Evaluation of a Quantitative Magnetic Resonance Method for Mouse Whole Body Composition Analysis

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Abstract

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Objective: To evaluate applicability, precision, and accuracy of a new quantitative magnetic resonance (QMR) analysis for whole body composition of conscious live mice.

Research Methods and Procedures: Repeated measures of body composition were made by QMR, DXA, and classic chemical analysis of carcass using live and dead mice with different body compositions. Caloric lean and dense diets were used to produce changes in body composition. In addition, different strains of mice representing widely diverse populations were analyzed.

Results: Precision was found to be better for QMR than for DXA. The coefficient of variation for fat ranged from 0.34% to 0.71% compared with 3.06% to 12.60% for DXA. Changes in body composition in response to dietary manipulation were easily detected using QMR. An increase in fat mass of 0.6 gram after 1 week ($p < 0.01$) was demonstrated in the absence of hyperphagia or a change in mean body weight.

Discussion: QMR and DXA detected similar fat content, but the improved precision afforded by QMR compared with DXA and chemical analysis allowed detection of a significant difference in body fat after 7 days of consuming a diet rich in fat even though average body weight did not significantly change. QMR provides a very precise, accurate, fast, and easy-to-use method for determining fat and lean tissue of mice without the need for anesthesia. Its ability to detect differences with great precision should be of value when characterizing phenotype and studying regulation of body composition brought about by pharmacological and dietary interventions in energy homeostasis.

Key words: quantitative magnetic resonance, DXA, body composition, nuclear magnetic resonance, body fat

Introduction

Obesity was initially defined by body weight, but that concept was later refined by BMI, which is weight in kilograms divided by height in meters squared (1). However, the amount of total body mass that is derived from adipose mass is the variable that is best associated with risk for pathology (2), and the better the characterization of this mass, the more accurate the assessment of such risk factors. Newer technologies that precisely and accurately calculate adipose mass may eventually replace BMI or simple body weight determination.

Measurement of body density by plethysmography and hydrostatic weighing assesses whole body fat content expressed as a percentage of body weight (3). Modern instrumentation designed for humans allows for a high degree of precision in volume measurement, but inconsistencies in body density, the necessity for lung volume correction, variation in skeletal mass, and degree of hydration are not accounted for by these methods. Widely used techniques for fat assessment based on body bioelectrical impedance analysis or electrical conductivity have not been implemented in broad populations (4), largely because of inaccuracy and poor specificity of the technology. Measurement of body composition of experimental animals by plethysmography, hydrostatic weighing, bioelectrical impedance, and electrical conductivity is not practical. DXA is more accurate and used with a variety of species (5–13). Precision of DXA differs with instrument, species, software, and methods em-

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ployed. Magnetic resonance imaging (MRI)[1] and computerized tomography have potential to determine spatial properties of body fat. Magnetic resonance spectroscopy has also been employed to measure whole body fat and water of mice (14). This paper describes in detail a new quantitative magnetic resonance (QMR) method (EchoMRI; Echo Medical Systems, Houston, TX) that previously has been described only in brief communications (15,16). In addition to advantages described herein regarding precision and accuracy, measurements are fast (<1 minute) and do not require anesthesia.

Research Methods and Procedures

Body Composition Analysis

Experiment 1. To compare QMR technology with DXA and chemical analysis, we examined mice comprised of different body compositions. Eighteen wild-type male C57BL/6NHsd mice (Harlan, Indianapolis, IN) were maintained on Purina 5008 diet (16.7% of calories from fat; Purina Mills, Inc., St. Louis, MO) to provide a standard chow. To study adiposity resulting from a caloricly dense diet (diet-induced obesity), 18 male C57BL/6NHsd mice were maintained on a diet comprised of 40% calories from fat (TD95217; Harlan Teklad, Madison, WI). To examine morbidly obese mice, 15 male C57BL/6-Lep$^{ob/ob}$ mice maintained on Purina 5008 were studied. Access to both diet and water were ad libitum. All mice were measured by QMR and DXA, both live and postmortem. Ten replicate measurements were made by QMR on live, free-moving mice and on postmortem mice. QMR measurements were made by placing live mice into a thin wall plastic cylinder (4.7 cm, inside diameter; 0.15 cm thick), with freedom to turn about but limited to ~4-cm vertical movements by a plastic insert. After 2 minutes, when the measurement was completed, the mice were returned to their home cage. Frozen mice were thawed by placing them into a 37 °C water bath and placing them in the same cylinder. Mice measured postmortem were repositioned between measurements. Each mouse was measured once by DXA while under methoxyflurane anesthesia (3%) to eliminate locomotor activity and assayed five times by DXA postmortem. After QMR and DXA analysis, mice were frozen, placed in plastic bags, and shipped on dry ice to Covance (Madison, WI) for body composition analysis by standard chemical carcass analytical methods.

