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Validation of Quantitative Magnetic Resonance Body Composition Analysis for Infants Using Piglet Model

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ABSTRACT: A study was conducted to validate the use of a quantitative magnetic resonance (QMR) device for measuring the body composition of infants and neonates weighing <12 kg using the pig as a model. A total of 25 piglets weighing between 2 and 12 kg were studied. Body composition was assessed by QMR, dual-energy x-ray absorptiometry (DXA), and whole-body chemical analysis (CA) of carcass for lipid and water content. The precision, mean and SD of repeated measurements, of QMR to estimate fat mass (FM), lean mass (LM), and total body water (TBW) for five consecutive scans with reposition was 12.5, 32.0, and 36.0 g, respectively. QMR measures of FM, LM, and TBW were highly and significantly correlated with CA of carcass. In terms of accuracy, mean difference between QMR and CA (percent of mean value for CA), QMR overestimated FM by 40 g (4.7%), overestimated LM by 114.9 g (2.1%), and underestimated TBW by 134.6 g (3.1%). This study concludes that QMR provides precise and accurate measures of FM, LM, and TBW in piglet weighing up to 12 kg. These results suggest that QMR can provide valuable body composition data in longitudinal studies in infants. **(*Pediatr Res* 69: 330–335, 2011)**

Accurate assessment and tracking of infant body composition is useful in evaluating 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).

A large variety of nondestructive 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) (8), total-body electrical conductivity (TOBEC) (9), total body potassium (TBK) (10), isotope dilution (11), skin-fold thickness measurements (SFT) (12), multicompartment models (13), computed tomography (CT) (14), MRI (15), magnetic resonance spectroscopy (MRS) (16), and quantitative magnetic resonance (QMR) (17).

Common shortcomings include complexity and time needed to obtain results (multicomponent models, TBK, isotope dilution, MRI, and MRS), immobilization requirement (MRI, MRS, CT, and DXA), expensive equipment (MRI, MRS, CT, DXA, QMR, and TOBEC), reliability and correspondence of relations between measured quantities and body components (BIA, TOBEC, ADP, and SFT), radiation exposure (CT and DXA), and potential vulnerability to oversimplification (ADP and SFT).

QMR devices stand out in that they are fast and very easy to use, require no sedation or anesthesia, and are free of radiation, while capable of unsurpassed precision and high accuracy. Typical scan times range from <1 min to <4 min in different specific devices and applications, and less than half an hour of training is sufficient for a typical user.

The QMR method is a branch of nuclear magnetic resonance (NMR), for whole body measurement of fat, lean tissues, free water (not bound in various tissues), and total body water (TBW, water contained in all the liquids and in tissues) of live animals including humans. QMR differs from MRI in that the processed signal is obtained from the whole body at once (without spatial encoding) and it differs from MRS in that the time domain signal (rather than spectrum) is processed directly. Principles of QMR are described elsewhere (17–22). Briefly, this system generates a signal that modifies the spin patterns of hydrogen atoms within the subject and uses an algorithm to evaluate the resulting T1 and T2 relaxation curves specific to each of the four components measured: fat mass (FM), lean muscle mass equivalent, TBW, and free water. Each component is estimated based on an individually derived T1/T2 relaxation curve fractionated from the total returned signal.

Success in using QMR in animals of different sizes (flies, mice, rats, birds, dogs, pigs, infants, children, and adult humans) has been comprehensively reported (17–22). This study addresses its potential for infants, using piglets as a generally accepted research model for infant body composition (23,24).

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Abbreviations: ADP, air-displacement plethysmography; **BCA,** body composition analysis; **BF,** body fat; **BMC,** bone mineral content; **CA,** chemical analysis; **CT,** computed tomography; **CV,** coefficient of variation; **DXA,** dual energy X-ray absorptiometry; **FM,** fat mass; **LM,** lean mass; **MRS,** magnetic resonance spectroscopy; **QMR,** quantitative magnetic resonance; **RMSE,** root mean square error; **SFT,** skin-fold thickness measurements; **TBW,** total body water; **TOBEC,** total-body electrical conductivity; **4-C,** four-compartment

There have been many studies of pig BCA, in particular, comparing DXA with chemical analysis (CA) (25–27) and comparing DXA with tissue dissection (28). This study validates, at the same time, QMR and DXA precision and accuracy using CA as the reference BCA method.

