Reduction of subcutaneous mass, but not lean mass, in normal fetuses in Denver, Colorado

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Denver, Colo, and Milan, Italy

OBJECTIVE: To test the hypothesis that reduced birth weight in normal fetuses born at moderately high altitude (Denver), compared with the birth weight in normal fetuses born at sea level (Milan), is caused by a reduction in both lean mass and subcutaneous fat mass.

STUDY DESIGN: Ninety-four normal singleton pregnancies (46 in Denver, 48 in Milan) had serial ultrasonographic axial images obtained to assess subcutaneous tissues of fetuses as a measure of body fat. The abdominal wall thickness and mid upper arm and mid thigh were examined. The equation was: Subcutaneous tissue equals total cross-sectional area minus bone and muscle area. Lean mass included the area of muscle and bone, head circumference, and femur length.

RESULTS: Gestational age at delivery was similar between groups. Birth weight was less at Denver’s altitude (2991 ± 79 g versus 3247 ± 96 g; P = .04). Abdominal wall thickness, mid upper arm, and mid thigh subcutaneous tissues measurements were significantly reduced at Denver’s altitude and increased further in significance with advancing gestational age. Lean mass measurements were similar between groups.

CONCLUSIONS: The reduced birth weight of the newborns in Denver was the result of a reduction in fetal subcutaneous fat tissue and not lean mass. Ultrasonography can be used to follow subcutaneous measurements longitudinally and to detect differences, and potentially disease processes, in study populations. (Am J Obstet Gynecol 2001;185:839-44.)

Key words: Fetus, altitude, subcutaneous tissue, fat, intrauterine growth restriction

A number of human and animal studies have shown that high altitude exposure and its associated relative hypoxia induce changes in the hematologic and hemodynamic profiles of adults and fetuses.1-7 Human studies showing a decrease in birth weight at high altitude have been performed in Leadville, Colo (altitude, 3100 m) and in Tibet (altitude, 3658 m).8-9 Reduced birth weight has also been reported at moderately high altitude (Denver, 1600 m) compared with sea level10. In this study, although birth weight was reduced by approximately 200 g, there were no differences in peripheral or central circu-

lotion Doppler flow velocity waveform profiles. This suggests that, in healthy fetuses, either there is no fetal hypoxia effect at this modest altitude or there is an adaptation to altitude that is expressed in body weight without an effect on peripheral or central vascular resistance. The reason for the decrease in birth weight at moderate altitude or even high altitude is unknown. It is possible that either fat or lean mass is affected by altitude.

Bernstein et al11 previously used ultrasonography to assess anthropometric measurements of fetal body composition. In healthy fetuses, they compared fat and lean body mass measurements across gestation and showed significant correlations with both birth weight and estimates of neonatal lean and fat mass. The purpose of the current study was to determine whether this decrease in birth weight at moderately high altitude is the result of a decrease in fat deposition or lean mass deposition, or both. We hypothesized that the reduced birth weight of healthy fetuses at moderately high altitude (Denver) is the result of a reduction in both lean mass and subcutaneous fat mass compared with healthy fetuses at sea level (Milan, Italy).

Material and methods

Study population. This longitudinal study included 94 patients (46 in Denver, 48 in Milan) who had single intrauterine pregnancies, no medical complications, and
no history of tobacco use. All pregnancies underwent a detailed survey of fetal anatomy, and no fetal anomalies were detected. The study was approved by the Institutional Review Boards at the University of Colorado Health Sciences Center and at ISBM L. Sacco and San Paolo Hospital of the University of Milan. Gestational age was determined by last menstrual period and confirmed with a mid-second–trimester ultrasonography.

**Ultrasonography.** Ultrasonographic examinations were performed at 3- to 4-week intervals with commercially available unmodified ATL 5000 (Milan) and ATL 3000 (Denver) ultrasound machines with 3.5- or 5-MHz transducers (ATL Ultrasound, Bothell, Wash). To obtain fat mass and lean mass, several measurements were obtained. Routine ultrasonographic biometric parameters were obtained, including head circumference, abdominal circumference, and femur length. We used the technique of Bernstein et al.\(^\text{11}\) to measure the fat and lean body mass areas on axial ultrasound images of the mid upper arm and leg.\(^\text{11}\) Briefly, a longitudinal view of the long bone and extremity in the middle of the ultrasound screen was obtained with an angle of 0 degrees to the transducer. The transducer was then rotated 90 degrees to obtain the axial view of the extremity. The fat mass was measured by taking the total cross-sectional limb area and subtracting the central lean area that consisted of muscle and bone. Fat mass and lean mass were obtained on cross-sectional images of the abdomen and proximal arm and leg (Fig 1). The fat mass of the abdomen was determined by measuring the thickness of the anterior abdominal subcutaneous tissue on the same axial image on which the abdominal circumference is obtained. This has also been previously reported by Gardeil et al.\(^\text{12}\)

**Reproducibility and precision.** The intra- and interobserver reproducibilities of the abdominal subcutaneous thickness, and extremity and lean mass subcutaneous tissue area measurements were tested in 20 different images. Two operators (HG and SR) performed three measurements on each of the images. Precision was assessed as the coefficient of variation of abdominal subcutaneous thickness, and extremity and lean mass subcutaneous tissue areas.

