Sonographic Evaluation of Fetal Growth and Body Composition in Women With Different Degrees of Normal Glucose Metabolism

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OBJECTIVE — To investigate the maternal demographic and metabolic factors contributing to the growth of fetal lean and fat body mass in women whose degree of glucose intolerance is less than that defining gestational diabetes in comparison with women with normal glucose metabolism.

RESEARCH DESIGN AND METHODS — Longitudinal sonographic examinations of 66 singleton fetuses without anomalies of nonobese mothers with abnormal oral glucose challenge test (GCT) results and without gestational diabetes (group 1) were compared with those of 123 singleton fetuses without anomalies of nonobese mothers with normal GCT values (group 2). Lean body mass measurements included head circumference, femur length, mid–upper arm, and mid-thigh central areas. Fat body mass measurements included the anterior abdominal wall thickness, the subscapular thickness, and the mid–upper arm and mid-thigh subcutaneous areas. All the women performed a 24-h glucose profile on the day preceding the ultrasound scan. Multivariate logistic regression analysis established best-fit equations for fetal sonographic measurements of fat and lean body mass. Independent variables included groups 1 and 2, maternal age, parity, prepregnancy BMI, gestational age, weight gain during pregnancy, fetal sex, and the following averaged 24-h profile maternal capillary blood glucose values: preprandial, 1-h postprandial, and 2-h postprandial.

RESULTS — No difference was found between the two groups with respect to fetal lean body mass parameters; the factors that contributed significantly and most frequently were gestational age and fetal sex (male). With respect to fetal fat body mass, all the measurements were significantly higher in group 1 than in group 2. In all instances, the significantly contributing factors were gestational age and maternal 1-h postprandial glucose values, whereas another frequent contributor was prepregnancy BMI.

CONCLUSIONS — Our study suggests the possibility of using sonographically determined fetal fat and lean mass measurements as indicators of body composition. The assessment of these parameters, achievable in a noninvasive and reproducible fashion in pregnancies complicated by glucose intolerance, might enable the real-time detection of fetal overgrowth and disproportion, thus opening the possibility of exploring interventions to limit fetal fat accretion, birth weight, and potential resulting morbidity.

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Gestational alterations in maternal metabolism provide nutrients in excess of those required for normal fetal growth and for maternal and fetal energy requirements. In this context, the presence of any degree of abnormal glucose tolerance, even if less than that conventionally required for the diagnosis of gestational diabetes, represents an altered environment for the growth of the fetus (1–7).

The excess supply of nutrients caused by altered glucose tolerance is most easily discerned through fetal fat body mass, which has been shown to be more closely associated with maternal glucose control than birth weight (8). In adults, there is a direct correlation between fat mass and energy stores, and therefore a nutritional assessment of a subject may be made by means of the distinction between fat and lean body mass. In newborns, 46% of variance in birth weight can be explained by fat body mass, despite the fact that fat is responsible for only 12–14% of total birth weight (9). These findings suggest the potential usefulness of ultrasonographically generated estimates of fetal fat for the determination and evaluation of growth abnormalities.

Recent advances in ultrasound measurement of fetal body composition with respect to lean and fat body mass make it possible to evaluate the intruterine nutritional state. In addition, longitudinal estimation of fetal body composition makes it possible to distinguish the effects on fetal growth by different maternal metabolic conditions. Bernstein et al. (10) studied healthy fetuses, comparing fat and lean body mass measurements during gestation and showed significant correlations between these and both birth weight and estimates of neonatal lean and fat mass.

The aim of this study was to compare the maternal demographic and metabolic factors associated with the growth of fetal lean and fat body mass in women with

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Abbreviations: GCT, glucose challenge test; GTT, glucose tolerance test.
A table elsewhere in this issue shows conventional and Systeme International (SI) units and conversion factors for many substances.
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levels of glucose intolerance less than those defining gestational diabetes with those of women with normal glucose metabolism.

