Published in final edited form as:

Ultrasound Obstet Gynecol. 2009 April ; 33(4): 427–440. doi:10.1002/uog.6319.

Fractional limb volume – a soft tissue parameter of fetal body composition: validation, technical considerations and normal ranges during pregnancy

W Lee^{*,†,‡}, M Balasubramaniam[§], RL Deter[¶], SS Hassan^{†,‡}, F Gotsch^{†,‡,**}, JP Kusanovic^{†,‡}, LF Goncalves^{†,‡}, and R Romero^{†,‡,**}

^{*}Division of Fetal Imaging Department of Obstetrics and Gynecology, William Beaumont Hospital, Royal Oak, MI

[§]Division of Biostatistics, William Beaumont Hospital Research Institute, Royal Oak, MI

[¶]Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston TX

[†]Perinatology Research Branch, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services, Bethesda, MD

[‡]Department of Obstetrics and Gynecology, Wayne State University, Detroit, MI, USA

Abstract

Objectives—The main goals were to provide normal reference ranges for fractional limb volume as a new index of generalized fetal nutritional status, to evaluate the reproducibility of fractional fetal limb volume measurements during the second and third trimesters of pregnancy, and to demonstrate technical considerations for this technique.

Methods—This was a prospective, cross-sectional study of gravid women during mid to late pregnancy. Fractional limb volumes were based on either 50% of humeral or femoral diaphysis length. Each partial volume was subdivided into five equidistant slices that were centered along the mid-arm or mid-thigh. Slices were traced manually to obtain fractional arm (AVol) or fractional thigh (TVol) volume. Reproducibility studies were performed, using Bland-Altman plots, to assess blinded interobserver and intraobserver measurement bias and agreement. Selected images were chosen to demonstrate technical factors for the acquisition and analysis of these parameters. Reference charts were established to describe normal ranges for AVol and TVol.

Results—Three hundred and eighty-seven subjects were scanned to include 380 AVol (range, 1.1–68.3 mL) and 378 TVol (Range, 2.0–163.2 mL) measurements between 18.0 and 42.1 weeks' menstrual age. No gender differences were found in these soft tissue measurements (AVol, $P=0.90$; TVol, $P=0.91$; Mann-Whitney test). Intraobserver mean bias $\pm$ SD and 95% limits of agreement (LOA) for fractional limb volumes were: $2.2 \pm 4.2\%$ (95% LOA, -6.0 to 10.5%) for AVol and $2.0 \pm 4.2\%$ (95% LOA, -6.3 to 10.3%) for TVol. Interobserver bias and agreement were $-1.9 \pm 4.9\%$ (95% LOA, -11.6 to 7.8%) for AVol and $-2.0 \pm 5.4\%$ (95% LOA, -12.5 to 8.6%) for TVol. Technical factors were related to image optimization, transducer pressure, fetal movement, soft tissue compression and amniotic fluid volume.

Corresponding Author: Dr. Wesley Lee, Division of Fetal Imaging, Department of Obstetrics and Gynecology, William Beaumont Hospital, 3601 West Thirteen Mile Road, Royal Oak, MI, wlee@beaumont.edu.

**The contribution of F. Gotsch and R. Romero to their article was prepared as part of their official duties as United States Government employees.

Conclusions—Fractional limb volume assessment may improve the detection and monitoring of malnourished fetuses because this soft tissue parameter can be obtained quickly and reproducibly during mid to late pregnancy. Careful attention should be placed on technical factors that can potentially affect optimal acquisition and analysis of these volume measurements.

Keywords

fetal growth; fractional arm volume; fractional thigh volume; soft tissue

INTRODUCTION

Fetal growth abnormalities clearly increase the risk for adverse neonatal outcomes and Cesarean delivery. Furthermore, the role of low birth weight, as an important predictive factor for chronic diseases in adulthood is supported by data from epidemiological and clinical studies for hypertension, diabetes and cardiovascular diseases.¹⁻⁵ Current obstetric practice is based on an estimation of fetal size as a common means of evaluating prenatal nutritional status by classifying weight as being small (< 10th percentile), appropriate (10-90th percentile) or large (> 90th percentile) for gestational age.⁶ This traditional approach, however, does not precisely separate malnourished fetuses from those that are constitutionally small or large, but otherwise normal.

Although the clinical interpretation of estimated weight percentiles has contributed greatly to our understanding of prenatal growth, more robust techniques are required for the early detection and monitoring of malnourished fetuses. Beattie and Johnson⁷ have proposed supplementary approaches for the evaluation of neonates (e.g. ponderal index, skinfold thickness and mid-arm circumference measurements) to improve nutritional assessment beyond the use of birth weight along. Complementary methods for the prenatal assessment of generalized nutritional status may also be possible beyond the use of estimated fetal weight as well. For example, fetal soft tissue evaluation has been described for thigh circumference,⁸ cheek-to-cheek diameter,^{9,10} buttock,¹¹ and subcutaneous measurements of the abdomen or limbs.¹²⁻¹⁸ The reproducibility of these measurements between examiners, however, has not been well established throughout pregnancy. Many of these studies are also based on a single two-dimensional image, as a distance or area measurement, neither of which may correlate well with actual soft tissue volume.

