Dual-energy X-ray absorptiometry: analysis of pediatric fat estimate errors due to tissue hydration effects

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There is increasing interest in measuring body compartments, particularly fat mass, in infants and young children (11). Although cross-sectional studies are valuable, a key focus is monitoring body composition changes over time (30). Evaluating body composition in infants and young children, particularly with growth and development, places constraints on compartment measurement methodology (9). The most important development over recent years is the introduction of dual-energy X-ray absorptiometry (DXA), which is a relatively practical method of quantifying body fat in pediatric subjects (11, 30).

DXA can be considered a “two-compartment” method that separates “pixel” mass into either soft tissue and bone mineral in pixels with bone or into lean and fat soft tissues in pixels without bone (23, 24). Two X-ray photon energy peaks generated by the DXA system X-ray source and filter penetrate tissues to varying degrees (18). Relative photon attenuation, expressed as the ratio of low to high energy (R value), is a function of tissue elemental content (19). Fat, lean soft tissue, and bone mineral body components have relatively high contents of hydrogen, oxygen, and calcium, respectively. In concept, most DXA systems are based on assumed specific constant R values for the three body components. The two body components found in any pixel can be separated when the pixel’s R value is known. When soft tissue overlies bone, the soft tissue R value is estimated by using interpolation and other mathematical procedures (20).

R values for fat and bone mineral can be considered highly stable, because these components are composed primarily of two molecular level species, triglycerides and calcium hydroxyapatite, respectively. On the other hand, lean soft tissue consists of at least three molecular-level components: water, protein, and soft tissue mineral. Stability of the lean soft tissue R value depends, at least in part, on the constancy of constituent water, protein, and soft tissue mineral proportions. Any change in these proportions that leads to an actual change in the lean soft tissue R value will lead to errors in DXA fat estimation (24).

An important concern is that the high and rapidly changing tissue hydration of infants and young children may render DXA inaccurate as a means of quantifying body fat (6), particularly in longitudinal studies. According to this hypothesis, developmental changes in fat-free mass hydration alter the R value of lean soft tissue, leading to inaccurate soft tissue partitioning. Alternatively, special age-specific calibrations may be needed for DXA studies of young subjects. For example, skeletal muscle has a water content of ~90% in infancy and declines to the adult value of ~80% by age 20–30 yr (4, 8, 27). The hydration of fat-free mass is highest at birth, ~81% (8), and declines thereafter to the adult
value of $\sim$73\% (24). A number of studies, carried out in both humans and animals, raise broad concerns regarding the accuracy of DXA body composition measurements in infants and young children (2, 3, 5, 6, 16, 22, 26, 31).

The aim of the present study was to provide a comprehensive analysis of lean soft tissue hydration and other maturational effects on DXA percent (%)/fat estimates as they might apply in the pediatric population.

PROTOCOL AND THEORETICAL CONSIDERATIONS

The specific aim of the study was to quantify the magnitude of error arising in DXA %fat estimates with tissue hydrations typical of those observed in infants and young children. It was assumed that current DXA systems are now calibrated for adult human tissues.

The study included two linked phases, the first providing the foundation for the second (Fig. 1). The first phase was experimental, consisting of a series of three linked in vitro studies. The primary aim of phase 1 was to establish experimentally the physical basis of DXA fat-estimation models. Once validated, these models then allowed us to formulate a series of theoretical calculations in phase 2, leading to an estimate of %fat errors emanating from previously reported soft tissue calculations in newborns, infants, and children. This two-phase approach permitted a high-resolution analysis of small-magnitude pediatric DXA %fat errors, which would be extremely difficult to demonstrate in vivo or in phantoms.

