Evaluation of a Quantitative Magnetic Resonance Imaging System for Whole Body Composition Analysis in Rodents

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We evaluated the EchoMRI-900 combination rat and mouse quantitative magnetic resonance (QMR) body composition method in comparison to traditional whole-body chemical carcass composition analysis (CCA) for measurements of fat and fat-free mass in rodents. Live and postmortem (PM) QMR fat and lean mass measurements were obtained for lean, obese and outbred strains of rats and mice, and compared with measurements obtained using CCA. A second group of rats was measured before and after 18 h food or water deprivation. Significant positive correlations between QMR and CCA fat and lean mass measurements were shown for rats and mice. Although all live QMR fat and lean measurements were more precise than CCA for rats, values obtained for mice significantly differed from CCA for lean mass only. QMR performed PM slightly overestimated fat and lean values relative to live QMR but did not show lower precision than live QMR. Food deprivation reduced values for both fat and lean mass; water deprivation reduced estimates of lean mass only. In summary, all measurements using this QMR system were comparable to those obtained by CCA, but with higher overall precision, similar to previous reports for the murine QMR system. However, PM QMR measurements slightly overestimated live QMR values, and lean and fat mass measurements in this QMR system are influenced by hydration status and animal size, respectively. Despite these caveats, we conclude that the EchoMRI QMR system offers a fast *in vivo* method of body composition analysis, well correlated to but with greater overall precision than CCA.

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INTRODUCTION

In studying the neural, physiological and endocrine mechanisms underlying propensity for weight gain, it is often desirable to quickly obtain accurate, repeated measurements of body composition. Because many of the comorbidities associated with obesity are specifically caused by excess body fat, rather than excess weight, assessment methods such as weight or BMI that estimate but do not specifically measure body fat are not adequate for use in primary research or clinical studies (1). A number of methods have been developed to determine body composition in experimental animal models used in obesity research. Traditional methods of determining body fat and lean mass, such as whole-body carcass composition analysis (CCA), are time-consuming and terminal procedures (2), precluding longitudinal repeated studies. Newer methods such as bioelectrical impedance analysis (BIA) and dual-energy X-ray absorptiometry (DXA) allow repeated measures in live subjects, however both methods require the use of anesthesia to immobilize study animals. In addition, BIA estimates of body composition show a greater magnitude of error in obese subjects (3,4), whereas DXA can take between 5 and 35 min per subject, depending on the size of the animal and the desired scan resolution (5). Body composition scanning using quantitative magnetic resonance (QMR) appears to be superior to CCA, BIA, and DXA methods in that QMR offers rapid measurement of body composition in live, unanesthetized animals. Although BIA directly measures only total water and DXA directly measures only two variables (fat and nonfat), QMR measurements utilize inherent differences in the nuclear magnetic resonance properties of hydrogen atoms and hydrogen density in fluids and tissues to derive estimates of fat mass, lean mass, total body water, and free water (body fluids not bound

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in tissues). Devices using these QMR methods are available for use with both human and animal subjects (6–11).

Although whole-body QMR systems designed specifically for mice and humans have previously been evaluated (9,11), no similar comparison has been performed for a QMR system designed for use with both rats and mice. To address this issue, we evaluated the precision and accuracy of QMR in comparison to traditional whole-body CCA, using the EchoMRI-900 (Echo Medical Systems, Houston, TX), a device designed to analyze both rats and mice, to address four goals: First, to compare data obtained from QMR and CCA analyses for outbred, lean, and obese strains of rats and mice; second, to determine whether postmortem (PM) and live QMR estimates of fat and lean mass are comparable; third, to compare the precision of QMR and CCA methods; and fourth, to determine the effects of short periods of food or water deprivation on QMR body fat and lean mass estimates.

