Noninvasive measurements of body composition and body water via quantitative magnetic resonance, deuterium water, and dual-energy x-ray absorptiometry in cats

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Objective—To compare quantitative magnetic resonance (QMR), dual-energy x-ray absorptiometry (DXA), and deuterium oxide (D_2O) dilution methods for measurement of total body water (TBW), lean body mass (LBM), and fat mass (FM) in healthy cats and to assess QMR precision and accuracy.

Animals—Domestic shorthair cats (58 and 32 cats for trials 1 and 2, respectively).

Procedures—QMR scans of awake cats performed with 2 units were followed by administration of D_2O tracer (100 mg/kg, PO). Cats then were anesthetized, which was followed by QMR and DXA scans. Jugular blood samples were collected before and 120 minutes after D_2O administration.

Results—QMR precision was similar between units (coefficient of variation < 2.9% for all measures). Fat mass, LBM, and TBW were similar for awake or sedated cats and differed by 4.0%, 3.4%, and 3.9%, respectively, depending on the unit. The QMR minimally underestimated TBW (1.4%) and LBM (4.4%) but significantly underestimated FM (29%), whereas DXA significantly underestimated LBM (9.2%) and quantitatively underestimated FM (9.3%). A significant relationship with D_2O measurement was detected for all QMR ($r^2 > 0.84$) and DXA ($r^2 > 0.84$) measurements.

Conclusions and Clinical Relevance—QMR was useful for determining body composition in cats; precision was improved over DXA. Quantitative magnetic resonance can be used to safely and rapidly acquire data without the need for anesthesia, facilitating frequent monitoring of weight changes in geriatric, extremely young, or ill pets. Compared with the D_2O dilution method, QMR correction equations provided accurate data over a range of body compositions. (*Am J Vet Res* 2013;74:721–732)

In recent years, DXA has been one of the standard methods used for in vivo determination of body composition. This technique is based on the differential attenuation of x-rays by bone, lean tissue, and fat tissue, which in turn allows the calculation of the proportion of each of these structural components with respect to total body composition. The accuracy and precision of DXA have been established primarily through validation against the criterion-referenced standard of chemical analysis of carcasses, and authors of several reports[1–4] have discussed the use and limitations of DXA for assessing body composition. In companion animals such as dogs and cats, only 1 study[5] has provided data for validation of DXA analysis, compared with results for chemical composition of carcasses. Those researchers[5]

Abbreviations

BCS	Body condition score
CV	Coefficient of variation
D_2O	Deuterium oxide
DXA	Dual-energy x-ray absorptiometry
FM	Fat mass
LBM	Lean body mass
QMR	Quantitative magnetic resonance
TBW	Total body water

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found that the mean estimates of body mass, lean tissue mass, and FM were strongly correlated, with coefficients of 0.999 for lean tissue mass and 0.982 for FM. Even with minimal validation of the use of DXA to assess body composition components in cats, DXA has been effectively used in studies on feline body composition and weight loss[6–9] and evaluation of BCS systems.[10–12]

Recently, a new technology, referred to as QMR, has been developed for assessment of body composition and measurement of TBW and body composition of lean and fat mass.[13] Evaluation of the QMR method across multiple species, including rodents,[14–17] pigs,[18,19] humans,[20–22] and chickens,[23] has revealed that it can be used to quickly and easily obtain accurate and precise data, without the need for sedation or anesthesia of subjects. These positive attributes provide a considerable advantage over the use of DXA for assessing and frequently monitoring body composition changes during nutrition studies and in veterinary medical patients. Use of QMR also would eliminate health concerns and a decrease in food intake associated with anesthesia, particularly in extremely young or old animals in which the health risks associated with anesthesia are greater.

Functionally, QMR is a technology that uses hydrogen (proton)-nuclear magnetic resonance principles. The response of a hydrogen proton is determined by its chemical bonding; thus, hydrogen protons in fat behave differently than the hydrogen protons in lean tissue. Therefore, QMR can specifically distinguish lean tissue mass from fat tissue mass.

The initial report[13] on use of QMR to assess in vivo body composition in animals stated a CV of 0.34% to 0.71% for FM in mice, compared with 3.06% to 12.6% with DXA. Studies to evaluate QMR data and data for carcass analysis in mice, rats, chickens, and pigs have all indicated that determination of TBW, LBM, and FM is highly related ($r^2 > 0.9$) between QMR and carcass analysis. Investigators of a study[18] in pigs ranging in weight from 3 to 49 kg found precision for QMR estimates of FM, LBM, and TBW to be high, with CVs of 1.3%, 0.9%, and 0.9%, respectively. Investigators of that study[18] also found that QMR overestimated FM by 2% and DXA overestimated FM by 15%, compared with results for carcass analysis. In humans, several studies[14,15,20] have confirmed the high precision of QMR measurements and indicated that simulated changes in body composition are accurately measured.[20] To accurately and precisely assess body composition in humans, the currently accepted reference method is use of a 4-compartment model[24] that combines body mass, bone mineral mass, TBW mass, and body volume.[25] Consequently, the 4-compartment model has been used to validate accuracy of the QMR method in humans.[20–22,26] However, the use of D_2O dilution as a reference method to determine body composition is more commonly used and relies on a 2-compartment model in which the body is partitioned into LBM and FM fractions.[22] The D_2O dilution method can be used to determine TBW mass, which enables the calculation of LBM on the basis of an LBM hydration constant and, subsequently, FM. This method is not without error, but it is the method of choice to generate the TBW component of the 4-compartment model and a hydration constant.

Analysis of the literature indicates that QMR measurements can overestimate or underestimate actual carcass values, depending on the animal species. Therefore, validation within target species is required, and prediction equations for each measurement to correct for method bias appear to be necessary. The objectives of the study reported here were to compare measurements of LBM, FM, and TBW for QMR and DXA with data generated via D_2O dilution as the reference method in cats with a range of ages and BCSs, to compare the precision and accuracy of the QMR method in awake and sedated cats, and to compare body composition measurements obtained with 2 QMR units that had body weight scales with different ranges.

Materials and Methods

Animals—Cats of various body weights and ages were used in 2 trials. In trial 1, 58 cats were scanned with a QMR unit[a] that had a body weight scale that ranged between approximately 4 and 50 kg. In trial 2, 32 cats were scanned with an infant QMR unit[b] that had a body weight scale that ranged between approximately 1 and 12 kg. Trial 1 was conducted in accordance with approved animal care and use committee protocols at the pet-care facilities of CanCog Technologies. Trial 2 was conducted in accordance with approved animal care and use committee protocols at the pet-care facilities of Nestlé Purina PetCare.

QMR and DXA accuracy, compared with results for the D_2O dilution method (trial 1)—Trial 1 was conducted to compare measurements of LBM, FM, and TBW in cats for QMR and DXA with data generated by use of D_2O dilution.

Animals, housing, and care

Domestic shorthair cats (58 cats for QMR and 51 for DXA) were evaluated. Kittens (age, 9 to 12 months; n = 15) and adult cats (age, 2 to 9 years; 43) were selected on the basis of age and BCS. For the DXA analysis, there were 11 kittens (6 males and 5 females) and 40 adult cats (7 males and 33 females). The group of kittens was balanced on the basis of sex, whereas the group of adult cats was not. The BCS was assigned on the basis of a 9-point scale, with a score of 4 or 5 representing an ideal body condition (characterized as a well-proportioned body, observable waist caudal to the ribs, ribs palpable with a slight covering of fat, and a minimal abdominal fat pad).[10,12] A BCS < 4 represented an assessment of diminished body fat tissue (thin category), and a BCS > 6 represented the overweight category.