**RESEARCH DESIGN AND METHODS** — In the period between January 1999 and December 2001, a population of pregnant women was screened for gestational diabetes between the 24th and 28th weeks of pregnancy with a 50-g, 1-h oral glucose challenge test (GCT) at the Perinatal Medicine Unit of the University of Florence. Subjects who met the inclusion criteria were invited to participate in this longitudinal study. These inclusion criteria were Caucasian, singleton pregnancies without congenital malformations, gestational age confirmed by first trimester ultrasound, absence of gestational diabetes based on the criteria of Carpenter and Coustan (11), two or more previous deliveries, pregestational BMI between 19 and 25 kg/m², and absence of tobacco use, chronic hypertension, and previous gestational diabetes.

The study protocol was approved by the hospital institutional review board, and written informed consent was obtained from each subject before participation in the study protocol. A total of 228 subjects were recruited for the study.

For both the GCT and glucose tolerance test (GTT), plasma glucose determinations were performed in the clinical laboratory of the hospital with the glucose oxidase method, using an automated system (Aeroset; Abbott Laboratories, Abbott Park, IL) with inter- and intra-assay coefficients of variation [CVs] of 0.8%.

Following the GCT, subjects were seen at 3-week intervals in the period between 24 and 38 weeks. Women were asked to have three main meals at 8:00 A.M., noon, and 8:00 P.M., and to perform a 24-h glucose profile on the day preceding the ultrasound scan using a memory-based reflectance meter (One Touch Profile; Lifescan, Milpitas, CA) with tested precision and accuracy (CV 3.0–4.0% and coefficient of correlation 0.981, respectively) without modifying their lifestyle or following any dietary restriction. Measurements were obtained before meals, 1 and 2 h after meals, and every 2 h in the afternoon and during the night.

At each visit, an ultrasound scan was performed, and the 24-h capillary glucose profile obtained by the subjects at home was presented. Ultrasound scans were performed using a commercially available, unmodified Teknos Esaote ultrasound machine (Esaote, Genoa, Italy) with a 3.5-MHz transducer.

Lean body mass measurements included head circumference, femur length,
Maternal weight gain (kg) 11.1 (8–14)
1-h GCT value (mg/dl) 156.3 (110–253)
Maternal blood glucose values (mg/dl)
Preprandial 76.9 ± 11.2
1-h postprandial 120.9 ± 12.8
2-h postprandial 100.5 ± 10.8
Compliance (%) 94.3 ± 2.3
Gestational age at delivery (weeks) 39.2 ± 1.6
Neonatal sex (% boys) 34 (51.5)
Birth weight (g) 3,561.8 ± 351.3
AGA 47 (71.2)
LGA 16 (24.3)
SGA 3 (4.5)
Ponderal index (units) 2.71 ± 0.21

Data are median (range), means ± SD, or n (%). *Patient compliance with self-monitoring of blood glucose was defined as the percentage of the 30 glucose measurements prescribed by the protocol during the 4 weeks before delivery that were actually performed (20). AGA, appropriate for gestational age; LGA, large for gestational age; SGA, small for gestational age.

and mid–upper arm and mid-thigh central areas. Fat body mass measurements included anterior abdominal wall thickness, subscapular thickness, and mid–upper arm and mid-thigh subcutaneous areas. Other routine sonographic parameters (i.e., head circumference and femur length) were also obtained. We used the technique of Bernstein and colleagues (10,12) to measure the fat and lean body mass areas on axial ultrasound images of the mid–upper arm, the mid–upper leg, and the anterior abdominal wall thickness. Briefly, mid–upper arm fat mass, mid–upper lean mass, mid–thigh fat mass, and mid–thigh lean mass were obtained as follows (Fig. 1A). A longitudinal view of the long bone was obtained and used to identify the midpoint with an angle of 0° to the transducer. The transducer was then rotated 90° to obtain the cross-sectional view of the mid-limb. The fat mass was measured by taking the total cross-sectional limb area and subtracting the central lean area, which consisted of muscle and bone. At least two measurements were made for each parameter at each observation. The mean value of each set of observations was used in the analysis. The anterior abdominal wall thickness was measured at the level of the abdominal circumference (Fig. 1B), between the midaxillary lines. At least four measurements were made, and the mean value was used in analysis. Subscapular subcutaneous thickness was evaluated longitudinally on the fetal trunk, visualizing the entire longitudinal section of the scapula between the skin surface and the subcutaneous tissue at the interface with the super-spinous and infra-spinous muscles (Fig. 1C). At least two measurements were made, and the mean value was used in analysis. The sonographers did not know to which group the women belonged.

