Evaluation of dual-energy X-ray absorptiometry for body-composition assessment in piglets and term human neonates1–3

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ABSTRACT The reproducibility, accuracy, and precision of dual-energy X-ray absorptiometry (DXA) was assessed by scanning 13 piglets (1471–5507 g) in triplicate. In four piglets, fat content was increased with porcine lard around the abdomen; additional measurements were performed on these animals. Reproducibility in DXA measurements from the animals without added fat was 0.09% for body weight, 1.95% for bone mineral content (BMC), and 5.35% for fat content. DXA estimates of body weight, BMC, and fat content were significantly correlated with scale body weight, ash weight, chemical calcium, and chemical fat. Body weight was measured accurately but fat content was overestimated by DXA. Mean BMC estimated by DXA represented 48% of ash weight and 215% of calcium content. The precision of DXA was 0.23% for body weight, 10.99% for ash weight, and 4.44% for calcium content. The precision of DXA for fat content was poor. However, for measurements performed in piglets with >250 g fat, the precision was 8.85%. Thirty appropriate-post-gestational-age term human neonates (birth weight: 3188 ± 217 g) were scanned once during the first week of life. BMC and fat content were 54 ± 6 and 470 ± 92 g, respectively, which corresponded to 26.4 ± 2.6 g calcium and 427 ± 82 g fat. These were close to the reference values previously determined by chemical analysis. This study suggests that DXA is accurate and reliable for measurement of calcium and fat contents in human neonates. Further refinements would be beneficial for determining fat content in preterm human infants. Am J Clin Nutr 1996;63:157–63.

KEY WORDS Body composition, bone mineral content, dual-energy X-ray absorptiometry, human neonates, piglets, total body fat

INTRODUCTION

There is a need for a rapid, safe, and reliable method for determining whole-body composition in human neonates, thus permitting the evaluation of short- and long-term effects of nutritional and treatment regimens. In adults, the assessment of body composition by dual-energy X-ray absorptiometry (DXA) provides results comparable to those of other methods (1–8). DXA performed on large pigs showed that this method is accurate for body composition measurement in subjects weighing 35–95 kg (9). In infants, DXA measurements of lumbar bone mineral content (BMC) have been validated (10, 11) and whole-body measurements of body composition and BMC have been developed. Brunton et al (12) evaluated a preliminary whole-body measurement developed by Hologic Inc (Waltham, MA) and known as the pediatric whole-body program (version 6.01, 1992). They found that the pediatric whole-body software was satisfactory for large (6-kg) piglets but that it underestimated BMC and overestimated fat content in small (1.6-kg) piglets. This experience and other experimental data led the DXA manufacturer to develop the infant whole-body application (Hologic Inc, version 5.64, 1993).

The main objectives of the present study were to evaluate the precision and accuracy of the infant whole-body application by comparing DXA estimates of body weight, BMC, and fat content with the results of carcass analysis in small piglets. To determine the suitability of this application for use in preterm and term human infants, coats of porcine lard were added in four piglets to approximate the fat content reported in human infants (13–17). The relevance of the DXA measurements of body weight, BMC, and fat content was assessed in term human infants by comparing the DXA data with body composition reference values for human infants.

METHODS

Animal study

Body composition was measured by DXA in 13 piglets (Landrace Pietrain; Veterinary School, University of Liège, Belgium) aged <1 mo and weighing between 1471 and 5507 g. After a 12-h fast, piglets were killed with an overdose of pentobarbital (300 mg/kg body wt) according to a protocol approved by the Veterinary School of the University of Liège and were scanned within 2 h after death. To increase the range of fat content, we applied coats of porcine lard 0.3-cm thick around the abdomen in four of the piglets. The initial body weights of the four fat-padded piglets were 1712, 2211, 2626,
and 3739 g. Each piglet was scanned with three to five different porcine lard pads so that 17 different fat contents were scanned.