Prior to the study, cats were housed in groups on the basis of compatibility and sex. Cats were housed indoors with natural lighting and exposure to natural light cycles in environmentally controlled rooms (3.1 × 3.1 × 3.1 m). Cats were provided environmental enrichment consisting of multiple perches, access to toys, and direct interaction with caretakers on a daily basis. Cats had ad libitum access to food and water; however, food and water were removed the night before the trial in preparation for impending sedation of the cats.

QMR

On the day of the trial, a physical examination was performed on each cat to confirm good health status, and body weight was recorded to the nearest 0.01 kg. An initial QMR scan was performed on each cat, and a jugular blood sample then was collected and used to determine background concentrations of deuterium. A dose of D_2O[c] (100 mg/kg; D_2O as 99.8% deuterium) was administered orally as a one-tenth dilution in sterile water via a sterile syringe. Care was used (slow and intermittent administration of the diluted D_2O) to ensure that all of the solution was swallowed by each cat. The volume administered was designed to provide a dose of 1 mL/kg; syringes were weighed before and after administration of D_2O to determine the exact dose administered. Jugular blood samples were collected 120 minutes after D_2O administration and evaluated to ensure equilibration. Serum was separated from blood samples and stored at –80°C until analyzed for deuterium content.

Immediately after collection of the blood sample at 120 minutes after D_2O administration, cats were sedated by IM administration of a combination of dexmedetomidine (0.023 mg/kg), ketamine hydrochloride (2.94 mg/kg), and butorphanol tartrate (0.23 mg/kg). A second QMR scan was performed within 15 minutes after cats were sedated. Immediately after the second QMR scan, the dexmedetomidine was reversed by administration of atipamezole (0.115 mg/kg, IM).

For both scans (awake and sedated), cats were placed in a polymethylmethacrylate crate that allowed only limited movement. The crate was placed within the magnet bore of the QMR unit, which was designed for scanning of animals weighing < 50 kg. Cats were positioned at the magnet isocenter such that their long axis was perpendicular to the long axis of the magnet bore. Data were collected as a standard 3-minute fat and water acquisition in accordance with the manufacturer's protocol.

D_2O dilution method

The D_2O dilution method involves enrichment of body water with the stable (natural and nonradioactive) hydrogen isotope deuterium, followed by determination of the deuterium concentration in a physiologic fluid such as serum. The stable hydrogen isotope contents of the serum samples were measured via gas-isotope ratio mass spectrometry by means of previously validated procedures.[27,28,d] Briefly, the deuterium in 10 µL of serum was converted to H_2 via the zinc reduction method.[27,28] The H_2 was introduced via the automated sample inlet system directly into a Finnigan instrument for measurement of the hydrogen isotope ratio. The precision (ie, SD) for the deuterium assay was 0.10% for samples with natural concentrations of deuterium and 0.18% for samples with enriched concentrations of deuterium.[28]

The isotopic results were normalized against 2 international water standards: Vienna standard mean ocean water and standard light Antarctic precipitation.[29] The isotope dilution space for deuterium was calculated by means of the following equation:

$$N_H \text{ (mol)} = (d \bullet A \bullet E_\alpha)/(\alpha \bullet E_d \bullet 18.02)$$

where N_H is the isotope dilution space for deuterium, d is the dose of D_2O in grams, A is the amount of laboratory water (in grams) used in the dose dilution, E_α is the increase in deuterium concentration in the laboratory water after the addition of the isotopic water, α is the amount of D_2O (in grams) added to the laboratory water in the dose dilution, and E_d is the increase in deuterium concentration in the serum samples at isotopic equilibrium at 120 minutes after D_2O administration. The use of a dose dilution in the calculation of isotope dilution space was recommended by the International Dietary Energy Consultancy Groups to assure accuracy of the isotope dilution calculations.[30]

The value for N_H was converted to TBW by means of the following equation:

$$\text{TBW (mol)} = N_H/1.03$$

where 1.03 is the correction factor to account for incorporation of deuterium into organic molecules during biosynthesis. Lean body mass is assumed to contain 73.2% moisture; therefore, by use of the hydration constant (0.732) for LBM, TBW was converted to LBM via the following equation: LBM = TBW/0.732. Consequently, FM was calculated as body weight minus LBM.

DXA

Because of unanticipated technical issues with the DXA unit, scans could not be performed on the same day as the QMR and D_2O methods. Consequently, DXA was performed on 51 cats within 7 days after QMR and D_2O data collection. Body weight of each cat was recorded, and the cats were sedated as described previously. Sedated cats were placed in sternal recumbency in a DXA unit.[e] Data acquisition was conducted by use of the manufacturer's pediatric small algorithm. The associated software was subsequently used to determine the proportions of LBM, FM, and bone mineral. Sedation was reversed immediately after data collection.

Comparison of infant QMR unit and DXA (trial 2)—Trial 2 was conducted with a separate group of adult cats and involved the use of an infant QMR unit designed to assess animals ranging in body weight up to 12 kg.

Animals, housing, and care

Adult domestic shorthair cats (n = 32) were evaluated. Cats were selected on the basis of age (range, 1 to 13 years), sex, and BCS. Assessment of BCS was similar to that in trial 1. Prior to the study, cats were housed separately or in groups (depending on compatibility and sex) in environmentally controlled rooms. All cats were monitored daily, had access to toys, and had direct interaction with caretakers on a daily basis.

QMR

On the day of the trial, a physical examination was performed on each cat to confirm a good health status. Body weight was recorded to the nearest 0.01 kg. An initial QMR scan was performed in each awake cat; data were collected as a standard set of 3 scans for fat and water content in accordance with the manufacturer's protocol. Cats then were sedated or anesthetized, and a

second set of 3 scans was performed. Nongeriatric (< 7 years old) cats were sedated by IM administration of a combination of medetomidine (0.02 mg/kg), ketamine (5 mg/kg), and butorphanol (0.1 mg/kg). At the end of the QMR data collection, atipamezole (a volume equal to the volume of medetomidine) was administered IM to reverse the sedation. Geriatric (≥ 7 years old) cats were sedated with a combination of buprenorphine (0.01 mg/kg) and glycopyrrolate (0.01 mg/kg) administered SC, which was followed 20 to 30 minutes later by administration of propofol (4 to 6 mg/kg, IV); anesthesia was maintained by administration of isoflurane. Geriatric cats were allowed to recover from anesthesia immediately after data collection.

Similar to trial 1, cats were placed in a polymethylmethacrylate crate for both scans (awake and sedated or anesthetized) and placed within the magnet bore of an infant QMR unit, which was designed for infants and children or other animals weighing < 12 kg.

Assessment of the precision of the QMR method

In a separate experiment, 10 cats were used to assess precision of the QMR method. Five scans were performed for each cat; cats were repositioned between subsequent scans. All 5 scans were performed within approximately 20 minutes. Data were acquired in awake cats and after cats were sedated. Precision of the technique was determined by calculating the CV for each cat and then determining the root mean square for the group.[31]

DXA

On the day of the QMR measurements and immediately following the QMR scan of the sedated or anesthetized cats, the cats were placed in sternal recumbency in a DXA unit.[e] Data acquisition was conducted by means of the manufacturer's small animal algorithm. The associated software was subsequently used to determine the proportions of LBM, FM, and bone mineral.