METHODS AND PROCEDURES

To compare data obtained from QMR with CCA analyses, we examined adult male rats and mice of varying body adiposity and genetic background. For each species, we examined one outbred, one lean, and one obese strain. For rats, Fisher rats (F344/NCr, National Cancer Institute, Frederick, MD; $n = 6$) were used as an outbred strain, and obesity-prone (OP, $n = 6$) and obesity-resistant (OR, $n = 5$) Sprague–Dawley rats (OP-CD and OR-CD, Charles River, Wilmington, MA) were used as obese and lean animals, respectively. For mice, we examined BALB/cJ outbred, ($n = 12$), B6.V-*Lep*^{ob}/J obese ($n = 12$), and black six C57BL/6J lean mice ($n = 12$) (Jackson, Bar Harbor, ME). For food and water deprivation studies, an additional seven Sprague–Dawley rats (Charles River) were used. Animals were housed individually (rats) or in groups (mice) in cages with a 12:12 light–dark cycle, with lights on at 0700 hours. Rodent chow (8604; Harlan Teklad, Madison, WI) and water were allowed *ad libitum*, except as described below for deprivation studies. All studies described here were approved by the local Institutional Animal Care and Use Committee at the Veterans Affairs Medical Center and the University of Minnesota.

All QMR measurements were made during the light phase (0700–1900 hours). Scans were performed by placing animals into a thin-walled plastic cylinder (mice: 1.5 mm thick, 4.7 cm inner diameter; rats: 3 mm thick, 6.8 or 8.2 cm inner diameter, based on body weight), with a cylindrical plastic insert added to limit movement. While in the tube, animals were briefly subjected to a low-intensity (0.05 Tesla) electromagnetic field to measure fat, lean mass, free water, and total body water. The general theoretical background and specific technical details describing the basic functionality of this system is well-described by Tinsley *et al.* (9). 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, lean muscle mass equivalent, total body water, and free water. It is important to note that the system used by Tinsley was an earlier model than the Echo device used in the previous study; in current generation systems, all four components are obtained from individual relaxation curves, whereas in previous models total body water was a derived estimate. Because each component is estimated based on an individually derived T1/T2 relaxation curve fractionated from the total returned signal, we consider each estimate to be a direct measurement. For rats, QMR scans were performed with accumulation times of 2 min. All mice in this study were scanned using a 4-min accumulation. This longer scan time for mice is recommended by the manufacturer for this combination machine; whereas short scan times are possible for mice, our initial investigations showed significantly higher variability for QMR fat mass values vs. CCA in mice at 2-min accumulation times (data not shown). For deprivation studies, rats were scanned once both before and after 18 h deprivation of either food or water, and scanned again 24 and 48 h after food or water was returned. For QMR measurements used for comparison with CCA, three sets of scans were performed in triplicate on each animal: Live scans, PM scans on intact carcasses, and postpreparation (PP) scans on shaved, eviscerated carcasses. Live subjects were returned to their cages between scans. For PM and PP scans, animals were warmed to 37 °C (verified by an internal temperature probe) prior to each scan, and repositioned between scans.

PM and PP QMR scans were performed to mimic the procedure used by investigators that ship animals to another location for QMR measurements. Following live QMR measurements, animals were killed by CO_2 asphyxia, enclosed individually in airtight plastic bags, and cooled to 4 °C for 24–48 h. For PM scans, intact carcasses were warmed to 37 °C in a hybridization oven prior to PM QMR measurements but were not removed from plastic bags to limit water loss. For PP scans, subjects were prepared for CCA analysis by removal of body hair and gut contents. After rewarming to 37 °C, PP QMR scans were performed on the eviscerated carcasses, and carcasses were stored frozen at −70 °C.

For CCA analysis, thawed carcasses were homogenized in double-distilled water. Aliquots of homogenate were used to determine total lipid content by chloroform extraction (12), and a separate set of aliquots were placed in an ashing oven to determine protein content (13). Three samples from each animal were analyzed for lipid content. For protein content, three samples per rat were analyzed, whereas for mice, three samples were obtained where possible, however only two samples were available for three mice (two black six, one BALB/c) due to small carcass size. For CCA, a two-compartment model was used, with fat mass defined as total lipid weight, and lean mass measurements defined as water weight plus ash and protein weights. A bootstrap procedure that calculated all potential combinations of variables was used to generate CCA lean mass values.

For comparisons between QMR and CCA, fat and lean mass measurements were compared using Deming regressions, an analysis that assumes error in both methodologies without assuming either method is superior (14). Linear regressions against body weight were also performed for all QMR and CCA values. Regression slopes were compared using a two-tailed *t*-test (15). The precision of each method was determined by calculating the coefficient of variation (CV) for QMR and CCA measurements. Arcsine transformed CV values were compared using one-way repeated measures ANOVA. Two-way repeated measures ANOVA was used to analyze differences in CV values by animal strain. For deprivation studies, data were analyzed using one-way repeated measures ANOVA over time. GraphPad Prism 5.0 (GraphPad Software, San Diego, CA) was used for regressions, statistical comparisons and to generate all graphs.

