Introduction

Accurate assessment and tracking of infant body composition is useful in evaluation of the amount and quality of weight gain, which can provide key information in both clinical and research settings. Body composition analysis (BCA) can be used to monitor and evaluate infant growth patterns, efficacy of nutritional and medical interventions, progression of chronic disease, and recovery from malnutrition [1-5].

The most recognized reference BCA method for animals is chemical analysis however its major drawback is the necessity to sacrifice the animal. A large variety of non-destructive BCA methods have been developed, each with its advantages and shortcomings, such as air displacement plethysmography (ADP) [6], bioelectrical impedance analysis (BIA) [7], dual energy X-ray absorptiometry (DXA or DEXA) [8], total-body electrical conductivity (TOBEC) [9], total body potassium (TBK) [10], isotope dilution [11], skinfold thickness (SFT) measurements [12], multi-compartment models [13], computed tomography (CT) [14], magnetic resonance imaging (MRI) [15], magnetic resonance spectroscopy (MRS) [16], and magnetic resonance T2 relaxometry (MRR or T2-MRR) [17].

Common shortcomings include complexity and time needed to obtain results (multi-component models, TBK, isotope dilution, MRI), understanding of full relations between measured quantities and body components (BIA, TOBEC, SFT), small but relevant for multiple repetitions radiation exposure (DXA), and potential vulnerability to oversimplification (ADP, SFT).

A study was conducted to appraise a new EchoMRI™ device for body composition analysis (BCA) of infants and to compare it with dual energy X-ray absorptiometry (DXA), using chemical analysis as a reference method.

The calibration part of the study included cross-validation comparisons between EchoMRI™ measurements of awake, anesthetized and dead piglets of the calibration set. It also included comparison of two different approaches to refining the calibration of EchoMRI™, by low- or by high-dimensional linear regressions. Only the low-dimensional approach was applied to DXA.

The validation part yielded EchoMRI™ accuracy of 27 g and 70 g for fat and total water, respectively, on piglets scanned while anesthetized, as compared with 24 g and 57 g, respectively, for DXA.

EchoMRI™ precision was found to be 4 g and 7 g for fat and total water, respectively, for anesthetized piglets, as compared to 16 g and 14 g, respectively, for DXA. The differences between fat measurements of awake, anesthetized and dead piglets can be statistically significant, but are comparable in magnitude to random errors.

To summarize: Characterization of random errors by CV, especially that of fat, is not suitable for BCA, whereas absolute errors or errors relative to total body weight can be applicable. Low- and high-dimensional regressions offer nearly the same accuracy improvements. Improved DXA and EchoMRI™ offer nearly the same accuracy, within 1% of weight in fat, while EchoMRI™ has better precision, within 0.2% of weight in fat for anesthetized and dead piglets as compared to DXA’s 0.5-0.6%.

Keywords: Body composition, infant, pig, QMR, DXA, chemical analysis, accuracy, precision, body fat, body lean, total body water

*Mention of a trade name does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may be suitable.
EchoMRI™ devices stand out in that they are fast and very easy to use without the need for sedation or anesthesia and free of radiation, body invasion, or other health issues, while capable of unsurpassed precision and high accuracy [18-23]. Typical scan times range from less than one minute to less than four minutes in different specific devices and applications, and less than half hour training is sufficient for a typical user.

EchoMRI™ devices employ a nuclear magnetic resonance method for measuring the masses of fat, lean tissues, free water, (not bound in various tissues, usually mostly urine), and total water (also known as total body water, TBW, contained in all the liquids and in a bound state in tissues) in a whole body of a live animal including human. It is also known as quantitative magnetic resonance (QMR) [18]. QMR differs from MRI [24] in that the signal processed is obtained from the whole body at once, it differs from T1-MRI in that both T1 and T2 are engaged, and it differs from MRS in that the time domain signal (rather than spectrum) is processed directly.

The essence of the QMR method is that a scan produces a record of nuclear magnetic resonance responses (NMR echoes) to a sequence of radio pulses. The sequence is composed of several periodic Carr-Purcell-Meiboom-Gill parts (CPMG segments) [25] separated by pauses of different durations. The lengths of the periodic parts and the durations of the pauses are designed to catch all the relevant characteristic (relaxation) time scales of the NMR responses (transverse, “T2”, and longitudinal, “T1”, relaxation) typical for fat, lean and free water. The signal from a body is a linear combination of fat, lean, and free water contributions, and the differences between the relaxation rates of the three basic substances make it possible to use linear regressions to calculate the amounts of fat, lean tissues, and free water. These regressions are found empirically from measurements of phantoms made of canola oil to represent fat, chicken breast (small animals) or lean pork (larger animals) to represent lean, and tap water to represent free water. The algorithm for optimizing these regressions is a variant of multivariate calibration (common in chemometric analysis) employing partial least squares optimization combined with principal component analysis for high-dimensional regressions [26].

Total water is calculated from the same records but in a different way, using the fact that at the time scales employed by QMR the contribution of protons tied in proteins and other substantially “solid” materials is negligible. Therefore the “lean” signal comes essentially from water bound within the lean tissues. There is also a substantial contribution from protons in fat molecules. The difference between an estimate of the total amount of protons participating in the record and an estimate of fat found by the regression analysis yields an estimate of the amount of total water included in lean and in free water together.

Success in using EchoMRI™ in animals of different sizes (flies, mice, rats, birds, dogs, pigs, and adult humans) has been reported [18-23] and now we address its potential for infants, using piglets as a generally accepted research model for infant body composition [27, 28]. There have been many studies of pig BCA [27-29], in particular some of them comparing DXA with chemical analysis [28, 29] and some comparing DXA with tissue dissection [27]. Here we calibrate and validate both DXA and EchoMRI™ using chemical analysis as the reference BCA method.

In comparative studies, EchoMRI™ was found to have tighter repeatability (precision) than all the other methods (usually including DXA) considered in these studies [18-23]. The ability of EchoMRI™ to detect precisely small longitudinal differences and monitor changes in body composition is particularly valuable.