Statistical analysis—All statistical analyses were conducted with statistical software.[32,f] To characterize body composition measurements obtained via the reference method (D_2O dilution) versus animal demographics for trial 1, an ANOVA was performed to assess the main effects of age, body weight, and BCS on the dependent variables of TBW, LBM, and FM determined via D_2O dilution. Regression coefficients and prediction equations were generated via linear regression analysis. Paired *t* tests were used to assess differences in mean values for body composition components (LBM, FM, and TBW), as measured via the various techniques (QMR [n = 58] or DXA [51]) versus the reference method (D_2O dilution). Significance was determined at a value of $\alpha = 0.05$.

To evaluate the predictive value of the correction equations generated in trial 1 to estimate body composition, a cross-validation (jackknife) analysis was performed as described elsewhere.[33] Briefly, data for each cat were removed from the data set and a correction equation was generated for the remaining 57 cats for QMR or 50 cats for DXA. The QMR or DXA values for each cat were entered into the equations to generate predicted measures of that particular cat's LBM, FM, and TBW. This analysis was performed for each cat, which allowed for a predicted measurement to be generated for each body composition measure for each cat. Paired *t* tests were used to compare predicted values to observed values obtained with the D_2O dilution method. Significance was determined at a value of $\alpha = 0.05$.

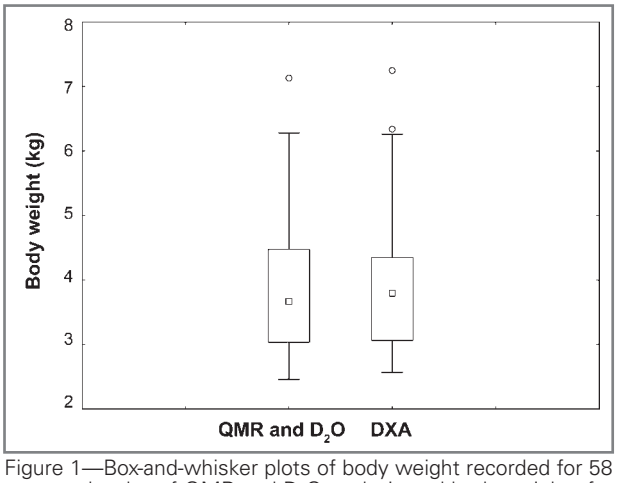

Figure 1—Box-and-whisker plots of body weight recorded for 58 cats on the day of QMR and D_2O analysis and body weights for 51 cats within 7 days after QMR and D_2O data collection on the day of DXA analysis. Each box represents the interquartile range (IQR; 25th to 75th percentiles), the square within each box represents the median, whiskers represent ± 1.5 times the upper or lower IQR, and circles represent outliers (> 1.5 × IQR). Body weights differed by < 5% for all cats, except for 2, between the days on which analyses were performed.

Table 1—Demographics of 58 cats and TBW of those cats determined via the D_2O dilution method.

Group*	Body weight (kg)	Age (y)	BCS†	TBW mass (kg)	TBW as percentage of body weight	
					Mean ± SD	Range
Ideal weight kittens (n = 15)	4.0 ± 0.6	0.9 ± 0.1	5	2.5 ± 0.4	61.9 ± 3.0	58–67
Adult cats						
Thin (n = 2)	2.7 ± 0.1	3.5 ± 1.2	3	1.8 ± 0.02	67.8 ± 1.9	66–69
Ideal weight (n = 23)	3.1 ± 0.4	3.5 ± 1.8	4–5	1.9 ± 0.2	62.2 ± 3.8	56–68
Overweight (n = 18)	5.0 ± 1.1	4.8 ± 2.8	6–8	2.6 ± 0.5	52.0 ± 6.9	46–63‡

Values reported are mean ± SD or range.

*Ideal weight kittens comprised 7 females and 8 males, thin adult cats comprised 2 females, ideal weight adult cats comprised 23 females, and overweight adult cats comprised 10 females and 8 males. †The BCS was scored on a scale of 1 to 9; ideal body weight was a BCS of 4 or 5, thin was a BCS < 4, and overweight was a BCS > 6. ‡Two cats in the overweight category had extreme values (38.3% and 67.4%) that were considered outside the range for this group; however, the values for these 2 cats were included for calculation of the mean ± SD for TBW as a percentage of body weight.

Table 2—Mean ± SD values for body composition determined during trial 1 via the D_2O dilution method, QMR with a QMR unit (scan was obtained with each cat awake but placed in a polymethylmethacrylate crate to minimize movement or sedated to eliminate movement), and DXA.

		QMR			
Body composition component	**D_2O (n = 58)**	**Awake (n = 58)**	**Sedated (n = 58)**	**D_2O (n = 51)**	**DXA (n = 51)**
TBW (kg)	2.27 ± 0.46	2.24 ± 0.40	2.33 ± 0.39	2.26 ± 0.47	NA
LBM (kg)	3.11 ± 0.63	2.97 ± 0.54	3.07 ± 0.54	3.09 ± 0.64[a]	2.80 ± 0.54[b]
FM (kg)	0.83 ± 0.62[a]	0.59 ± 0.59[b]	0.61 ± 0.61[b]	0.83 ± 0.64	0.75 ± 0.63
Free water (kg)	NA	0.032 ± 0.041	0.053 ± 0.048	NA	NA
LBM hydration estimate (%)*					
Excluding free water	NA	74.5 ± 2.7	74.7 ± 2.8	NA	NA
Including free water	76.7 ± 6.3†	75.7 ± 2.3	76.4 ± 2.5	80.7 ± 7.1‡	NA

*Lean body mass hydration estimate determined by excluding the value for free water was calculated as 100•([TBW – free water]/LBM). The LBM hydration estimate determined by including the value for free water was calculated as 100•(TBW/LBM). †Calculated with TBW determined via D_2O dilution in awake cats and LBM determined via QMR in awake cats. ‡Calculated with TBW determined via D_2O dilution and LBM determined via DXA.

NA = Not applicable.

[a,b]Within a row, values with different superscript letters differ significantly ($P < 0.05$).

Results

QMR and DXA accuracy (trial 1)—Results for QMR and DXA were compared with results for the D_2O dilution method.

Relationships between BCS, body weight, and age of cats

The study population included both kittens and adult cats, and cats were grouped on the basis of BCS and mean age and body weight (categories of thin, ideal weight, or overweight). Body weights differed by < 5% for all cats, except for 2, between the days on which scans for QMR and DXA were performed (**Figure 1**).

A significant relationship ($r^2 = 0.671$; $P < 0.001$) was detected between body weight and BCS, but body weight was not related to age ($r^2 = 0.006$). Mean TBW content for body weight and the corresponding TBW as a percentage of body weight, both of which were determined by use of the D_2O method, were summarized (**Table 1**). For all cats, TBW was significantly related to body weight ($r^2 = 0.827$; $P < 0.001$) and BCS ($r^2 = 0.396$; $P < 0.001$). No relationship was detected between age and TBW ($r^2 = 0.005$; $P = 0.54$). Determination of LBM and FM via the D_2O method indicated that LBM was not related to age ($r^2 = 0.005$) and minimally related to BCS ($r^2 = 0.40$). Fat mass also was not related to age ($r^2 = 0.048$) but was significantly related to BCS ($r^2 = 0.74$; $P < 0.001$).