In comparative studies, QMR was found to have higher precision than all the other methods considered in these studies (17–22). The ability of QMR to precisely detect minute longitudinal differences in body composition is particularly valuable. When measuring the FM in rats (21), the coefficient of variance (CV) % for QMR was 0.94 compared with 5.12 for CA; in mice (19), it was 1.58 for QMR compared with 2.2 for DXA; and in pigs (20), it was 1.3 for QMR compared with 3.7 for DXA.

DXA and CT can sometimes achieve repeatability comparable with that of QMR; however, these BCA methods require immobilization and various degree of x-ray irradiation, both undesirable in most applications and unacceptable in applications to infants. As DXA is the closest BCA method to QMR in terms of precision, speed, and convenience of use, this validation study of QMR usually includes some comparison with DXA (17–22). This study follows that tradition and, in addition, examines by regression analysis the corrections that are usually needed for DXA and can be used in the same way for QMR. This validation study was a part of more extensive study which will be fully described in the future. Future studies will include the use of QMR to evaluate the changes in body composition during growth of both the piglet and the human infant.

MATERIALS AND METHODS

Animals and procedures. Twenty-five piglets weighing <12 kg were used to validate the QMR device. Each piglet was scanned by QMR (EchoMRI-Infants) five times and by DXA (Lunar Prodigy) three times while anesthetized and then subjected to CA. As DXA requires anesthesia, for better comparability, piglets were anesthetized for QMR as well. The calibration of both the QMR and the DXA was checked each day immediately before scanning. All devices were installed at USDA Animal Bioscience and Biotechnology Laboratory, Beltsville, MD.

The piglets were sedated using an intramuscular injection of a mixture of ketamine, Telazol (tiletamine + zolazepam), 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 piglets were killed by an intracardiac injection of pentobarbital. Immediately after scanning, the piglet carcass was sealed in a plastic bag and frozen at −15°C until processing for CA.

All QMR scans, DXA scans, and carcass analysis for all the piglets was performed by the same person. Experimental protocols used in this study were approved by the Beltsville Area Institutional Animal Care and Use Committee.

BCA methods. QMR EchoMRI-Infants (Echo Medical Systems, Houston, TX) device was used for subjects weighing up to 12 kg. The device weight is ~1000 kg and has external dimensions of $L \times W \times H$ is $120 \times 60 \times 180$ cm^3. The resistive magnet generates a static magnetic field of ~0.0145 Tesla in a bore size of $L \times W \times H$ is $120 \times 30 \times 30$ cm^3. The field of view is a 25-cm diameter, 60-cm long cylinder in the center of the bore and the system is self-shielded. The operating system is based on Windows XP Professional Edition. Measuring time is typically <3 min, with three repeat measurements taking <10 min; there is a recommended daily system test in the most recent software. The system output includes FM, lean tissue mass, free water, and total water in units of grams.

DXA Lunar Prodigy (GE Lunar, Madison, WI) device was tested as supplied by manufacturer. Details of piglet measurement by DXA are described elsewhere (29). Scans were performed and analyzed using the Small Animal Mode (version 8.10). The quantities measured by DXA are FM, lean mass (LM), and bone mineral content (BMC).

Chemical analysis. For CA, the entire body was homogenized, and then samples were analyzed for total water and lipid (fat) content. Lipid content was measured by chloroform/methanol extraction (30) and water content was measured by lyophilization. The quantities measured by CA are FM and TBW. Particular details are the following.

Carcass preparation. Piglets weighing >5 kg were homogenized three times using a whole-body grinder (Autio Model 810M GH, Astoria, OR). Piglets weighing ≤5 kg were autoclaved for 2 h at 121°C, cooled to 3°C, and then homogenized for 1 min using a food processor (Robot Coupe, Model R10, Jackson, MS). Samples were stored at −15°C before analysis.

Water analysis. A single sample from each piglet was weighed (sample size was ~400 g), frozen, and then lyophilized in a freeze dryer (Virtis, Model 100 SRC-6, Gardiner, NY) for 14 d. The samples were weighed again immediately after removal 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 h in a 125-mL separatory funnel containing 60 mL of chloroform:methanol (2:1, vol/vol). After 24 h, 12 mL of 0.88% potassium chloride in water was added and then mixed by shaking for 10 s. The sample was allowed to set for another 24 h to permit phase separation. The lower phase was then drained into preweighed 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). The vials were allowed to cool and then reweighed to determine the amount of lipid extracted.