Experiment 2. To monitor linear changes in body composition, 20 male C57BL/6NHsd mice were maintained on TD95217 diet since weaning, whereas another 20 mice of that strain were fed Purina 5001 diet (12% of calories from fat). After initial measurements of body composition by QMR and DXA, 10 mice from each dietary group were switched to other diet. Body composition of all mice was analyzed in duplicate at weekly intervals by QMR until a significant change in body composition was detected.

The Eli Lilly & Co. Research Laboratories Institutional Animal Care and Use Committee approved all animal protocols used in these studies. Mice were individually housed in micro-isolator (Labproducts, Seaford, DE) cages and were maintained at 25 °C in a room programmed with lights off from 10:00 AM to 10:00 PM. Access to both diet and water were ad libitum.

Experiment 3. A group of wild-type mice (25 males and 27 females) from different backgrounds were provided by Dr. Andrzej Bartke (Department of Physiology, Southern Illinois University) to represent a heterogeneous population of mice with body compositions ranging from lean to obese. Mice were frozen after death at Southern Illinois University, placed in plastic bags, and shipped to Eli Lilly on dry ice for determination of body composition postmortem by both DXA and QMR. Carcasses were thawed to room temperature for measurement by DXA and warmed to 37 °C by placing carcasses in a water bath for QMR analysis.

Optimization of QMR

Fat, muscle, and tissue-free body fluids generate different signals in response to various radio frequency pulses at distinct static magnetic fields. These signals depend on many material and environmental parameters, chiefly temperature. Principles of our method may be demonstrated at any temperature. Room temperature was chosen for convenience and sample stability. Hydrogen density and relaxation behavior of water (saline; Walgreen's, Houston, TX), canola oil, olive oil, corn oil, and chicken muscle trimmed of all visible fat (all fats and chicken were purchased from Randal's, Houston, TX) were examined at room temperature at applied static magnetic field corresponding to radio frequency of 20 MHz. A brief description of nuclear magnetic resonance (NMR) techniques used in this study is given in the Discussion section. More specifics can be found elsewhere (17,18).

Free induction decay (17) of all five pure samples had a dead time of ~50 μs (Figure 1). Assuming hydrogen density of 1 gram of water to be unity, 1 gram of chicken muscle had 90% signal amplitude (10% less of "liquid" hydrogen nuclei) at 0.9 units/g. Thus, the hydrogen density per 1 gram of canola oil, olive oil, corn oil, and skeletal muscle was 1.27, 1.34, 1.25, and 0.90, respectively.

Preliminary studies indicated that the difference between water and oil (fat) was observed to be mainly in T1 relaxation at high resonance frequencies. At frequency of 20 MHz and below, T2 relaxation differences play a substantial role in fat and lean differentiation. Therefore, the most

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[1] Nonstandard abbreviations: MRI, magnetic resonance imaging; QMR, quantitative magnetic resonance; NMR, nuclear magnetic resonance; IR, inversion recovery; CPMG, Carr-Purcell-Meiboom-Gill; TE, time-to-echo; CV, coefficient of variation; WBC, whole body composition.

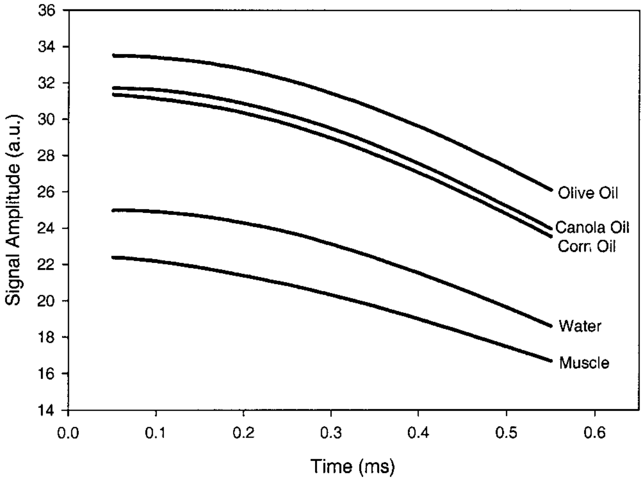

Figure 1: Free induction decay for 1 gram of water, olive oil, canola oil, corn oil, and chicken muscle having dead time of 50 μs. a.u., arbitrary units.