QMR assessment of awake and sedated cats

Quantitative magnetic resonance was used to determine TBW, LBM, and FM in awake cats and subsequently when the cats were sedated. The TBW, LBM, and FM determined via QMR did not differ between awake and sedated cats and differed by 3.9%, 3.4%, and 4.0%, respectively (**Table 2**). Regression analysis of TBW determined via QMR in awake versus sedated cats indicated a significant relationship ($r^2 = 0.891$; $P < 0.001$), with a slope of 0.951 and an intercept of 0.212, which were not significantly different from 1 and 0, respectively. Similarly, regression analysis of LBM determined via QMR in awake versus sedated cats revealed that there was a significant relationship ($r^2 = 0.932$; $P < 0.001$), with a slope of 0.956 and an intercept of 0.238. The slope did not differ significantly ($P = 0.36$) from 1; similarly, the intercept did not differ significantly ($P = 0.068$) from 0. Regression analysis of FM determined via QMR in awake versus sedated cats also revealed a significant relationship ($r^2 = 0.992$; $P < 0.001$), with a slope of 1.032, which differed significantly ($P = 0.001$) from 1, and an intercept of 0.0097, which did not dif-

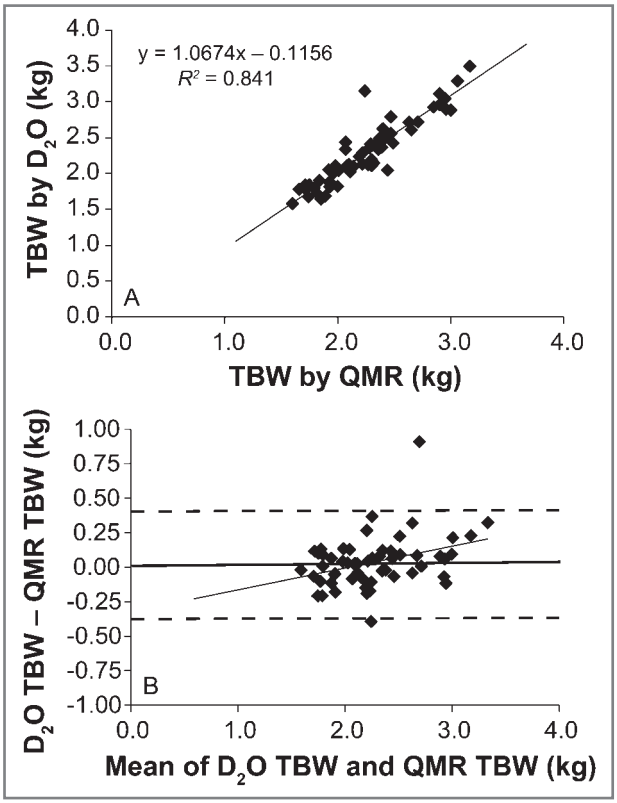

Figure 2—Graph of the relationship for TBW determined via D_2O dilution and QMR in 58 awake cats (A) and a Bland-Altman plot of TBW estimated by use of D_2O dilution and QMR (B). In panel A, each symbol represents results for 1 cat. Notice the line of best fit for the data (thin solid line). In panel B, the thin solid line represents the line of best fit for the data, the thick solid horizontal line represents the mean difference (bias), and the dashed lines represent the limits of agreement (mean difference ± 1.96 SD).

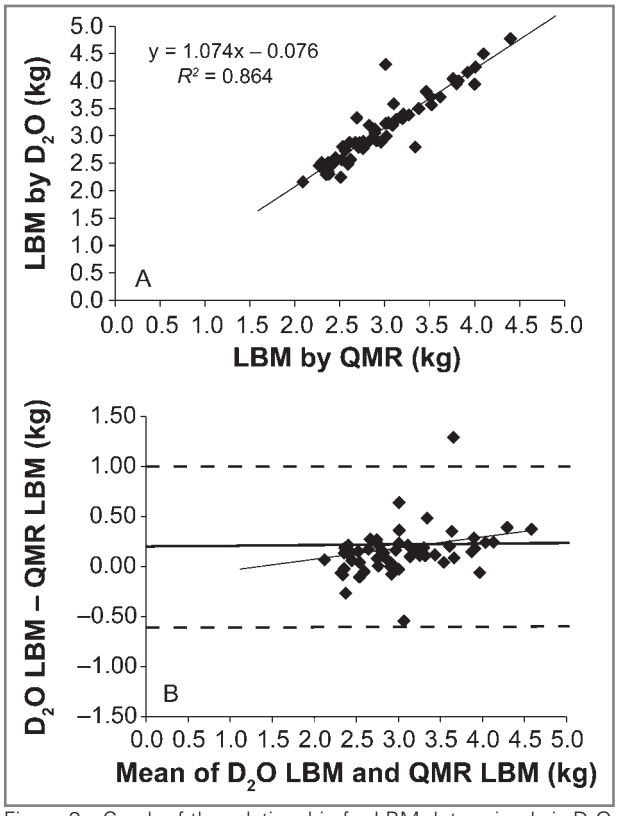

Figure 3—Graph of the relationship for LBM determined via D_2O dilution and QMR in 58 awake cats (A) and a Bland-Altman plot of LBM estimated by use of D_2O dilution and QMR (B). *See* Figure 2 for remainder of key.

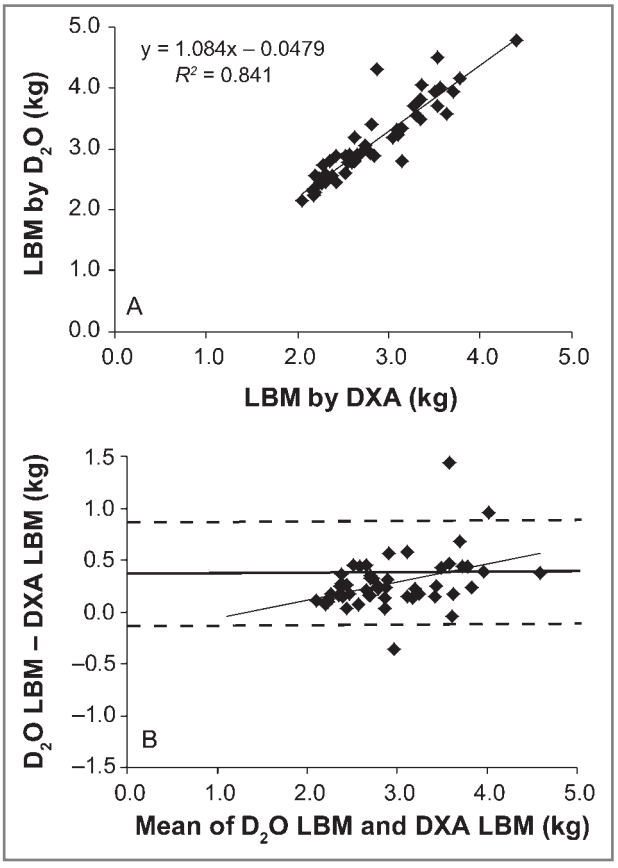

Figure 4—Graph of the relationship for LBM determined via D_2O dilution and DXA in 51 awake cats (A) and a Bland-Altman plot of LBM estimated by use of D_2O dilution and DXA (B). *See* Figure 2 for remainder of key.

fer significantly (P = 0.50) from 0. Because all body composition measures did not differ for QMR acquired with the cats awake or sedated, all subsequent analyses reported for TBW, LBM, and FM for trial 1 used QMR data recorded in awake cats.

The lean tissue hydration constant was calculated with and without the value for free water (Table 2). Although the mean estimate of LBM water content was not different between awake or sedated cats, linear regression analysis of the data from awake versus sedated cats revealed that no relationship (r^2 = 0.02) existed, regardless of the method (with or without the value for free water) of calculating the hydration constant.

Comparison of methods for assessment of TBW

Quantitative magnetic resonance minimally underestimated TBW by 1.4%, compared with the value determined via D_2O dilution (Table 2). Regression analysis of TBW revealed a significant relationship (r^2 = 0.841; P < 0.001) between results obtained with QMR and D_2O dilution (**Figure 2**). Analysis of a Bland-Altman plot did not reveal a significant bias for QMR to overestimate or underestimate TBW with increasing TBW.