Calculations and statistics. Statistics were done using custom software. The results of QMR, DXA, and CA analysis were correlated, and precision and accuracy were evaluated.

Validity was evaluated by comparing the QMR variables with chemical carcass analysis variables by paired *t* tests, and linear regression analysis to determine whether the results passed through the origin and had a slope that did not differ from unity. In addition, the root mean square error (RMSE) for each equation was calculated to assess performance. For regression analysis, the mean absolute error (MAE) was calculated as the average value of the residuals. The measurement of fat content by QMR was also compared with that by CA by plotting the difference between the two measurements against the average of the two measurements (Bland-Altman plot) (31).

RESULTS

The mean values for the measurement of total body fat, lean, and water by QMR, DXA, and CA are shown in Table

Table 1. *Mean [plusmn] SD values for measurement of body composition components by CA, QMR, and DXA*

Body composition component	CA		QMR		DXA	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Fat (g)	854 ± 428	95–1447	894 ± 448*	123–1557	1034 ± 515*	162–1724
Fat (%)	12.6 ± 3.3	4.8–17.1	13.2 ± 3.3*	6.2–17.9	15.6 ± 4.4*	8.3–22.6
Lean (g)	5391 ± 2099‡	1482–9032	5506 ± 2135	1956–9359	5130 ± 2050	1767–8927
Water (g)	4395 ± 1730	1486–7343	4257 ± 1593‡	1485–7016		

QMR and DXA values were compared with CA by paired *t* test.

* $p < 0.001$.

† CA lean = BWT − (CA fat + DXA BMC).

‡ $p < 0.01$.

1. Compared with CA, both QMR and DXA significantly overestimated total body fat, QMR by 4.7% and DXA by 21%. The RMSE for the prediction of FM by QMR was calculated as 65 g, and the RMSE by DXA was 215 g.

Although not significant, the QMR measurement of total body lean was 2.1% less than CA while DXA was 4.8% less. The QMR measurement of TBW was only 3.1% less that the CA measurement, yet significantly lower based on the paired *t* test ($p < 0.05$). The differences between the measurements by QMR and DXA and those by CA, including the RMSE, are shown in Table 2.

The relationship between the CA for total body fat, lean, and water and the measurements obtained by QMR and DXA are shown in Figures 1–3. In all cases, there results were highly correlated. For both fat and lean, the slope of the regression line was closer to 1.0 with QMR than with DXA. By regression analysis, the mean absolute error for the prediction FM was 30 g for QMR and 54 g for DXA. For the prediction of LM, the mean absolute error was 123 g for QMR and 108 g for DXA.

Precision of fat/QMR in terms of SD ranged from 4.15 to 24.47 g with a mean and SD of 11.67 ± 5.19 g, resulting in mean CV of 1.31%. Precision of lean/QMR, TBW/QMR, fat/DXA, and lean/DXA in terms of SD are presented in Table 3. A plot of the SD for repeatability measurement of FM for each piglet measured by QMR relative to the body weight of the pig is shown in Figure 4. The relationship between precision of fat/QMR in terms of CV ranged from 0.44 to 8.61%, with a mean and SD of 1.80 ± 1.86%. Precision of lean/QMR, TBW/QMR, fat/DXA, and lean/DXA in terms of CV are presented in Table 4. A plot of the CV for repeatability measurement of FM for each piglet measured by QMR relative to the body weight of the pig is shown in Figure 5. In Figure 6, the difference between the percentage of fat measured by QMR and CA is plotted against the average of the percentage of fat for the two methods (Bland-Altman plot). The relationship between the amount of total body fat measured by QMR and the body weight of the piglet is shown in Figure 7.

DISCUSSION

In studies designed to validate the use of various approaches for measuring human body composition, a variety of reference methods have been used, and in many cases, more than one. DXA has been used extensively in studies of both infant and adult human body composition and is frequently used as a reference method for validating other methods. However, the most recognized reference BCA method for animals is CA (30). This study compares QMR to both CA and DXA, thus evaluating it relative to a well-defined reference method and to a method that is commonly used in human studies.