RESULTS

QMR live scan vs. CCA

Mean fat and lean mass values for all groups are presented in **Table 1**. For both rats and mice, Deming regressions showed positive significant correlations between fat mass as determined by QMR and CCA (rats: fat mass slope = 0.927 ± 0.0168, **Figure 1a**; mice: fat mass slope = 1.038 ± 0.0044, **Figure 1b**). Differences in the y-intercept suggest an overestimation of fat mass for QMR in rats relative to CCA. Linear regression against body weight shows no difference in slope between QMR and CCA fat mass measures for both rats (**Figure 2a**) and mice (**Figure 2b**). For lean mass, Deming regressions showed positive correlations between lean mass as determined by QMR and CCA measurements for both rats and mice (rats: lean mass slope = 0.860 ± 0.017, **Figure 1c**; mice: lean mass slope = 0.551 ± 0.110, **Figure 1d**). Similar to fat mass in rats, the regression y-intercept shows that lean mass in mice appears

Table 1 Comparison of fat and lean mass values

	CCA[a]	Live[a]	PM[a]	PP[a]
Fat mass				
Rats (all)[b]	59.88 ± 22.78	73.87 ± 27.99*	81.94 ± 25.97*,†	77.21 ± 25.68*,†
Outbred	36.45 ± 7.83	45.28 ± 9.99	56.61 ± 10.29	51.77 ± 9.35
Obese	83.78 ± 15.50	104.17 ± 17.22	110.19 ± 17.24	104.82 ± 17.61
Lean	59.33 ± 4.19	71.81 ± 5.39	78.44 ± 4.33	74.60 ± 4.31
Mice (all)[b]	6.87 ± 7.69	7.83 ± 8.74*	8.14 ± 9.08*	7.73 ± 8.87*
Outbred	1.88 ± 0.26	2.21 ± 0.32	2.18 ± 0.34	1.89 ± 0.29
Obese	17.41 ± 0.83	19.82 ± 0.73	20.60 ± 0.62	19.90 ± 0.57
Lean	1.31 ± 0.14	1.45 ± 0.17	1.64 ± 0.17	1.40 ± 0.21
Lean mass				
Rats (all)	363.87 ± 81.37	322.24 ± 69.98*	324.66 ± 71.47*	315.85 ± 71.57*,†
Outbred	268.84 ± 30.57	239.51 ± 27.89	240.80 ± 28.05	232.18 ± 26.87
Obese	444.01 ± 13.57	388.61 ± 10.47	393.43 ± 10.67	385.53 ± 10.77
Lean	381.75 ± 38.78	341.90 ± 33.55	342.77 ± 35.36	332.62 ± 36.10
Mice (all)	16.88 ± 1.91	16.83 ± 1.19	16.32 ± 1.16	15.15 ± 1.11*,†
Outbred	16.34 ± 1.03	16.93 ± 0.81	16.76 ± 0.74	15.73 ± 0.71
Obese	18.82 ± 0.72	17.20 ± 0.72	16.08 ± 0.79	14.80 ± 0.80
Lean	15.48 ± 1.91	16.37 ± 1.80	16.13 ± 1.74	14.92 ± 1.55

Values are mean ± s.e. (g).
[a]CCA: destructive carcass composition; Live: quantitative magnetic resonance (QMR) measurement on live, unanesthetized animal; PM: postmortem QMR, warmed to 37 °C; PP: postmortem QMR, prepared for CCA and warmed to 37 °C. [b]Outbred (Fisher, BALB/c), obese (SD-DIO obesity-prone, B6.V-Lepob) and lean (SD-DR obesity-resistant, C57BL/6) strains of rats and mice, respectively. Mean values for all rats or all mice are presented first, followed by individual averages for each strain. Significance for strain averages not reported.
*$P \leq 0.05$ vs. CCA. †$P \leq 0.05$ vs. Live.

to be overestimated by QMR relative to CCA. When regressed against body weight, QMR and CCA lean mass measurements show significantly different slopes for both rats ($F_{1, 30} = 11.045$, $P = 0.0024$, **Figure 2a**) and mice ($F_{1, 32} = 8.516$, $P = 0.0064$, **Figure 2b**); slopes for fat mass did not differ significantly in either species (**Figures 2a,b**).