Phase 1

The first experiment established optimum phantom thickness for carrying out later phase 1 studies. The physical background for this experiment was as follows. A photon beam with incident intensity $I_0$ passing across tissues is attenuated and lowered beam intensity (I) is recorded (21). Fractional change (i.e., $\Delta$ or d) of photon intensity is proportional to the tissue’s linear attenuation coefficient ($\mu$) and path length change (dL):

$$-d(I/I_0) = \mu \times dL,$$

and thus

$$I = I_0 \times e^{-\mu \times L} \quad (1)$$

In studying tissues that differ in physical density ($\rho$), the mass attenuation coefficient ($\mu/\rho$) is substituted for the linear attenuation coefficient (28). Photon attenuation of a multielemental substance can then be calculated as

$$I = I_0 \times e^{-\sum_i f_i \times \mu_{mi} \times M_i} \quad (2)$$

where $f_i$ is mass fraction of the ith component as heterogeneous absorber, $I$ is transmitted photon intensity, $I_0$ is initial photon intensity, $M$ is absorber mass, and $\mu_{mi}$ is mass attenuation coefficient for object or material i.

Equation 2 can be rearranged so that attenuation is expressed as the measurable transmitted-to-incident photon ratio

$$\ln(I/I_0) = \Sigma(-f_i \times \mu_{mi} \times M) \quad (3)$$

Two photon energies are used with DXA systems, and the attenuation of each beam (i.e., $I_{0L}$) by soft tissues can be measured as the ratio (R) of low (L) to high (H) energy, calculated as

$$R = \ln(I/I_0)_L/\ln(I/I_0)_H \quad (4)$$

$$= \Sigma(-f_i \times \mu_{mi} \times M)_L/\Sigma(-f_i \times \mu_{mi} \times M)_H$$

$$= \Sigma/[f_i \times (\mu_{mi})_L]/\Sigma/[f_i \times (\mu_{mi})_H] \quad (5)$$

Although, in theory, R values at any two given energies should be constant, beam hardening occurs with the polenergetic DXA X-ray source, and path length (i.e., tissue thickness) R value dependency is recognized (10, 15, 20). Beam hardening occurs when polychromatic X-ray photons pass through tissues and low-energy components are attenuated more rapidly than are higher energy components (28). The beam “hardens” as the low-energy photons are selectively removed and mean photon energy increases. Commercial DXA systems adjust for beam-hardening and tissue-thickness effects.

The first experiment was designed to evaluate the relationship between R value and phantom thickness with the aim of selecting a reference phantom thickness for subsequent experiments. A 4-cm-diameter plastic tube was sealed with parafilm at one end. The tube was placed upright, with the sealed end at the bottom, and then filled with varying levels of distilled water or corn oil. The DXA system laser was used to center the tube, and scans were then made at each level of water or oil. The mean intensity (I) at each photon energy was calculated from three 60-s successive readings. The tube was also scanned empty, thus establishing $I_0$. Relative attenuation at each water/corn oil level and energy was calculated as $I/I_0$. The R value at each level was also calculated by using Eq. 4.
DXA systems are usually calibrated against two-component standard phantoms representing fat and lean soft tissue. The basis for this approach is as follows. For a mixture of n components, the mass attenuation coefficient for heterogeneous mixtures can be calculated from fractional mass and mass attenuation coefficient of components present as

\[ \mu_m = \sum (f_i \times \mu_{mi}) \]  

(6)

Respective R values for elemental and complex absorbers for DXA systems producing photon beam energies L and H can be calculated using Eq. 6 and known mass attenuation coefficients. For a two-component (1 and 2) mixture, a simplified R value formula can be derived, which assumes that \( \mu_m \) values for the two components at energy H are approximately equal

\[ R_T = f_1 \times R_1 + f_2 \times R_2 \]  

(7)

and

\[ f_i = (R_T - R_2)/(R_1 - R_2) \]  

(8)

where \( R_T \) is total R value for the component mixture.