**DXA scan procedure**

DXA measurements were performed with a QDR-2000 instrument (Hologic Inc). The principle of DXA has been described previously (9-11). The system makes use of a dual-energy source that is pulsed between 70 and 140 kV synchronously with the line frequency. The X-ray source is mounted beneath the subject and generates a thin, dual-energy X-ray beam that passes first through a calibration disk containing bone- and tissue-equivalent materials and then through the patient. The transmitted X-rays strike a detector mounted above the subject. All piglets were positioned as recommended for the neonate, with the subject lying supine on an infant table pad specifically designed for infant whole-body measurements, with an interposing paper sheet. A tissue-equivalent step phantom was placed alongside the subject to provide a calibration of the body composition measurements. Scan time was ~6 min and analysis with the infant whole-body software (Hologic Inc, version 5.64) required ~30 s. Body composition was analyzed assuming that the hydration of the lean body mass is 83.3%. To determine whether the DXA analysis was robust with respect to this assumption, we reanalyzed our data with the chemically determined value of water content in lean body mass. The body composition analysis produced measurements of body weight (g), bone area (cm²), bone mineral content (g), lean mass (g), and fat content (g). Reproducibility was determined by scanning the 13 piglets three times consecutively while the piglets were supine and without repositioning; from these data, the percentage CV due to instrument error was calculated.

**Electronic scale and whole-carcass analysis**

To assess the accuracy of DXA measurements, the piglets were weighed before and after DXA analysis and their mean weights were compared with the DXA results. After DXA analysis, whole-body chemical analysis was performed in each piglet to assess the accuracy of BMC and DXA measurements. The carcass was homogenized with a butchery grinder and residues (small pieces of bone with small amounts of meat) were carefully collected; these residues weighed between 142 and 489 g. The grinder was rinsed with chloroform after homogenization to avoid fat losses. More than 95% of the piglets' total body weight was recovered after homogenization. Total fat content was calculated as the sum of fat content in the homogenized carcass and in residues. The fat content of the homogenized carcass was determined by chemical fat extraction (6-g samples, in triplicate) with chloroform-methanol (18). The mean CV (± 1 SD) of the chemical fat measurement was 3.0 ± 1.7%. The fat content of residues and of porcine lard was determined by a gravimetric method after fat extraction with chloroform (19). In piglets with additional porcine lard, the total fat content was calculated as the sum of the fat content in the homogenized carcass and in residues plus the amount of fat added.

Total ash weight and calcium content were calculated as the sum of ash and calcium contents in the homogenized carcass and in residues. Ash weight and calcium content were determined after 3-g samples were burned in triplicate at 550 °C for ≥ 14 h; calcium content was determined by atomic absorptiometry of the ash. Ash and calcium contents in the residues were determined in the same fashion: the residues were burned completely at 550 °C for ≥ 24 h until complete calcination and the obtainment of a fine powder. Mean CVs of chemical ash and calcium determinations were 6.9 ± 6.0% and 4.6 ± 3.0%, respectively.

Total water content was calculated as the sum of water content in the homogenized carcass and in residues. Water content was calculated by weighing samples (three samples of 50, 100, and 200 g) before drying at 100 °C and repetitively after drying until a constant weight was obtained. After deducing the fat content, we recalculated the mean water content of the lean body mass. Then we reanalyzed the piglet DXA data with the chemically measured value of hydration of lean body mass to evaluate the influence of this factor on the assessment of soft-tissue composition by DXA.

**Statistical analysis**

Linear-regression analysis and the Pearson product-moment correlation coefficient (r) were used to compare DXA measurements with chemical analyses. Blind use of the data obtained from regression analysis has some limitations in clinical practice: a high correlation coefficient does not mean that the two methods agree in absolute terms (20). In a relation with the zero intercept, the difference between the slope of the regression line and the identity represents the accuracy or the systematic deviation from the identity line (21). Data conversion is necessary to express DXA accuracy as the dispersion of DXA values obtained below and above the reference value. In our study, this dispersion was called precision and can be expressed as the mean difference in percentage of reference between the converted DXA value and the reference value, according to Bland and Altman (20). Conversion formulas for body weight, BMC, and fat content were generated by the inverse relation (chemical analysis versus DXA measurements). All data are given as the mean ± 1 SD.