PM and PP QMR scans

One-way repeated measures ANOVA showed significant differences between fat mass values obtained during live, PM and PP scans ($F = 27.99$, $P < 0.0001$, **Table 1**). Bonferroni-adjusted *post hoc* comparisons showed that all QMR values were significantly different than CCA ($P < 0.001$), and that PM (but not PP) values differed from live measurements ($P < 0.01$). Deming regressions against CCA values indicate significantly different slopes for live, PM and PP fat mass values for both rats (Live: 0.9277 ± 0.0168; PM: 0.9174 ± 0.0163; PP: 0.8130 ± 0.02032; $F_{2, 45} = 12.91$, $P < 0.0001$, **Figure 1a**) and mice (Live: 1.038 ± 0.0044; PM: 1.014 ± 0.0050; PP: 0.8792 ± 0.0069; $F_{2, 48} = 241.54$, $P < 0.0001$, **Figure 1b**). For lean mass, one-way repeated ANOVA also indicated significant differences between live, PM and PP scan values ($F = 38.45$, $P < 0.0001$, **Table 1**). Bonferroni-adjusted *post hoc* comparisons showed that while QMR scan values did not differ significantly, all were significantly different than CCA ($P < 0.001$). For both rats and mice, Deming regressions against CCA lean mass values showed no significant differences in slope for live, PM or PP measures; however, there were significant differences in the intercepts in both groups (rats: $F_{2, 47} = 20.73$, $P < 0.0001$, **Figure 1c**; mice: $F_{2, 50} = 14.77$, $P < 0.0001$, **Figure 1d**).

Precision of QMR scans vs. CCA

Mean CV values for all groups are presented in **Table 2**. Values were arcsine transformed prior to analysis. Overall, one-way repeated measures ANOVA showed significant differences between QMR live, PM, PP, and CCA values for both rats (fat mass: $F = 10.54$, $P < 0.0001$; lean mass: $F = 20.84$, $P < 0.0001$) and mice (fat mass: $F = 3.46$, $P = 0.0229$; lean mass: $F = 4.30$, $P = 0.0089$). For rats, Bonferroni-adjusted *post hoc* comparisons showed no significant differences between live, PP or PM CV values for either fat or lean mass, but indicated that all were significantly ($P < 0.001$) lower than the CV for CCA (**Table 2**). For mouse lean mass, *post hoc* comparisons were similar to rats, with live, PP, and PM CV values all significantly lower than CCA CV values ($P \leq 0.05$). However, *post hoc* comparisons for fat mass in mice showed significant differences only between PM and CCA CV values ($P \leq 0.05$). Although no significant differences in the CV were observed between rat strains for either method of analysis, two-way repeated measures ANOVA showed significant effects of mouse strain for fat measurements only ($F = 5.11$, degrees of freedom = 2, $P = 0.0203$).

