# Estimation of Total Body Fat and Subcutaneous Adipose Tissue in Full-Term Infants Less Than 3 Months Old

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ABSTRACT. Both fetal and neonatal nutrition may influence the body fat content of infants. Epidemiologic field studies would be facilitated by a simple method of measuring total body fat (TBF). The accuracy of a method, based on skinfold measurements, of estimating TBF was evaluated in 22 infants by comparing the results with those obtained by the body water dilution technique. Because the accuracy was poor, a modification was evaluated in 14 of the infants. The measurements were used to calculate the amounts of subcutaneous and nonsubcutaneous body fat. Estimates of the thickness of subcutaneous adipose tissue made with calipers and ultrasonography were compared with each other and with those obtained with Futrex 5000, a device based on the principle of near-infrared interactance. The composition of the adipose tissue in biopsy specimen from 38 infants was analyzed and contained a mean of 0.66 g of fat/cm<sup>3</sup>. Results obtained by Futrex 5000 correlated with subcutaneous adipose tissue thickness only when the latter was thin. Estimates of adipose tissue thickness by calipers were significantly higher than estimates by ultrasonography at the forearm, thigh, and calf, whereas the opposite was found at the triceps, biceps, umbilicus, and nipple. The amount of nonsubcutaneous fat, in relation to body weight minus subcutaneous fat, increased with age and was higher in girls than in boys. Skinfolds were poor predictors of TBF. However, it may be possible to predict TBF from anthropometric measurements if appropriate knowledge about the growth and development of adipose tissue in infancy are available. (Pediatr Res 34: 448-454, 1993)

## Abbreviations

BWD, body water dilution

TBF, total body fat

TBW, total body water

- BFD, TBF measured with the method of Dauncey et al. using caliper readings
- BFD/2, TBF measured with the method of Dauncey *et al.* using caliper readings divided by 2
- BFMD, TBF measured with a modification of the method of Dauncey *et al.*

BFBWD, TBF estimated with BWD technique

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FFM, fat-free mass NII, near-infrared interactance

In the evaluation of the nutritional status of infants, an estimate of TBF is often desirable. There are several ways of measuring TBF, but the method of underwater weighing, often used as a reference method in adults (1), is unfortunately not applicable in infants. The BWD technique of estimating TBF from body weight and TBW is a possible alternative because the technique of measuring TBW has recently been improved (2). However, this method is complicated and expensive and thus not suitable in field studies. Durnin and Womersley (1) developed a simple and widely used method of estimating TBF in adults based on estimates of skinfolds with calipers. Skinfolds are often used in studies of infants but, as pointed out by Dietz (3), a correlation between skinfolds and TBF has not been established for infants. In fact, Davies and Lucas (2) were unable to find any correlation between skinfolds (triceps and subscapular) and TBF in infants. A simple method, also based on skinfolds, for estimating TBF in infants was developed by Dauncey et al. (4) and applied in studies on infants (5, 6). This method has not, however, been compared with a more accurate method of estimating TBF. Using their method, Dauncey et al. (4) found healthy full-term newborns to contain 11-13% fat. Other ways to estimate infant fatness have given similar figures. Thus according to Widdowson (7), full-term newborns contain about 16% fat, whereas the corresponding figure according to Fomon et al. (8) is 14%. Swedish newborn infants contained 15.9% fat (5) when studied using the method of Dauncey et al. (4) and assuming that a skinfold equals the thickness of the subcutaneous adipose tissue layer corrected for skin thickness. Data from children and adults (9-11) indicate that a skinfold measures a double rather than a single layer of adipose tissue, but we could find no comparable data for infants.

Ultrasonography has been used for estimating the thickness of subcutaneous adipose tissue in adults (12). Comparison between estimates obtained by calipers and ultrasonography thus represents a means to evaluate whether skinfolds in infants measure a single or a double layer of adipose tissue. Dauncey *et al.* (4) assumed that triceps and subscapular skinfolds represent the average thickness of subcutaneous adipose tissue in infants, but we know of no data confirming this. They also assumed that each cm<sup>3</sup> of infant adipose tissue contains 0.9 g of fat (4). This seems unlikely because the corresponding value in adults is about 0.77 g/cm<sup>3</sup> (13). Nevertheless, the method of Dauncey *et al.* (4) is intuitively sound, and if sufficient justification could be found for the assumptions made or if these could be replaced by more appropriate ones, the method may be useful in studies of body fat in infants. However, Dauncey *et al.* (4) assumed that subcu-

taneous fat equals TBF, because in their method nonsubcutaneous fat is not considered. The truth of this assumption can be checked if reasonable justification can be found for the statement that the method of Dauncey *et al.* (4), or an appropriate modification of that method, measures the amount of subcutaneous fat, because apparently the difference between subcutaneous fat and TBF estimated by the BWD technique represents the amount of nonsubcutaneous fat.