Comparison of methods for assessment of LBM and FM

Both QMR and DXA underestimated LBM, compared with results obtained with the D_2O method (Table 2). Use of QMR resulted in underestimation of body mass by 4.4%, which did not differ significantly (P = 0.21) from the value for the D_2O method. However, DXA underestimated LBM by 9.2%, which differed significantly (P = 0.017) from the value for the D_2O method. Regression analysis of LBM comparing results for QMR and D_2O dilution indicated a significant relationship (r^2 = 0.864; P < 0.001 [**Figure 3**]). The slope and intercept were not significantly different from 1 (P = 0.69) and 0 (P = 0.85), respectively. Analysis of a Bland-Altman plot revealed that no significant (P = 0.25) bias existed for QMR to overestimate or underestimate LBM with increasing LBM. Similarly, regression analysis of LBM comparing results for DXA and D_2O dilution also revealed a significant relationship (r^2 = 0.841; P < 0.001 [**Figure 4**]). The slope and intercept were not significantly different from 1 (P = 0.31) and 0 (P = 0.73), respectively. Analysis of a Bland-Altman plot revealed a significant (P = 0.005) bias in the relationship between DXA and D_2O determinations of LBM, with the amount of underestimation becoming greater with increasing LBM.

Quantitative magnetic resonance significantly (P = 0.04) underestimated FM by 28.9%, compared with results for the D_2O dilution (Table 2). However, a significant relationship (r^2 = 0.892; P < 0.001) was detected between results for the QMR and D_2O methods (**Figure 5**). Analysis of the regression variables indicated that the intercept was significantly (P < 0.001) different from 0, but the slope was not significantly (P = 0.15) different from 1. Although FM was significantly un-

derestimated by QMR, analysis of a Bland-Altman plot indicated that no significant bias existed in the determination of FM between the QMR and D_2O methods with increasing FM.

Fat mass determined with DXA did not differ significantly ($P = 0.53$), compared with the value determined with the D_2O method, and minimally underestimated FM by 9.3% (Table 2). Similar to results for the QMR, regression analysis of FM determined by DXA and D_2O dilution revealed a significant relationship ($r^2 = 0.867$; $P < 0.001$ [**Figure 6**]). The intercept was significantly ($P = 0.025$) different from 0, but the slope was not significantly ($P = 0.082$) different from 1. Analysis of a Bland-Altman plot revealed no bias for DXA to overestimate or underestimate FM with increasing FM.

The significant relationships between values determined with D_2O dilution and QMR as well as D_2O dilution and DXA allowed for the generation of correction equations for TBW, LBM, and FM via linear regression analysis (**Table 3**). The prediction models were evaluated via a cross-validation procedure and indicated that LBM, FM, and TBW in the cats could be predicted from

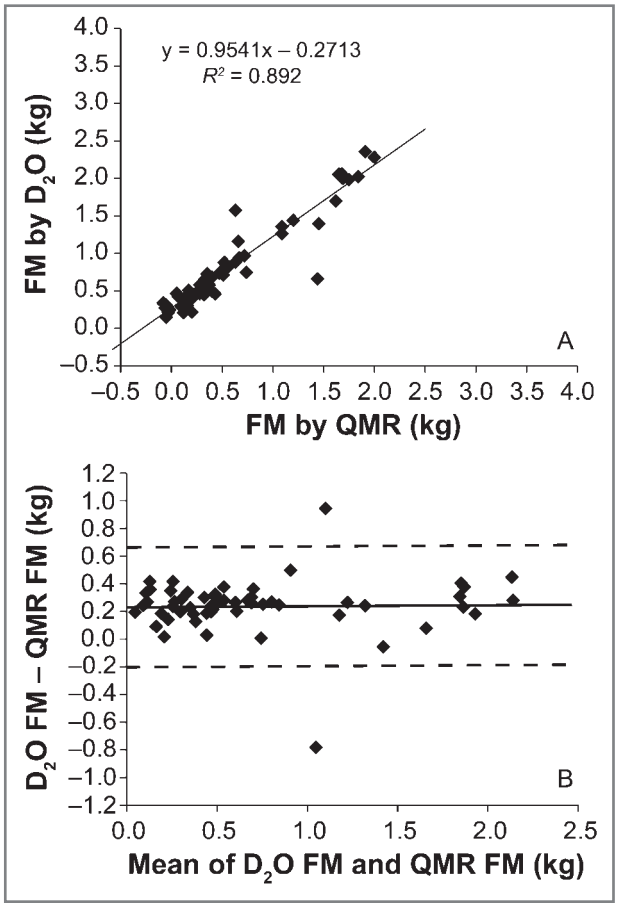

Figure 5—Graph of the relationship for FM determined via D_2O dilution and QMR in 58 awake cats (A) and a Bland-Altman plot of FM estimated by use of D_2O dilution and QMR (B). *See* Figure 2 for remainder of key.

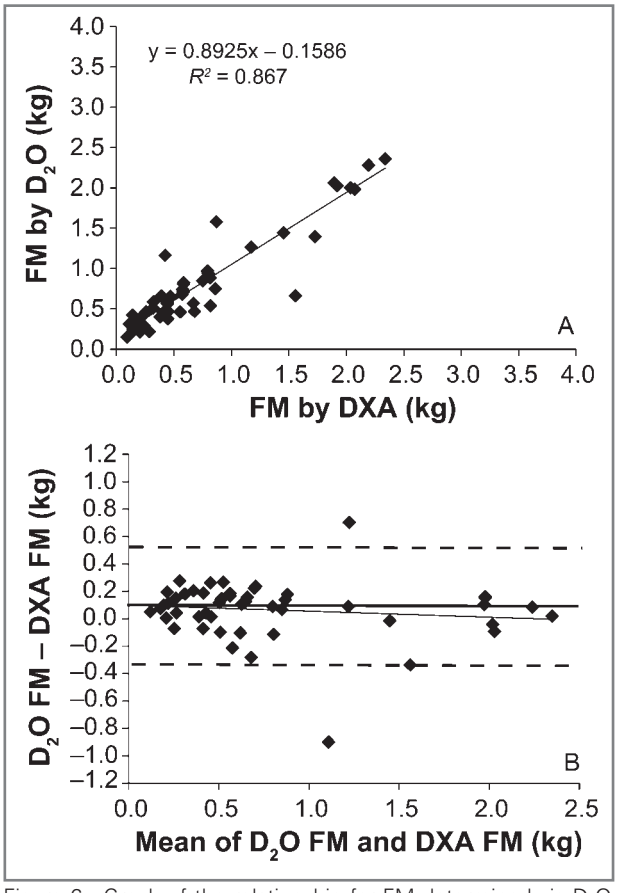

Figure 6—Graph of the relationship for FM determined via D_2O dilution and DXA in 51 awake cats (A) and a Bland-Altman plot of FM estimated by use of D_2O dilution and DXA (B). *See* Figure 2 for remainder of key.

Table 3—Correction equations for TBW, LBM, and FM prediction for DXA and QMR (scan obtained with each cat awake but with minimal movement) on the basis of results for the D_2O dilution method.