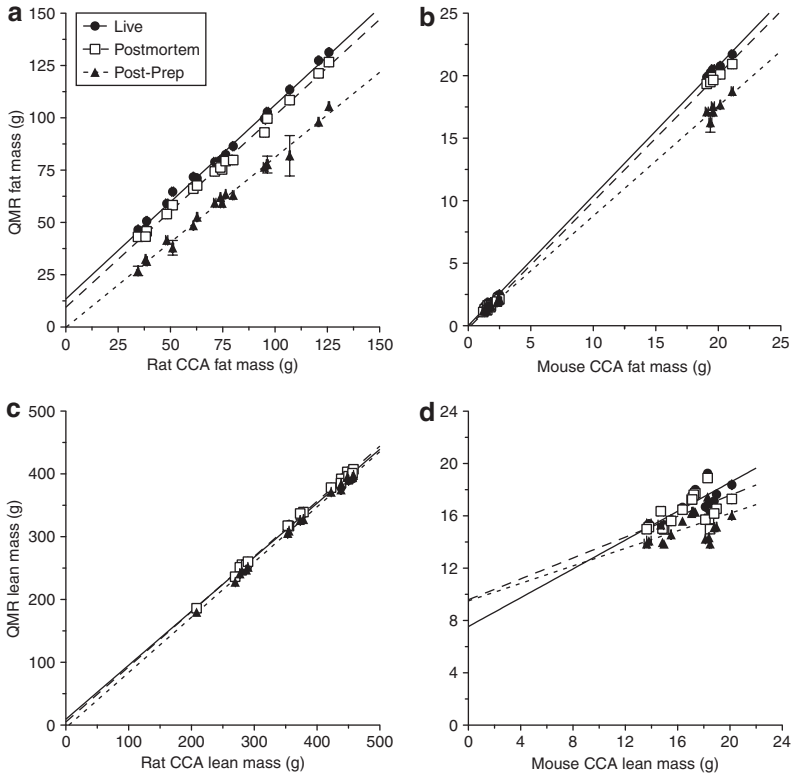

Figure 1 Regression analysis of QMR and CCA body composition measures. Deming regression analysis of fat and lean mass values obtained by quantitative magnetic resonance (QMR) regressed against chemical carcass composition analysis (CCA) measurements. Fat mass regressions: (**a**) rats, (**b**) mice. Lean mass regressions: (**c**) rats, (**d**) mice. Live: QMR scans (unanesthetized animal) indicated by solid circles, Postmortem QMR (warmed to 37 °C) by open circles, and Postpreparation QMR (gut contents removed, warmed to 37 °C) by solid triangles. Standard error for both QMR and CCA values indicated by error bars.

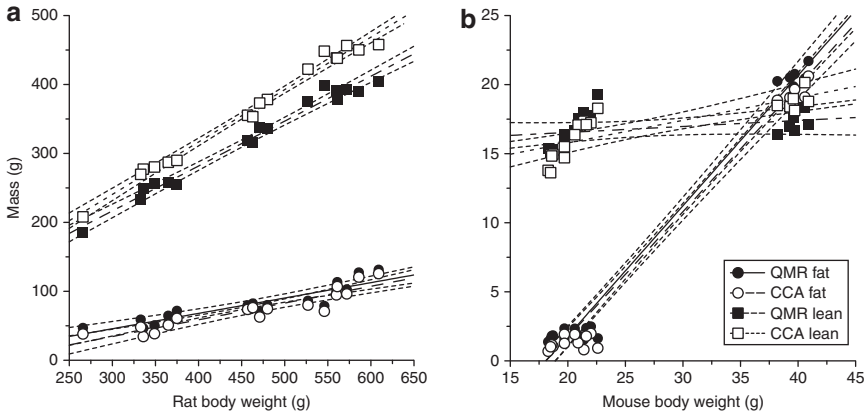

Figure 2 QMR and CCA fat and lean mass regressed against body weight. Fat and lean mass values obtained by quantitative magnetic resonance (QMR) and chemical carcass composition analysis (CCA) regressed against body weight in (**a**) rats and (**b**) mice. Solid symbols represent QMR measurements, open symbols represent CCA. 95% Confidence intervals for all measurements indicated by thin broken lines.

Deprivation studies

One-way repeated measures ANOVA showed that 18 h food deprivation significantly reduced body weight ($F = 103.8$, $P < 0.0001$), fat mass ($F = 52.62$, $P < 0.0001$), lean mass ($F = 37.74$, $P < 0.0001$), and total water weight ($F = 91.05$, $P < 0.0001$) relative to starting values (Figure 3a); no significant change in free water was found. Bonferroni-adjusted *post hoc* comparisons indicated that all reductions due to deprivation were significantly different than baseline values (mean changes: weight = −24.33 g; fat = −4.605 g; lean = −14.70 g; total water = −14.67 g; all $P < 0.001$), and that all measures returned to a level not significantly different from initial values after 24 h refeeding.

Although one-way repeated ANOVA showed a significant main effect of 18 h water deprivation for body weight ($F = 109.4$, $P < 0.0001$), fat mass ($F = 137.7$, $P < 0.0001$) lean