Limb volume is another soft tissue parameter that has been described for the valuation of fetal nutritional status.¹⁹ Three-dimensional ultrasonography (3DUS) provides a versatile method for evaluating the soft tissue of fetal limbs. Nonetheless, earlier attempts to measure soft tissue by 3DUS were hampered by the extended time necessary to allow manual tracing of surface borders along the length of an entire limb.²⁰ Acoustic shadowing of these borders near the joints also posed additional technical limitations that hindered the practical implementation of this approach in obstetric care. The concept of fractional limb volume addresses these technical limitations for fetal weight estimation and growth assessment.²¹⁻²³ (Figure 1). This soft tissue parameter is derived from a central portion of the limb diaphysis (Figures 2 and 3). Transverse slices of the mid-limb are more likely to display the sharpest soft tissue borders for manual tracing (Figure 4). Measuring times are substantially reduced because the examiner needs only to trace a few equidistant slices within the partial limb volume.

Our investigation establishes normal reference ranges for fractional limb volume, examines the reproducibility of these soft tissue measurements during the second and third trimesters of pregnancy, and describes technical advantages and pitfalls of this approach.

METHODS

This prospective, cross-sectional study of gravid women was approved by the Institutional Review Boards at William Beaumont Hospital, Wayne State University, and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, and participants gave their informed consent. Included women were in their second and third trimesters of pregnancy. The protocol excluded multiple gestations, infants found to have structural anomalies, and fetuses with poorly visualized limbs owing to technical factors. Subjects with maternal risk factors of abnormal fetal growth, such as hypertension, diabetes and smoking, were also excluded. Pregnancy age was based on the first day of the last normal menstrual period or menstrual age confirmed by a first- or early second-trimester dating scan. Maternal age, gravidity, menstrual age at time of scan, gender, ethnicity and presence of obstetric complications were documented.

Fetuses were examined using conventional sonography for standard two-dimensional biometry. Each pregnancy was scanned only once. Volume datasets were acquired using hybrid mechanical and curved-array abdominal ultrasonic transducers (RAB 4-8P, RAB 2-5P; Voluson 730 Expert, GE Healthcare, Milwaukee, WI, USA). Image depth and magnification were adjusted for a volume of interest that filled at least two-thirds of the display screen. The acoustic focal zone was placed near the femoral or humeral diaphysis. System gain was adjusted to optimize the signal-to-noise ratio. Each volume acquisition, lasting approximately 10s, was taken from a sagittal sweep of the limb diaphysis during the maternal breath-hold. Image data were archived on digital media for subsequent offline analysis. Multiplanar images were selected to demonstrate technical approaches and potential pitfalls for fractional limb volume measurements.

Fractional limb volumes were calculated using commercially available software (4D View 5.0, GE Healthcare). Volume measurements were based on either 50% of humeral diaphysis (AVol) or 50% of femoral diaphysis (TVol) length. Each partial volume was subdivided into five equidistant slices that were centered along the mid-arm or mid-thigh.²¹⁻²³ Images were magnified to fill at least two-thirds of the display. Soft tissue borders were enhanced by use of a color filter (sepia) with additional gamma curve adjustments for brightness and contrast (Figure 5). Fractional limb volumes were calculated after each of the five slices was manually traced from a transverse view of the extremity (Videoclip S1).

Statistical analysis

Distribution of fractional limb volume measurements was initially assessed graphically and numerically, using standard tests for normality (i.e. Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von Mises and Anderson-Darling tests). The scatterplots indicated curvilinear exponential growth curves for both AVol and TVol exponential growth curves for both AVol and TVol over time, with increasing variability at later menstrual ages (heteroscedasticity). Box-Cox transformations were used to identify the most useful linear transformations of the raw data.²⁴ Based on these results, a natural logarithmic transformation was applied to menstrual age, AVol and TVol.

The transformed data became linearized to correct for non-normality of error terms and unequal error variances. Age-related percentile tables were developed using weighted least-squares regression and absolute residuals.^{25,26} After residual analyses had been completed and normality of error terms confirmed, the fitted percentile data were then transformed back into their original units. Within each menstrual age group, the mean response at each percentile (5th, 10th, 25th, 50th, 75th, 90th, 95th), with its corresponding average deviation score for these parameters, was developed to establish normal ranges during mid to late pregnancy. Statistical analysis for regression analysis and the development of percentile

charts was performed using the SAS System for Window, version 9.1.3, Service Pak 2 (SAS, Cary, NC, USA).

After removal of subject identifiers, 40 arm and 40 thigh volume datasets were reviewed to investigate the reproducibility of these measurements. These datasets were selected to represent a broad range of menstrual ages. Two different examiners independently manipulated these datasets and made fractional limb volume measurements in a blinded manner. The first examiner replicated these fractional limb volume measurements twice in a blinded manner. A second examiner analysed the same volume datasets once only. Bland-Altman plots (% difference vs. average) were used to determine 95% limits of agreement (LOA) and bias for a single examiner and between examiners (GraphPad Prism, version 4.0b for Macintosh, GraphPad Software, San Diego, CA, USA).²⁷ Unpaired comparisons between AVol or TVol measurements were performed between male and female fetuses using the Mann-Witney test. *P* values less than an α of 0.05 (probability of Type 1 error) were considered statistically significant.