DXA and CT can sometimes achieve repeatability comparable to that of EchoMRI™ however these BCA methods require immobilization and X-ray irradiation, both undesirable in applications to infants. As DXA is the closest BCA method to QMR in terms of precision, speed, and convenience of use, a validation study of QMR usually includes some comparison with DXA [18-23]. We follow this tradition, and in addition examine also the corrections by regression analysis which are usually needed for DXA and can be used in the same way for QMR.

Subjects and methods

Subjects

Twenty-five piglets ranging in weight between 1720 g and 4070 g were used for calibration during the period between 2008.01.11 and 2008.02.27. Then a series of validation measurements were performed on another set of twenty-five piglets ranging in weight between 2000 g and 4300 g during the period between 2008.07.17 and 2008.09.18.

Experimental animal protocols used in this study were approved by the Beltsville Area Institutional Animal Care and Use Committee.

Calculations and statistics

Statistics were addressed using Matlab Statistics Toolbox and propriety Matlab scripts.

Body composition analysis methods

EchoMRI™ – Infants device based on a permanent magnet with constant field of ≈ 0.021T (Larmor frequency ≈ 880 kHz) was designed for live subjects in mass range from 1 kg to 4 kg. According to CDC weight charts for 50th percentile [http://www.cdc.gov/growthcharts/clinical_charts.htm], this range roughly corresponds to the age range of human infants from those born premature to normal infants of 2 weeks age. The device was provisionally calibrated on traditional phantoms representing fat, lean, and free water, namely canola oil, pork loins with a known fat content (measured on smaller samples in a different but previously validated
Chemical (carcass) analysis (CA) – For CA the entire body was homogenized then samples analyzed for total water content and fat identified with lipid content. Lipid content was measured by chloroform/methanol extraction [30] and water content was measured by lyophilization. The quantities measured by CA are fat and total water. Particular details are the following:

Carcass preparation: Pig carcasses were autoclaved for 2 hr at 121 °C, cooled to 3 °C, and then homogenized for 1 minute using a food processor (Robot Coupe, Model R10). Samples were stored at -15 °C prior to analysis.

Water analysis: A single sample from each pig was weighed (sample size was approximately 400 g), frozen, and then lyophilized in a freeze dryer (Virtis, Model 100 SRC-6) for 14 days. The samples were weighed again immediately after removing from freeze dryer and the weight difference between the two weightings was assumed to be due to water loss.

Lipid analysis: Quadruplicate 3 to 5 g samples of the wet homogenate were extracted for lipid analysis by the method of chloroform/methanol extraction [30]. Each sample was extracted for 24 hr in a 125 ml separatory funnel containing 60 ml of chloroform:methanol (2:1, v/v). After 24 hr, 12 ml of 0.88% potassium chloride in water was added and then mixed by shaking for 10 sec. The sample was allowed to set for another 24 hr to permit phase separation. The lower phase was then drained into pre-weighted vials and the solvent evaporated off at 70°C under a stream of nitrogen in a sample concentrator [Sybron SC248 Sample Concentrator, Brinkman Instruments (Canada) LTD]. The vials were allowed to cool and then reweighed to determine the amount of lipid extracted.

Ash analysis: Triplicate samples (approximately 2 g, each) of the freeze dried sample were weighed into pre-weighed vials then placed in a muffle furnace. The samples were allowed to combust for 10 hr at 520 °C. The cooled vial was reweighed to determine ash content.

Refined calibration methods

Low-dimensional linear regressions – Systematic errors (accuracy) can be affected by various conditions of evaluation experiments, if for instance different reference BCA methods are used for calibration and validation, or if different kinds of test subjects are used which may have some differences in actual chemical compositions. A practical approach to bridge such differences is to employ low-dimensional linear regressions [22, 23, 31-34] which reinterpret the already interpreted quantities so as to get unbiased fits to the desired reference method for the desired class of subjects. This approach has the advantage that any researcher can do it independently of the manufacturer, and it is tested in this study for both EchoMRI™ and DXA.

Although one dimensional regressions are often used [22, 23, 33], for instance to predict chemical fat from measured fat, such regressions are not really applicable for BCA methods, because the errors in fat have contributions from both fat tissues and fat-free tissues, and some similar complexity would affect errors in any body component (unlike in measurements of fundamentally single-component quantities such as total weight). A reasonable model of systematic errors should include in its sources at least the dominant body component of the lean tissue mass [31, 32, 34]. In addition, if other components are available, dependent or independent of fat and lean but such that their determination involved means not used for fat and lean, as are the EchoMRI™ total water or DXA’s BMC, it may make sense to use these components for predictions as well.

To distinguish from high-dimensional regressions addressed further on, we designate these regressions as low-dimensional. We test a low-dimensional regression for EchoMRI™ defined as matrix \( L_e \) in linear prediction equation

\[
\begin{pmatrix}
\text{ChemFat} \\
\text{ChemTotalWater}
\end{pmatrix}
= L_e
\begin{pmatrix}
\text{FatEchoMRI} \\
\text{LeanEchoMRI} \\
\text{TotalWaterEchoMRI}
\end{pmatrix}
\]

obtained by minimizing the sum of squares of absolute errors in predicting CA fat and total water from EchoMRI™ fat, lean, and water. In the same way we test a low-dimensional regression for DXA defined as matrix \( L_x \) in linear prediction equation

\[
\begin{pmatrix}
\text{ChemFat} \\
\text{ChemTotalWater}
\end{pmatrix}
= L_x
\begin{pmatrix}
\text{FatDXA} \\
\text{LeanDXA} \\
\text{BMC}
\end{pmatrix}
\]

The linear fits (LE) and (LX) do not include the intercept (constant) also often employed when incomplete [22, 33] as well as complete [31] regressions are used. The reason for not including the intercepts is that the intercepts have meaning when the linear fits are applied to relations between quantities which are either substantially non-linear or approach zero substantially not simultaneously. EchoMRI™ devices do
have a possibility of some non-linearity caused by small inhomogeneity of the static magnetic field. However, these inhomogeneities are not prominent as is seen from the results reported below, and it is known that fat, lean, and total water are within the precision range from zero when the device is empty. Similar considerations apply to DXA. Under these circumstances the addition of an intercept would essentially be an addition of an extra parameter to improve the fit without physical justification for its existence.