Although QMR measurement does not require anesthesia, all piglets were measured in the anesthetized state as required

Table 2. *Accuracy of measuring body composition by QMR and DXA with reference to chemical analysis*

	QMR			DXA	
Measuring method	Fat	Lean	TBW	Fat	Lean
Body composition component					
Average difference (g)	40	115	−138	180	−261
Average difference as % of average CA	4.7	2.1	−3.1	21.1	−4.8
RMSE (g)	67.7	204.0	274.5	224.5	306.5

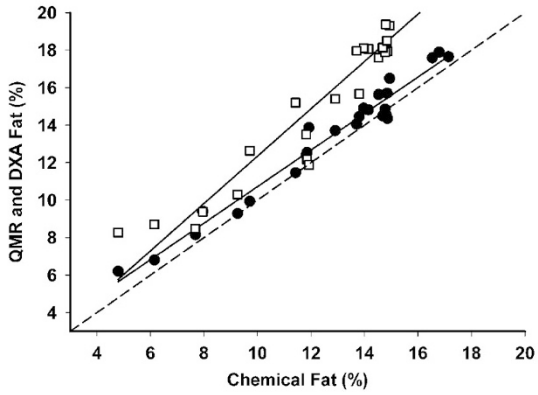

Figure 1. Total body FM measured by QMR (●) and DXA (□) *vs.* CA of carcass for piglets weighing <12 kg. QMR, $Y = 6.75 + 1.040X$, $R^2 = 0.988$; DXA, $Y = 20.73 + 1.186X$, $R^2 = 0.968$; --- indicates line of identity.

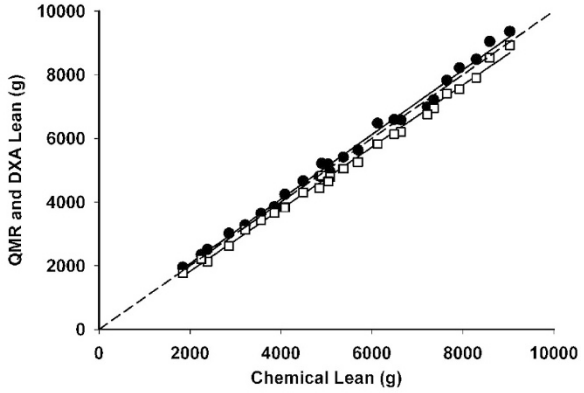

Figure 2. Total body LM measured by QMR (●) and DXA (□) *vs.* CA of carcass for piglets weighting <12 kg. QMR, $Y = 31.7 + 1.015X$, $R^2 = 0.994$; DXA, $Y = -126 + 0.975X$, $R^2 = 0.996$; --- indicates line of identity.

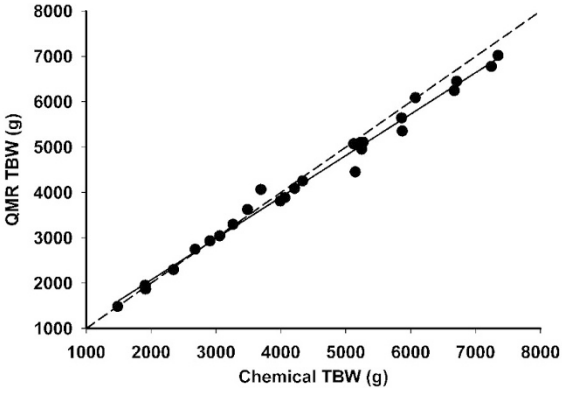

Figure 3. TBW measured by QMR (●) *vs.* CA of carcass for piglets weighting <12 kg. QMR, $Y = 238 + 0.915X$, $R^2 = 0.988$; --- indicates line of identity.

Table 3. *Precision of measuring body composition by QMR and DXA in terms of SD in units of grams*

Measuring method	QMR			DXA	
	Fat	Lean	TBW	Fat	Lean
Body composition component					
Mean (SD) g	11.67	29.61	29.09	27.08	27.38
SD (SD) g	5.19	14.76	29.07	14.93	15.03
Range (min) g	4.15	8.11	6.77	6.11	5.16
Range (max) g	24.47	66.19	157.98	58.03	57.80

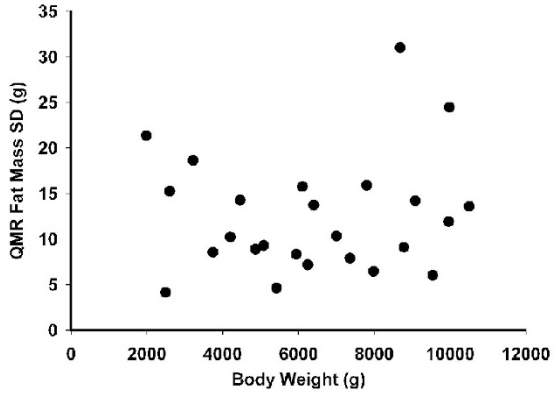

Figure 4. Standard deviation for repeatability measurement of FM for each piglet measured by QMR as a function of body weight.