Problems associated with caliper measurements include variations in the compressibility of adipose tissue and the need for skilled staff to do the measurements. Another possibility to measure body fat is the NII technique, which has been used in adults with reasonable success (14). This technique relies on the principles of light absorption and reflection. The NII signal penetrates the subcutaneous tissue and the electromagnetic radiation is reflected, absorbed, or transmitted depending on the composition of the tissue. A device (Futrex 5000, Futrex, Inc., Gaithersburg, MD) based on this principle is available commercially and is, according to the manufacturer, capable of providing an estimate reflecting the amount of subcutaneous fat. Futrex 5000 provides noninvasive measurements without any health hazards and is simpler to use than a caliper, but so far the technique has been little used in infants.

The specific goals of this study were 1) to compare estimates of subcutaneous adipose tissue thickness made by calipers and ultrasonography; 2) to find out whether Futrex 5000 can be used to estimate the amount of subcutaneous fat in infants; 3) to analyze the composition of adipose tissue in infants; 4) to assess the precision and accuracy of the method of Dauncey *et al.* (4) for estimating TBF of infants; 5) to investigate whether this accuracy and precision could be improved by modifying the method of Dauncey *et al.* (4); 6) to estimate the amount of nonsubcutaneous fat in infants; and 7) to identify correlations between anthropometric measurements and TBF, estimated by the BWD technique.

### MATERIALS AND METHODS

Anthropometric Measurements of TBF and Adipose Tissue in Infants. TBF measured by method of Dauncey et al. using caliper readings (BFD). The volume of subcutaneous adipose tissue was calculated from total length, crown-rump and arm lengths, and circumferences of head, trunk, mid-thigh, and calf, as well as from triceps and subscapular skinfolds minus 2 mm. The weight of fat in that volume was calculated by multiplying by 0.9.

TBF measured by method of Dauncey et al. using caliper readings divided by two (BFD/2). The volume of subcutaneous adipose tissue and the weight of fat in that volume were measured as described for BFD above, but skinfolds were divided by 2, and 1 mm, representing a single layer of skin, was deducted before the calculations were made.

TBF measured by a modification of method of Dauncey et al. (BFMD). The following modifications were made to BFD when measuring the volume of subcutaneous adipose tissue: Trunk circumference was the mean of hip and chest circumferences. Arm circumference was the mean of upper arm and forearm circumferences. Skinfolds from 10 body sites were taken, divided by 2, and 1 mm, representing a single layer of skin, was deducted from the estimate. The mean of the 10 skinfolds was taken as the thickness of the subcutaneous adipose tissue layer. Subcutaneous adipose tissue was assumed to contain 0.66 g of fat/cm<sup>3</sup> (see below).

Caliper measurements. Skinfolds were measured, always by the same person, in triplicate on the left side of the body by a Harpenden skinfold caliper (Hemco Corp., Holland, MI) with calibrated pressure to the nearest 0.1 mm. They were measured at triceps, biceps, forearm, subscapular, umbilicus, and front of thigh (15). Skinfolds were also taken 1 cm to the left of the nipple, at the back of the thigh, midway between the fold below the buttock and the popliteal space, and on the back of the calf midway between the popliteal space and the ankle. On the buttock, a skinfold was measured vertically at the center. Unless otherwise indicated, skinfolds were not corrected for skin thickness.

Ultrasonography. Thickness of subcutaneous adipose tissue was estimated with an Acuson 128 Computed Sonography System (Acuson Corporation, Mountain View, CA) by an experienced radiologist. Images perpendicular to the skin that showed the tissue beneath the site of study were projected on a screen, and the thickness of subcutaneous adipose tissue including skin, was measured. Photographs were taken of the images and used to confirm the measurements.

*NII.* By following the instructions given by the manufacturer (Futrex Inc.), an estimate of the amount of subcutaneous fat was made using the Futrex 5000.

Other anthropometric measurements. The body weight of the nude infant was recorded to the nearest 5 g using an infant balance (Stathmos/Lindell personal scale, model 304, Stathmos AB, Stockholm, Sweden). Total length and crown-rump length were recorded to the nearest cm on a measuring board. Circumferences and other lengths were measured with a tape measure to the nearest 0.5 cm. Arm length was the distance between acromion and caput radii. Circumferences were taken as follows: Upper arm, midway between the olecranon and the acromion; forearm, midway between the olecranon and caput radii; chest, at the level of the nipples; head, as described by Weiner and Lourie (16); hip, the maximal circumference at the hips; thigh, midway between the fold below the buttock and the popliteal space; and calf, midway between the popliteal space and the ankle.