RESULTS

A prospective cross-sectional study of 387 pregnant women was performed between April 2002 and February 2008. Ultrasound scans were performed between 18.0 and 42.1 weeks' menstrual age.

Study population – descriptive statistics

The median maternal age was 29.4 (interquartile range (IQR), 25.0-34.0) years and 104 of these subjects (26.9%) were primigravidae. The major racial distribution was 58.7% White, 32.0% Black, 5.9% Asian and 1.3% Hispanic. Three hundred and eighty-seven infants were delivered with a median birth weight of 3241 (IQR, 2755-3716) g at 39.0 (IQR, 37.7-39.7) weeks. Gender distribution was 51.2% females and 48.8% males.

Fractional limb volume – normal ranges during pregnancy

A non-parametric distribution was observed for both the AVol (range, 1.1-68.3 mL; $n = 380$) and TVol (range 2.0-163.2 mL; $n = 378$) (Figure 6). No significant differences were observed in either AVol ($P = 0.90$) for TVol ($P = 0.91$) on the basis of gender for this cross-sectional population. Fractional limb volumes demonstrated a linear relationship to menstrual age during early pregnancy, from approximately 18 weeks until 28-29 weeks. Soft tissue accretion appeared to increase exponentially after this time. The mean $\pm$ SD TVol/AVol ratio was 2.16 ± 0.36 . A small linear increase in this ratio was noted throughout the entire study period ($R^2 = 0.20$, $P < 0.0001$) (Figure 7). Normal fitted percentile ranges for AVol and TVol are summarized in Tables 1 and 2. An online calculator is also available that allows the plotting of fractional limb volume measurements against percentiles fitted to gestational age; this can be found at <http://www.obsono.org>.

The reproducibility of fractional limb volume measurements was summarized using Bland-Altman plots for two independent examiners (Figure 8). Volume datasets were obtained between 18.1 and 39.9 weeks' menstrual age. Mean intraobserver bias $\pm$ SD and agreement for fractional limb volume measurements were documented for a single examiner; values were $2.2 \pm 4.2\%$ (95% LOA, -6.0 to 10.5%) for AVol and $2.0 \pm 4.2\%$ (95% LOA, -6.3 to 10.3%) for TVol. Satisfactory interobserver bias and agreement were also noted for blinded measurements between different examiners: $-1.9 \pm 4.9\%$ (95% LOA, -11.6 to 7.8%) for AVol and $-2.0 \pm 5.4\%$ (95% LOA, -12.5 to 8.6%) for TVol.

DISCUSSION

Human fetuses have the greatest proportion of body fat among mammalian species and an enormous capacity for altering this body compartment as a result of intrauterine growth.^{28,29} Sparks et al.³⁰ used carcass analysis to estimate that fat deposition represents over half of the calorie accretion from the 27th menstrual week until term, and constitutes up to 90% of this metabolic requirement before delivery. In this regard, we have also performed longitudinal growth studies that demonstrated accelerated soft tissue accretion of the fetal limb after approximately 28 weeks in pregnancies with normal growth outcome.^{22,23} Disruption of this fundamental growth process will lead to malnourished fetuses with excessive or reduced soft tissue development. Unfortunately, the current use of head, abdominal and femoral diaphysis length for weight estimation does not also include an evaluation of fetal soft tissue status.

Pediatric studies have applied soft tissue evaluation of newborn infants for decades. Mid-arm circumference measurements have been used as indirect measures of subcutaneous fat and lean body mass in newborn infants.³¹⁻³⁴ Excler and coworkers³⁵ found mid-arm circumference to be more useful than birth weight, length and head circumference for identifying growth restriction in French infants. The mid-arm circumference to head circumference ratio has also been applied to neonatal nutrition studies to identify which fetuses were at greatest risk for metabolic abnormalities associated with intrauterine growth restriction.³⁶ These postnatal investigations suggest that soft tissue assessment should provide additional insight into generalized fetal nutritional status.

Several reports have attempted to quantify fat and lean body mass in fetuses, although the interobserver reproducibility of these measurements has not been well characterized throughout pregnancy. For example, Bernstein and colleagues³⁷ correlated ultrasound images of fetal limbs with neonatal body composition in 25 pregnant women for fat and lean body mass. Axial images were taken of the fetal arms (mid-arm fat and lean mass, cm²), legs (mid-thigh fat and lean mass, cm²) and subscapular (subscapular fat mass, mm) areas.^{38,39} Abdominal fat thickness (mm) was also measured.⁴⁰ Limb fat content was calculated as total cross-sectional limb area minus central lean area (including bone and muscle). The intraobserver reliability of limb fat and lean mass area measurements was determined in only 10 subjects, and the corresponding menstrual ages were not specified. Coefficients of variation for lean body mass of the thigh (4.9%) and arm (7.5%) were less than those observed for fat mass of the thigh (12.1%) and arm (10.8%). The same group also reported that limb fat measurements were associated with an intraobserver coefficient of variation of 28%.³⁸ The relatively large variability was attributed to distortion of the proximal limbs from external compression. Others have used similar techniques to demonstrate reduction of subcutaneous mass, but not lean mass, in normal term fetuses that were born at high altitude.¹⁵ Measurement reproducibility was expressed as the coefficient of variation in 20 fetuses for abdominal subcutaneous thickness (intraobserver, 2.6%; interobserver, 11.1%) and extremity subcutaneous fat (intraobserver, 4.1%; interobserver, 8.2%). Similar comparisons were made for lead body mass. Larciprete et al.¹⁷ have applied these soft tissue parameters to define reference values of fetal subcutaneous tissue thickness in 218 healthy pregnancies and in 85 women with gestational diabetes. Fetal fat mass was greater in diabetic women, especially during late pregnancy. Measurement reproducibility was examined for 20 images that were not specified for menstrual age. Coefficients of variation for those between examiners ranged from 7.0% to 10.9%.