**Statistical analysis.** Maternal age, parity, gestational age at delivery, and birth weights were compared by the F test for equal variance and by the Student t test. Categorical data were tested with either the \(\chi^2\) or Fisher exact tests. SAS Proc Mixed (SAS Institute, Inc, Cary, NC) was used to compare fetal biometry, abdominal subcutaneous thickness, and the lean mass and subcutaneous areas of the proximal upper and lower extremities of patients from Denver with those from Milan. Correlation coefficients were calculated for each of the subcutaneous and lean mass areas versus estimated fetal weight. Nongraphical data are presented as mean ± SE. A \(P\) value of < .05 was considered significant.

**Results**

The demographic data of the study population are shown in Table I. The maternal age at delivery for patients in Denver was significantly younger compared with the patients in Milan. Gestational age at delivery was similar between groups. Denver birth weights were significantly less than those of Milan with a difference in mean birth weight of 255 g. There were also a greater number of nulliparous patients in Denver. Nulliparous patients generally have smaller babies compared with multiparous patients. Therefore, we stratified the birth weights on the
basis of parity, and the differences in birth weights remained significant (Table I).

There was a similar effect of gestational age on lean mass and subcutaneous indices of patients studied in Denver and those studied in Milan. There were no differences between the groups of Denver and Milan for head circumference, abdominal circumference, or femur length (Figs 2, 3, and 4). The anterior abdominal subcutaneous tissue became significantly greater in the patients from Milan at 26 weeks, and this difference persisted for the remainder of gestation (Fig 5). The lean mass of the proximal upper arm was similar between the groups in Denver and Milan. However, the subcutaneous fat area of the proximal arm became significantly greater in the patients from Milan at 30 weeks, and this difference persisted for the remainder of gestation (Fig 6). A similar pattern of reduced subcutaneous tissue area and similar lean mass area was seen for the proximal leg (Fig 7). Regression lines are shown for Figs 2-7.

For the abdominal subcutaneous thickness, the intraobserver coefficient of variation was 2.6%, while the

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### Table I. Maternal and neonatal characteristics at delivery

<table>
<thead>
<tr>
<th></th>
<th>Denver</th>
<th>Milan</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age at delivery (y)</td>
<td>24.1 ± 0.9</td>
<td>30.1 ± 0.8</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Gestational age at delivery</td>
<td>39.3 ± 0.4</td>
<td>39.2 ± 0.1</td>
<td>n.s.</td>
</tr>
<tr>
<td>Nulliparous patients (n)</td>
<td>15/46</td>
<td>14/48</td>
<td>.06</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>2991.6 ± 77</td>
<td>3246.7 ± 96</td>
<td>&lt; .05</td>
</tr>
<tr>
<td>Nulliparous birth weights</td>
<td>2881.4 ± 106</td>
<td>3295.8 ± 59</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Multiparous birth weights</td>
<td>2973.8 ± 147</td>
<td>3387.9 ± 72</td>
<td>&lt; .05</td>
</tr>
</tbody>
</table>

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**Fig 2.** Head circumference versus gestational age for Denver and Milan patients. Regression equations and correlation coefficients: \( y = 7.9535x + 37.368; R^2 = 0.89 \) (Milan); \( y = 8.641x + 16.032; R^2 = 0.96 \) (Denver).

**Fig 3.** Abdominal circumference versus gestational age for Denver and Milan patients. Regression equations and correlation coefficients: \( y = 9.8706x - 43.178; R^2 = 0.96 \) (Milan); \( y = 10.035x - 46.479; R^2 = 0.96 \) (Denver).

**Fig 4.** Femur length versus gestational age for Denver and Milan patients. Regression equations and correlation coefficient: \( y = 2.2828x - 11.51; R^2 = 0.95 \) (Milan); \( y = 2.235x - 11.147; R^2 = 0.96 \) (Denver).

**Fig 5.** Abdominal subcutaneous thickness versus gestational age for Denver and Milan patients. Regression equations and correlation coefficient: \( y = 0.0631e^{0.0582x}; R^2 = 0.74 \) (Milan); \( y = 0.0607e^{0.0513x}; R^2 = 0.31 \) (Denver). Abdominal subcutaneous thickness differences (asterisk) became significant at 26 weeks’ gestation, and this difference persisted.
interobserver coefficient of variation was 11.1%. For the extremity subcutaneous area, the intraobserver coefficient of variation was 4.1%, while the interobserver coefficient of variation was 8.2%. For the lean mass area, the intraobserver coefficient of variation was 2.3%, while the interobserver coefficient of variation was 5.2%.