BFBWD. TBF was estimated using the BWD technique as body weight minus FFM, the latter being TBW/0.80 (8). For each infant, a urine sample was taken to give a baseline value for isotope enrichment and then weighed amounts of <sup>18</sup>O-labeled water  $[0.14 (0.02) \text{ g H}_2^{18}\text{O/kg TBW}]$  were given by a nasogastric tube or p.o. The container and the tubing or feeding device were rinsed with tap water, which was also fed to the infant. Urine produced during the following 6 h was collected in a plastic bag applied over the urethra. The amount was measured and a sample saved for isotope analysis. Six h after dosing, a new plastic bag was applied, and as soon as the infant had passed urine, a sample was collected. The infants were allowed to feed normally during the urine collection. For 15 infants, another four urine samples were collected during the following 2 wk by the mother, who had been given appropriate instructions in advance. The enrichments of <sup>18</sup>O in the dose and in the urine samples were measured at the Bureaux of Stable Isotope Analysis in London with a mass spectrometer (VG Sira II, VG Isotech, Middlewich, England) that was equipped with ISOPREP 18 by which the <sup>18</sup>O/ <sup>16</sup>O ratio was measured using a shaking water/carbon dioxide equilibration system. The results were reproducible to within 0.7 ppm. The <sup>18</sup>O-space was calculated (17) assuming maximum enrichment of isotope in body water about 6 h after dosing and by deducting the amount of isotope excreted in the urine during the first 6 h after dosing (plateau method). When possible, <sup>18</sup>Ospace was also calculated (17) assuming that enrichment at time zero represents the maximum enrichment of isotope in body water (extrapolation method). This latter calculation was based on isotope enrichments in the four urine samples collected during 2 wk after the dose had been given assuming that <sup>18</sup>O is excreted according to single-pool kinetics (18). When estimates based on both methods were available, <sup>18</sup>O-space is given as the mean of the two measurements. When only the plateau method was used, <sup>18</sup>O-space is this result multiplied by 0.985. TBW is calculated as <sup>18</sup>O-space divided by 1.01.

Composition and Density of Adipose Tissue. Biopsies were analyzed for fat, fat-free solids, and water. Fat content was determined by extraction with petroleum ether (boiling point  $40-60^{\circ}$ C) (Soxtec System Ht, Höganäs, Sweden), and fat-free solids were determined by drying the extracted residue to constant weight at 105°C. Water content was the difference between the weight of the biopsy and the sum of its contents of fat and fat-free solids. Assuming adipose tissue to contain (wt/wt, in percent) fat (x), fat-free solids (y), and water (z), its fat content (g/cm<sup>3</sup>) was calculated as: x/(x/0.9 + y/1.4 + z/1.012). Densities (g/cm<sup>3</sup>) for fat, fat-free solids, and water are 0.9, 1.4, and 1.012, respectively (13).

Distribution of Body Fat Between Subcutaneous and Nonsubcutaneous Sites. Estimates of BFMD were also considered to represent the amount of subcutaneous fat. The amount of nonsubcutaneous fat was calculated as BFBWD – BFMD.

Infants and Study Design. Three groups of full-term infants were studied. The protocol was approved by the ethical committees of Huddinge Hospital and the Karolinska Institute, Stockholm, Sweden.

Group I (n = 8; Table 1). The staff of a pediatric ward asked parents of infants less than 3 mo old, admitted to the hospital for minor illnesses, if they would agree to let their infants participate in the study. If so, further information was given and consent requested from both parents. The study was conducted while the infant remained in the ward. BFD, BFD/2 and BFBWD were estimated for the infants in this group.

Group 2 (n = 14; Table 1). The staff of child health centers asked mothers of healthy infants less than 3 mo old if they would allow their infants to participate in the study. If so, further information was given by one investigator (N.K.), who visited the mother at home. Consent from both parents was requested. The measurements were made during 1 d when the mother brought her infant to the hospital. BFD, BFD/2, and BFBWD were estimated for the infants in this group. Also, thickness of subcutaneous adipose tissue was measured by both ultrasonography and calipers at 10 body sites, and an estimate of the amount of subcutaneous fat was obtained by Futrex 5000 at these 10 sites. Circumferences of hip and forearm were measured and BFMD was calculated. The distribution of body fat between subcutaneous and nonsubcutaneous fat was also investigated.