Table 2 Precision of QMR and CCA estimates

	Fat mass				Lean mass			
CV (%)	CCA[a]	Live[a]	PM[a]	PP[a]	CCA	Live	PM	PP
Rats (all)[b]	5.12	0.94‡	0.31‡	0.39‡	0.97	0.22‡	0.09‡	0.09‡
Outbred	7.14	1.18	0.32	0.42	0.81	0.23	0.10	0.10
Obese	5.89	0.67	0.23	0.28	1.10	0.17	0.09	0.06
Lean	2.33	0.97	0.38	0.47	1.03	0.27	0.07	0.11
Mice (all)[b]	4.33	3.47	1.82*	2.76	2.80	0.85*	0.71*	0.62*
Outbred	5.12	2.78	1.36	2.74	2.44	0.84	0.46	0.54
Obese	2.75	0.74	0.51	0.40	2.06	0.68	0.81	0.71
Lean	5.13	6.89	3.60	5.13	3.89	1.03	0.86	0.61
Overall[b]	4.72	2.21†	1.07‡	1.57‡	1.91	0.55†	0.41‡	0.36‡

Coefficient of variation (CV) % values used as an estimate of precision.
[a]CCA: destructive carcass composition; Live: QMR measurement on live, unanesthetized animal; PM: postmortem QMR, warmed to 37 °C; PP: postmortem QMR, prepared for CCA and warmed to 37 °C. [b]Outbred, obese, and lean strains of rats and mice are the same as described in **Table 1**. Mean values for all rats or all mice are presented first, followed by individual averages for each strain, with overall mean CV for all animals reported last.
*$P \leq 0.05$ vs. CCA. †$P \leq 0.01$ vs. CCA. ‡$P \leq 0.001$ vs. CCA.

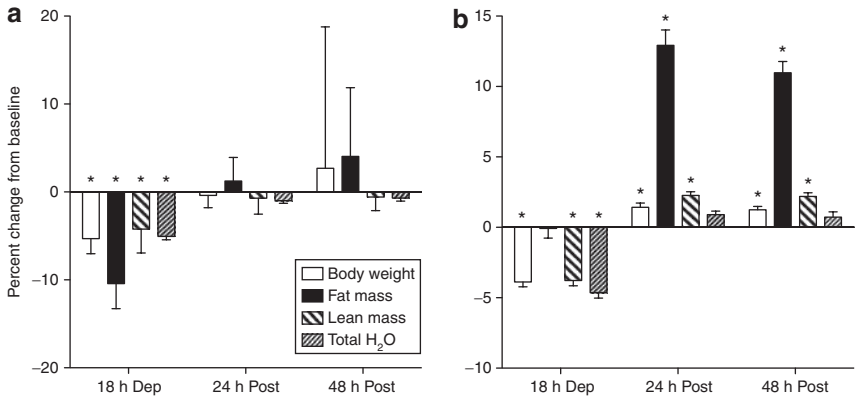

Figure 3 Effects of food or water deprivation on QMR measures. Effects of (**a**) 18 h food or (**b**) water deprivation on quantitative magnetic resonance estimates of body composition measurements in live, unanesthetized rats. Measurements are percent change (±s.e.m.) from predeprivation values, performed after 18 h deprivation and repeated 24 and 48 h after return of food or water. Significant changes relative to baseline indicated by asterisk (*).

mass ($F = 134.4$, $P < 0.0001$), and total water ($F = 91.05$, $P < 0.0001$), Bonferroni-adjusted *post hoc* comparisons indicate that the pattern of change was different than that seen for food deprivation (**Figure 3b**). Fat mass measures after water deprivation were not significantly different than initial values, but did increase significantly (mean increase: 6.301 g, $P < 0.001$) 24 h after water was returned, and this difference persisted for at least 48 h following deprivation. Postdeprivation body weight and lean mass measures were significantly decreased relative to baseline (mean change: weight = −18.41 g; lean = −13.34 g; both $P < 0.001$), and both were significantly increased relative to baseline following both 24 and 48 h of water availability (mean 24 h increase: weight = 25.21 g; lean = 8.061 g; both $P < 0.001$). Total water decreased significantly after deprivation (mean change = −13.77 g, $P < 0.001$) but returned to initial values within 24 h of water availability. As was observed for food deprivation, water deprivation did not significantly affect free water measurements.