A similar question can be asked of using the total water in equation (LE) or of the BMC in equation (LX). In this case however there are physical justifications, as for instance hydration is known to affect the NMR relaxation properties of lean and the measurement of the total water involves a part which is independent of the measurement of fat and lean.

**High-dimensional regressions** – Specific for EchoMRI™, the application of low-dimensional regression analysis on top of high-dimensional regressions is essentially a doubly repeated regression analysis, and so it is possible to do the same in a single act, that is to re-tune the initial high-dimensional regressions to obtain required predictors at least as good as or better than the low-dimensional regression predictors. Similar data was not available for DXA and so the re-tuning of high-dimensional regressions was done only for EchoMRI™.

A basic difference between low-dimensional and high-dimensional regression recalibrations is in that the input of the latter has, in principle, more information. Much of it may be irrelevant for the quantities measured, but in general there is always a possibility that it has some relevant information that disappears from the quantities measured using the provisional calibration.

**Characterization of errors**

**Accuracy** – As the corrective regressions are obtained in this study by minimizing mean-square-error (MSE) between the reference method (chemical analysis) and the predictions from the method being corrected (EchoMRI™ or DXA) averaged over the repetitions (5 and 3, respectively), the natural characterization of the goodness of fit would be the lack of fit (LOF) which is the difference between MSE and pure error [35]. The pure error can also serve as a measure of precision, and since it happens to be substantially smaller than MSE, the MSE can serve as a close upper bound approximation of LOF. In addition LOF is not formally applicable to characterize the errors before regressions are applied, whereas the point of interest is in comparing the errors before and after the corrections. For these reasons, the RMSE calculated after the measurements are averaged over repetitions is used in this study to characterize accuracy. Since no more fitting is done at validation, the accuracy of validation results can also be characterized by error as fraction of body weight (RMSE-as-FBW). In addition, of some interest can sometimes be the mean residuals from comparison between measured or predicted quantities and the reference chemical analysis values. Also of interest can sometimes be the mean residuals from comparison between measured or predicted quantities and the reference chemical analysis values. Also of interest can

**Precision** – Precision is often addressed in the literature by means of the coefficient of variation (CV). However the use of CV is questionable for BCA methods, for the same reasons as discussed above regarding one-dimensional regressions. For fat measurements in particular, if the measured subject affects the precision at all, the dominant source of errors is likely to be the lean tissues as in many types of animals the amount of fat is substantially smaller than the amount of lean. In addition, specifically for EchoMRI™ in cases of high static magnetic field homogeneity, the random errors which determine the precision may not depend on the subject at all.

An example of a diligent model of fat repeatability errors in fat (similar models may apply to lean or other components) can be

\[
\text{Err}_{\text{ra} \cdot \text{Fat}} = X_{\text{empty}} + X_{\text{fat}} \cdot \text{FatMass} + X_{\text{lean}} \cdot \text{LeanMass} \ (\text{ESEFLa})
\]

where the three coefficients \(X_{\text{empty}}, X_{\text{fat}}, X_{\text{lean}}\) are random variables, and ESEFL stands for “error sources empty, fat, and lean”. If the random variables are assumed to be normally distributed with zero mean, the full characterization of repeatability errors would require measuring the 6 components of the full covariance matrix between coefficients \(X_{\text{empty}}, X_{\text{fat}}, X_{\text{lean}}\), from the experiments and relation

\[
E[(\text{Err}_{\text{ra} \cdot \text{Fat}})^2] = E[X_{\text{empty}}^2] + E[X_{\text{fat}}^2] \cdot \text{FatMass}^2 + E[X_{\text{lean}}^2] \cdot \text{LeanMass}^2 + 2E(X_{\text{empty}} X_{\text{fat}}) \cdot \text{FatMass} + 2E(X_{\text{fat}} X_{\text{lean}}) \cdot \text{LeanMass} + 2E(X_{\text{empty}} X_{\text{lean}}) \cdot \text{FatMass} \cdot \text{LeanMass} \ (\text{ESEFLb})
\]

In comparison, the use of CV implies error model

\[
\text{Err}_{\text{ra} \cdot \text{Fat}} = X_{\text{ra}} \cdot \text{FatMass} \ (\text{ESF})
\]

and CV = SD(X_{\text{ra}}). A simple evidence for model (ESF) to be faulty is the dependence of CV on the percentage of body fat, %BF, and in fact it is so observed [22].

Measuring all the 6 unknown model parameters needed by model (ESEFL) can be unrealistic in a small study. Even for its simplified version

\[
\text{Err}_{\text{ra} \cdot \text{Fat}} = X_{\text{empty}} + X_{\text{body}} \cdot \text{BodyMass} \ (\text{ESEBa})
\]

where ESEB stands for “error sources empty and (total) body (mass)” and the respective relation

\[
E[(\text{Err}_{\text{ra} \cdot \text{Fat}})^2] = E[X_{\text{empty}}^2] + E[X_{\text{body}}^2] \cdot \text{BodyMass}^2 + 2E(X_{\text{empty}} X_{\text{body}}) \cdot \text{BodyMass} + 2E(X_{\text{empty}} X_{\text{body}}) \cdot \text{BodyMass} \ (\text{ESEBb})
\]

requires the measurement of only 3 parameters, the current study is too small and yields excessive uncer-
tainties for the three parameters, as is seen below in the results.