Group 3 (n = 38). The staff of a pediatric surgery department asked parents of infants less than 1 y old for permission to take adipose tissue samples from the infants during hernia operations.

| Table | 1. Age, | sex, | body   | weight,  | and  | length | of infar | us |
|-------|---------|------|--------|----------|------|--------|----------|----|
|       |         | i    | n groi | ups 1 ar | 1d 2 |        |          |    |

|                      |           |     | Body wt    | Length |  |
|----------------------|-----------|-----|------------|--------|--|
| Infant no.           | Age (mo)  | Sex | (g)        | (cm)   |  |
| Group 1 $(n = 8)$    |           |     |            |        |  |
| 1                    | 1.1       | F   | 3920       | 54     |  |
| 2                    | 1.2       | F   | 4000       | 54     |  |
| 3                    | 1.3       | F   | 4500       | 54     |  |
| 4                    | 1.3       | F   | 3640       | 51     |  |
| 5                    | 2.0       | Μ   | 5760       | 59     |  |
| 6                    | 0.6       | F   | 3500       | 50     |  |
| 7                    | 1.5       | F   | 4610       | 57     |  |
| 8                    | 0.9       | Μ   | 4510       | 56     |  |
| Mean (SD)            | 1.2 (0.4) |     | 4300 (720) | 54 (3) |  |
| Group 2 ( $n = 14$ ) |           |     |            |        |  |
| 9                    | 2.6       | F   | 6150       | 61     |  |
| 10                   | 2.1       | М   | 5500       | 59     |  |
| 11                   | 1.5       | Μ   | 5750       | 58     |  |
| 12                   | 2.4       | F   | 6150       | 60     |  |
| 13                   | 1.5       | F   | 5170       | 57     |  |
| 14                   | 2.8       | F   | 4980       | 58     |  |
| 15                   | 0.8       | М   | 4440       | 56     |  |
| 16                   | 0.9       | Μ   | 4000       | 53     |  |
| 17                   | 2.6       | Μ   | 6110       | 61     |  |
| 18                   | 1.6       | F   | 4500       | 55     |  |
| 19                   | 2.5       | F   | 5270       | 48     |  |
| 20                   | 2.6       | Μ   | 5500       | 59     |  |
| 21                   | 2.5       | Μ   | 5880       | 59     |  |
| 22                   | 2.5       | F   | 4600       | 59     |  |
| Mean (SD)            | 2.1 (0.7) |     | 5290 (700) | 57 (3) |  |

If the parents agreed, a biopsy of the subcutaneous adipose tissue was done during surgery, and the specimen was placed in an airtight container and kept at  $-20^{\circ}$ C until its composition was analyzed. The infants were 5.5 ± 3.7 mo old, 63 ± 8 cm tall, and weighed 6840 ± 2010 g.

Statistics. Linear regression and t test (paired and unpaired) were used (19). Results for BFD, BFD/2, and BFMD were compared with results for BFBWD according to Bland and Altman (20). Results are expressed as mean (SD) except where otherwise stated.

# RESULTS

Subcutaneous adipose tissue thickness by calipers and ultrasonography. Table 2 shows the thickness of subcutaneous adipose tissue measured by ultrasonography and by calipers at 10 body sites. Measurements by ultrasonography were higher than skinfolds divided by 2 at five sites, whereas the opposite was found for the other five sites. The differences were in most cases significant. However, there was no overall significant difference between the two kinds of measurements when all sites were combined.

Evaluation of Futrex 5000. Figure 1a shows the association between thickness of subcutaneous adipose tissue estimated by calipers on the x axis and measurements obtained by Futrex 5000 on the y axis, whereas Figure 1b shows the corresponding association between the Futrex measurements (y) and the thickness of subcutaneous adipose tissue measured by ultrasonography (x). In both cases, there was apparently a weak association between NII units and adipose tissue thickness when the latter was less than approximately 5 mm. For adipose tissue thickness above 5 mm, the estimate obtained by Futrex 5000 did not increase as the thickness of adipose tissue increased.

Adipose tissue composition and density. Infant adipose tissue contained 71% (SD 9%) fat (range 47–89%), 26% (SD 8%) water (range 9–44%), and 4% (SD 3%) fat-free solids (range 1–17%) corresponding to 0.66 (SD 0.07) g of fat/cm<sup>3</sup> adipose tissue (range 0.46–0.82 g/cm<sup>3</sup>). There was no association between age and the content of fat or water in adipose tissue.

*TBW.* Table 3 shows <sup>18</sup>O-space, TBW, and elimination rate for <sup>18</sup>O for infants in groups 1 and 2.