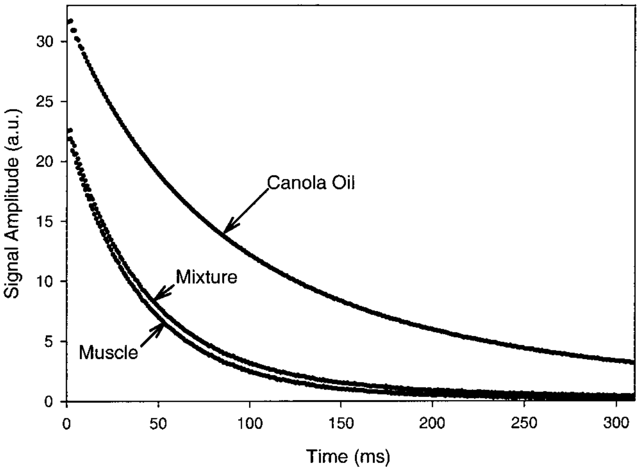

Figure 2: Three T2 relaxation curves correspond to three 1-g samples comprised of canola oil (fat), chicken lean muscle, and a mixture sample of 7.1% canola oil and 92.9% of chicken lean muscle. T2 relaxation measured by a CPMG sequence having time-to-echo (TE) = 1 ms. a.u., arbitrary units.

appropriate NMR method to run is fast-inversion-recovery (18) phase-alternated pulse sequence consisting of up to 30 inversion times spaced logarithmically from 10 ms to 2 seconds and 90° read-out pulse in a standard inversion recovery (IR) sequence, replaced by a Carr-Purcell-Meiboom-Gill (CPMG) sequence having time-to-echo (TE) of 1 ms and ~200 to 500 echoes. Additional reasons for using this type of sequence are that fast T1 measurements actually require CPMG to cancel the longitudinal component of the magnetization; changes in T1 and T2 values are in opposite direction as a function of temperature, which makes the measurement of fat-to-lean ratio more stable; measurement of 20 to 30 phase-alternated points on the T1 relaxation curve takes about 1 minute, and measurement of 500 phase-alternated points on the T2 relaxation curve takes only 1 second. Furthermore, each delay in this sequence was optimized for maximum signal-to-noise of differences among fat, muscle, and free body fluids.

Experiments with Pure Samples and Their Mixtures

Three T2 relaxation curves shown in Figure 2 correspond to canola oil (fat), chicken lean muscle, and mixture sample having 7.1% canola oil and 92.9% of chicken lean muscle. The T2 relaxation curves (signal amplitude and decay rate) of all three samples are different. More specifically, oil may be represented by a uni-exponential function (two independent parameters − amplitude and decay rate), whereas chicken lean muscle may be represented by a bi-exponential function (four independent parameters − two amplitudes and two decay rates). Differences in signal amplitude are caused by differences in hydrogen density in these materials. Thus, canola oil has ~40% more hydrogen per unit weight compared with chicken lean muscle. Similar to hydrostatic weighing method for whole body composition analysis, measurement of NMR signal amplitude alone may be sufficient to determine composition of the two components in mixtures of oil and lean tissue.

Differences in T1 relaxation curves measured by IR method for canola oil, chicken lean muscle and a mixture of the two having 7.1% canola oil and 92.9% of chicken lean muscle were greater (Figure 3) compared with T2 relaxation curves. Thus, our preferred measurement method is based primarily on differentiating T1 relaxations but also includes elements of T2 relaxations.