The second phase 1 experiment was designed to confirm the linear correlation previously reported between the measured R value (i.e., \( R_T \) in Eq. 8) and the mass fraction of lipid (i.e., \( f_1 \)) in a two-component lipid-lean system. The tube level established in experiment 1 was set as 100% total mass. Varying fractions of corn oil and corn oil (i.e., lipid) were created by weighing amounts of each while always maintaining the total fluid level constant. The electrolyte solution present in Ringer lactate (i.e., lean) and corn oil (i.e., lipid) were commercially prepared and ingredient amounts were provided by the manufacturer (McGaw, Kendall, Ontario, Canada). The 150 mM KCl solution was prepared using distilled water and crystalline KCl. Commercial corn oil (Hunt Wesson Foods, Fullerton, CA) was used in undiluted form. These phantoms were all scanned at the tube level established in experiment 1, and the mean of three readings, after adjustment for empty tube readings, was used to calculate respective R values.

Fresh lean beef and fatback were obtained locally and minced, and six varying mixtures of the two were prepared to vary the percentage fat of the phantom. The lean beef and fatback were then analyzed, using conventional extraction measures (7, 24), for total fat content. The measured R values were plotted against %fat, and R values extrapolated to 0% and 100% were taken as “pure” lean beef (protein) and “pure” fatback (fat) R values, respectively.

Corresponding theoretical R values were calculated from the elemental proportion of each phantom component (Table 1). Corn oil and beef fat R values were calculated by assuming the composition of a reference lipid (C55H102O6), and only small differences in calculated R appear when lipid saturation is varied (23). The pure beef R value was calculated by assuming the composition reported by Pietrobelli et al. (23, 24).

A critical assumption of our phase 2 modeling efforts is that theoretical R values calculated for complex chemical mixtures, such as ECF, are equivalent to those actually measured experimentally and in vivo.

In the third experiment, we compared theoretically derived and actually measured R values for a series of liquid and semisolid mixtures, including Ringer lactate, normal saline (0.9% NaCl), 150 mM KCl, 5% dextrose in distilled water, corn oil, Crisco (i.e., semisolid vegetable fat), and beef fat and lean. Ringer lactate, normal saline, and 5% dextrose were commercially prepared and ingredient amounts were provided by the manufacturer (McGaw, Kendall, Ontario, Canada). The 150 mM KCl solution was prepared using distilled water and crystalline KCl. Commercial corn oil (Hunt Wesson Foods, Fullerton, CA) was used in undiluted form. These phantoms were all scanned at the tube level established in experiment 1, and the mean of three readings, after adjustment for empty tube readings, was used to calculate respective R values.

Table 1. Mass attenuation coefficients at 40 keV and 80 keV and calculated theoretical R values for 13 elements and major molecular level components found in humans

<table>
<thead>
<tr>
<th>Element/Compound</th>
<th>Atomic Number</th>
<th>Atomic/Molecular Weight</th>
<th>( \mu_m ) 40 keV</th>
<th>( \mu_m ) 80 keV</th>
<th>( R_T )</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>1</td>
<td>1.008</td>
<td>0.3458</td>
<td>0.3091</td>
<td>1.1187</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>12</td>
<td>0.2047</td>
<td>0.1609</td>
<td>1.272</td>
</tr>
<tr>
<td>N</td>
<td>7</td>
<td>14</td>
<td>0.2246</td>
<td>0.0658</td>
<td>1.371</td>
</tr>
<tr>
<td>O</td>
<td>8</td>
<td>16</td>
<td>0.2533</td>
<td>0.1677</td>
<td>1.510</td>
</tr>
<tr>
<td>Na</td>
<td>11</td>
<td>23</td>
<td>0.3851</td>
<td>0.1793</td>
<td>2.1478</td>
</tr>
<tr>
<td>Mg</td>
<td>12</td>
<td>24.3</td>
<td>0.4704</td>
<td>0.3235</td>
<td>4.5783</td>
</tr>
<tr>
<td>P</td>
<td>15</td>
<td>31</td>
<td>0.7784</td>
<td>0.2684</td>
<td>4.9836</td>
</tr>
<tr>
<td>S</td>
<td>16</td>
<td>32.1</td>
<td>0.9507</td>
<td>0.1367</td>
<td>4.9271</td>
</tr>
<tr>
<td>Cl</td>
<td>17</td>
<td>35.5</td>
<td>1.100</td>
<td>0.1946</td>
<td>2.417</td>
</tr>
<tr>
<td>K</td>
<td>19</td>
<td>39.1</td>
<td>1.484</td>
<td>0.2315</td>
<td>3.3624</td>
</tr>
<tr>
<td>Ca</td>
<td>20</td>
<td>40.1</td>
<td>1.792</td>
<td>0.2576</td>
<td>3.6906</td>
</tr>
<tr>
<td>Fe</td>
<td>26</td>
<td>56</td>
<td>3.601</td>
<td>0.5918</td>
<td>6.0848</td>
</tr>
<tr>
<td>H2O</td>
<td>18.016</td>
<td>0.3150</td>
<td>0.2651</td>
<td>1.46109</td>
<td></td>
</tr>
<tr>
<td>Protein*</td>
<td>2,258.7</td>
<td>0.2835</td>
<td>0.2283</td>
<td>1.3678</td>
<td></td>
</tr>
<tr>
<td>Fat†</td>
<td>860.8</td>
<td>0.2954</td>
<td>0.2546</td>
<td>1.2798</td>
<td></td>
</tr>
<tr>
<td>Glycogen‡</td>
<td></td>
<td>0.2835</td>
<td>0.2381</td>
<td>1.38007</td>
<td></td>
</tr>
</tbody>
</table>