Method of Dauncev et al. (4) for estimating TBF. Estimates of BFD, BFD/2, and BFBWD for infants in groups 1 and 2 are given in Table 4. With TBF expressed as percent of body weight, these infants contained 20.7% (SD 4.4%) BFD, 10.4% (SD 2.2%) BFD/2, and 16.2% (SD 5.6%) BFBWD. Figure 2a shows differences between percent BFD and percent BFBWD plotted against TBF in percent. This difference was 4.5% (SD 5.6%). No signif-

Table 2. Thickness (mm) [mean (SD)] of subcutaneous adiposetissue measured by ultrasonography and by calipers at 10 sitesfor 14 infants in group 2

| Site                  | Ultrasono-<br>graphy | Calipers*  | Difference† | Intermethod<br>difference‡ |
|-----------------------|----------------------|------------|-------------|----------------------------|
| Triceps               | 4.4 (1.1)            | 3.7 (0.7)  | 0.7 (1.1)   | <i>p</i> < 0.05            |
| Biceps                | 4.4 (1.6)            | 2.8 (0.5)  | 1.6 (1.5)   | <i>p</i> < 0.01            |
| Forearm               | 3.5 (0.7)            | 4.3 (0.9)  | -0.8 (0.9)  | <i>p</i> < 0.01            |
| Subscapula            | 4.4 (1.4)            | 4.0 (0.7)  | 0.5 (1.2)   | <i>p</i> > 0.05            |
| Umbilicus             | 4.1 (1.5)            | 3.4 (0.9)  | 0.7 (1.1)   | <i>p</i> < 0.05            |
| Nipple                | 4.5 (1.7)            | 3.5 (0.7)  | 1.1 (1.6)   | <i>p</i> < 0.05            |
| Thigh, front          | 6.1 (1.5)            | 7.6 (1.7)  | -1.6 (1.6)  | <i>p</i> < 0.01            |
| Thigh, back           | 5.4 (1.3)            | 8.4 (1.2)  | -2.9 (1.5)  | <i>p</i> < 0.001           |
| Calf                  | 5.2 (1.5)            | 7.2 (1.8)  | -2.0 (2.2)  | <i>p</i> < 0.01            |
| Buttock               | 9.9 (2.2)            | 11.5 (2.5) | -1.6 (3.0)  | p > 0.05                   |
| All sites $(n = 140)$ | 5.2 (2.3)            | 5.6 (3.0)  | -0.4 (2.2)  | p > 0.05                   |

\* Value obtained by calipers divided by 2.

† Value obtained by ultrasonography minus value obtained by calipers divided by 2.

‡ Paired t test (19).



Fig. 1. *a*, Association between the thickness of subcutaneous adipose tissue estimated by calipers (skinfolds divided by 2) (mm) (*x* axis) and estimates of the amount of subcutaneous fat by Futrex 5000 (NII units) (*y* axis). The data are from 10 sites in 14 infants.  $\star$ , triceps;  $\blacktriangle$ , biceps;  $\bigcirc$ , forearm;  $\blacksquare$ , subscapula;  $\blacklozenge$ , umbilicus;  $\bigstar$ , nipple;  $\triangle$ , front thigh;  $\bigcirc$ , back thigh;  $\Box$ , calf;  $\bigcirc$ , buttock, *b*, Association between the thickness of subcutaneous adipose tissue estimated by ultrasonography (mm) (*x* axis) and estimates of the amount of subcutaneous fat by Futrex 5000 (NII units) (*y* axis). The data are from 10 sites in 14 infants. Symbols are the same as in *a*.

icant relationship between this difference and TBF in percent was found. Figure 2b shows differences between percent BFD/2 and percent BFBWD plotted against TBF in percent. This difference was -5.8% (SD 5.1%). There was a significant linear relationship between the difference between BFD/2 and BFBWD and percent TBF (r = -0.75, p < 0.001).

Modification of method of Dauncey et al. for estimating TBF. Table 4 also shows estimates of BFMD and BFBWD for infants in group 2. With TBF expressed as percent of body weight, these infants contained 12.2% (SD 2.4%) BFMD and 17.4% (SD 5.1%) BFBWD. Figure 2c shows differences between percent BFMD and percent BFBWD plotted against TBF in percent. This difference was -5.2% (SD 4.9%). A significant linear relationship between BFMD – BFBWD and TBF in percent was found (r = -0.66, p < 0.01).

Distribution of body fat between subcutaneous and nonsubcutaneous sites. The values in Table 4 describe the distribution of body fat: BFMD and BFBWD – BFMD represent the amounts of subcutaneous and nonsubcutaneous fat, respectively. Furthermore, Table 4 shows BFBWD – BFMD expressed in percent of body weight minus BFMD, thus representing the fat content of the infant's body when the fat in subcutaneous adipose tissue is disregarded. The infants contained 281% (SD 260) g of nonsubcutaneous fat, and when expressed in percent of body weight minus BFMD, 5.9% (SD 5.5%). This figure was significantly higher (p < 0.02) for girls [9.2% (SD 3.8%)] than for boys [2.6% (SD 5.1%)]. A significant correlation between this percentage (y) and age (x) (mo) of the infant was also found: y = 5.14x - 4.72; r = 0.63, p < 0.02, n = 14.