			Cross-validation*	
Dependent variable	**Regression equation**	**Model r^2**	**Absolute difference (kg)**	**Percentage difference**
TBW				
QMR	$(1.0674 \bullet QMR_{TBW\ [in\ kg]}) - 0.1156$	0.841†	2.28 ± 0.06	5.5 ± 0.7
LBM				
QMR	$(1.074 \bullet QMR_{LBM\ [in\ kg]}) - 0.076$	0.864	3.11 ± 0.08	4.5 ± 0.7
DXA	$(1.084 \bullet DXA_{LBM\ [in\ kg]}) + 0.0479$	0.841	3.09 ± 0.08	5.2 ± 0.7
FM				
QMR	$(0.9541 \bullet QMR_{FM\ [in\ kg]}) + 0.2713$	0.892	0.83 ± 0.07	18.8 ± 3.6
DXA	$(0.8925 \bullet DXA_{FM\ [in\ kg]}) + 0.1586$	0.867	0.83 ± 0.08	23.3 ± 3.7

*Mean ± SE values for the absolute difference and the percentage difference between predicted and actual measurements for the D_2O dilution method. †Value of the model r^2 was significant ($P < 0.001$).

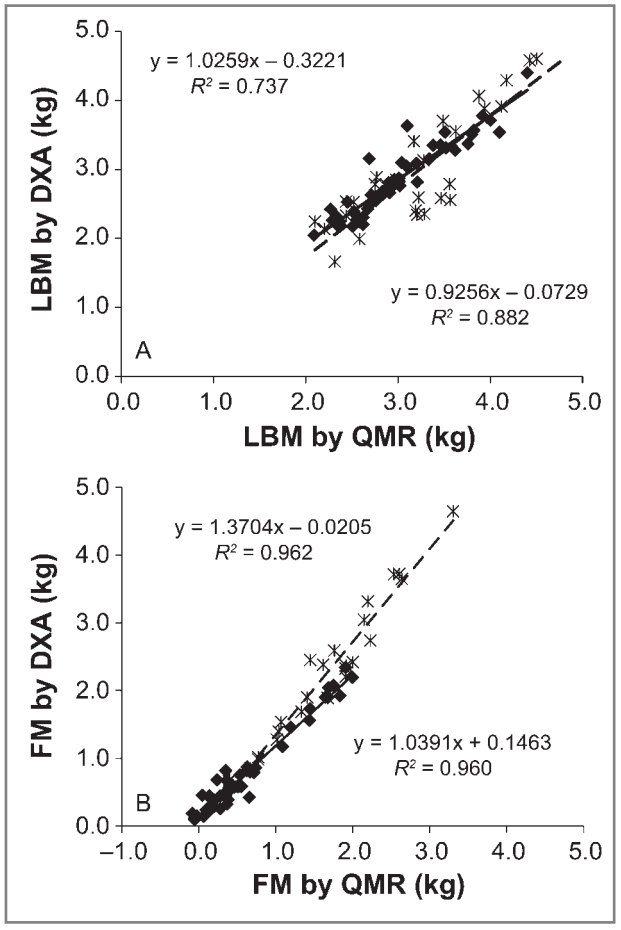

Figure 7—Graph of the relationship of LBM (A) and FM (B) determined via DXA and QMR by use of a QMR unit (diamonds) or an infant QMR unit (crosses). Notice the line of best fit for the DXA-QMR unit (solid line) and the DXA–infant QMR unit (dashed line). The top regression equation in each panel represents results for data generated with the infant QMR unit, whereas the bottom regression equation in each panel represents results for data generated with the QMR unit.

QMR and DXA results. The mean errors of QMR-measured LBM, FM, and TBW were 4.5%, 18.8%, and 5.5%, respectively, whereas the mean errors of DXA-measured LBM and FM were 5.2% and 23.3%, respectively. For each body composition component, the mean of the predicted values did not differ significantly ($P > 0.7$ for all analyses) from observed values.

Comparison of QMR and DXA for determining LBM and FM

Although a direct comparison between QMR and DXA was not the original intent of the study, LBM and FM data obtained via these 2 methods were compared. There was not a significant difference in LBM ($P = 0.11$) or FM ($P = 0.11$) determined via QMR, compared with results determined with DXA; however, QMR overestimated LBM by 5.4% and underestimated FM by 22%. Lean body mass values determined via QMR and DXA were significantly related ($r^2 = 0.883$; $P < 0.001$ [**Figure 7**]). The slope was 0.926, and the intercept was 0.073. Analysis of the regression variables indicated that the slope was significantly ($P = 0.045$) different from 1 but the intercept was not significantly ($P = 0.38$) different from 0. Fat mass determined with QMR did not differ significantly ($P = 0.11$), compared with the FM determined with DXA; however, these means differed by 24%. Although QMR underestimated FM, there was a significant relationship ($r^2 = 0.960$; $P < 0.001$), with a slope of 1.04 and intercept of 0.146. Analysis of the regression variables indicated that the slope was not significantly ($P = 0.07$) different from 1 but the intercept was significantly ($P < 0.001$) different from 0.

Table 4—Mean ± SD values for body composition components determined via QMR and DXA in 32 cats during trial 2.

Body composition component	QMR		DXA
	Awake	Sedated or anesthetized	
TBW (kg)	2.25 ± 0.44	2.25 ± 0.50	NA
Free water (kg)	0.030 ± 0.016	0.038 ± 0.022	NA
Lean mass (kg)	3.15 ± 0.67	3.08 ± 0.66	2.94 ± 0.77
FM (kg)	1.42 ± 0.81[a]	1.42 ± 0.81	1.92 ± 1.12[b]
Hydration estimate (%)			
Excluding free water	70.8 ± 2.8	71.9 ± 5.8	NA
Including free water	71.8 ± 2.9	73.2 ± 5.7	NA

See Table 2 for key.

Comparison of infant QMR unit and DXA (trial 2)—Trial 2 was conducted with a separate group of adult cats and involved the use of an infant QMR unit designed to assess animals ranging in body weight up to 12 kg.

Relationships between BCS, body weight, and age

The cats included only adult cats (mean ± SE age, 7.6 ± 0.6 years), with a mean body weight of 5.0 ± 0.2 kg and BCS that ranged from 3 to 9. For all cats, a significant relationship ($r^2 = 0.577$; $P < 0.001$) was detected between body weight and BCS (data not shown); however, there was not a significant relationship ($r^2 = 0.002$; $P = 0.81$) between body weight and age (data not shown).

QMR precision

Body fat, LBM, and TBW were determined only in awake cats. The precision for body fat, LBM, and TBW determination was high, with a CV of 2.9%, 2.0%, and 2.9%, respectively. Precision for determination of free water was extremely low (CV, 73%). The hydration constant was calculated with and without the inclusion of the value for free water, and the precision was high (CV, 3.0%), regardless of the calculation method used.

QMR assessment of awake and sedated or anesthetized cats

In trial 2, QMR was again used to determine TBW, LBM, and FM in both awake and sedated or anesthetized cats. In contrast to trial 1, scans were acquired in triplicate without removing the cat from the crate. Means for body composition components were summarized for the initial scan (for comparison with data obtained in trial 1) and triplicate data (**Table 4**). Total body water, LBM, and FM did not differ significantly between awake and sedated or anesthetized cats; values for these 3 components differed by 1.2%, 1.8%, and

0.8%, respectively (on the basis of data obtained during the initial scan), or 0.2%, 2.0%, and 0.2%, respectively (on the basis of the mean of the triplicate data). The triplicate data were used for subsequent reporting of significant differences and regression analyses. Regression analysis of TBW determined via QMR in awake and sedated or anesthetized cats indicated a significant relationship (r^2 = 0.890; $P < 0.001$), with a slope of 1.059 and an intercept of –0.129 that were not significantly different from 1 and 0, respectively. Similarly, regression analysis of LBM determined via QMR in awake and sedated or anesthetized cats revealed that values were significantly related (r^2 = 0.983; $P < 0.001$), with a slope of 0.976 and an intercept of 0.014 that were not different from 1 and 0, respectively. In addition, regression analysis of FM determined via QMR in awake and sedated or anesthetized cats also revealed a significant relationship (r^2 = 0.997; $P < 0.001$), with a slope of 0.991 and intercept of 0.010 that did not differ from 1 and 0, respectively. Subsequent analysis of LBM and FM to compare QMR and DXA results used the QMR data recorded in awake cats.