3DUS has also been used to quantify soft tissue of the fetal limbs. Chang and colleagues⁴¹ studied soft tissue volume measurements of the fetal extremities for a Taiwanese population. Predicted values were developed for fetal thigh volume in 225 singleton fetuses between 20

and 40 weeks' menstrual age. Axial slices of the fetal thigh were measured along the entire length of the femoral diaphysis in 3-mm steps, using a process that took 10-15 min per limb. Thigh volumes ranged from a mean of 9.0 mL at 20 weeks to 129 mL at 40 weeks. Although no reproducibility studies were initially reported, a subsequent publication included an intraobserver analysis for a single examiner that included 20 fetuses between 20 and 40 weeks' gestation. The sensitivity of fetal thigh volume for predicting birth weight below the 10th percentile was 86% in a Taiwanese population.⁴² A third publication used similar techniques and found the sensitivity of fetal arm volume for predicting birth weight below the 10th percentile to be 97.5%.⁴³ maternal ages were not reported for these comparisons. Similar to their thigh volume study, repeated measurements for only one examiner were evaluated in 20 fetuses during the second half of pregnancy.

Careful attention must be given to technical factors that could influence the reproducibility of measuring fractional limb volumes that contain fetal subcutaneous fat, lean muscle mass and bone (Figure 9). Although the precise degree of examiner experience remains to be defined, adequate training will optimize the likelihood of accurate and reproducible measurements. As a quality control benchmark, a trained examiner should be able to blindly measure two sets of fractional limb volume measurements ($n = 40$ for each set). For AVol, 95% of the percentage differences should be between -6.0% and 10.5%; for TVol, 95% of the percentage differences should be between -6.3% and 10.3%. A single examiner should be able to reliably measure fractional limb volumes once this level of proficiency has been achieved.

Although most fractional limb volumes were easily measured within a couple of minutes, the initial volume data acquisition procedure may be affected by several technical factors. Image quality influenced how well soft tissue borders were visualized around the fetal limb. Increased maternal wall thickness hindered optimal delineation of soft tissue borders, although adjustments in transducer frequency, harmonic imaging, compound imaging, color filtering and speckle reduction were found to be helpful. The best results were achieved by considering the following issues.

1. Excessive abdominal transducer pressure can obliterate the small fluid layer that helps to differentiate soft tissue borders from the adjacent fetal limb and uterine wall (Figure 10). Although increased fluid can improve visualization of soft tissue borders, decreased amniotic fluid volume can make fractional limb volume measurement more difficult.
2. An inadequate sweep angle or suboptimally centered placement of the volume probe on the maternal abdomen can lead to an incomplete scan of the fetal limb (Figure 11). This problem is especially relevant for macrosomic fetuses as the three-dimensional sweep angle for a sagittal sweep can reach the technical limits of commercially available probes. For AVol, the optimal Y-plane sweep angle setting was usually less than 50° at 18 weeks and this requirement increased at 85° by term. For TVol, the optimal Y-plane sweep angle setting was greater than 40° at 18 weeks and this value also increased to 85° by term. Occasionally, fractional thigh volume can be difficult to obtain for a macrosomic fetus. Under these circumstances, one may attempt to acquire serial images from transverse views of the limb that will allow a technically adequate sweep.
3. Movement artifacts, resulting from maternal respiration, can be minimized by breath holding during volume acquisition. After the sweep sequence has been completed, the examiner should immediately scroll through transverse limb volume slices to identify movement artifacts or poorly differentiated soft tissue borders before saving the data (Figure 12)

4. Compression by adjacent structures may be caused by a folded limb at the elbow or knee and is more likely to occur with decreased amniotic fluid volume (Figure 13). When the former occurs, improved acquisitions may be possible after fetal movement or transabdominal manipulation of the fetus has occurred.
5. Upper and lower limbs can have very similar sonographic appearances. Inadvertent confusion between the arm and leg can be minimized if transducer orientation is understood in relation to fetal lie. A cross-sectional view of the fetal arm is smaller than the thigh and is positioned more towards the head. The adjacent ribs and thorax should also be easily identified in a volume dataset that contains AVol. As early as 18 weeks, a characteristic indentation can be seen that separates the deltoid and triceps muscles of the fetal arm (Figure 14). A similar skin surface indentation is not found in the fetal thigh.