Correlations between TBF estimated by BWD and anthropometric measurements. A significant correlation was found between the subscapular skinfold (mm) and BFBWD (%) (r = 0.63, p < 0.01, n = 22). Also, BFBWD (%) correlated with forearm skinfold (mm) (r = 0.60, p < 0.05, n = 14) and with calf skinfold (mm) (r = 0.68, p < 0.01, n = 14). There was no correlation between BFBWD (%) and triceps skinfold (mm) or between BFBWD (%) and any of the other skinfolds (mm) measured. A significant linear relationship was found between weight (g)/ height (cm) (x) and BFBWD (%) (y): y = -8.55 + 0.284x; r = $0.6\overline{0}, p < 0.01, n = 22$ . There were also significant correlations between BFBWD (g) (y) and BFMD (g) (x) (y = 1.098x + 216, r = 0.63, standard error of the estimate = 270, p < 0.02, n = 14), as well as between BFBWD (g) (y) and BFBWD - BFMD (g) (x) (y = 1.055x + 642, r = 0.82, standard error of the estimate = 199, p < 0.001, n = 14).

# DISCUSSION

Our results show that caliper measurements, divided by 2, give lower figures than ultrasonography when the subcutaneous adipose tissue is thin (i.e. triceps, biceps, subscapular, umbilicus, and nipple) and higher estimates where the adipose tissue is thick (i.e. thigh, calf, and buttock). Possibly, in infants, thin skinfolds are more easily compressed by the caliper than are thick ones. It needs to be emphasized that neither of the two methods necessarily gives the true figure for adipose tissue thickness. Ultrasonography requires a correct identification of the border between adipose tissue and muscle, which may be difficult because adipose tissue is sometimes present in layers separated by membranes that are visible on the screen. Studies in adults (10) comparing caliper measurements to direct measurements of subcutaneous adipose tissue indicated good agreement between the two, although the caliper tended to underestimate the true adipose tissue thickness. From our results we conclude that, in infants, a caliper measurement divided by 2 is likely to give a better estimate of the thickness of subcutaneous adipose tissue than the initial caliper reading alone.

Our results showed that the NII technique gave rather similar values at all body sites although both ultrasonography and calipers indicated that the thickness of subcutaneous adipose tissue varied. It thus seems unlikely that measurements made with the Futrex 5000 represent valid estimates of the amount of subcutaneous fat in infants. Although the NII technique may be useful, the Futrex 5000 device requires improvement before it can be adopted for studies of infants.

Our findings with respect to adipose tissue composition are in agreement with data published by Baker (21) and suggest that the assumption by Dauncey *et al.* (4) about the density of infant adipose tissue needs modification. Our finding that the lipid content of adipose tissue does not change with age is in contrast to Baker's data because he showed (21) that the amount of fat in adipose tissue tended to increase with age during early life.

We have assumed that the BWD technique provides valid estimates of TBF in infants. This requires an accurate assessment of TBW and a correct calculation of FFM. Two approaches could be used when TBW is estimated using <sup>18</sup>O. In the extrapolation method, <sup>18</sup>O-space is the time zero distribution space calculated from the rate at which the isotope disappears. In the plateau method, <sup>18</sup>O-space is calculated from the isotope enrichment observed shortly (usually about 4 to 6 h) after the dose is given, assuming that no isotope is lost or that all isotope lost during the equilibration period can be accounted for. Because the plateau method tends to overestimate <sup>18</sup>O-space and the extrapolation method tends to give too low results (17), we used the average of the two for estimating <sup>18</sup>O-space. When the extrapolation method was not used, 18O-space was assumed to equal 98.5% of the estimate obtained by the plateau method because our values obtained by the extrapolation method were, on average, 97% of values obtained by the plateau method. The elimination rate of <sup>18</sup>O and TBW have been studied previously