The lean tissue hydration constant was calculated with and without the value for free water (Table 4). The mean estimate of LBM water content was not significantly different between awake and sedated or anesthetized cats. In contrast to results for trial 1, there was low relative agreement (r^2 = 0.32) between awake and sedated or anesthetized cats following a linear regression analysis, regardless of the method of calculating the hydration constant. It is worth mentioning that the SE of the data for awake cats was 50% lower, compared with the SE when the data were obtained for sedated or anesthetized cats.

Comparison of QMR and DXA to determine LBM and FM

Quantitative magnetic resonance quantitatively overestimated LBM by 7.1%, compared with the value determined via DXA (Table 4). However, LBM determined via QMR and DXA was significantly related (r^2 = 0.728; $P < 0.001$ [**Figure 7**]). The slope (0.97; P = 0.83) and intercept (–0.127; P = 0.72) were not significantly different from 1 and 0, respectively. Analysis of a Bland-Altman plot indicated that a significant bias existed for the relationship between QMR and DXA determinations of LBM, with the overestimation being significantly (P = 0.007) greater with increasing LBM.

A greater difference was observed between QMR and DXA for the determination of FM, and QMR significantly (P = 0.045) underestimated the value by 26%, compared with the value obtained with DXA. Similar to LBM, a significant relationship was detected for FM measurements between QMR and DXA (r^2 = 0.962; $P <$ 0.001). The slope was 1.350 and was significantly ($P <$ 0.001) different from 1, but the intercept was 0.003 and was not significantly (P = 0.97) different from 0.

Discussion

Several methods have been established for determining body composition components,[24] but each method has advantages and disadvantages as well as differences in precision and accuracy. Excessive body weight and an increase in obesity among cats and dogs is a growing concern, and veterinary clinicians rely primarily on subjective assessment of body fat through body condition scoring, which has been successfully validated against DXA in several studies.[10–12,34,35] Although DXA is not readily available for clinical use, DXA has been used by veterinarians and animal scientists in research settings at specialty clinics or teaching institutions to more objectively assess changes in body weight. However, DXA requires that an animal be anesthetized to prevent movement, which increases the health risks for extremely young, old, or diseased animals, which consequently limits routine use of DXA to only healthy and adult animals.

Use of a technology such as QMR can allow veterinarians and animal nutritionists at a research or teaching institution to safely and more routinely assess loss of adiposity as well as evaluate maintenance of LBM after interventions. This would be particularly beneficial in older or diseased pets that are underweight or overweight because it would not require the need for anesthesia (and the accompanying risks) and could provide a promising advancement for studies on weight loss, weight management, or aging.

Initially, evaluation of the QMR method in the present study revealed the precision and accuracy of QMR to measure TBW, LBM, and FM in both awake and sedated or anesthetized cats. Consequently, this study validated the use of the QMR method as an effective means of rapidly and accurately measuring body composition in awake cats.

In trial 2 of the present study, the infant QMR unit (body weight range, 1 to 12 kg) had a mean CV < 3.0% when awake cats were scanned. Precision of the QMR unit used in trial 1 (body weight range, 4 to 50 kg) was assessed in awake and sedated cats in another study,[36] and a mean CV < 6.3% for precision was found for all measures when evaluating cats ranging from 3.7% to 32% body fat. The precision of both instruments was similar, except for body fat for that QMR unit, for which the CV was 6.3% when including all cats and 3.8% when 2 cats with < 5% body fat were excluded. Precision data generated via that QMR unit were only slightly better when cats were sedated in that other study,[36] and the difference in CV was < 0.2%. Consequently, this justified assessing precision with only awake cats via the infant QMR unit in trial 2. With either unit, precision of the technique was sufficiently high and indicated that accurate data can be obtained during in vivo studies with awake cats.

The precision for QMR in the present study was less than that in other studies. The CV of 2.9% for FM obtained in this study was higher (which indicated less precision) than that in other species (eg, 0.2% to 1.4% in humans,[20–22] < 1.0% in rats,[15] 1.3% in pigs,[18] and 1.3%[37] and 1.5%[14] in live mice). For LBM, the CV of 2.0% was not as good as that for rats (< 0.4%) in another study,[15] but it generally was similar to CVs attained in studies in live mice (0.8%[14] or 3.0%[37]) and pigs (0.9%).[18] Finally, for TBW, the CV of 2.9% indicated less precision than for rats (< 0.74%)[15] or baby pigs (0.9%).[18] To assess method precision in data recorded from cats, QMR in the present study was more precise for determination of

FM, compared with previously reported data obtained by use of DXA (CV, 5.6%),[6] but slightly less precise for determination of LBM than was DXA (CV, 0.9%).[6] Some of the difference may be attributed to the method used in the DXA study[6] because precision was determined by 4 to 10 repeated scans without repositioning in a group of 5 cats.

We used 2 QMR units to measure TBW, LBM, and FM in cats that were awake or not moving (sedated or anesthetized). Although both QMR units resulted in similar, accurate LBM and TBW measurements, the differences between measurements while awake and without movement were greater with the QMR unit designed for animals between 4 and 50 kg than with the infant QMR unit. This suggested that variation was increased when obtaining data from animals near the lower body weight range for the QMR unit with a range between 4 and 50 kg, but variation was also likely impacted by acquiring only a single scan for each cat. As might be expected, accuracy was improved for measurements when collecting triplicate scans with the infant QMR unit. The percentage difference decreased between body composition means for awake versus sedated or anesthetized cats, and the r^2 values improved the greatest for TBW and the LBM hydration constant when 3 scans were used instead of 1. Specifically, the r^2 for TBW improved from 0.81 to 0.89 and the LBM hydration constant improved from 0.01 to 0.32. Coefficients for LBM (r^2 = 0.98) and FM (r^2 = 0.997) also improved, but they were already high (r^2 = 0.97 and 0.98, respectively). On the basis of these results, it was likely that accuracy of the QMR unit with a weight range of 4 to 50 kg would be improved if triplicate scans had been performed during trial 1. Regardless, a high degree of confidence can be placed in the accuracy of data obtained in awake cats with either QMR unit.

Analysis of the literature indicated that the underestimation or overestimation of FM, TBW, and LBM by QMR, compared with results for the reference methods, differs substantially by species. In the present study, QMR measurements for awake cats underestimated all 3 body composition components, compared with results for D_2O dilution. Quantitative magnetic resonance was highly accurate for measurement of TBW, with a difference of only 1.4%, compared with an underestimation of 5%[15] or 3.1%[19] in rats and 6.4% in baby pigs[18] and an overestimation of 4% in chickens,[23] which were all evaluated via chemical carcass analysis. In the present study, the accuracy of determining LBM for cats via QMR (4.4%) was similar, compared with that in other species. Lean body mass was underestimated by 12.5% in rats,[15] 7.8% in mice,[14] 3.6% in baby pigs,[18] and 1.1% in chickens[23] and overestimated by 2.1% in other baby pigs.[19]