Fractional limb volumes may improve the detection and monitoring of abnormal fetal growth because they can be traced rapidly and reproducibly over a broad range of pregnancy. Rapid changes in soft tissue accretion, especially during the third trimester of pregnancy, could provide the basis for a sensitive biomarker of fetal growth aberrations by allowing direct comparisons between fractional limb volume measurements and cross-sectional standards from the present study. Alternatively, at least one fractional limb volume measurement could be acquired during a screening examination and combined with a subsequent second-trimester measurement for individualized fetal growth assessment. This approach simplifies the interpretation of a growth parameter for an individual fetus because population-based standards inherently contain a high degree of biological variability. Normal growth is assumed for the Rossavik analysis if the 'start point' of the growth model and growth velocity of a given parameter (coefficient *c*) are within the normal range during the second trimester. Second-trimester growth velocity changes for fractional limb volumes have been used to establish Rossavik model coefficients for predicting expected third-trimester growth trajectories, using each fetus as its own control.^{22,23} Finally, fractional limb volume measurements have also been used with conventional two-dimensional parameters to improve the precision of fetal weight estimation in term pregnancies.²¹

Intrauterine growth results from complex hormonal and physiological interactions between the mother, placenta and fetus.⁴⁴ Low birth weight and poor prenatal nutrition are associated with altered fat distribution, reduced muscle mass and strength, and low bone mineral content in adults. Potential mechanisms include a direct effect on cell number, altered stem cell function, and resetting of regulatory hormonal axes as a result of fetal programming.^{2,45} The clinical application of a reproducible soft tissue parameter may improve our understanding of how the intrauterine environment can influence health in adult life for growth-restricted and macrosomic infants. A companion article in this issue of the Journal describes the relationship of fractional limb volume to fetal growth parameters, birth weight and infant body composition. The application of soft tissue parameters will be examined subsequently for fetal weight estimation. Fractional limb volumes, either alone or in combination with Doppler blood flow studies, may improve our ability to detect and monitor malnourished fetuses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors wish to acknowledge the technical assistance of Melissa Powell, RDMS, and Beverley McNie, BS, CCRP. This research was supported partially by the Perinatology Research Branch, Division of Intramural

Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services.

REFERENCES

1. Hales CN, Barker DJ. The thrifty phenotype hypothesis. *Br Med Bull.* 2001; 60:5–20. [PubMed: 11809615]
2. Barker D. The midwife, the coincidence, and the hypothesis. *BMJ.* 2003; 327:1428–1430. [PubMed: 14684636]
3. Reyes L, Manalich R. Long-term consequences of low birth weight. *Kidney Int Suppl.* 2005; 97:S107–S111. [PubMed: 16014086]
4. McMillen IC, Robinson JS. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. *Physiol Rev.* 2005; 85:571–633. [PubMed: 15788706]
5. Plagemann A. Perinatal nutrition and hormone-dependent programming of food intake. *Horm Res.* 2006; 65(Suppl 3):83–89. [PubMed: 16612119]
6. Lubchenco LO, Hansman C, Dressler M, Boyd E. Intrauterine growth as estimated from liveborn birth-weight data at 24 to 42 weeks of gestation. *Pediatrics.* 1963; 32:793–800. [PubMed: 14075621]
7. Beattie RB, Johnson P. Practical assessment of neonatal nutrition status beyond birth weight: an imperative for the 1990s. *Br J Obstet Gynaecol.* 1994; 101:842–846. [PubMed: 7999684]
8. Deter RL, Rossavik IK, Cortisoz C, Hill RM, Hadlock FP. Longitudinal studies of thigh circumference growth in normal fetuses. *J Clin Ultrasound.* 1987; 15:388–393. [PubMed: 3134437]
9. Abramowicz JS, Sherer DM, Bar-Tov E, Woods JR. The cheek-to-cheek diameter in the ultrasonographic assessment of fetal growth. *Am J Obstet Gynecol.* 1991; 165:846–852. [PubMed: 1951542]
10. Abramowicz JS, Sherer DM, Woods JR. Ultrasonographic measurements of cheek-to-cheek diameter in fetal growth disturbances. *Am J Obstet Gynecol.* 1993; 169:405–408. [PubMed: 8362956]
11. Matsumoto M, Yanagihara T, Hata T. Three-dimensional qualitative sonographic evaluation of fetal soft tissue. *Hum Reprod.* 2000; 15:2438–2442. [PubMed: 11056149]
12. Hill LM, Guzik D, Boyles D, Merolillo C, Ballone A, Gmitter P. Subcutaneous tissue thickness cannot be used to distinguish abnormalities of fetal growth. *Obstet Gynecol.* 1992; 80:268–271. [PubMed: 1635742]
13. Petrikovsky BM, Oleschuk C, Lesser M, Gelertner N, Gross B. Prediction of fetal macrosomia using sonographically measured abdominal subcutaneous tissue thickness. *J Clin Ultrasound.* 1997; 25:378–382. [PubMed: 9282803]
14. Chauhan SP, West DJ, Scardo JA, Boyd JM, Joiner J, Hendrix NW. Antepartum detection of macrosomic fetus: clinical versus sonographic, including soft-tissue measurements. *Obstet Gynecol.* 2000; 95:639–642. [PubMed: 10775720]
15. Galan HL, Rigano S, Radaelli T, Cetin I, Bozzo M, Chyu J, Hobbins JC, Ferrazzi E. Reduction of subcutaneous mass, but not lean mass, in normal fetuses in Denver, Colorado. *Am J Obstet Gynecol.* 2001; 185:839–844. [PubMed: 11641662]
16. Padoan A, Rigano S, Ferrazzi E, Beaty BL, Battaglia FC, Galan HL. Differences in fat and lean mass proportions in normal and growth-restricted fetuses. *Am J Obstet Gynecol.* 2004; 191:1459–1464. [PubMed: 15507983]
17. Larciprete G, Valensise H, Vasapollo B, Novelli GP, Parretti E, Altomare F, Di Pierro G, Menghini S, Barbati G, Mello G, Arduini D. Fetal subcutaneous tissue thickness (SCTT) in healthy and gestational diabetic pregnancies. *Ultrasound Obstet Gynecol.* 2003; 22:591–597. [PubMed: 14689531]
18. Larciprete G, Valensise H, Di Pierro G, Vasapollo B, Casalino B, Arduini D, Jarvis S, Cirese E. Intrauterine growth restriction and fetal body composition. *Ultrasound Obstet Gynecol.* 2005; 26:258–262. [PubMed: 16116565]
19. Jeanty P, Romero R, Hobbins JC. Fetal limb volume: a new parameter to assess fetal growth and nutrition. *J Ultrasound Med.* 1985; 4:273–282. [PubMed: 3892024]