| Infant no. | $\begin{array}{c} {}^{18}\text{O-space}\\ \text{extrapolation (g)}\\ (n=15) \end{array}$ | <sup>18</sup> O-space<br>plateau (g)<br>(n = 22) | TBW (g) $(n = 22)$ | TBW (% of body wt)<br>( $n = 22$ ) | $k_{18_O}$<br>(24 h <sup>-1</sup> )<br>(n = 15) |
|------------|------------------------------------------------------------------------------------------|--------------------------------------------------|--------------------|------------------------------------|-------------------------------------------------|
| 1          | 2507                                                                                     | 2612                                             | 2534*              | 65                                 | 0.251                                           |
| 2          |                                                                                          | 2996                                             | 2922†              | 73                                 |                                                 |
| 3          |                                                                                          | 3228                                             | 3148†              | 70                                 |                                                 |
| 4          | 2532                                                                                     | 2587                                             | 2534*              | 70                                 | 0.261                                           |
| 5          |                                                                                          | 3575                                             | 3487†              | 61                                 |                                                 |
| 6          |                                                                                          | 2573                                             | 2510†              | 72                                 |                                                 |
| 7          |                                                                                          | 3099                                             | 3022†              | 66                                 |                                                 |
| 8          |                                                                                          | 3459                                             | 3374†              | 75                                 |                                                 |
| 9          | 3866                                                                                     | 3849                                             | 3820*              | 62                                 | 0.221                                           |
| 10         | 3699                                                                                     | 4120                                             | 3871*              | 70                                 | 0.328                                           |
| 11         | 3887                                                                                     | 4042                                             | 3926*              | 68                                 | 0.258                                           |
| 12         |                                                                                          | 4005                                             | 3906†              | 63                                 |                                                 |
| 13         | 3344                                                                                     | 3426                                             | 3352*              | 65                                 | 0.243                                           |
| 14         | 2743                                                                                     | 3176                                             | 2931*              | 59                                 | 0.303                                           |
| 15         | 3335                                                                                     | 3223                                             | 3247*              | 73                                 | 0.250                                           |
| 16         | 2867                                                                                     | 2911                                             | 2861*              | 72                                 | 0.309                                           |
| 17         | 3736                                                                                     | 3974                                             | 3817*              | 62                                 | 0.283                                           |
| 18         | 3032                                                                                     | 3126                                             | 3049*              | 68                                 | 0.289                                           |
| 19         | 3208                                                                                     | 3319                                             | 3232*              | 61                                 | 0.310                                           |
| 20         | 3716                                                                                     | 3717                                             | 3680*              | 67                                 | 0.327                                           |
| 21         | 4158                                                                                     | 3764                                             | 3922*              | 67                                 | 0.267                                           |
| 22         | 2960                                                                                     | 3261                                             | 3080*              | 67                                 | 0.239                                           |
| Mean (SD)  | 3306 (524)                                                                               | 3366 (475)                                       | 3283 (472)         | 67 (5)                             | 0 276 (0 034)                                   |

Table 3. <sup>18</sup>O-space by extrapolation and plateau methods, respectively, TBW, and elimination rate of <sup>18</sup>O ( $k_{180}$ ) for infants in groups 1 and 2

\* {[<sup>18</sup>O-space (extrapolation method) + <sup>18</sup>O-space (plateau method)]  $\div$  2}/1.01.

† 98.5% of <sup>18</sup>O-space (plateau method)/1.01.

|                    | BFD (g)    | BFD/2 (g) | BFMD (g)† | BFBWD (g) | (BFBWD – BFMD) |           |
|--------------------|------------|-----------|-----------|-----------|----------------|-----------|
| Infant no.         |            |           |           |           | g              | %‡        |
| 1                  | 898        | 453       |           | 752       |                |           |
| 2                  | 595        | 302       |           | 347       |                |           |
| 3                  | 758        | 379       |           | 565       |                |           |
| 4                  | 642        | 324       |           | 472       |                |           |
| 5                  | 1710       | 860       |           | 1401      |                |           |
| 6                  | 746        | 376       |           | 362       |                |           |
| 7                  | 881        | 441       |           | 832       |                |           |
| 8                  | 767        | 392       |           | 293       |                |           |
| 9                  | 1621       | 811       | 1115      | 1375      | 260            | 5 2       |
| 10                 | 985        | 493       | 638       | 661       | 23             | 0.5       |
| 11                 | 1633       | 817       | 754       | 842       | 88             | 1.8       |
| 12                 | 1278       | 639       | 730       | 1267      | 537            | 9.9       |
| 13                 | 1199       | 599       | 644       | 980       | 336            | 74        |
| 14                 | 795        | 398       | 632       | 1316      | 684            | 15.7      |
| 15                 | 941        | 471       | 579       | 381       | -198           | -5.1      |
| 16                 | 682        | 341       | 321       | 424       | 103            | 2.8       |
| 17                 | 1420       | 710       | 681       | 1339      | 658            | 12.1      |
| 18                 | 700        | 350       | 407       | 689       | 282            | 69        |
| 19                 | 1024       | 512       | 629       | 1230      | 601            | 12.9      |
| 20                 | 1563       | 782       | 754       | 900       | 146            | 31        |
| 21                 | 1308       | 654       | 832       | 977       | 145            | 29        |
| 22                 | 783        | 391       | 483       | 750       | 267            | 6.5       |
| Mean (SD) (n = 22) | 1042 (361) | 523 (180) |           | 825 (371) | 201            | 0.5       |
| Mean (SD) (n = 14) | 1138 (341) | 569 (170) | 657 (192) | 938 (334) | 281 (260)      | 5.9 (5.5) |

Table 4. TBF obtained using variations of method of Dauncey et al. (4) (BFD, BFD/2, and BFMD) and using BFBWD\*

\* The distribution of body fat between subcutaneous fat (BFMD) and nonsubcutaneous fat (BFBWD - BFMD) is also shown.