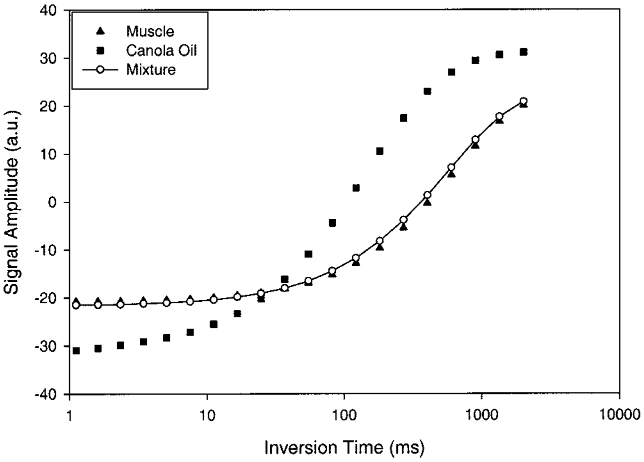

Figure 3: Three T1 relaxation curves correspond to three 1-g samples comprised of canola oil (fat), chicken lean muscle, and a mixture sample of 7.1% canola oil and 92.9% of chicken lean muscle. T1 relaxation measured by an IR phase-alternated pulse sequence having 20 inversion times spaced logarithmically from 10 ms to 2 seconds. a.u., arbitrary units.

For validation of three-component composition (fat/lean/free body fluid) analysis of mice, 30 mixture samples were prepared from pure fat, muscle trimmed of visible fat, and free body fluids. Average sample weight was 19.5 grams (range, 8.3 to 27.0 grams), and average fat fraction was 18.4% (7.5% to 33.0%). Each sample component was weighed to the nearest 0.001 gram using a Mettler balance. Canola oil was the surrogate for pure fat, and saline was used for free body fluids. Average quantity and ranges for mixture components were 3.6 grams (range, 1.4 to 8.7 grams), 14.9 grams (range, 5.8 to 22.6 grams), and 1.0 gram (range, 0.6 to 1.8 gram) for canola oil, fat-free chicken muscle, and saline, respectively. Oil was added to the mixture after chicken muscle and before saline to minimize water absorption by chicken muscle. Mixtures were sealed in glass vials to prevent water evaporation from chicken muscle and saline. All samples were kept at 37 °C for at least 1 hour before NMR measurement.

For validation of two-component composition (fat/lean) analysis of small sample sizes such as dissected organs, 18 mixture samples were prepared from canola oil and chicken muscle. Average sample weight was 3.2 grams (range, 2.1 to 4.1 grams), and average fat composition was 29.4% (range, 7.5% to 65.2%). Average quantity for canola oil was 0.8 gram (range, 0.3 to 1.9 gram) and for chicken muscle was 2.3 grams (range, 0.7 to 3.4 grams). Mixtures were sealed in glass vials and were kept at 37 °C for at least 1 hour before NMR measurement. Two-component calibration was used for experiments 1 and 2, but three-component calibration was used for experiment 3. Mass identified as free fluid in experiment 3 was considered part of the lean mass.

Data Analysis

Sampled NMR time domain data were analyzed using custom software. Non-negative least-squares algorithm of Lawson and Hanson (19) was used for relaxation time distribution determination. Components of mixture samples and whole body composition analysis were performed using a method of chemometric analysis, specifically, multivariate calibration by partial least-squares regression (20).

Calibration

Automatic tuning and calibration of the instrument parameters using rape seeds maintained at 37 °C as a standard sample were conducted daily for quality control. These parameters include resonance frequency, temperature, signal amplitude, and decay of a reference sample.

DXA

Measurement by DXA was performed using a Norland p-DXA (Fort Atkinson, WI) calibrated daily according to the manufacturer's instructions. Mice were scanned at 20 mm/s with a resolution of 0.5 mm × 0.5 mm. The Norland p-DXA, operating in the research mode, allows the histogram averaging width threshold to be set for the size of the animal. A simple linear regression formula (threshold $=$ body wt $\times$ $0.0005 + 0.017$) was used to determine the appropriate threshold setting. Live mice were placed into a custom built Plexiglass rectangular box (10.4 cm wide × 24.3 cm long × 8 cm high; 0.3-cm-thick panels) that permits anesthesia gas to flow in one end and out the opposite end to a vent. Anesthetized mice were positioned to be prone with forelimbs cephalic and hind limbs directed caudally. The tail was wrapped to one side. Dead mice were positioned in the same manner and placed into the anesthesia chamber, but gas was not used. Mice were repositioned between DXA measurements.