*Protein, C100H159N26O22S0.7; †Fat, C55H102O6; ‡Glycogen, (C6H10O5)n.
The DXA system-measured R values were then empirically calibrated by scanning three primary fluids (distilled water, methanol, and ethanol) and then establishing the relationships between measured and theoretical R values. Empirical adjustments are required because the two main effective photon energies provided by the polyenergetic X-ray source and filter may differ slightly from those assumed in deriving the theoretical R values. The resulting regression formula was used to calculate an adjusted measured R value for each of the phantom test substances. Theoretical and measured R values for the eight test substances were then compared by using Eq. 5.

Phase 2

The elemental composition of intracellular fluid (ICF), ECF, protein, and glycogen were obtained from previous studies: the elemental composition of fluid compartments was based on the analysis by Maffy (17). Protein and glycogen elemental content are based on literature reference standards (Table 1). The theoretical R values of these components were then calculated. Two data sets for reference human values were used, one for boys and girls reported by Fomon et al. (8) and the other a summary of literature on human tissue and organ composition (4).

The elemental composition values for reference male and female children reported by Fomon provided similar results, and only male values are reported for convenience because adult reference male values were also available for comparative purposes (27). The collected body composition data (i.e., ICF, ECF, protein, and glycogen) as a function of age were then used to calculate the corresponding theoretical lean soft tissue R values by assuming that lean soft tissue is represented by the sum of these four components. The elemental composition and R value for fat (Table 1) was assumed constant and independent of age.

Statistical Methods

All results are presented as means ± SD. Analyses were carried out by using the statistical program SAS Release 7.0 (Statistical Analysis System, SAS Institute, Cary, NC). The developed hydration model is based on the assumption that R values follow physical rules related to photon attenuation and biological substance component proportions. The conceptual basis of these models was validated by comparison of theoretically derived to actually measured DXA R values. Simple linear regression analysis and means ± SD were used as the basis of these analyses.