DISCUSSION

The use of a nuclear magnetic resonance-based device such as the EchoMRI QMR system for measures of whole-body composition allows for reduced time and effort spent to obtain data, fewer number of subjects needed per study, and the ability to track changes in individual subjects over time. Although the advantages for both researcher and subject are clear, there are potential drawbacks that must be considered. First, to be useful to investigators, QMR must be shown to be comparable to traditional CCA to allow comparisons between studies performed using the different methods. If there is no meaningful, consistent relationship between measurements, conclusions drawn from CCA studies might not agree with those from studies implementing QMR. Second, precision of measurements must be considered. A technically superior yet less precise method may not be appropriate for some experiments. Third, as many researchers do not have direct access to a QMR machine, in some cases scans are performed PM at remote facilities offering QMR services, making it especially important to determine the

accuracy of scanning nonliving animals. Finally, gut contents and hydration status of subjects can vary due to differences in food or water intake, experimental manipulations, or in the case of PM scans from evaporative water loss. It is thus necessary to determine whether these changes affect QMR measurements, especially because previous studies suggest that tissue hydration can affect the accuracy of other noninvasive scan methods (16,17). We have attempted to address all of these concerns in our present study.

The data presented here indicate a positive correlation between CCA and QMR body composition measurements for both fat and lean mass in rats and mice. For fat mass, we have shown a linear correlation in values obtained by each method (**Figure 1a,b**), although a noticeable bias is apparent in rat fat estimates as evidenced by the regression intercepts. Consistent with previous studies (9), lean mass estimates for QMR were consistently lower than those obtained using CCA (**Table 1**). Although lean mass measurement correlations were thus not as strong as those for fat mass, a weaker correlation between these measurements is expected because the components used to define lean mass differ between methods. The two-compartment CCA method used in the present study defines lean mass as fat-free lean mass plus ash and water, whereas QMR is calibrated to a signal most closely correlated to lean skeletal muscle only. However, the Deming regression analysis used in this study shows that, despite differences in methodology, a linear, predictive relationship exists between CCA and QMR composition measurements for both fat and lean mass (**Figure 1**). This statistical analysis is a structural relationship model which accounts for variability in the error of each method, and does not assume that either is superior (14). We used the Deming methodology rather than the Bland–Altman analysis because the Deming analysis was more appropriate for our goals: First, to compare methods of measurement, in which each method is assumed to have an unknown amount of measurement error, and second, to determine whether it would be possible to predict or convert data between measurement methods for future meta-analyses. A structural relationship model such as the Deming regression is appropriate for these goals, as Bland and Altman originally stated (18), but mathematical complexity of the Deming regression made it difficult to implement until more recent advances in computerized statistical programs.

In the present study we use CV values (the ratio of the standard deviation to the mean) from repeated measurements as an estimate of precision. Previous investigations using a QMR machine designed specifically for mice have shown that QMR is significantly more precise than both CCA and DXA measurements for fat mass, reporting QMR CV values of 0.86–3.70% for live mice (6,9). Although the CV values for fat mass in the present study are within the range reported previously, averaging 0.94% for rats and 3.47% for mice (**Table 2**), it is important to note that the duration of each scan was longer in this study than the 1-min scans performed using the mouse-specific QMR device in the previous study (9). Pilot data for the combination rat and mouse system used in this study showed that the CV for fat mass in mice was higher when a shorter (2 min) accumulation time was used, averaging around 4.8% (data not shown). However, the shorter 2-min accumulation scan was adequate to obtain low CV values for rats. The variability in fat mass measurements in this study was greatest in animals with smaller body sizes, especially in the lean mouse strains, despite longer accumulation times. Although DXA fat estimates are expected to be more variable in very lean animals, QMR estimates are not (9). Although we do not replicate these findings here, it is important to note that the mouse-specific system used by Tinsley *et al.* may have greater accuracy for small animals than the QMR system used here. Overall these findings suggest that for fat mass, the combination rat and mouse QMR system is fairly optimal for rats even at the shortest scan durations, whereas for mice, longer scan durations or repeated scans may be necessary for the highest precision, especially in very small or very lean individuals. Although the previous study showed strong correlations between QMR lean mass measures and body weight (9), no estimate of precision was provided for lean mass. We have shown here that the QMR lean mass measurements are more precise than CCA for both rats and mice (**Table 2**). The precision in lean mass measurements was very high, with CV values lower than those obtained for fat mass in all animals examined. Additionally, pilot data suggest that unlike fat mass values, lean mass measurements in mice are less strongly affected by shorter scan durations. Because the QMR machine used is specifically calibrated against lean muscle (6,9), the high precision could allow rapid monitoring of even small changes in muscle mass in study subjects.