Table II shows the correlation coefficients between the various subcutaneous tissues and lean mass area measurements versus the estimated fetal weights at the time those areas were measured. The correlation coefficients for Denver and Milan patients are shown separately. There was a positive correlation between abdominal subcutaneous tissue thickness and increasing estimated fetal weights. This correlation was lower in Denver ($R^2 = 0.28$) than in Milan ($R^2 = 0.7$). In contrast, there was a strong correlation coefficient for the proximal upper and lower extremity lean mass and subcutaneous areas with estimated fetal weights in both the Denver and Milan populations (all $R^2 > 0.6$).

We found that standard morphometric biometry of the fetus (head circumference, abdominal circumference, and femur length) followed previously described charts. Bernstein et al. have previously reported significant correlations with estimates of neonatal lean and fat mass and birth weight. Similarly, we found a strong correlation of estimates of neonatal lean and fat mass with estimated fetal weights. This provides some insight on the relationship of lean and fat mass to fetal weights across the second half of pregnancy. While the abdominal fat thickness correlated poorly with estimated fetal weight, proximal arm and leg subcutaneous tissue followed an exponential pattern with increasing estimated fetal weight. This is consistent with human data showing an exponential increase in fat deposition during the third trimester.

The accuracy and reproducibility of subcutaneous area measurements on axial views of the extremities have been previously reported. Our coefficients of variation for these measurements are comparable with that of Bernstein et al. For determination of abdominal subcutaneous tissue thickness, we measured in linear fashion the
counted for by body fat and that subcutaneous fat accounts for 12% to 14% of term newborn birth weight. It has been shown that the interobserver coefficient of variation at 11%, while the other measurements of area were 8% or less.

In our study, birth weights of infants born in Denver (2991.6 ± 77 g) were significantly lower than in Milan (3246 ± 96 g). The difference in birth weights between the 2 cities was similar to that in our previous study. The difference exists even though there was no history of maternal medical or obstetric complications or tobacco use. We speculate that the higher multiparity in Milan could be partially responsible for these differences, the birth weight differences persisted when groups were stratified for parity. An interesting and unexpected finding was that the subcutaneous tissues, as depicted by the abdominal wall area measurements and estimated fetal weight for Denver and Milan patients

<table>
<thead>
<tr>
<th></th>
<th>Denver</th>
<th>Milan</th>
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<tbody>
<tr>
<td>Abdominal subcutaneous thickness</td>
<td>0.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Proximal arm lean mass area</td>
<td>0.82</td>
<td>0.68</td>
</tr>
<tr>
<td>Subcutaneous area</td>
<td>0.76</td>
<td>0.82</td>
</tr>
<tr>
<td>Proximal leg lean mass area</td>
<td>0.63</td>
<td>0.82</td>
</tr>
<tr>
<td>Subcutaneous area</td>
<td>0.67</td>
<td>0.89</td>
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Table II. Correlation between subcutaneous and lean area measurements and estimated fetal weight for Denver and Milan patients

There is a difference in altitude of approximately 1600 m between Denver and Milan. While altitude can have an impact on fetal oxygenation and growth, these effects have been described at much higher altitudes (3100-3600 m). Chronic high-altitude exposure (3100 m) is associated with an increased risk of preeclampsia and fetal growth restriction. Our patients were not hypertensive and had normally grown fetuses, and thus, those complications do not explain the differences in birth weight. The small difference in PO2 between the 2 study sites is unlikely to explain the difference in birth weights. Denver’s study population was acclimated to the altitude; and, in a previous study, vascular resistance in several fetal vessels were similar in Denver and Milan. Other possible explanations for the reduction in fat mass in Denver’s population may be issues of maternal ethnicity, socioeconomic status, and nutritional status.

One theory that could account for the differences in subcutaneous fat mass and birth weights between Denver and Milan is maternal age. Milan patients were significantly older than those in Denver. As pointed out by Bernstein et al, maternal age is an independent contributor to the accretion of fetal fat. Increased maternal age is a known risk factor for the development of gestational diabetes. Catalano et al have previously reported on the contribution of maternal insulin resistance to neonatal fat accretion. Although not clinically diabetic, the older mothers in Milan may have a relative increase in insulin resistance compared with mothers in Denver that leads to increased fat accumulation.

We conclude that the reduced birth weight in Denver’s population is caused primarily by reduction in fetal subcutaneous fat tissue and not lean mass. Although the reasons for the difference in subcutaneous fat remain uncertain, we conclude that ultrasonography can be used to evaluate the dynamic growth of fetal subcutaneous tissue and fat mass. It can be used to detect differences in utero between different populations and potentially to detect disease processes in longitudinal fashion that would otherwise only be evaluated by 1 end point at the end of pregnancy, in the newborn period. We also conclude that fetal lean mass remains the same regardless of the causes for decrease in fetal fat mass. Studies are in progress to assess the impact of maternal age, weight, ethnicity, height and body mass index, pregnancy weight gain, paternal height, and finally to make further assessments of fat accretion, such as the determination of leptin levels.

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