Simple linear regression analysis was used to develop a DXA fat fraction prediction formula based on R values as the independent variable. Two of the samples were the lean beef and lard. Lean beef and lard were then thoroughly mixed to form three additional mixtures of varying fat fraction. Fat was measured by the method of Folch et al. (7). Homogenized sample and methanol were added to an Erlenmeyer flask. The sample was stirred, chloroform was added at 30 min, and the mixture was set aside overnight. The homogenate mixture next was filtered, and the flask was rinsed with 2:1 chloroform-methanol. The volume of liquid in the tubes was then compared with a preprepared capped tube of known volume. The volume of sample was then made up with 2:1 chloroform-methanol, and 0.88% KCl solution was added as the wash. The liquid mixture was homogenized, and samples were then centrifuged. The top layer was aspirated, and the tubes were placed in a heated water bath until volume decreased to ~4 ml. Tubes were then dried in an oven for several hours until only an oily lipid layer remained. Tubes were then cooled, and fat weight per sample was obtained by taking the difference between the conical tube weight with and without fat. Triplicate values of total fat weight per gram of homogenate were averaged.
The corresponding measured R values as a function of phantom thickness are presented for water and corn oil in Fig. 3. The two developed functions were similar, with a steep R value rise at lower phantom thickness and a relatively stable plateau reached at ~10 cm and continuing to 20 cm. On the basis of these observations, we chose a phantom thickness at the plateau midpoint of 15 cm for all subsequent experiments.

Experiment 2: Relative attenuation of a two-component mixture. The relationship between measured R value and fraction of a Ringer lactate-corn oil solution as oil is presented in Fig. 4. There was an inverse linear correlation between R value and oil fraction \( R = 0.0838 \times (\text{oil fraction}) + 1.223; R = 0.9934; P < 0.005 \). The developed function predicts an R value of 1.223 and 1.140 at oil fractions of 0 and 100%, respectively. This experimental phase supports the two-compartment soft tissue (i.e., lean and lipid) DXA model, as defined by Eq. 7. That is, %oil of a lean-oil phantom is a predictable and linear function of the measured R value.

Experiment 3: Comparison of measured and theoretical R values. The calibration of R values with water and alcohol compounds (methanol and ethanol) produced the following relationship: theoretical \( R = 3.363 \times (\text{measured R}) - 2.54 \) \( r = 0.99, \text{SEE} = 0.002, P < 0.0001 \). This formula was then used to adjust measured R values.

The adjusted R values of components mimicking fat (i.e., corn oil, Crisco, animal fat), glycogen (i.e., 5% dextrose), and lean (i.e., Ringer lactate, 150 mM KCl, 0.9% NaCl) are plotted in Fig. 5 against their respective theoretical counterparts. The measured R values for fat and lean were 1.136 and 1.196, extrapolated from beef phantoms \( R = -0.060 \times (\text{fat fraction}) + 1.196, r = 0.96, P < 0.001 \). Adjusted fat and lean R values were 1.280 and 1.482, respectively. The adjusted measured R values were highly correlated with the calculated theoretical values \( r = 0.97; P < 0.0001 \), and there was no significant difference between the measured \( 1.42 \pm 0.11 \) and theoretical \( 1.41 \pm 0.11 \) R values. Bland-Altman analysis (1) failed to disclose a significant correlation between R value difference and R value mean.

This experimental phase strongly supports the underlying DXA theoretical foundation: pixel R value is a predictable function of tissue elemental components. Mixtures of two components, as in experiment 2, have R values consistent with the individual component R values. These assumptions provide the fundamental background for phase 2 modeling experiments.
Phase 2

Experiment 1: Lean soft tissue R value as a function of age. The reference body composition for boys varying in age from birth to 120 mo was used to calculate theoretical R values on the basis of reported protein, glycogen, ECF, and ICF. Lean soft tissue R values as a function of age are plotted in Fig. 6: R values were maximal at birth and declined rapidly within a few months; R values continued to decline gradually thereafter. The R value changes with greater age correspond to a small relative decrease in total water fraction and rise in protein fraction (Fig. 7). The lean R value for reference man, 1.452, is similar to that of the older reference boys.

Experiment 2: Modeling %fat error when DXA is applied to subjects varying in age. The developed lean soft tissue R values were used in the %fat error analysis. The R value for animal fat, 1.2798, was assumed independent of age and constant. We used a single R value, 1.42 or ~20%fat, to make all of the error calculations (same at any fat level). The resulting errors in DXA %fat estimates as a function of age are plotted in Fig. 8. The maximum “error” of ~0.8% was at birth with a rapid decline thereafter to ~0.25% by 120 mo of age. Hence the maximum modeled error was <1% with near-zero error by about age 60 mo or 5 yr.