20. Chang FM, Liang RI, Ko HC, Yao BL, Chang CH, Yu CH. Three-dimensional ultrasound-assessed fetal thigh volumetry in predicting birth weight. *Obstet Gynecol.* 1997; 90:331–339. [PubMed: 9277639]
21. Lee W, Deter RL, Ebersole JD, Huang R, Blanckaert K, Romero R. Birthweight prediction by 3D ultrasonography: fractional limb volume. *J Ultrasound Med.* 2001; 20:1283–1292. [PubMed: 11762540]
22. Lee W, Deter RL, McNie B, Go calves LF, Espinoza J, Chaiworapongsa T, Romero R. Individualized growth assessment of fetal soft tissue using fractional thigh volume. *Ultrasound Obstet Gynecol.* 2004; 24:766–774. [PubMed: 15586365]
23. Lee W, Deter RL, McNie B, Go calves LF, Espinoza J, Chaiworapongsa T, Balasubramaniam M, Romero R. The fetal arm: individualized growth assessment in normal pregnancies. *J Ultrasound Med.* 2005; 24:817–828. [PubMed: 15914686]
24. Box GEP, Cox DR. An analysis of transformations. *J R Stat Soc Ser B.* 1964; 26:211–246.
25. Altman DG. Charts of fetal size: 1. Methodology. *Br J Obstet Gynaecol.* 1994; 101:20–34.
26. Neter, J.; Kutner, MH.; Nachtsheim, CJ.; Wasserman, W. *Applied Linear Regression Models.* 3rd edn. The McGraw-Hill Companies; New York: 1996.
27. Bland MJ, Altman DG. Measuring agreement in method comparison studies. *Stat Methods Med Res.* 1999; 8:135–160. [PubMed: 10501650]
28. Widdowson E. Chemical composition of newly born mammals. *Nature.* 1950; 166:26–29. [PubMed: 15439095]
29. Ziegler EE, O'Donnell AM, Nelson SE, Fomon SJ. Body composition of the reference fetus. *Growth.* 1976; 40:329–341. [PubMed: 1010389]
30. Sparks JW, Girard JR, Battaglia FC. An estimate of the caloric requirements of the human fetus. *Biol Neonate.* 1980; 38:113–119. [PubMed: 7407269]
31. Meadows NJ, Till J, Leaf A, Hughes E, Jani B, Larcher V. Screening for intrauterine growth retardation using ratio of midarm circumference to occipitofrontal circumference. *Br Med J (Clin Res Ed).* 1986; 292:1039–1040.
32. Sasanow SR, Georgieff MK, Pereira GR. Mid-arm circumference and mid-arm/head circumference ratios: standard curves for anthropometric assessment of neonatal nutritional status. *J Pediatr.* 1986; 109:311–315. [PubMed: 3734968]
33. Patterson RM, Pouliot MR. Neonatal morphometrics and perinatal outcome: who is growth retarded? *Am J Obstet Gynecol.* 1987; 157:691–693. [PubMed: 3509974]
34. Golebiowska M, Ligenza I, Kobierska I, Zielinska E, Wlazlowski J, Sikora P, Haladaj K. Use of mid-arm and head circumference to estimate gestational age and nutritional status of newborn. *Ginekol Pol.* 1992; 63:221–226. [PubMed: 1304513]
35. Excler JL, Sann L, Lasne Y, Picard J. Anthropometric assessment of nutritional status in newborn infants. Discriminative value of mid arm circumference and of skinfold thickness. *Early Hum Dev.* 1985; 11:169–178. [PubMed: 4029053]
36. Georgieff MK, Sasanow SR, Mammel MC, Pereira GR. Midarm circumference/head circumference ratios for identification of symptomatic LGA, AGA, and SGA newborn infants. *J Pediatr.* 1986; 109:316–321. [PubMed: 3734969]
37. Bernstein IM, Goran MI, Amini SB, Catalano PM. Differential growth of fetal tissues during the second half of pregnancy. *Am J Obstet Gynecol.* 1997; 176:28–32. [PubMed: 9024084]
38. Bernstein IM, Catalano PM. Ultrasonographic estimation of fetal body composition for children of diabetic mothers. *Invest Radiol.* 1991; 26:722–726. [PubMed: 1917407]
39. Winn HN, Holcomb WL. Fetal nonmuscular soft tissue: a prenatal assessment. *J Ultrasound Med.* 1993; 4:197–199. [PubMed: 8497023]
40. Gardeil F, Greene R, Stuart B, Turner JM. Subcutaneous fat in the fetal abdomen as a predictor of growth restriction. *Obstet Gynecol.* 1999; 94:209–212. [PubMed: 10432129]
41. Chang CH, Yu HC, Chang FM, Ko HC, Chen HY. Threedimensional ultrasound in the assessment of normal fetal thigh volume. *Ultrasound Med Biol.* 2003; 29:361–366. [PubMed: 12706186]