**Study of term human neonates**

Thirty well-nourished, appropriate-for-gestational-age, term human neonates (mean birth weight, 3188 ± 217 g; mean gestational age, 39.4 ± 1.1 wk; and mean postnatal age, 3 ± 1 d) were scanned once during their first week of life in the same way as for the piglets. The infants were lying supine on the infant table pad with an interposing paper sheet. They were naked and wrapped in a paper sheet to avoid movement during the measurements and to obtain abduction of legs. No sedation was necessary for these newborn infants. This study was approved by the Ethical Committee of University of Liège and informed parental consent was obtained.

**RESULTS**

**Animal study**

In the piglets (Figure 1 and Figure 2) total ash content ranged between 46 and 196 g and calcium accounted for 25.9 ± 2.8% of ash values. Fat content calculated chemically ranged from 30 to 580 g, representing between 17% and 12.5% of body weight. Fat content of the additional porcine lard represented 87.8 ± 2.0% of the lard weight and in the four fat-
padded piglets, the upper value of fat content reached 2116 g (35.9% of body weight).

Total body water determined by chemical analysis was 72 ± 3% in the 13 piglets. This corresponded, after deduction of the fat content, to a mean water content of the lean body mass of 78 ± 1%. DXA-measured percentage fat was increased by only 0.32 ± 0.026% after piglet DXA results were reanalyzed with the chemically measured value of hydration for lean body mass instead of the assumed value of 83.3%.

Reproducibility (CV%) of the DXA measurements was, respectively, 0.09 ± 0.05% for body weight, 1.95 ± 1.28% for BMC, and 5.35 ± 3.74% for fat content. When all data (n = 30) were pooled, the reproducibility of the fat measurement was 4.20 ± 3.0% and was independent of the amount of fat measured.

Body weight determined by DXA in 13 piglets was highly correlated (r = 0.999) with scale body weight (Figure 1A). BMC (g) determined by DXA in 13 piglets was significantly correlated with ash weight (r = 0.955) (Figure 1B) and total calcium content (r = 0.992) (Figure 1C). BMC calculated by DXA was 48% of ash weight and 215% of calcium content, respectively. DXA-measured fat content in 13 piglets was significantly correlated (r = 0.971) with chemically measured fat content (Figure 2A). Fat content was overestimated by DXA in 9 of 13 piglets. When the 17 DXA scans in the fat-padded piglets were added, the correlation between fat content calculated by DXA and chemically measured fat content was improved (r = 0.993) (Figure 2B). However, overestimation of fat content by DXA remained at these higher percentages of fat.

Formulas for data conversion of DXA measurements according to the relations between chemical analysis and DXA were established: y = 0.986x + 28.27 for body weight, y = 1.892x + 0.89 for ash weight, y = 0.456x + 1.56 for calcium content, and y = 0.887x + 10.41 for fat content. These formulas were
applied to all DXA measurements of body weight, BMC, and fat content to determine the precision of the DXA estimates (16).

The mean difference between the converted DXA value and the reference value (scale body weight and carcass analysis data) was 0.01 ± 0.23% for body weight (Figure 3A), 0.99 ± 10.99% for ash weight (Figure 3B), 0.05 ± 4.44% for calcium content (Figure 3C), and 22.11 ± 90.91% for fat content (Figure 4A). Therefore, precision of DXA measurements was 0.23% for body weight, 10.99% for ash weight, 4.44% for calcium content, and 90.91% for fat content. For the 22 measurements performed in the 10 piglets with a fat content > 250 g, the difference in percentage of chemical fat between the converted value of DXA-measured fat and chemically measured fat was improved from 22.11 ± 90.91% to 1.54 ± 8.85% (Figure 4B), and therefore the precision from 90.91% to 8.85%

FIGURE 3. Estimation of the precision of dual-energy X-ray absorptiometry (DXA) measurements; difference between the converted values of DXA measurements and the reference values in percentage of the measured values in the piglets. A: scale body weight (BW), B: ash content, C: calcium content.