From the recruited population, we selected 189 women who had uneventful pregnancies, did not receive drugs known to affect glucose metabolism (e.g., steroids and β2-sympathomimetics) throughout gestation, and delivered term (37–42 weeks completed) live-born infants. Among the 39 women excluded, 25 decided to discontinue the study protocol, 5 did not deliver at our clinic, and 9 had a spontaneous preterm delivery.

For the selected women, 848 ultrasonographic examinations and 848 24-h glucose profiles (mean number per subject 4.5, range 3–6 for both) were performed. Two operators (E.P. and L.C.) performed the ultrasound examinations. The intra- and interobserver reproducibilities of the measurements were tested in 20 different images. Precision was assessed as the CV of previous measurements. For anterior abdominal wall thickness and subscapular thickness, the intraobserver CVs were 2.2 and 2.5%, respectively, whereas the interobserver CVs were 5.2 and 6.1%, respectively. For the lean body areas of the thigh and arm, the intraobserver CVs were 1.9 and 2.3%, respectively, while the interobserver CVs were 3.9 and 5.6%, respectively. For the fat body areas of the thigh and arm, the intraobserver CVs were 2.9 and 4.4%, respectively, while the interobserver CVs were 5.9 and 7.3%, respectively.

On the basis of GCT results, the 189 selected women were separated into two groups. Group 1 comprised 66 women with an abnormal GCT value (plasma glucose level ≥135 mg/dl after 1 h) (13) followed by a negative 100-g GTT (11). Group 2 comprised 123 women with a normal GCT value.

Infants were considered appropriate for gestational age when their birth weight ranged between the 10th and 90th percentiles. They were large for gestational age when birth weight was ≥90th percentile and small for gestational age when birth weight was ≤10th percentile on the basis of standard growth development for our population (14).

The following anthropometric parameters were evaluated within 24 h after birth: birth weight and ponderal index, which is the ratio between 100 times the weight in grams and the cube of length in centimeters (15). Continuous variables
were compared with either Student’s t or Mann-Whitney U tests. Categorical variables were tested with either χ² or Fisher’s exact test. Multivariate logistic regression analysis established best-fit equations for fetal sonographic measurements of fat and lean body mass.

Independent variables included groups (group 1 = 0 and group 2 = 1), maternal age (years), parity (nulliparous = 0 and parous = 1), prepregnancy BMI, gestational age at sonographic measurements (weeks), weight gain during pregnancy (kilograms), fetal sex (girl = 0 and boy = 1), and the following averaged maternal capillary blood glucose values from 24-h profiles (milligrams per deciliter): preprandial, 1-h postprandial, and 2-h postprandial. A P < 0.05 was considered statistically significant. Statistical analysis was performed by Stata statistical software release 5.0 (Stata, College Station, TX).

RESULTS — Maternal and neonatal characteristics are listed in Table 1. No differences were found in regard to the maternal characteristics between the study subgroups and the 39 excluded women (data not shown).

The women in group 1 had significantly higher levels of preprandial (P < 0.003), 1-h postprandial (P < 0.001), and 2-h postprandial (P < 0.002) capillary glucose values. Infant birth weights and ponderal indexes were significantly higher in group 1 than in group 2 (P < 0.04 and P < 0.01, respectively). The incidence of infants who were large for gestational age was significantly higher in group 1 than in group 2 (P < 0.004), while the incidence of infants who were of appropriate size for gestational age was significantly lower in group 1 than in group 2 (P < 0.04). In group 1, 12 women (18.2%) had one elevated GTT value (fasting, n = 2; 1 h, n = 5; 2 h, n = 4; and 3 h, n = 1).

With respect to all parameters used to evaluate fetal lean body mass, no difference was observed between groups 1 and 2. The factors that were significantly associated with head circumference were gestational age and sex (male) (Fig. 2A). Gestational age was the only independently associated factor for femur length (Fig. 2B). For the central lean area of the mid–upper arm, independently associated factors were gestational age and sex (male) (Fig. 2C). For the central lean areas of the mid-thigh, independently associated factors were gestational age, sex (male), and maternal 2-h postprandial glucose values (Fig. 2D).