Chemical Analysis

Mice were frozen, placed into individual plastic bags, and shipped on dry ice to Covance (Madison, WI) for body composition analysis by standard chemical methods. Covance monitors precision periodically using standard preparations. A whole peanut preparation was used as a fat standard, and a cereal preparation was used as a protein standard. Fat was determined by petroleum ether extraction, and protein was determined by the Kjeldahl method for organic nitrogen. Lean mass was defined as the mass of moisture combined with the mass of protein and ash. A homogenized carcass aliquot was dried to stable weight in a 100 °C vacuum oven to determine water mass. A separate aliquot was combusted at 550 °C to provide measurement of body ash.

Statistics

Coefficient of variation (CV) from repeated measures was calculated to provide a measure of precision. Pearson product moment correlations were performed using Sigmastat to compare methods for body composition. Student-Newman-Keuls post hoc test (Sigmastat) was used to compare mean changes in body composition in response to dietary change over time. For comparison of individual changes over time, one-way repeated measures ANOVA was combined with the above post hoc test. Estimation of caloric efficiency for experiment 2 was based on the values generated by Flatt (21).

Results

Testing of Prepared Samples

QMR analysis of 30 prepared mixtures of chicken muscle, canola oil, and saline was in excellent agreement with the component masses, which were weighed with an analytical balance (Figure 4). Outstanding agreement between balance weight and QMR values ($R^2 = 1.00$ for lean and fat) was also achieved with small mass (2.1 to 4.1 grams) mixtures of canola oil and chicken muscle (Figure 5).

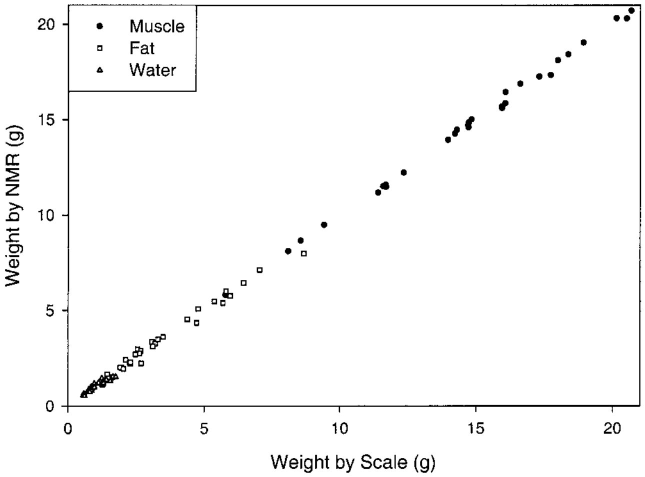

Figure 4: Correlation of muscle, fat, and water mass determined by analytical weighing to that measured by QMR. Average sample weight was 19.5 grams (range, 8.3 to 27.0 grams), and average fat fraction was 18.4% (range, 7.5% to 33.0%). Each sample component was weighed to the nearest 0.001 gram using a Mettler balance. Canola oil was the surrogate for pure fat, and saline was used for free body fluids. Average quantity and ranges for mixture components were: 3.6 grams (range, 1.4 to 8.7 grams), 14.9 grams (range, 5.8 to 22.6 grams), and 1.0 gram (range, 0.6 to 1.8 gram) for canola oil, fat-free chicken muscle, and saline, respectively A fast-inversion-recovery phase-alternated pulse sequence was used. It consisted of 28 inversion times spaced logarithmically from 10 ms to 2 seconds and 90° readout pulse followed by a CPMG sequence having TE = 1 ms and 120 echoes.