FIGURE 4. Estimation of the precision of dual-energy X-ray absorptiometry (DXA) measurements; difference between the converted values of fat measured by DXA and fat content determined chemically in percentage of the reference value in piglets without (□) and with (▲) additional porcine lard (n = 30) (A) and in those with > 250 g fat content (n = 22) (B).

Study of term human neonates

In term, appropriate-for-gestational-age human neonates (n = 30), mean body weight (± SD) at the time of DXA analysis was 3065 ± 239 g and mean body weight estimated by DXA was 3053 ± 202 g. For these infants, average BMC and fat content as estimated by DXA were, respectively, 54 ± 6 and 470 ± 92 g, corresponding to 26.4 ± 2.6 g calcium and 427 ± 82 g fat content after the conversion formulas to estimate calcium content and to correct fat overestimation by DXA (as suggested by the study in piglets) were applied. Mean lean body mass as calculated by DXA (body weight − BMC − fat mass) was 2529 ± 168 g. Mean bone area scanned during DXA analysis was 279 ± 16 cm².

DISCUSSION

DXA could be a practical tool in assessment of whole-body composition and weight-gain composition. The radiation exposure during an infant whole-body scan with the infant table pad is low (3 μSv, or 0.3 mrem) (22). In adults, DXA has been compared with other methods such as hydrodensitometry, skinfold-thickness measurement, bioelectrical impedance analysis,
deuterium oxide dilution, and total body potassium (3–7), but the only real validation study was recently performed in 35–
161 kg pigs (8).
Assessment of body composition by DXA in infants and neonates has already been reported (23), but a precise validation of DXA is necessary before reliable clinical application in neonates. After a validation study in piglets in which pediatric whole-body software (Hologic Inc, version 6.01, 1992) was used, Brunton et al (12) concluded that further improvements were necessary before reliable measures of BMC and fat content in small infants were attainable. Therefore, the manufacturer developed a new infant whole-body software program (Hologic Inc, version 5.64, 1993), which we evaluated.
According to the manufacturer, the new infant whole-body software uses a “moving box” algorithm that divides the image into numerous small rectangular regions and then identifies the bone points and evaluates BMC by using tissue from the small local region. Thus, the earlier pediatric whole-body software used a global approach whereas the infant whole-body software uses a regional or local approach. The infant table pad was specifically designed for infant whole-body measurements. It filters the low-energy beam to improve system linearity in small subjects and to reduce the radiation dose (22).
Piglets were used as a model because they were used in the previous validation studies (12–22) and their body weights and compositions are close to those of human neonates. The 13 piglets in the present study (body weight, 1471–5507 g) represented the weight range of preterm and term infants. The piglets had 1.7–12.5% of body weight as fat and 12.1–48.5 g calcium, which is in agreement with the published results of carcass analysis in piglets (12, 15, 22, 24, 25) and is close to fat and calcium contents observed previously in human fetuses (13–17, 26, 27). By adding porcine lard we extended the upper figure of fat content to 35% of body weight.
DXA reproducibility has been evaluated by duplicate scans in human adults (9) and infants (22). Triplicate measurements would provide a more robust estimate of DXA reproducibility; however, such experiments are generally performed in animals for ethical reasons. The CV obtained in the present experiment was similar to that observed previously (9, 12, 22, 23); it was lower for body weight (<0.5%), intermediate for BMC (1.5–2.5%), and higher for fat content (2.5–6.5%). Because the piglets were scanned three times consecutively without repositioning, the CV was related directly to the pure instrument error.
The only method for a reliable evaluation of DXA accuracy and precision is the comparison of body weight, BMC, and fat content values estimated by DXA with measurements from weighing and chemical analysis. These comparisons have been performed in pigs (9, 28) and in piglets (12, 22). These studies measured only the correlation coefficient between the methods to determine DXA accuracy; precision as defined in the present study was not calculated.