With respect to fetal fat body mass, the growth of the anterior abdominal wall thickness was significantly greater in group 1 than in group 2 from the 26th week to the end of pregnancy. The factors that were significantly and independently associated with this measurement were gestational age and maternal 1-h postprandial glucose values (Fig. 3A). The growth of the subscapular thickness was significantly greater in group 1 from the 26th week to the end of pregnancy. The factors that were significantly and independently associated with this measurement were gestational age, maternal 1-h postprandial glucose values, and prepreg-
nancy BMI (Fig. 3B). The growth of the mid-upper arm subcutaneous area was significantly greater in group 1 from the 25th week to the end of pregnancy. The factors that were significantly and independently associated with this measurement were gestational age and 1- and 2-h postprandial glucose values (Fig. 3C). The growth of the mid-thigh subcutaneous area was significantly greater in group 1 from the 25th week to the end of pregnancy. The factors that were significantly and independently associated with this measurement were gestational age, 1-h postprandial glucose values, and prepregnancy BMI (Fig. 3D). The proportions of variance for which each of the independent variables were responsible are listed in Table 2.

**CONCLUSIONS** — It is known that macrosomic fetuses resulting from a diabetic pregnancy often show asymmetric macrosomia, that is, a tendency of insulin-sensitive tissues to overgrow in spite of a normal growth pattern in non-insulin-sensitive tissues (16–18). Despite this observation, the neonatal outcome of diabetic pregnancies has often been, and indeed still is, commonly evaluated only in terms of neonatal birth weight rather than by the assessment of anthropometric characteristics suggestive of growth disproportion (19).

Recent studies (1–7) have shown that some characteristics of offspring of diabetic mothers are present, although to a lesser extent, in cases of minor degrees of glucose intolerance in pregnancy. This concept has been further expanded by the demonstration that glycemia in pregnancy can be regarded as a continuum ranging from normal glucose metabolism to overt diabetes, as has been shown in the correlation between fetal abdominal circumference and 1-h postprandial glucose values in women with normal glucose metabolism (19).

In our study, glycemic values were significantly higher in group 1 than in group 2, thus confirming that even in cases of minor alteration of glucose metabolism, there can be an excess of nutrients for the fetus, as revealed by both the higher rate of infants who were large for gestational age and greater ponderal index in group 1 infants. Glycemic values found in group 2 with normal glucose metabolism are similar to those previously reported by our team (19). In our study population, the rate of women with a positive GCT was 35%, a value similar to that reported in a previous investigation (1).

With respect to the parameters of fetal lean mass, there were no significant differences between the two groups; gestational age was the only factor that significantly contributed in a constant fashion, whereas another relevant contributing factor was fetal sex, though not significantly for femur length. Overall, the only metabolic factor contributing to lean mass was the 2-h postprandial glucose value, which was weakly, yet significantly, associated with mid-thigh lean mass and accounted for only 4% of variance. Conversely, all of the parameters of fetal fat mass were significantly greater in group 1 than in group 2; again, gestational age was found to be a significant contributor to all parameters along with 1-h postprandial glucose values. Apart from gestational age, which, as expected, contributed significantly to both fetal lean and fat body mass, it can be seen that glucose values at the 1-h postprandial time point are of absolute relevance in their contribution to fat body mass. Therefore, this finding suggests that in women with minor degrees of glucose intolerance, the peak postprandial glucose value can initi-
ate a cascade of events that finally leads to fetal body disproportion, a sequence that relies on the same pathophysiological mechanism fully expressed in cases of fetal asymmetric macrosomia, which are found in diabetic pregnancies (16–18). Previous studies have shown that the 1-h postprandial glycemic peak is significant for fetal overgrowth in diabetic pregnancies (20–26) and that there is a correlation between fetal abdominal circumference and 1-h postprandial glucose levels in nondiabetic women (19). In this context, our results may confirm that minor degrees of glucose intolerance in pregnancy are associated with overgrowth of insulin-sensitive tissues. However, these results take us a step further toward understanding the complex relationship between glucose metabolism and fetal growth, revealing that 1-h postprandial glucose levels are significant contributors to fat body mass not only in GCT-positive nondiabetic women, but also in women with absolutely normal glucose metabolism (group 2). These results may therefore strengthen the suggestion that 1-h postprandial glucose values should be taken into account when optimizing treatment for glucose-intolerant pregnant women in an attempt to disrupt this cascade and possibly prevent fetal overgrowth and disproportion.