As a practical simplified approach, we describe precision by two alternative characterizations, the root-mean variance (RMV)

$$\text{RMV} = \{E[(\text{Err}_{\text{fat}})^2]\}^{\frac{1}{2}}$$

which in case of negligible $X_{\text{body}}$ approximates

$$\text{RMV} = \{E[(\text{Err}_{\text{empty}})^2]\}^{\frac{1}{2}}$$

and the root-mean variance as fraction of body weight (RMV-as-FBW)

$$\text{RMV-as-FBW} = \left( \frac{\text{Err}_{\text{fat}}}{\text{BodyMass}} \right)^{\frac{1}{2}}$$

which in case of negligible $X_{\text{empty}}$ would approximate

$$\text{RMV-as-FBW} = \{E[(X_{\text{body}})^2]\}^{\frac{1}{2}}$$

**Study design**

**Measurements** – All devices were installed at USDA Animal Bioscience and Biotechnology Laboratory, Beltsville, MD next to the baby pig nursery.

Each piglet was scanned by EchoMRI™ in 3 states: awake, anesthetized, and dead; scanned by DXA in 2 states: anesthetized and dead; and finally subjected to chemical analysis. Each EchoMRI™ scan was repeated 5 times and each DXA scan was repeated 3 times. When repeated, the mean of results of repetitions was taken as final. The calibration of both the EchoMRI and the DXA was checked each day immediately prior to scanning.

The sequence of measurements was as follows. Each pig was scanned 5 times awake (not sedated) on the EchoMRI, then sedated (asleep, anesthetized) and scanned 5 times by EchoMRI, the sedated pig was immediately scanned 3 times by DXA, the pig was then euthanized and immediately scanned 5 more times by EchoMRI, and finally, the dead pig was immediately scanned 3 more times by DXA. The time interval between EchoMRI and DXA scanning was approximately 2 minutes. The same routine was followed for all pigs.

The pigs were sedated using an intramuscular injection of a mixture of ketamine, Telazol (tiletamine + zolapam), and xylazine (5.0 mg ketamine, 0.8 mg tiletamine, 0.8 mg zolazepam and 3.3 mg xylazine per kg body weight). While still sedated the pigs were killed by an intracardiac injection of pentobarbital. Experimental protocols used in this study were approved by the Beltsville Area Institutional Animal Care and Use Committee.

Immediately after scanning the pig carcass was sealed in a plastic bag and frozen at -15°C until processed for chemical analysis.

The same user performed all the DXA scans and carcass analysis for all the pigs.

**Calibration and validation** – The two approaches, the low- and high-dimensional refined calibrations were tested and compared using the EchoMRI™ data from the calibration set of 25 piglets. In both approaches the QMR device had provisional calibration prior to this study, and animal-specific, refined calibration was developed during the calibration stage. Only the low-dimensional refined calibration was performed for DXA. All the calibrations were performed on the measurements of anesthetized piglets and cross-validated on the measurements of the same piglets awake (EchoMRI™) and dead (DXA and EchoMRI™).

Finally, the validation set of 25 piglets measured six months later was used to compare the refined calibration (high-dimensional for EchoMRI™ and low-dimensional for DXA) with chemical analysis directly.

**Results**

**Provisional calibration results versus chemical analysis**
There were substantial systematic errors in the provisionally calibrated DXA results for fat and EchoMRI™ results for fat and total water relative to the results of chemical analysis, as can be seen in Figures 1a-c and Table 1.

The significant correlation coefficients between systematic residuals of provisional values with respect to reference chemical values and both weight and chemical fat suggested the possibility of corrections by predictions based on low-dimensional regressions applied to the outputs of both DXA and EchoMRI™.

**Corrections by low-dimensional linear regressions**
The least square optimization (using Matlab) on the training set composed of all the anesthetized piglet measurements yielded

$$L_e = \begin{pmatrix}
0.88 & [0.71, 1.06] \\
-0.57 & [-0.80, -0.35] \\
0.63 & [0.41, 0.86]
\end{pmatrix}
\begin{pmatrix}
-0.33 & [-0.95, 0.29] \\
1.08 & [0.29, 1.87] \\
-0.04 & [-0.82, 0.75]
\end{pmatrix}$$

and

$$L_e = \begin{pmatrix}
0.78 & [0.71, 0.85] \\
0.05 & [0.02, 0.07] \\
-2.03 & [-3.27, -0.79]
\end{pmatrix}
\begin{pmatrix}
-0.04 & [-0.21, 0.13] \\
0.77 & [0.70, 0.83] \\
3.74 & [0.73, 6.74]
\end{pmatrix}$$

where the values in square brackets are the 95% confidence intervals. The results of self-testing on the training set and of the cross-validation on awake and dead piglets measurements can be seen in Figures 2a-d and Tables 2a-b.

**Calibration by refined high-dimensional regressions and cross-validation for EchoMRI™**
Three alternative permutations for refined calibration and cross-validation were tested: taking for the training set either awake, anesthetized, or dead piglet sets and testing the resulting calibration on the remaining two sets. All the three permutations yielded similar
results. The recalculated results obtained with calibration based on the anesthetized piglets (direct results from the refined high-dimensional regressions rather than low-dimensional linear regression corrections) are shown in Figures 3a-b and Table 3.

Full calibration of EchoMRI™
Finally, the high-dimensional regressions were optimized for fat and lean on all the data available (awake, anesthetized, and dead) from the calibration set of 25 piglets. The EchoMRI™ results reported from here on were obtained with these (refined) regressions.
Table 1. Statistics of fat and total water residuals versus chemical analysis for provisional calibrations of EchoMRI™ and DXA.

<table>
<thead>
<tr>
<th>Residual</th>
<th>EchoMRI™ Fat</th>
<th>EchoMRI™ TBW</th>
<th>DXA Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awake</td>
<td>Asleep</td>
<td>Dead</td>
<td>Awake</td>
</tr>
<tr>
<td>RMSE* (g)</td>
<td>107</td>
<td>100</td>
<td>95</td>
</tr>
<tr>
<td>Mean residual (g)</td>
<td>5</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>CCRW** (P-value)</td>
<td>0.74</td>
<td>0.75</td>
<td>0.74</td>
</tr>
<tr>
<td>CCRF*** (P-value)</td>
<td>(&lt;10^-4)</td>
<td>(&lt;10^-4)</td>
<td>(&lt;10^-4)</td>
</tr>
</tbody>
</table>

*RMSE is root-mean-square error; **CCRW is coefficient of correlation between residuals and weight of the subject; ***CCRF is coefficient of correlation between residuals and chemical fat.