42. Chang CH, Yu CH, Ko HC, Chen CL, Chang FM. The efficacy assessment of thigh volume in predicting intrauterine fetal growth restriction by three-dimensional ultrasound. *Ultrasound Med Biol.* 2005; 31:883–887. [PubMed: 15972193]
43. Chang CH, Yu CH, Ko HC, Chen CL, Chang FM. Fetal upper arm volume in predicting intrauterine growth restriction: a three-dimensional ultrasound study. *Ultrasound Med Biol.* 2005; 31:1435–1439. [PubMed: 16286022]
44. Murphy VE, Smith R, Giles WB, Clifton VL. Endocrine regulation of human fetal growth: the role of mother, placenta, and fetus. *Endocr Rev.* 2006; 27:141–169. [PubMed: 16434511]
45. Sayer AA, Cooper C. Fetal programming of body composition and musculoskeletal development. *Early Hum Dev.* 2005; 81:735–744. [PubMed: 16081228]

Figure 1.

Fractional limb volume. Fractional arm (AVol) and thigh (TVol) volumes are based on 50% of the humeral (a) or femoral (b) 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 2.

Fractional arm volume (AVol). A sonographic view of the fetal arm is analyzed at 20 weeks' menstrual age. By placing two electronic cursors (arrowheads, upper left window) along the humeral diaphysis, the software defines a partial volume that is based on 50% of the bone length. Five equidistant axial limb slices display soft tissue borders that are traced manually for each image slice (green dots) and the AVol measurement is calculated automatically (not shown).

Figure 3.

Fractional thigh volume (TVol). A sonographic view of the fetal thigh is analyzed at 20 weeks' menstrual age. By placing two electronic cursors (arrowheads, upper left window), one at each end of the femoral diaphysis, the software defines a partial volume that is based on 50% of the bone length. Five equidistant axial limb slices are used to display soft tissue borders that are traced manually for each image slice (green dots) and the TVol measurement is calculated automatically (not shown).

Figure 4.

Ultrasound images demonstrating how transverse slices of the mid-limb improves visualization of the soft tissue borders. Sagittal views of the fetal arm are displayed (left) with their corresponding transverse views (right). (a) Placement of the reference dot at the center of the limb enables soft tissue borders to be visualized clearly in the transverse view. (b) When the reference dot is very close to the shoulder, soft tissue borders are no longer clearly discernible in the transverse view.

Figure 5.

Image postprocessing improves visualization of fetal soft tissue. (a) Original axial image of a fetal thigh at term. (b) Adjustment of brightness and contrast levels, as well as the addition of a sepia color filter, improves delineation of the soft tissue borders.

Figure 6. Scatterplots of fractional arm volumes (AVol; $n = 380$) (a) and fractional thigh volumes (TVol; $n = 378$) (b) with fitted percentiles (5th, 50th and 95th) during the second and third trimesters of pregnancy.

Figure 7.

Fractional thigh volume (TVol) to arm volume (AVol) ratio. A very small linear increase occurred with advancing pregnancy; TVol values were approximately two times greater than their corresponding AVol measurements. $y = 0.22x + 1.46$ ($R^2 = 0.20$, $P < 0.0001$).

Figure 8.

Reproducibility of fractional limb volume measurements. Bland-Altman plots indicate an acceptable degree of intraexaminer (a and c) and interexaminer (b and d) bias and agreement for fractional arm volume (AVol; a and b) and thigh volume (TVol; c and d) measurements. Dashed horizontal lines summarize the average measurement bias in terms of percentage differences. Solid horizontal lines represent the 95% limits of agreement for these percentage differences.

Figure 9.