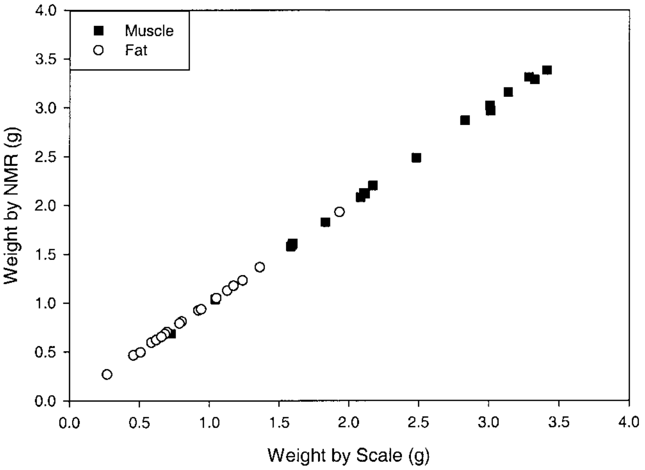

Figure 5: Correlation of fat and muscle mixture masses determined by analytical weighing to component masses determined by QMR. Average sample weight was 3.2 grams (range, 2.1 to 4.1 grams). Average fat composition was 29.4% (range, 7.5% to 65.2%). Average quantity for canola oil was 0.8 gram (range, 0.3 to 1.9 gram) and for chicken muscle was 2.3 grams (range, 0.7 to 3.4 grams). A fast-inversion-recovery phase-alternated pulse sequence was used. It consisted of 28 inversion times spaced logarithmically from 10 ms to 2 seconds and 90° readout pulse followed by a CPMG sequence having TE = 1 ms and 120 echoes.

Body Composition of Obese Mice

Experiment 1. Multiple measures of body composition from three phenotypes were determined by QMR, DXA, and chemical carcass analysis. Precision of QMR measurements was better than that for DXA (Table 1). The CVs for fat mass of mice fed the high-fat diet measured postmortem by QMR and DXA were 0.34% and 9.59%, respectively. Some loss of QMR precision was observed when mice were analyzed live and free moving. However, live measurements by QMR were less variable than those analyzed postmortem by DXA. The CV for chemical carcass analysis was not provided, but the CVs for standard preparations of fat, protein, moisture, and ash were 3.4%, 0.9%, 2.3%, and 2.5%, respectively.

Fat mass determined postmortem or live by DXA and QMR was similar and not significantly different within phenotype. Fat mass of Purina 5008–fed C57BL/6NHsd mice measured live by DXA was significantly less than that determined by QMR (Table 2) and not different from chemical analysis. Postmortem fat mass measured by chemical analysis was always significantly less than that measured by QMR and DXA ($p < 0.02$; Table 2). Fat mass of the three phenotypes was differentiated statistically by each methodology ($p < 0.001$). The correlation coefficients (R^2) and p values comparing fat by QMR to chemical analysis were 0.74 ($p < 0.001$) for 5008-fed C57BL/6NHsd mice, 0.84 ($p < 0.001$) for TD-fed C57BL/6NHsd mice, and 0.94 ($p < 0.001$) for Lep$^{ob/ob}$ mice. The corresponding values correlating QMR to DXA were 0.81 ($p < 0.001$), 0.92 ($p < 0.001$), and 0.60 ($p = 0.02$), respectively, whereas those correlating DXA to chemical analysis were 0.59 ($p = 0.01$), 0.89 ($p < 0.001$), and 0.57 ($p = 0.03$).

Lean mass is more difficult to compare because each methodology measures different components of body composition. The values obtained by chemical analysis were greatest but include masses of carcass water, ash, and protein (Table 3). The lowest lean mass was measured by QMR, but it is the mass with physical characteristics identical to lean chicken breast muscle that was measured. Measurement of lean mass by DXA is calculated from density of all tissue that is greater than fat and less than bone. Lean mass for the three phenotypes was similar when measured by chemical analysis. Lean mass of the C57BL/6NHsd mice fed either diet was similar when measured by QMR or DXA. However, lean mass of Lep$^{ob/ob}$ mice was less ($p < 0.001$) when determined by either noninvasive method than when measured by chemical methods. Lean mass measured by DXA was better correlated to lean mass determined by chemical analysis (5008, 0.51 $p = 0.03$; TD, 0.70 $p = 0.001$; Lep$^{ob/ob}$, 0.83 $p < 0.001$) than lean mass defined by QMR (5008, 0.37 $p = 0.13$; TD, 0.65 $p = 0.004$; Lep$^{ob/ob}$, 0.053 $p = 0.04$).

Table 1. CV (%) for fat measurement

Tissue group	QMR live* measure	QMR dead† measure	DXA dead‡ measure
5008§ diet—lean	2.19	0.58	7.55
TD¶ diet—lean	3.24	0.67	5.49
ob/ob 5008§—lean	3.70	0.98	5.80
5008 diet—fat	1.55	0.71	12.60
High fat diet—fat	1.43	0.34	9.59
ob/ob 5008 diet—fat	0.86	0.34	3.06

Lean is all tissues and fluids with a density greater than fat and less than bone when measured by DXA. Lean is apparent muscle mass when determined by QMR.

ob/ob is leptin-deficient $Lep^{ob/ob}$ mice on a C57BL6 genetic background. When not indicated, mice were C57BL6 strain.