Table 2a. Statistics of residuals versus chemical analysis for fat and total water predicted from EchoMRI™ by a low-dimensional regression on the first (calibration) set of 25 piglets.

<table>
<thead>
<tr>
<th>Residual</th>
<th>EchoMRI™ Fat</th>
<th>EchoMRI™ TBW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awake</td>
<td>Asleep</td>
<td>Dead</td>
</tr>
<tr>
<td>RMSE* (g)</td>
<td>27</td>
<td>21</td>
</tr>
<tr>
<td>Mean residual (g)</td>
<td>-6.8</td>
<td>0.3</td>
</tr>
<tr>
<td>CCRW** (P-value)</td>
<td>0.03 (0.89)</td>
<td>-0.02 (0.92)</td>
</tr>
<tr>
<td>CCRF*** (P-value)</td>
<td>-0.16 (0.43)</td>
<td>-0.22 (0.28)</td>
</tr>
</tbody>
</table>

*RMSE is root-mean-square error; **CCRW is coefficient of correlation between residuals and weight of the subject; ***CCRF is coefficient of correlation between residuals and chemical fat.

Table 2b. Statistics of residuals versus chemical analysis for fat and total water predicted from DXA by a low-dimensional regression on the first (calibration) set of 25 piglets.

<table>
<thead>
<tr>
<th>P</th>
<th>DXA Fat</th>
<th>DXA TBW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awake</td>
<td>Asleep</td>
<td>Dead</td>
</tr>
<tr>
<td>RMSE* (g)</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Mean residual (g)</td>
<td>-0.8</td>
<td>-0.9</td>
</tr>
<tr>
<td>CCRW** (P-value)</td>
<td>0.21 (0.32)</td>
<td>0.07 (0.75)</td>
</tr>
<tr>
<td>CCRF*** (P-value)</td>
<td>-0.03 (0.88)</td>
<td>-0.02 (0.91)</td>
</tr>
</tbody>
</table>

*RMSE is root-mean-square error; **CCRW is coefficient of correlation between residuals and weight of the subject; ***CCRF is coefficient of correlation between residuals and chemical fat.

Figure 3. Predictions for fat (a) and total water (b), calculated for all the EchoMRI™ measurements of the 25 calibration set piglets using the refined high-dimensional regressions based on the measurements of anesthetized (asleep) piglets. Each point is the average of 5 repeated scans.
**Table 3.** Statistics of residuals versus chemical analysis for fat and total water obtained from EchoMRI™ recalibrated by a high-dimensional regression on the first (calibration) set of 25 piglets.

<table>
<thead>
<tr>
<th>Residual</th>
<th>Awake</th>
<th>EchoMRI™ Fat</th>
<th>Dead</th>
<th>Awake</th>
<th>EchoMRI™ TBW</th>
<th>Dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMSE* (g)</td>
<td>24</td>
<td>23</td>
<td>25</td>
<td>96</td>
<td>91</td>
<td>89</td>
</tr>
<tr>
<td>Mean residual (g)</td>
<td>2</td>
<td>9</td>
<td>11</td>
<td>9</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>CCRW** (P-value)</td>
<td>0.20 (0.35)</td>
<td>0.09 (0.66)</td>
<td>0.01 (0.95)</td>
<td>-0.15 (0.47)</td>
<td>-0.11 (0.59)</td>
<td>-0.11 (0.61)</td>
</tr>
<tr>
<td>CCRF*** (P-value)</td>
<td>0.34 (0.10)</td>
<td>0.25 (0.22)</td>
<td>0.13 (0.52)</td>
<td>-0.28 (0.18)</td>
<td>-0.13 (0.52)</td>
<td>-0.14 (0.50)</td>
</tr>
</tbody>
</table>

*RMSE is root-mean-square error; **CCRW is coefficient of correlation between residuals and weight of the subject; ***CCRF is coefficient of correlation between residuals and chemical fat.

**Figure 4.** Fat (a) and total water (b) validation for EchoMRI™. Each point is the average of 5 repeated scans.

**Table 4.** Statistics of fat and total water residuals of EchoMRI™ validation versus chemical analysis on the second (validation) set of 25 piglets.

<table>
<thead>
<tr>
<th>Residual</th>
<th>Awake</th>
<th>EchoMRI™ Fat</th>
<th>Dead</th>
<th>Awake</th>
<th>EchoMRI™ TBW</th>
<th>Dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMSE* (g)</td>
<td>34</td>
<td>27</td>
<td>26</td>
<td>76</td>
<td>64</td>
<td>71</td>
</tr>
<tr>
<td>Mean residual (g)</td>
<td>-20</td>
<td>-11</td>
<td>-10</td>
<td>35</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>RMSE-as-FBW**</td>
<td>0.010</td>
<td>0.008</td>
<td>0.008</td>
<td>0.023</td>
<td>0.020</td>
<td>0.022</td>
</tr>
<tr>
<td>CCRW*** (P-value)</td>
<td>-0.48 (0.0148)</td>
<td>-0.55 (0.0041)</td>
<td>-0.47 (0.0167)</td>
<td>0.63 (0.0007)</td>
<td>0.53 (0.0064)</td>
<td>0.53 (0.0060)</td>
</tr>
<tr>
<td>CCRF**** (P-value)</td>
<td>-0.02 (0.92)</td>
<td>-0.17 (0.43)</td>
<td>-0.11 (0.62)</td>
<td>0.24 (0.24)</td>
<td>0.11 (0.61)</td>
<td>0.09 (0.65)</td>
</tr>
</tbody>
</table>

*RMSE is root-mean-square error; **RMSE-as-FBW is root-mean-square error as fraction of body weight; ***CCRW is coefficient of correlation between residuals and weight of the subject; ****CCRF is coefficient of correlation between residuals and chemical fat.

