and painless measurements. They do not involve any needles or blood tests.
Thank you for agreeing to take part in INTERGROWTH-21st, our study of normal fetal and infant growth. We would now like to invite you to take part in a follow-up study to INTERGROWTH-21st. The aim is to study babies' growth from the womb to the age of 2. Before you decide whether to continue your involvement with INTERGROWTH-21st, we would like you to understand why the research is being done and what it would involve for you. Please read this information sheet carefully. One of our team will go through it with you and answer any questions you have. We suggest this should take about 15 minutes. Talk to others about the study if you wish. Part 1 tells you the purpose of this study and what will happen if you take part. Part 2 gives you more detailed information about the conduct of the study. Please ask us if anything is not clear. Oxford Maternal & Perinatal Health Institute Green Templeton College Woodstock Road Oxford OX2 6HG Nuffield Department of Obstetrics & Gynaecology University of Oxford Women's Centre, John Radcliffe Hospital Oxford OX3 9DU Page 1 of 6	Page 2 of 6

REC reference 08/H0606/139 What will happen to me if I take part (cont'd)? We would like to take extra tape measurements around your baby's mid-arm, leg, and tummy, to help us better understand how babies grow. We would also like to estimate the amount of fat on your baby's body. To do this, we would use a specialised instrument (callipers) to measure the skinfold thickness. This can be done with your baby in your arms, in the cot or on the measuring table. We would also like to find out the relative amount of fat and other tissues (body composition) in your baby's body after birth. This is done using a 'PEA POD', which is a computerised weighing scale and measuring machine. Your baby would be undressed, weighed and then placed in a cot for 2-3 minutes. The cot has a window (see picture) so that you can see your baby and he/she can see you. These techniques have been used thousands of times and babies are usually calm when being measured. In the unlikely event that your baby became distressed for any reason then we would stop straight away and would only continue if you were happy. The whole procedure should take no longer than 30 minutes. This includes time to undress and re-dress your baby, prepare the PEA POD, and perform repeated measurements to ensure they are as accurate as possible. We would then like to follow-up all children at the time of their 1st and 2nd birthdays (although we can see them up to two weeks either side of these dates). We would contact you, and your GP, by letter about these appointments nearer the time, to invite you to attend. The two follow-up appointments would be with our follow-up Measurement Team at the Women's Centre, John Radcliffe Hospital, and should last no longer than 30 minutes. At these appointments, we would repeat the measurements described above, except for the PEA POD measurements that are only carried out in younger babies. If your baby is born prematurely we would also like to take weight; length; head, arm, tummy and leg circumferences, and skinfold thickness every two weeks for the first eight weeks after your baby is born, and then every month until your baby is 8 months old. We would also like to measure body composition with the PEA POD machine at each visit until your baby weighs more than 9kg (the limit of the machine). These appointments will be with the team at the hospital as described above. We will then invite you to attend the one- and two-year appointments as described above. Expenses and payments You may claim back travel expenses for these additional appointments.	REC reference 08/H0606/139 What are the possible disadvantages and risks of taking part? If you choose to take part in this additional study, we would ask you to bring your baby to the hospital for the extra appointments. The measurements themselves are safe and carry no risk to your baby. Babies do occasionally become upset during measurements, as explained above. In the unlikely event that your baby became distressed for any reason then we would stop straight away. We expect most of the babies we follow up to be entirely healthy and to have normal growth. If, however, our measurements showed any problems with your baby's growth then we would report these to your GP who would arrange appropriate monitoring of your baby. What are the possible benefits of taking part? Taking part in this study would not usually benefit you or your baby directly. The only situation where there might be direct benefit would be if we detected growth problems, which might allow earlier monitoring and/or investigations by your GP. However, the information obtained from this study will enable us to create unique fetal and infant growth charts for the first time. These will be used internationally and potentially save the lives of many babies and newborns. What if there is a problem? Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. The detailed information on this is given in Part 2. Will my taking part in the study be kept confidential? Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. The details are included in Part 2. This completes Part 1. If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 (next pages) before making any decision.
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REC reference 08/H0606/139 V 01 26.04.11 PART 2: More detailed information about the conduct of the study What will happen if I don't want to carry on with the study? You may withdraw from the study at any time. It would not affect the care you receive in any way. If you withdraw from the study, any stored data that identifies you would be destroyed. What if there is a problem? If you have concerns about the way you have been approached or treated during the course of the study, you can contact Dr Stephen Kennedy, Clinical Director, Women's Services, John Radcliffe Hospital and Head of the Nuffield Department of Obstetrics & Gynaecology, on 01865 221003. If you remain unhappy and wish to complain formally, you may also follow the normal NHS complaints procedure. Please see: www.nhsdirect.nhs.uk/articles/article.aspx?articleid=569 for more information. In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against University of Oxford but you may have to pay your legal costs. Will my taking part in this study be kept confidential? Our data manager, who has a duty of confidentiality to you and your baby as research participants, is the only person able to identify you and your baby. If you choose to continue with the study, your and your baby's results and pictures will be held anonymously in a secure database to which only the research team will have access. Authorised representatives of the University of Oxford may look at the anonymised results to check that the study is being performed correctly. No individual participants will be identified when the results of the study are published. The data will be retained for 20 years after the end of the study in the event that future discussions about fetal/infant growth require the data to be reanalysed. After 20 years, the data will be disposed of securely. You have the right to check the accuracy of data held about you and your baby, and to correct any errors. Involvement of the General Practitioner/Family doctor (GP) In clinical research such as this, it is our responsibility to inform your GP that you have agreed to take part in this study. This is to ensure that your baby is healthy at the time of participation and remains well during the course of the study. If the study did show any problems with your baby's growth we would also inform your GP of this so that he/she could monitor and/or investigate this as appropriate.	REC reference 08/H0606/139 V 01 26.04.11 What will happen to the results of the research study? The results will be prepared for worldwide distribution, publication in scientific journals and presentation at international meetings. We can provide you with a copy of the papers after publication if you wish. Your name will not appear in any report or publication. Some of the data from the study may be included in the PhD thesis of one or more of the researchers. Your identity will be protected at all times. Who is organising and funding the research? The research is being carried out by the Nuffield Department of Obstetrics & Gynaecology and it is being funded by the Bill and Melinda Gates Foundation. The doctors involved in the research are not being paid to include you in the study and have no conflicts of interest with regards to the study. Who has reviewed the study? Research is reviewed by an independent group of people called a Research Ethics Committee to protect your interests, safety, rights, well-being and dignity. This study has been reviewed and given favourable opinion by the South Central – Oxford C Research Ethics Committee. Further information and contact details If you have any questions, please do not hesitate to contact Fenella Roseman, our Research Midwife by: Telephone: 07837846234 Email: fenella.roseman@obs-gyn.ox.ac.uk Post: NDOG, University of Oxford, John Radcliffe Hospital, Women's Centre, Level 3, Headington, Oxford OX39DU You can find our website at www.INTERGROWTH21.org.uk This completes Part 2. You may keep this information leaflet for your records. If you have any questions, please contact us as above. If you wish to take part in the study we will ask you to sign a consent form. We will give you a signed copy to keep for your records
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This consent form relates to the INTERGROWTH-21st (REC reference o8/Ho606/139)
FOLLOW UP: INFANT GROWTH Patient Information Leaflet version 01.26.04.11