A similar pattern of results, with some exceptions, was observed for specific lean tissue analyses, with maximum error (~1.5%) in newborns and declining to <1% in 4- to 7-mo-old infants.

DISCUSSION

The present study results support the hypothesis that lean soft tissue R values are not constant in children but instead decrease with greater age. The most pronounced changes appear to be early in life and are secondary to shifts in the composition of tissues as protein and water. The result of these age-related soft tissue changes is DXA %fat estimation errors, assuming that systems are calibrated to a single adult lean standard.

Although errors in DXA %fat estimates are predicted by our models, the magnitude of these effects is extremely small and typically <1%. Predicted errors were maximal in newborns and decreased to <1% in infants and young children. Hence, for practical purposes, it would appear unlikely that there exists a need for special pediatric soft tissue calibrations for systems used on a wide age range of subjects. Moreover, there exist other pediatric calibration issues such as body size, shape, thickness, and skeletal characteristics that may be more important than the soft tissue hydration effects detected in this investigation (19, 20). However, we did confirm in a rigorously controlled series of experiments and developed models that DXA %fat estimates at the two applied energies are not free from influence by hydration changes characteristic of early childhood growth and development.

Our approach was to examine hydration effects using both experimental and theoretical analyses. It is also possible to develop experimental phantoms with varying hydration levels to directly evaluate hydration effects (24). However, as observed in the present study, these effects are exceedingly small and, in some cases, within the range of system and experimental error. This may be why some investigators report no apparent DXA errors on the basis of in vivo studies in which small changes in total body fluid are produced experimentally or through disease processes (12, 21, 29). Our combined experimental and theoretical approach provided a higher resolution analysis of hydration effects,
particularly the interesting temporal trends that appear during early life. The main observed cause of DXA %fat error relative to adult calibrations was a fall in the proportion of lean tissue as fluid and a corresponding increase in the proportion of protein. Fluids have higher R values than does protein, and the result is a gradual decline in lean soft tissue R value with greater age.

Our modeling strategy was based on the R value concept originally advanced by Mazess et al. (18). This concept is founded on well-established physical concepts for monoenergetic photons produced by radionuclide sources. A practical concern, and one demonstrated in the present study, is that polyenergetic photons produced by filtering an X-ray source are vulnerable to beam hardening (25, 32). The result is that measured R values can vary and deviate from theoretical estimates depending on several factors, including, as seen in phase 1 of this study, phantom and tissue thickness. Manufacturers may be moving to non-R-value approaches for composition analysis, although, conceptually, DXA operational principles must rely on concepts similar to those advanced in this report. Hence, our error analyses likely apply to whatever %fat estimation method is applied.

Our study was formulated on a combination of in vitro and theoretical experiments. We specifically chose this approach for practical reasons: the variables of interest could be systematically changed giving us good control over experimental conditions; the anticipated %fat errors were very small and potentially confounded by commonly encountered factors such as subject nutrition and hydration status; and in vitro experimentation avoids any radiation exposure to very young subjects. We found that experiments with the simple tubular phantom and stationary X-ray tube detector were far easier to conduct than were studies with the larger phantoms and more complex scanning configurations used in our previous experiments (24).

The present study presents a simple experimental paradigm for evaluating DXA concepts in vitro. Our findings clearly demonstrate the close association between theoretical DXA concepts and their experimentally derived counterparts. These strong associations provide confidence for the validity of theoretical hydration models applied to human reference whole body and isolated tissue data. Our findings support the hypothesis that errors in DXA %fat estimates arise if systems are calibrated using an adult lean soft tissue phantom. However, the modeled errors were extremely small in magnitude, generally <1%. Hence, it would appear unlikely that special calibration phantoms are needed when DXA systems are used in subject populations across the entire lifespan. These phantom considerations, however, may be important when systems are dedicated to pediatric or young animal use.

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