Table 4. *Precision of measuring body composition by QMR and DXA in terms of CV units of %*

Measuring method	QMR			DXA	
	Fat	Lean	TBW	Fat	Lean
Body composition component					
Mean CV %	1.80	0.60	0.85	3.09	0.56
SD (CV) %	1.86	0.39	1.12	1.98	0.32
Range (min) %	0.44	0.12	0.13	0.71	0.18
Range (max) %	8.61	1.66	5.75	8.12	1.35

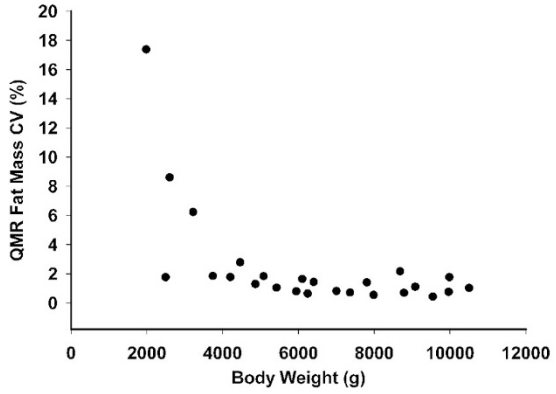

Figure 5. CV for repeatability measurement of FM for each piglet measured by QMR as a function of body weight.

by DXA so that conditions would be the same for both measurements. In addition, by 4 or 5 d after birth, the newborn piglet is quite robust and its level of activity, even if confined or restrained, would be far greater than would be observed with the human infant, thus the anesthetized piglet would be a

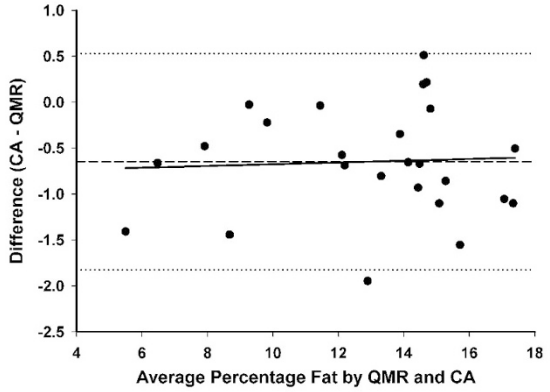

Figure 6. Bland-Altman plot comparing the average percentage of fat measured by QMR and CA to the difference in the values (CA-QMR) for the two methods. ---, mean difference; ·····, mean ± 2 SD; —, regression line.

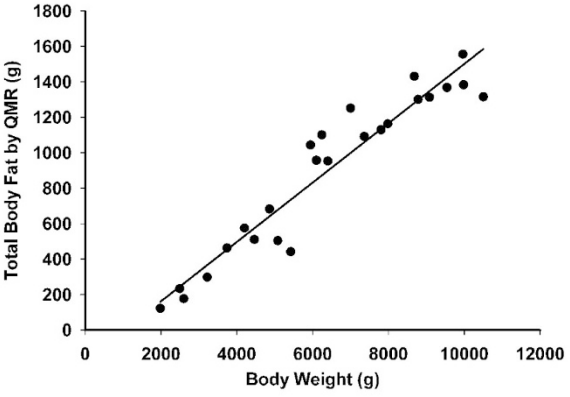

Figure 7. Relationship between FM measurement and body weight. $R^2 = 0.91$.

better model for the human infant. It was observed and reported elsewhere that precision for measuring anesthetized piglets by QMR is about twice better than for nonanesthetized. One of five repeats of QMR measurements for one of 25 animals (1 of 125 scans) was found to be outlier and was excluded from further analysis. One of three repeats of DXA measurements for one of 25 animals (1 of 75 scans) was found to be outlier and was excluded from further analysis. The Bland-Altman plot (Fig. 6) shows that compared with CA, QMR overestimated the percentage of total body fat in piglets by 0.6%. There is little or no indication of a trend for the difference between the methods to increase as the average increases (regression slope = 0.009). Furthermore, it does not appear that the scatter around the bias line increases as the average gets higher.