Fetal magnetic resonance images of the thigh from two different pregnancies during the third trimester (28.0 weeks (a) and 34.5 weeks (b)). Fractional limb volumes include fat, muscle and bone. Transverse views of a normal fetal thigh are demonstrated using T1 imaging sequences during the third trimester. Areas of high signal intensity (white arrows) represent the subcutaneous fat layer that surrounds muscle tissue and bone. Areas of intermediate signal intensity within the limb represent muscle tissue. Cross-sectional views of the femoral diaphysis are seen as low-intensity circular areas in the center of the limb muscle mass.

Figure 10.

Technical considerations – excessive transducer pressure. Limb tissue is better visualized when surrounding amniotic fluid separates the skin line from the uterine wall or placenta (a and b). Excessive manual pressure against the maternal abdomen can obliterate this fluid layer and may cause increased uncertainty for tracing soft tissue borders (c and d).

Figure 11.

Technical considerations – inadequate volume sweep. A sagittal volume acquisition sweep of the entire limb diaphysis (Panel A) is recommended for fractional limb volume measurements. In this macrosomic fetus, a transverse view of the limb (Panel B) indicates that the transducer sweep angle does not pass through the entire limb width. This problem is usually solved by centering the diaphysis in the middle of the image or by increasing the volume sweep angle. Excessive transducer pressure on the maternal abdomen should be avoided because of undesirable limb tissue compression effects (arrowhead).

Figure 12.

Technical considerations – movement during volume acquisition. This three-dimensional multiplanar view demonstrates a volume acquisition that has been performed in the presence of fetal movement. The original plane of volume acquisition (Panel A) displays a fetal arm without obvious evidence of image distortion. However, reconstructed orthogonal images (Panels B and C) reveal significant movement artifacts that were not apparent from the original sweep.

Figure 13.

Technical considerations – limb compression. The original plane of volume acquisition (a) does not indicate problems with the volume scanning procedure. The transverse view of the thigh (b) suggests that the fetal leg is actually bent and causes some uncertainty as to how best to trace soft tissue borders owing to the presence of acoustic shadowing (white arrow). The leg bent at the knee is best appreciated from a reconstructed view of the thigh and lower leg (c).

Figure 14.

Technical considerations – confusion of anatomical landmarks between the fetal arm and leg. The original plane of volume acquisition (Panel A) demonstrates a sagittal view of the fetal arm with a shallow surface indentation (white arrow). This indentation is caused by an overlying edge of the deltoid muscle that wraps around the triceps. Other important clues, not seen with the fetal thigh, include the presence of a chest wall and shoulder in another reconstructed image (Panel C).

Table 1

Fitted percentiles for fractional arm volume during second and third trimesters of pregnancy.

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

Table 2
Fitted percentiles for fractional thigh volume during second and third trimesters of pregnancy.

Menstrual age (weeks)	n	5 th	10 th	25 th	50 th	75 th	90 th	95 th	1 SD
18-18.9	16	2.4	2.6	2.9	3.4	3.9	4.4	4.7	1.23
19-19.9	12	2.9	3.2	3.6	4.1	4.7	5.4	5.8	1.23
20-20.9	11	3.7	4.0	4.6	5.3	6.1	6.9	7.4	1.23
21-21.9	17	4.5	4.9	5.6	6.4	7.4	8.4	9.1	1.23
22-22.9	15	5.3	5.7	6.5	7.5	8.6	9.8	10.6	1.24
23-23.9	12	6.9	7.4	8.4	9.7	11.2	12.8	13.8	1.24
24-24.9	10	7.6	8.2	9.4	10.8	12.5	14.2	15.4	1.24
25-25.9	14	9.3	10.1	11.5	13.3	15.3	17.5	18.9	1.24
26-26.9	13	11.1	12.0	13.7	15.8	18.3	20.8	22.5	1.24
27-27.9	12	13.0	14.0	16.0	18.5	21.3	24.3	26.3	1.24
28-28.9	14	14.9	16.1	18.3	21.2	24.5	27.9	30.2	1.24
29-29.9	11	17.2	18.6	21.2	24.5	28.4	32.3	35.0	1.24
30-30.9	14	20.1	21.8	24.8	28.7	33.2	37.9	41.0	1.24
31-31.9	13	23.1	25.0	28.6	33.1	38.3	43.7	47.2	1.24
32-32.9	15	26.9	29.2	33.3	38.5	44.6	50.9	55.1	1.24
33-33.9	14	31.0	33.5	38.3	44.3	51.4	58.7	63.5	1.24
34-34.9	9	33.3	36.1	41.2	47.8	55.4	63.2	68.4	1.24
35-35.9	13	38.0	41.2	47.0	54.5	63.2	72.2	78.2	1.25
36-36.9	13	43.6	47.2	54.0	62.6	72.6	83.0	89.9	1.25
37-37.9	15	49.1	53.1	60.8	70.5	81.8	93.5	101.3	1.25
38-38.9	45	56.2	61.0	69.7	80.9	93.9	107.4	116.4	1.25
39-39.9	49	60.6	65.7	75.1	87.2	101.3	115.9	125.6	1.25
40-40.9	9	66.6	72.2	82.6	95.9	111.4	127.5	138.2	1.25
41-41.9	13	76.7	83.2	95.2	110.7	128.6	147.2	159.6	1.25
42-42.9	8	80.2	87.0	99.6	115.8	134.5	154.0	167.0	1.25