The contribution of fetal sex, which was significant only for parameters of fetal lean mass, offers a confirmation of the hypothesis of Catalano et al. (27). It suggests that fetal lean body mass is primarily affected by genetic factors, whereas fetal fat body mass is mainly influenced by factors relating to the maternal metabolic environment, as demonstrated in our study by the roles of maternal 1-h postprandial values and prepregnancy BMI (Table 2).

A crucial issue addressed by our study is the technique used for fetal lean and fat mass assessment. As a matter of fact, most studies on the management of diabetic pregnancy refer to parameters of neonatal outcome, such as macrosomia and/or anthropometric features, thus enabling only a retrospective evaluation of treatment efficacy (20,24,28,29). On one hand, the unexpectedly high rate of macrosomia found in some of these studies highlights the need to revise glucose targets currently accepted in the treatment of diabetic pregnancy (26), yet it also suggests the need for a means of assessing fetal growth in relation to maternal metabolism during gestation so that appropriate treatment can be initiated before the end of pregnancy. The longitudinal sonographic examination of fetal lean and fat body mass can be regarded as an effective and noninvasive tool that can provide a careful evaluation of the fetal consequences of maternal hyperglycemia, shifting the interest to an indicator of fetal hyperinsulinemia.

In this study, the accuracy and reproducibility of both lean and fat mass measurements were similar to those reported by Bernstein et al. (10) and Galan et al. (30), thus suggesting that the technique is reliable. In our study we also considered the subscapular thickness, a parameter of fetal fat mass not taken into account in previous investigations, the assessment of

![Figure 3](#)

**Figure 3**—Fat body mass measurements plotted against gestational age in group 1 (○, solid regression line) and group 2 (○, dotted regression line). A: Anterior abdominal wall thickness. B: Subscapular thickness. C: Mid–upper arm subcutaneous area. D: Mid-thigh subcutaneous area. The asterisk indicates the gestational age at which group differences become significant.
which is easy to perform and highly reproducible.

It is understood that our results need confirmation and verification in glucose-intolerant pregnant women to legitimize possible clinical use. In addition, there are some limitations in our study that deserve particular consideration. First of all, the lack of fat mass measurements in the neonate does not allow comparison between sonographic findings and those obtained postnatally. In this respect, a significant correlation between these measurements has already been demonstrated by Bernstein et al. (10), while Crane et al. (31) documented that sonographic estimates of fat mass were not significantly different from those predicted by neonatal total body electrical conductivity, although with a mean absolute percent error of 11.8 ± 9.0%. Second, standard curves for lean and fat mass should be defined in a large normal population. It should also be noted that all women enrolled in the study were nonobese, thus ruling out a known limiting factor in fetal sonographic evaluation. It is possible that testing this technique in gestational diabetic women, who are often obese, may prove technically difficult. It is also possible that timing the glucose profile on the day before ultrasound scan may have introduced a bias in terms of the women’s diet and activity, even though the physicians had not advised any specific restriction.

In conclusion, our study suggests the possibility of using sonographically determined fetal fat and lean mass measurements as indicators of body composition. The assessment of these parameters, achievable in a noninvasive and reproducible fashion in pregnancies complicated by glucose intolerance, might enable the real-time detection of fetal overgrowth and disproportion, thus opening the possibility of exploring inter-

### Table 2—Stepwise, regression analysis: factors correlating with fetal body composition

<table>
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<th>Dependent and independent variables</th>
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<tr>
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<td>Head circumference</td>
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ventions that will limit fetal fat accretion, birth weight, and potential resulting morbidity.

References