The amount of fat was highly dependent on body weight (Fig. 7). By CA, the total body fat content of the pigs used in this study ranged from 95 to 1447 g, compared with 123 to 1557 g by QMR and 162 to 1724 g by DXA. On average, QMR overestimated FM by 40 g compared with an overestimation of 180 g by DXA. The RMSE for the prediction equation for FM was calculated as 67.7 g for QMR and 224.5 g for DXA. Not only was the QMR measurement of total body fat closer to the CA measurement than was DXA, but also the slope of the regression line for QMR fat was closer to unity (Fig. 1). The greater accuracy of QMR for measuring fat

content of piglets compared with DXA is in agreement with a recent study that reported that QMR overestimated FM by 2%, compared with 15% by DXA (20). QMR showed excellent precision for measuring FM, with a mean CV of 1.8% compared with 3.1% for DXA. This too is in close agreement with a recent study that reported CV ~1.5% for QMR and CV ~3.5% for DXA measurements of total body fat in pigs weighing between 3 and 50 kg (20). As can be seen from Figure 5, CV is a function of body weight—at low weight (<4 kg), there was a higher CV compared with high weight animal. In a previous study using QMR to measure the body composition of mice (19), it was reported that there was a significant relationship between the weight of the animal and the fat CV%. It is not certain that this is entirely a function of body weight. At least in this study, fat content itself was also a function of body weight, as shown in Figure 7. In fact, the relationship with CV% shown in Figure 5 is quite similar if BF% is substituted for body weight.

The accuracy of the prediction of total body lean is somewhat arbitrary since the CA did not directly measure LM. Instead, the chemical LM was defined as: chemical lean = BWT − (fat/CA + BMC/DXA), where BWT is the live body weight. Using this reference, the accuracy and precision for the prediction of LM was similar for QMR and DXA. The RMSE for the prediction equation for total body lean was calculated as 204 g for QMR and 306.5 g for DXA. As the LM is predominately water, the relationship between QMR and CA measurements of TBW provides a more direct comparison. Figures 1–3 demonstrate dependence of difference between QMR and CA on value of the parameter. This tendency is weaker for TBW. One reason for this is that sample sizes for CA are small; for FM measurement, 3–4 g sample is used; for TBW measurement, 400 g samples are used; then the result is multiplied by the animal weight. The precision of QMR for measuring TBW was very similar to that of LM.

Usage of SD or CV measures of spread is dictated by their usefulness and depends very much on the nature of measurement method and measured parameter. A goal of this study was to simply determine how to predict the precision for measuring, for example, fat in a piglet weighting 7 kg. From Figure 4, an SD of ~10 g was observed for animals between 2 and 12 kg; from Figure 6, the estimate for FM would be ~1 kg; therefore, CV is expected to be 1%. Recently, it was reported that with a smaller QMR instrument, DXA and QMR offered nearly the same accuracy, within 1% of weight in fat, while QMR had better precision, within 0.2% of weight in fat for anesthetized piglets compared with DXA's 0.5–0.6% (32). The same study noted that the 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. This conclusion was based on the significant correlations of CV with fat measurements.

ADP has been evaluated for measuring body composition of human infants (33,34). When ADP was compared with deuterium (^2H_2O) dilution, there was no difference in the mean percentage of BF (20.32% BF by ADP and 20.39% BF by ^2H_2O) and regression analysis gave an R^2 of 0.76 and a RMSE of 3.26 (33). Similarly, when ADP was compared with a four-compartment (4-C) model, there was no difference in the mean percentage of BF (16.9% BF by ADP and 16.3 by 4-C) and regression analysis gave an R^2 of 0.73 and a RMSE of 3.7 (33). By the same measure, in this study, when QMR was compared with CA, there was no difference ($p < 0.05$) in the mean percentage of BF (12.6% BF by QMR and 13.2% BF by CA) and regression analysis gave an R^2 of 0.96 and a RMSE of 0.61.

The results of this study indicate a strong potential for the use of the QMR method for measuring the body composition of human infant and warrant its evaluation in the clinical setting. The advantages of QMR over various other methods include accuracy, precision, insensitivity to movement, and no exposure to x-rays. Furthermore, the QMR is designed for easy calibration that can be customized and updated as needed. The ability of QMR to accurately and precisely measure total body fat and water could be a valuable tool for monitoring changes in body composition of the infant in either the healthy or diseased state.

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