**Figure 5.** Fat (a) and total water (b) validation for DXA. Each point is the average of 3 repeated scans.
Validation of EchoMRI™
The measurements on the validation set of 25 piglets were performed with the fully calibrated regressions. The direct comparison with CA results is shown in Figures 4a-b and Table 4.

Validation of DXA
A similar comparison of CA results with predictions made from DXA results using the low-dimensional regressions found from calibration data for anesthetized piglets, is shown in Figures 5a-b and Table 5.

Precision of EchoMRI™ and DXA
Table 6a shows repeatability statistics summarized for all the 50 piglets, each scanned 5 times with repositioning in the EchoMRI™ device. Table 6b shows correlations between repeatability and possible error sources for all the 50 piglets, each scanned 5 times with repositioning in the EchoMRI™ device. Table 6c shows least square fit results for expectations in equation (ESEBb) for fat, for all the 50 piglets, scanned dead, 5 times dead with repositioning in the EchoMRI™ device.

### Table 5. Statistics of fat and total water residuals of low-dimensional regression predictions from DXA validation measurements on the second (validation) set of 25 piglets versus chemical analysis.

<table>
<thead>
<tr>
<th>Residual</th>
<th>Fat predicted from DXA</th>
<th>TBW predicted from DXA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asleep</td>
<td>Dead</td>
<td>Asleep</td>
</tr>
<tr>
<td>RMSE* (g)</td>
<td>24</td>
<td>23</td>
</tr>
<tr>
<td>Mean Residual (g)</td>
<td>-12</td>
<td>-13</td>
</tr>
<tr>
<td>RMSE-as-FBW**</td>
<td>0.009</td>
<td>0.008</td>
</tr>
<tr>
<td>CCRW*** (P-value)</td>
<td>-0.29 (0.15)</td>
<td>-0.31 (0.13)</td>
</tr>
<tr>
<td>CCRF**** (P-value)</td>
<td>-0.02 (0.94)</td>
<td>0.00 (1.00)</td>
</tr>
</tbody>
</table>

*RMSE is root-mean-square error; **RMSE-as-FBW is root-mean-square error as fraction of body weight; ***CCRW is coefficient of correlation between residuals and weight of the subject; ****CCRF is coefficient of correlation between residuals and chemical fat.

### Table 6a. Repeatability statistics summarized for all the 50 piglets, each scanned 5 times with repositioning in the EchoMRI™ device.

<table>
<thead>
<tr>
<th></th>
<th>Awake</th>
<th>Asleep</th>
<th>Dead</th>
<th></th>
<th>Awake</th>
<th>Asleep</th>
<th>Dead</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td></td>
<td></td>
<td></td>
<td>Fat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean trend 5th-1st (g)</td>
<td>10.4</td>
<td>-33.1</td>
<td>-27.8</td>
<td>-0.1</td>
<td>-4.2</td>
<td>0.1</td>
<td>8.8</td>
</tr>
<tr>
<td>t-test P-value</td>
<td>0.06</td>
<td>0.0002</td>
<td>0.002</td>
<td>0.9</td>
<td>0.004</td>
<td>0.9</td>
<td>2·10^-10</td>
</tr>
<tr>
<td>Mean trend 5th-3rd (g)</td>
<td>-5.0</td>
<td>0.0</td>
<td>1.1</td>
<td>0.2</td>
<td>-1.5</td>
<td>0.4</td>
<td>5.1</td>
</tr>
<tr>
<td>t-test P-value</td>
<td>0.2</td>
<td>1.0</td>
<td>0.9</td>
<td>0.8</td>
<td>0.2</td>
<td>0.7</td>
<td>4·10^-6</td>
</tr>
<tr>
<td>RMV* (g)</td>
<td>24</td>
<td>39</td>
<td>42</td>
<td>4.1</td>
<td>5.8</td>
<td>6.5</td>
<td>5.7</td>
</tr>
<tr>
<td>RMV-as-FBW**</td>
<td>0.008</td>
<td>0.014</td>
<td>0.012</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*RMV is root-mean-variance; *RMV-as-FBW is root-mean-variance as fraction of body weight.

### Table 6b. Correlations between repeatability and possible error sources for all the 50 piglets, each scanned 5 times with repositioning in the EchoMRI™ device.

<table>
<thead>
<tr>
<th></th>
<th>Coefficient of correlation with weight squared</th>
<th>Coefficient of correlation with fat squared</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Awake (P-value)</td>
<td>Asleep (P-value)</td>
</tr>
<tr>
<td>Var(Fat) (P-value)</td>
<td>0.13 (0.37)</td>
<td>-0.11 (0.44)</td>
</tr>
<tr>
<td>Var(TBW) (P-value)</td>
<td>0.15 (0.30)</td>
<td>0.10 (0.49)</td>
</tr>
<tr>
<td>Var(Lean) (P-value)</td>
<td>0.03 (0.83)</td>
<td>0.08 (0.58)</td>
</tr>
<tr>
<td>CV Fat (P-value)</td>
<td>-0.31 (0.027)</td>
<td>-0.43 (0.002)</td>
</tr>
</tbody>
</table>

### Table 6c. Least square fit results for expectations in equation (ESEBb) for fat, for all the 50 piglets, scanned dead, 5 times dead with repositioning in the EchoMRI™ device.

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>E[XEmpty’y]</td>
<td>E[XEmpty’XBody]</td>
<td>E[XBody’y]</td>
</tr>
<tr>
<td>(g^2)</td>
<td>(g)</td>
<td></td>
</tr>
<tr>
<td>Value</td>
<td>-42</td>
<td>2E[XEmpty’y]</td>
</tr>
<tr>
<td>95% confidence interval</td>
<td>(-182, 97)</td>
<td>(-0.064, 0.131)</td>
</tr>
</tbody>
</table>
body mass (RMV-as-FBW) are the two characterizations of repeatability in error model ESE employed in this study.