Body weight calculations can be easily validated by body weight measurement on a scale. Body weight was accurately estimated by DXA because the difference between the slope of the regression line and identity was almost zero (1.014 – 1) (Figure 1A). The precision was also excellent (0.23%), thus confirming previous data in human adults and newborns (5, 8, 16) and in pigs and piglets (9, 12, 22).
BMC measurements by DXA are theoretically given in grams of hydroxyapatite, which is difficult to measure directly in whole-body carcass analysis. Therefore, validation of BMC measurements by DXA can be estimated by ash or calcium measurement, but it is unclear which chemical determination best defines BMC. In pigs, Svendsen et al (9) suggested that chemical ash weight measurement for the validation of DXA-measured BMC was inappropriate because they observed a high CV (54%) in replicate ash samples. Brunton et al (12), who analyzed ash content in two groups of 10 piglets, reported much better reproducibility; CVs in triplicate samples from each piglet were 7.0% in 1.6-kg piglets and 7.6% in 6.0-kg piglets, suggesting that ash weight is appropriate for BMC validation. However, it appears that the pediatric whole-body program used in that study was unable to precisely estimate ash weight in the two groups. By contrast, Chan (29) found a high correlation between BMC estimated by DXA and the ashed weight of fresh chicken; DXA estimates of BMC represented 50% of the ash values. Recently, Koo et al (22) found in piglets with body weights from 886 to 5526 g that the CV of ash weight determinations from triplicate samples of the pig homogenate was 4.7%. They observed a highly significant correlation (r = 0.992) between BMC measured by DXA and ash weight: BMC measured by DXA corresponded to 59% of the ash value.
In the present study, the CV of ash weight determinations was 6.9% and we observed a highly significant correlation between DXA estimates of BMC and total ash weight (Figure 1B); BMC measured by DXA represented 48% of the ash value. Furthermore, we calculated that ash values were determined by DXA with a precision of 10.99%. Therefore, we confirmed that ash weight determination could be used to validate BMC measurements. The ash weight measurements indicate that the improvements in the new infant whole-body program in the accuracy and precision of BMC determinations by DXA were satisfactory (10.99%).
On the other hand, Braillon et al (10) determined BMC, ash weight, and calcium content in six excised femurs from preterm stillborn infants of gestational age from 25 to 29 wk. The BMC measurement was calculated with lumbar spine software. Braillon et al observed a highly significant correlation between BMC measured by DXA and calcium content: BMC measured by DXA corresponded to 3.1 times the calcium content. The precision, as defined in our study, calculated from their data was 5.9%. Using infant whole-body software, we observed a significant correlation between BMC measured by DXA and calcium content (Figure 1B). The r value (0.992) and the precision (4.44%) were improved compared with values observed between BMC calculated by DXA and ash content (0.955 and 10.99%), suggesting that calcium determination is more appropriate than ash measurement to validate BMC determination by DXA. Taking into account that we obtained a ratio of DXA-measured BMC to calcium corresponding to 2.15, we suggest that measuring BMC with DXA is a useful and precise way to estimate whole-body calcium content.
Fat content determination can be validated by analysis of whole carcass fat. Chan (29) analyzed small chicken parts with a Norland system (Norland Corporation, Fort Atkinson, WI) and observed that the weight of fat dissected from chicken parts was highly correlated with the fat content determined by DXA (r = 0.98); the slope of the regression line was 0.95. Svendsen
et al (9) performed fat extraction in samples of homogenized whole carcasses from pigs weighing 35–95 kg and showed that fat measured by DXA was highly correlated with chemical fat ($r = 0.99$). The slope of the regression line was 0.90. In large piglets (6 kg), Brunton et al (12), using preliminary whole-body software, observed a significant correlation between DXA fat and chemical fat ($r = 0.83$); the slope was 0.87. In small piglets (1.6 kg), there was no significant correlation between fat measured by DXA and chemically ($r = 0.06$). When comparing DXA fat with chemical fat content, Brunton et al (12) concluded that DXA overestimated fat content by 35% in large piglets and by 250% in small piglets.