+ Represents TBF estimated using a modification of the method of Dauncey et al. (4) as well as subcutaneous fat.

‡ Expressed in percent of (body weight - BFMD).

§ Infants 9-22.



Fig. 2. *a*, Difference between BFD and BFBWD (BFD – BFBWD) plotted against percent TBF (average of BFD and BFBWD) for 22 infants. BFD – BFBWD = 4.5% (SD 5.6%). *b*, Difference between BFD/2 and BFBWD (BFD/2 – BFBWD) plotted against percent TBF (average of BFD/2 and BFBWD) for 22 infants. BFD/2 – BFBWD = -5.8% (SD 5.1%); r = -0.75, p < 0.001. *c*, Difference between BFMD and BFBWD (BFMD – BFBWD) plotted against percent TBF (average of BFMD and BFBWD) for 14 infants. BFMD – BFBWD = -5.2% (SD 4.9%); r = -0.66, p < 0.01.

in infants (22-25) and our results are in agreement with these studies.

A correct conversion of TBW to FFM requires that the degree of hydration of the latter is known. In human adults, FFM is assumed to contain 72-73% water, although the variation around this value is considerable in adult animals (26). Infants and children have a higher degree of hydration in FFM than adults, and the corresponding value for a fetus is even higher (7). The value that we used (80%), must be regarded as potentially inaccurate because it is based on limited data and because the degree of hydration in FFM may also be variable in infants. Assuming that the mean degree of hydration in FFM among the infants in this study was 82%, rather than 80%, the difference between BFD and BFBWD would be 2.4%, not 4.5%. Unfortunately, no better alternative to estimate TBF of infants *in vivo* is presently available.

The method of Dauncey et al. (4) seems to give inaccurate and

imprecise estimates of TBF irrespective of whether skinfolds were taken to represent single or double layers of adipose tissue. Assuming a more realistic fat content of adipose tissue than suggested by Dauncey et al. does not change this conclusion. Attempts to modify the method by including more skinfolds (as in BFMD) did not improve the accuracy and improved the precision only slightly. BFD overestimated TBF, whereas BFD/ 2 and BFMD underestimated it, probably because infants may have fat at sites other than subcutaneously, for example around the internal organs and within muscles. Therefore, BFD/2 and BFMD, which presumably assess only the amount of fat located subcutaneously, underestimate TBF; BFD is higher than TBF, although the nonsubcutaneous fat is not taken into account, probably because the amount of subcutaneous fat is overestimated. The underestimation of TBF by BFD/2 and BFMD increased as the TBF content of the infant increased, which suggests that fatter infants contain relatively more nonsubcutaneous fat than lean infants do.

Our estimates of nonsubcutaneous fat should be regarded with caution because their derivation was based on many assumptions. It is relevant to mention that a similar approach has been used to estimate "internal fat mass" in adults (27). Our data suggest that the amount of nonsubcutaneous fat varies considerably among infants and are thus in agreement with the suggestion that variation in the distribution of internal and external fat stores is a major factor behind the poor correlation between skinfolds and BFBWD in infants (2).

In agreement with previous results (2), we found no correlation between triceps skinfold and BFBWD. Although some of the skinfolds taken in this study did correlate with BFBWD, most of them did not. This agrees with findings by Deans *et al.* (28), who failed to show any relationship between the percentage of fat in fetuses in late pregnancy estimated by magnetic resonance imaging and TBF after birth calculated from caliper measurements. Correlations between BFBWD and BFMD as well as between BFBWD and BFBWD – BFMD were, however, found.

Although the above results may appear discouraging, we suggest that BFMD may, after further refinement, be potentially useful in field studies as a simple method of predicting TBF in infants. With more knowledge available about the growth and development of adipose tissue during infancy, it may be possible to establish an appropriate measuring procedure and develop adequate predictive equations. On the basis of the present results, we suspect that separate equations for boys and girls with the age of the infant included would increase the accuracy of such a method. Knowledge about how the amount of adipose tissue in different parts of the body changes during the first year of life could be obtained by the magnetic resonance imaging technique. Exploring changes in fat distribution during infancy is also of interest, because later in life the distribution of body fat is related to the development of chronic disease (29).

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