Table 6b shows coefficients of correlation between variances calculated for each piglet from the 5 repeated scans and the squares of their weight and fat.

Table 6c shows results of an attempt to determine the three parameters of error model (ESEB)

Although DXA does not give directly total water, it is possible to use the same kind of predictions for total water as for fat. Tables 7a-c show for DXA the same as Tables 6a-c for EchoMRI™ with the difference that there were only 3 scan repetitions for each subject.

Measurement of differences between awake, anesthetized and dead

Statistics of differences between measurements made on awake, asleep, and dead subjects, for EchoMRI™ and DXA, using the refined calibration for all the 50 piglets are shown in Table 8.

Discussion

The insignificance of misfits and their correlations in Tables 2a, 2b, and 3 indicate mostly the tightness of various linear fits made, but the real test of the calibrations found is the results of validation on another, independent set of validation piglets, in Tables 4 and

<table>
<thead>
<tr>
<th>Repeatability</th>
<th>Fat</th>
<th>Asleep</th>
<th>TBW</th>
<th>Dead</th>
<th>TBW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean trend 3rd-1st (g)</td>
<td>20</td>
<td>-20</td>
<td>19</td>
<td>-19</td>
<td></td>
</tr>
<tr>
<td>t-test P-value</td>
<td>3.10^13</td>
<td>1.10^4</td>
<td>3.10^13</td>
<td>1.10^12</td>
<td></td>
</tr>
<tr>
<td>Mean trend 3rd -2nd(g)</td>
<td>5.1</td>
<td>-4.8</td>
<td>5.5</td>
<td>-5.7</td>
<td></td>
</tr>
<tr>
<td>t-test P-value</td>
<td>0.004</td>
<td>0.053</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>RMV* (g)</td>
<td>15</td>
<td>18</td>
<td>13</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>RMV-as-FBW**</td>
<td>0.0049</td>
<td>0.0058</td>
<td>0.0045</td>
<td>0.0046</td>
<td></td>
</tr>
</tbody>
</table>

*RMV is root-mean-variance; **RMV-as-FBW is root-mean-variance as fraction of body weight.

<table>
<thead>
<tr>
<th>Coefficient of correlation with weight squared</th>
<th>Coefficient of correlation with fat squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asleep</td>
<td>Dead</td>
</tr>
<tr>
<td>Var(Fat) (P-value)</td>
<td>0.32 (0.02)</td>
</tr>
<tr>
<td>Var(TBW) (P-value)</td>
<td>0.27 (0.06)</td>
</tr>
<tr>
<td>CV Fat (P-value)</td>
<td>-0.30 (0.034)</td>
</tr>
<tr>
<td>Var(Fat)/(Weight^2)</td>
<td>0.10</td>
</tr>
<tr>
<td>(P-value)</td>
<td>-0.474</td>
</tr>
</tbody>
</table>

Table 7c. Least square fit results for expectations in equation (ESEBb) for fat, for all the 50 piglets, scanned asleep, 3 times with repositioning in the DXA device.

\[
\begin{align*}
\bar{\bX} = & \frac{1}{N} \sum_{i=1}^{N} x_i \\
\text{Value} & = 622 \\
95\% \text{ confidence interval} & = (-953, 2197) \\
\end{align*}
\]

Table 8. Mean values ± SEM for differences between measurements made on awake, anesthetized (asleep), and dead subjects, for EchoMRI™ and DXA, using the refined calibration for all the 50 piglets. Mean values of fat, total (body) water (TBW) and lean for EchoMRI™ and DXA are also provided for relative comparison.

<table>
<thead>
<tr>
<th>Repeatability</th>
<th>Fat</th>
<th>Asleep</th>
<th>TBW</th>
<th>Dead</th>
<th>TBW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean trend 3rd-1st (g)</td>
<td>20</td>
<td>-20</td>
<td>19</td>
<td>-19</td>
<td></td>
</tr>
<tr>
<td>t-test P-value</td>
<td>3.10^13</td>
<td>1.10^4</td>
<td>3.10^13</td>
<td>1.10^12</td>
<td></td>
</tr>
<tr>
<td>Mean trend 3rd -2nd(g)</td>
<td>5.1</td>
<td>-4.8</td>
<td>5.5</td>
<td>-5.7</td>
<td></td>
</tr>
<tr>
<td>t-test P-value</td>
<td>0.004</td>
<td>0.053</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>RMV* (g)</td>
<td>15</td>
<td>18</td>
<td>13</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>RMV-as-FBW**</td>
<td>0.0049</td>
<td>0.0058</td>
<td>0.0045</td>
<td>0.0046</td>
<td></td>
</tr>
</tbody>
</table>

*RMV is root-mean-variance; **RMV-as-FBW is root-mean-variance as fraction of body weight.

<table>
<thead>
<tr>
<th>Coefficient of correlation with weight squared</th>
<th>Coefficient of correlation with fat squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asleep</td>
<td>Dead</td>
</tr>
<tr>
<td>Var(Fat) (P-value)</td>
<td>0.32 (0.02)</td>
</tr>
<tr>
<td>Var(TBW) (P-value)</td>
<td>0.27 (0.06)</td>
</tr>
<tr>
<td>CV Fat (P-value)</td>
<td>-0.30 (0.034)</td>
</tr>
<tr>
<td>Var(Fat)/(Weight^2)</td>
<td>0.10</td>
</tr>
<tr>
<td>(P-value)</td>
<td>-0.474</td>
</tr>
</tbody>
</table>

Table 7b. Correlations between repeatability and possible error sources for all the 50 piglets, each scanned 3 times with repositioning in the DXA device.

\[
\begin{align*}
E[\bX_{\text{asleep}}^2] & = 622 \\
2E[\bX_{\text{asleep}} \times \bX_{\text{dead}}] & = -0.44 \\
E[\bX_{\text{dead}}^2] & = 9.7 \times 10^4 \\
95\% \text{ confidence interval} & = (-953, 2197) \\
& = (-1.54, 0.66) \\
& = (-8.7 \times 10^{-5}, 2.8 \times 10^{-4})
\end{align*}
\]
5. It is interesting to note that the validation accuracy in Tables 4 and 5 is quite close to the calibration accuracy in Tables 2a and 2b.