Oxford Maternal and Perinatal
Health Institute
Green Templeton College
University of Oxford
Woodstock Road
Oxford OX2 6HG



Nuffield Department of
Obstetrics & Gynaecology
University of Oxford
Level 3, Women's Centre
John Radcliffe Hospital
Oxford OX3 9DU



Oxford Radcliffe Hospitals
NHS Trust



**The International Fetal and
Newborn Growth Standards for the 21st Century
(INTERGROWTH-21st) STUDY**

FOLLOW-UP: INFANT GROWTH

CONSENT FORM

Centre Number: _____ Patient Identification Number for this trial: _____
Study Number: _____ Name of Researcher: _____

Please initial box

☐	☐	☐	☐
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1. I confirm that I have read and understood the information sheet dated 26.04.2011 (version 1) for the above study. I have had the opportunity to consider the information, ask questions and have received satisfactory answers.
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected.
3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from the University of Oxford, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.
4. I agree to my GP being informed of my participation in the study.
5. I agree to take part in the above study.

_____ Name of patient	_____ Signature	_____ Date
_____ Name of person taking consent	_____ Signature	_____ Date

When completed: 1 for participant; 1 for researcher site file; 1 (original) to be kept in medical notes.

Appendix F: Contributions to appendices

All protocols and documents within the appendices conceived and written by the author unless otherwise stated.

C: Fetal MRI study protocol

Written by the author, under the supervision of A.P.

C: Fetal MRI scanning protocol

Developed by G.Q and the author, with input from S.R. and A.P.
Written by G.Q. after further discussion with the author and S.R.
Second version (shown in appendix) edited by the author

C: Fetal MRI GP information letter

Written by the author
(A.P. was listed as P.I. as it had been thought the author may have left Oxford prior to completion of the study whereas A.P. would have still been in Oxford.)

D: 3D volume segmentation protocol for MATLAB tool

Written by M.A. and the author
Screenshots by M.A.