Although QMR was least accurate for the determination of FM in cats, underestimation by 29% is comparable to variation for determining FM in several other QMR studies. Specifically, QMR overestimated FM by 29% in mice[14] and underestimated FM by 34% in chickens.[23] Other studies have revealed that more accurate measures can be attained, with an overestimation of as little as 6% in rats[15] and 4.7%[19] or 2.1%[18] in baby pigs. In humans, FM was underestimated more in males (range of underestimation, 13% to 18%) than females (range of underestimation, 2% to 8%).[20,21] Depending on the reference method used for assessment in infants, FM was overestimated by 10%, compared with results for the 4-compartment model, whereas it was underestimated by 4%, compared with results for D_2O dilution.[22]

Overall, the correction equations developed in the present study will improve the accuracy of data obtained for feline body composition determined via QMR, particularly for determination of FM. The significant linear relationships between the QMR and D_2O dilution data indicated that QMR can be confidently used for in vivo studies in cats. A jackknife cross-validation procedure with QMR values was able to predict the TBW, LBM, and FM, with mean errors of 5.5%, 4.5%, and 18.8%, respectively (Table 3). Cross-validation of DXA also predicted LBM and FM with similar mean errors of 5.2% and 23.3%, respectively. These percentages represent the mean relative difference between the predicted body composition measurements, compared with the actual D_2O-generated values. Another study[33] to assess DXA measurements of snakes revealed similar but slightly better mean errors, with prediction of FM also being the least accurate. The increase in variation observed in the data for the present study was partially contributed by including small cats with a low percentage of body fat. However, the correction equations equally predict FM, regardless of measurements obtained with QMR or DXA. The high percentage difference detected with the cross-validation assessment of FM for the QMR data was largely influenced by 4 cats that had > 50% error between predicted and observed D_2O dilution–determined FM. Post hoc analysis of the percentage mean difference without these 4 cats reduced the mean value for QMR to 12.7%, which indicated that the QMR correction equation for FM predicted FM for 54 of 58 (93%) cats in the study within 87.3% of actual FM, as determined on the basis of D_2O dilution as the reference method. The QMR correction equations accurately predicted TBW or LBM for all cats in the study within at least 95% of actual TBW or LBM.

Analysis of the data indicated that compared with D_2O dilution as the reference method, both QMR and DXA underestimated FM (29% or 9.3%, respectively) and LBM (4.4% and 9.2%, respectively). Investigators in another study[5] determined that DXA underestimated FM by a mean of 2.0% and LBM by 2.6% in dogs and cats in a combined data set, compared with results for chemical carcass analysis, but that substantial interanimal variation existed. Other methods have been assessed for their ability to measure TBW in cats. Dual-energy x-ray absorptiometry resulted in a 1.6% difference from results for carcass analysis.[5] Bioelectrical impedance differed by < 0.1% to 6.5%, compared with results for D_2O dilution, with the variation depending on the placement of the electrodes on anesthetized cats.[38,39] Therefore, the similar underestimation of TBW by 1.4% with QMR in the present study, compared with results for D_2O dilution, supports the use of D_2O dilution to validate the use of QMR in cats.

Comparison between trials 1 and 2, which assessed different QMR units and evaluated separate popula-

tions of cats, revealed that results for QMR and DXA consistently differed, and QMR overestimated LBM by 5.4% (trial 1) or 7.1% (trial 2) and underestimated FM by 22% (trial 1) or 26% (trial 2). Despite this difference in FM determination, the QMR method appears to consistently underestimate FM determined via DXA across multiple species, including rats (approx 46%),[17] baby pigs (14.5%),[18] chickens (57%),[23] and adult human males (15.1%), but there is no difference for adult human females.[21] A notable limitation of trial 1 was the interval between the acquisition of QMR and DXA data, which was the result of unanticipated technical issues. However, given that the change in weight between the 2 time points was < 5% for all but 2 cats, there was no change in the distribution of the data between the 2 time points (Figure 1). Thus, the relative differences between DXA and QMR data for trials 1 and 2 were similar, and we believe that the effect on the conclusions was minimal.

Because QMR can be used to accurately determine TBW and LBM in cats, it appears feasible that this method may provide a quick and objective means of assessing LBM hydration status. This determination would be based on the same relationship that is routinely used with the D_2O method to estimate LBM content by use of the LBM water constant. The hydration constant associated with LBM is generally considered to be 73.2%.[31,32] The LBM hydration constant for each cat in trial 1 was derived from the QMR-based value for body water divided by the QMR-based value for LBM and yielded a mean estimate of 75.7% and 76.4% in awake and sedated cats, respectively. In contrast, trial 2 yielded a mean estimate of 71.8% and 73.2% in awake and sedated or anesthetized cats, respectively. In both trials, exclusion of the free water value generally resulted in a reduction of 1.5% in the estimated hydration constant. In comparison, a hydration constant was calculated with the D_2O dilution–based value for TBW, which was divided by the LBM value determined with DXA or QMR. In either calculation, the mean and variation were greater than the estimates generated with QMR (Table 2).

Importantly, data obtained with the larger QMR unit or infant QMR unit were used to confirm that the reliability of the hydration constant requires that the accuracy of the individual variables be extremely high, considering that no agreement was observed in the relationship of awake or sedated cats with the QMR unit for trial 1 data ($r^2 = 0.02$) when only a single scan was performed on the cats. The infant QMR unit had much better agreement ($r^2 = 0.32$), probably as a result of the much higher accuracy in generating the TBW and LBM measures for each animal. Most interesting, multiple scans apparently are required for ensuring an accurate assessment of LBM hydration, given that the precision data for the QMR unit precision data indicated that agreement improved considerably ($r^2 = 0.67$) when the mean of 5 scans was compared between values obtained for awake and sedated cats. This indicated that both high precision and multiple scans improve the reliability when measuring LBM hydration in awake cats.

The present study indicated that QMR is a useful technique for the determination of body composition in cats and is suitable for longitudinal studies, which is an improvement on the precision associated with evaluation of FM via DXA. Furthermore, QMR does not have the disadvantage of requiring anesthesia of subjects. Of particular advantage, QMR provided a safe, quick, and accurate method of assessing healthy awake adult cats. More importantly, it can be used in cats that are extremely young, extremely old, or have some degree of compromised health that would make them unsuitable for sedation or anesthesia procedures. Therefore, this allows for the ability to determine TBW and other body composition components in these groups of cats. These attributes make the QMR technique readily applicable to clinical practice, with distinct advantages over DXA and D_2O dilution, particularly the lack of the need to sedate or anesthetize subjects and the fact it is completely noninvasive. None of the subjects had been trained to be placed in the polymethylmethacrylate crate, and it is highly likely that this would be accepted by most client-owned cats, especially if they are accustomed to being transported in a pet carrier. Comparison with results for the D_2O dilution method allowed the development of correction equations to provide accurate data over a range of body composition values, although there were some limitations for cats with a FM less than approximately 5% of body weight when the QMR unit was used. Finally, the greater precision of QMR, compared with results for DXA (CV, 2% vs 6%[1,28]), implies that QMR will be a valuable technique for use in weight management of individual animals on a longitudinal basis. Although BCS determined by experienced personnel is a convenient and practical measure of body composition, it is qualitative in nature, whereas QMR provides a reliable quantitative measure.

a. EchoMRI-D QMR analyzer, Echo Medical Systems, Houston, Tex.
b. EchoMRI-Infants QMR analyzer, Echo Medical Systems, Houston, Tex.
c. D_2O, Sigma-Aldrich, St Louis, Mo.
d. Gas-Isotope-Ratio Mass Spectrometry Laboratory, USDA Agricultural Research Service Children's Nutrition Research Center, Baylor University, Waco, Tex.
e. Lunar DPX-IQ DXA unit, GE Healthcare, Waukesha, Wis.
f. SAS, version 9.1.2, SAS Institute Inc, Cary, NC.

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