Another curious observation is that the small value of $L_{y}(2,3)$ indicates that chemical total water is predicted mostly by provisional EchoMRI™ lean and fat, nearly excluding provisional total water. As regards predicting chemical fat, all the three quantities measured by provisional EchoMRI™ calibration, fat, lean, and total water make comparable contributions. The large values and confidence intervals of $L_{y}(1,3)$ and $L_{y}(2,3)$ are related to the fact that BMC is usually a small fraction of total mass and suggest that BMC does not play a substantial role in predicting chemical fat and total water.

The repeatability statistics in Tables 6a-b and 7a-b indicate that this study is too small to use error models more complex than ESEB. The significant correlations with fat indicate that CV is not well suited to characterize fat precision for awake, anesthetized, and dead piglets, for both EchoMRI™ and DXA. The significant correlations with weight indicate that root-mean variance as fraction of body mass (RMV-as-FBW) is not well suited to characterize EchoMRI™ fat precision for anesthetized piglets and DXA fat precision for dead piglets. The significant correlations with weight indicate that root-mean variance (RMV) is not well suited to characterize EchoMRI™ fat, lean, and total water precision for dead piglets and DXA fat and total water precision for dead and anesthetized piglets.

The larger standard deviations in awake piglets are likely associated with the motion of an animal inside the probe during the scan.

The significant trends found for both DXA and EchoMRI™ can have more than one source. In the case of dead piglets in EchoMRI™, a possible source of the trend may be the change of temperature in the body of a freshly killed piglet, consistent with the presence of the trend in both 5th-1st and 5th-3rd differentials. In the case of awake piglets in EchoMRI™, a possible source of the trend may be the stress experienced by a piglet when first placed into unfamiliar confinement. In the case of anesthetized piglets in EchoMRI™, a conceivable source of the trend in lean may be in the device itself possibly associated with some heating when scans follow each other very fast, although there is no significant trend in fat and total water for these piglets.

In the case of anesthetized and dead piglets in DXA, the trend is significant in all quantities, it is present in both 3rd-1st and 3rd-2nd differentials, and it appears to be a dominant reason for standard deviations of repeated DXA quantities to be several times larger than those of EchoMRI™. A conceivable source of the trend may be in the device itself, perhaps associated with some heating when the device is used continuously.

The large uncertainties in Tables 6c and 7c indicate that this study is not large enough to determine parameters of an ESEB model for random errors of both EchoMRI™ and DXA. Therefore the only random errors description available is the combination of RMV and RMV-as-FBW of which, in all cases, at least one is not correlated with sample properties and so is applicable.

The significant differences between quantities measured in awake, anesthetized, and dead piglets may conceivably result from changes associated with transitions from one state to another, such as evacuation.

There are a number of criteria to be considered when selecting the most suitable method for measuring the body composition of a particular group of subjects. In all cases, accuracy and precision are very important.

The notions of fat, lean, free water and total water may be simple to use intuitively when only rough estimates are required. However when accuracy is an issue, it is important to be aware of definitions and of reference method qualifications.

If BCA methods were employed without adaptive calibration by a reference method, differences in measurement results would be largely determined by the differences in definitions. For instance, what we regard as chemical analysis fat is in fact the mass of lipids [30] which may contain some constituents formally not classified as fat in chemistry (e.g. waxes, sterols, fat soluble vitamins, mono- and diglycerides, etc; a major contributor to these non-fat lipids would be the neural tissues). DXA fat is a function of a projection of a volume labeled as fat, and EchoMRI™ Fat can in principle contain contributions from arbitrary substances whose contributions to the measurement vector are not canceled out by the convolution with the fat regression.

However the calibration by a reference method makes DXA and EchoMRI™ into adaptive methods which can be used to predict the results of the reference method. For adaptive methods, the role of the differences in definitions is reduced, and only the power of prediction of the reference method results matters. This power depends not only on the adaptive method but also on the selected reference method, as for example it may happen that precision, sensitivity and specificity of the reference method may limit the accuracy of an adaptive method.

The DXA method of BCA was well studied on piglets, and some studies report precision and accuracy [29] (after low-dimensional regression corrections) comparable to or only slightly worse than what we observe in this study.

The results of this study indicate that with proper calibration, both DXA and EchoMRI™ can provide similar good accuracies and precisions in measurements of fat and total water content of piglets in the 2000 to 4000 g body weight range.

As it appears from the validation set of 25 piglets, the precision is 2-4 times better in the case of EchoMRI™ and the accuracy is 1.1-1.3 times better in the case of DXA.

Two additional major advantages of the QMR method over DXA are that QMR does not expose the subject to X-ray radiation and that QMR does not
necessarily require immobilization (i.e., restraint or anesthesia) of the subject. These two advantages are most critical for BCA of infants. An advantage of DXA is the possibility of scanning a part of a body. Another advantage of QMR is the fast scanning time: a typical scan in this study took less than 90 seconds.

The bottom line summary of the main results of this study is the following. Characterization of random errors by CV, especially that of fat, is not suitable for BCA, whereas absolute errors or errors relative to total body weight can be applicable. Low- and high-dimensional regressions offer nearly the same accuracy improvements. Improved DXA and EchoMRI™ offer nearly the same accuracy, within 1% of weight in fat, while EchoMRI™ has better precision, within 0.2 % of weight in fat for anesthetized and dead piglets as compared to DXA's 0.5-0.6%.

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References

8. Shypailo RJ, Butte NF, Ellis KJ. DXA: can it be used as a criterion reference for body fat measurements in children? Obesity (Silver Spring) 2008; 16(2): 457-62.