In our study, 30 DXA measurements in 13 piglets were used to evaluate the determination of fat content with the new infant whole-body program. The range of fat content measured was wide (30–2116 g) and there was a significant correlation between fat content estimated by DXA and that measured chemically (Figure 1D). The difference between the slope of the regression line and identity was positive (1.11 – 1), which represented an overestimation of $\approx 11\%$. The precision of the fat estimate by DXA was low in the 30 measurements because of the great difference observed between DXA and chemical measurements in the leanest two piglets (30 and 80 g chemical fat content). When fat content was $> 250$ g, precision was much better (8.85%; Figure 4). In addition, our study showed that fat determination by DXA was robust with respect to the hydration factor of lean body mass. A decrease of the hydration factor from 83.3% to 78% induced only an increase in fat percentage of 0.3%. These results represent an improvement in fat content assessment by DXA compared with results obtained with the pediatric whole-body program, but further refinements are necessary in small subjects with fat contents $< 250$ g.

In a 3500-g human term infant, reference values (13–17, 26, 27) for fat and calcium content were estimated to be 500 (14.2% of body wt) and 28.5 g, respectively. Because these studies were performed in live-born and stillborn infants, new indirect methods of evaluating the fat compartment in vivo were developed (30). Fat mass was calculated as the difference between body weight and fat-free mass (FFM). FFM was measured by total-body electrical conductivity (31–33) or by total-body water content [bioelectrical impedance analysis (34), deuterium (35), or doubly labeled water method (33)]. Unfortunately, the precision of individual fat estimates by these indirect methods is relatively poor. Indeed, in a 3500-g term infant, an error of 5% for FFM (3000 g) corresponds to 150 g. This error of 150 g amounts to an error of 30% in the calculated fat mass of 500 g (body weight – FFM). Estimation of FFM from total body water measurements [bioelectrical impedance analysis, deuterium, and oxygen-18] assumes a theoretical and fixed hydration factor for FFM, which is not appropriate in neonates and can lead to an underestimation of fat mass (33, 36) and therefore to additional error. In contrast, the use of DXA appears to be more appropriate for the determination of fat content. Fat mass is directly evaluated by DXA with relatively high precision, $\pm 8.85\%$ in our piglet study, and the estimation of fat mass by DXA is very robust with respect to the hydration factor of FFM.

The first application of the DXA method in infants was published recently by Venkataraman and Ahluwalia (23) in a group of term neonates (body weight: 3458 $\pm$ 91 g); they reported a mean BMC of 80.05 g and a mean fat content of 685 g, which corresponded to 18% of body weight. In our study, the average fat content in term infants was 15.4%, which was corrected to 14.1% when we applied the conversion formula calculated in piglets. The application of the conversion formula calculated for BMC made possible the estimation of the whole-body calcium content in newborns: BMC was $54 \pm 6$ g, which corresponded to 26.4 g calcium content. These values of fat and BMC contents were lower than those observed by Venkataraman and Ahluwalia (23) but were in the range determined previously by cadaver analysis (13–17, 26, 27). Therefore, conversion formulas determined in piglets were useful for evaluating DXA precision in piglets, for estimating calcium content in term human newborns, and for correcting the overestimation of fat content by DXA.

In conclusion, our data in piglets and our measurements in term human neonates by use of conversion formulas suggest that DXA is an accurate method for evaluating calcium and fat contents in human neonates. However, further refinements of the infant whole-body software (Hologic Inc, version 5.64, 1993) are necessary, especially for improving fat determination in small preterm infants. DXA technology is noninvasive and safe for use in newborns; it may become the standard for body composition analysis because other methods are not feasible (hydrodensitometry), not reliable (skinfold-thickness measurement) (37), or not precise enough (bioelectrical impedance analysis, total-body electrical conductivity, and stable isotope dilution) in human newborn infants.

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