To determine what, if any, changes in QMR readings might occur PM, we performed triplicate PM and PP QMR scans on all animals used for comparison with CCA. A previous study using a mouse-specific QMR machine showed an increase in precision in PM scans, suggesting that movement during scanning might affect accuracy (9). Our results do not replicate this finding for the combination rat and mouse QMR system. For both PM and PP scans, the precision of QMR fat and lean mass estimates are not significantly different than values obtained in live animals (**Table 2**). However, our data indicate there are systematic differences in the absolute values for both measurements. Specifically, scans performed on nonliving animals appear to overestimate fat and lean mass relative to scans performed in live subjects (**Figure 1**, **Table 1**). For lean mass, it is possible that differences observed in nonliving animals might be due to PM evaporative water loss. Only PP lean mass values significantly differed from live QMR estimates. These animals are presumably subject to greater evaporative water loss during removal of gut contents. Furthermore, although the absolute values for lean mass differed between scans, the regression slope values versus CCA did not (**Figure 1c,d**). This constant amplitude bias is consistent with an underestimation of lean mass as tissue hydration decreases. In contrast, the differences observed for fat mass measurements are not as easily explained. It is possible that the increased error in fat mass measurement in very small or lean individuals, or small inaccuracies due to animal movement during live QMR, could explain some PM differences in estimated fat mass. However, these explanations

would predict a convergence in fat estimates in larger animals due to decreased magnitude of effect as fat mass increases. Our results show that the difference between live and PM fat mass estimates increases along with body size, especially for mice. Although it is clear that some PM change affects QMR fat mass estimates, the exact cause of this change is still uncertain.

We were interested in whether short-term deprivation of food or water would affect QMR fat and lean mass measurements, both to ascertain whether such short manipulations could result in detectable differences in body composition and to determine whether changes in hydration state would affect estimates, as has previously been shown for BIA and DXA (3,16,17). Previous studies suggest that although both fat and protein are lost during short-term fasting, protein loss is minimal and the majority of weight lost from muscle is primarily glycogen and water (19–21). In the short period of food deprivation described here, QMR showed small but significant changes in fat, lean mass, and total body water (**Figure 3a**). As would be expected based on previous studies, all measured changes were returned to baseline values following refeeding. In contrast, during water deprivation, no change in fat mass was observed, whereas lean mass and total water estimates decreased significantly (**Figure 3b**). When water was returned, lean mass returned to initial values, but fat and total water estimates increased significantly relative to baseline. The change in fat mass during and after water deprivation is likely due to the concurrent reduction in food intake observed in water-deprived rats (22). Although total water decreased during deprivation, free water did not change, indicating that water lost was primarily that bound in tissues rather than from body fluids. Although we feel confident that the QMR system is sensitive enough to detect body composition changes due to short-term food manipulations, data from animals that do not have adequate access to water may be problematic as this QMR system appears to overestimate lean mass as water is lost. We suggest caution in interpreting data regarding water-deprived animals until the effects of tissue hydration on QMR measurements are better characterized.

We have shown here that body composition analysis using the rat and mouse combination EchoMRI QMR system produces fat and lean mass values comparable to those obtained by CCA. It is important to note that the components measured by this machine are not identical to those obtained using CCA, especially for lean mass. However, the consistent linear correlations between the two measurement methods suggest that studies using either method could be directly compared using a mathematical transformation. We have also shown that measurements using this QMR system have higher overall precision for both fat and lean mass than measurements obtained using CCA (mean QMR CV difference vs. CCA = 2.5% and 1.4% for fat and lean mass, respectively), and that for both rats and mice the precision of this machine is comparable to that of a similar machine designed specifically for mice. Although the precision of this machine is not significantly higher than that of CCA for fat mass in mice, the advantages of using a nonterminal method are obvious, especially for long-term studies. With respect to PM scans, we have provided evidence suggesting that this combination QMR machine yields fat and lean mass measurements that slightly overestimate those obtained from live animals, yet remain more precise than those obtained using CCA. We also show that this system is sensitive to very small changes due to short periods (<1 day) of food or water deprivation in rodents, and provide evidence that for lean mass in particular the hydration state of tissue affects body composition estimates. We conclude that the Model 900 EchoMRI QMR system offers a fast, nonterminal, and precise method of body composition analysis in both rats and mice, yielding measurements comparable to those obtained by CCA without the need for the time-consuming chemical analyses of the latter method.

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DISCLOSURE

The authors declared no conflict of interest.

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