* Live mice were not anesthetized.

† Dead mice were thawed and placed in a water bath at 37 °C for 1 hour before QMR.

‡ Dead mice were thawed and scanned at room temperature for DXA.

§ 5008 diet is Purina 5008 and is comprised of 16.7% calories from fat.

¶ TD95217 diet is a custom diet comprised of 40% calories from fat.

Experiment 2. The initial assessment of body composition for C57BL6NHsd mice fed 5001 chow from weaning until 10 weeks of age provided significantly different values for lean mass and fat mass obtained by DXA and QMR (Table 4). Body fat was 2% and 11%, respectively. There was no correlation between fat values determined by DXA and those measured by QMR (R^2, -0.12, $p = 0.61$). This is in marked contrast to the high correlation ($R^2 = 0.92$, $p < 0.001$) observed (Table 1) when mice presented with higher percentages of body fat. On the other hand, the greater percentages of lean mass measured by QMR and DXA were well correlated ($R^2 = 0.89$, $p < 0.001$), and those values were also correlated to body weight ($R^2 = 0.92$, $p < 0.001$ and 0.82, $p < 0.001$, respectively).

Mice switched from 5001 (12% fat) chow to the TD diet (40% fat) did not show a preference for the high-fat diet and ate fewer grams than did the group remaining on 5001 chow. However, when food consumption was converted to caloric intake and adjusted for efficiency of the mice to digest and adsorb nutrients from the diet, no difference was seen in weekly intake (weeks 1 to 3: 5001 fed, 74.3 ± 1.3, 68.9 ± 1.3, and 67.9 ± 1.1 kcal/wk; TD fed, 74.1 ± 1.7, 70.1 ± 1.4, and 69.6 ± 2.1 kcal/wk, respectively), and the cumulative caloric intake was not significantly different (5001 fed: 211.1 ± 2.3 kcal; TD fed: 213.8 ± 3.9 kcal). Average body weight for mice switched to the high-fat diet did not differ from those maintained on the 5001 diet (Figure 6). Interestingly, although caloric intake and body

Table 2. Comparison of fat mass values

	Postmortem			Live	
	QMR	DXA	Chemical	QMR	DXA
C57BL6*	12.8 ± 0.7§	12.6 ± 1.0	9.0 ± 0.6‡	12.0 ± 0.7	9.6 ± 0.7†
DIO*	18.0 ± 0.5	18.0 ± 0.5	14.0 ± 0.4‡	17.5 ± 0.5	16.8 ± 0.5
Obese*	30.1 ± 0.9	30.2 ± 1.3	24.1 ± 0.9‡	29.6 ± 1.1	31.3 ± 1.9

Values are mean ± SE (grams).

* C57BL/6NHsd strain was fed either Purina 5008 diet or TD95217 (DIO). $Lep^{ob/ob}$ mice were fed 5008 diet (obese) and were of the C57BL/6 genetic background.

† $p < 0.05$ compared with live measurement by QMR.

‡ $p < 0.05$ compared with postmortem measurements by QMR and DXA.

§ Fat mass was different for each phenotype within a method ($p < 0.001$).

Table 3. Comparison of lean mass values (postmortem)

	Postmortem			Live	
	QMR*	**DXA**	**Chemical**	**QMR***	**DXA**
C57BL/6†	18.4 ± 0.2	22.5 ± 0.5	25.3 ± 0.5§	19.3 ± 0.3	22.8 ± 0.6
DIO†	19.6 ± 0.3	22.0 ± 0.4	25.9 ± 0.4§	19.8 ± 0.3	22.3 ± 0.5
Obese‡	15.5 ± 0.9	17.2 ± 1.5	23.6 ± 0.6§	15.5 ± 0.4	16.1 ± 1.7

Values are mean mass ± SE (grams).

* QMR lean mass is not equivalent to non–fat mass.

† C57BL/6NHsd strain was fed either Purina 5008 diet or TD95217 (DIO).

‡ $Lep^{ob/ob}$ mice were fed 5008 diet (obese) and were of the C57BL/6 genetic background.

§ Significantly ($p < 0.001$) different than when measured by QMR or DXA.

weight were not altered by consuming a caloricly dense diet, body composition measured by QMR was altered. The increased fat mass of mice switched to 40% fat was significant ($p = 0.01$) by day 7, whereas mice fed only the 5001 diet (12% fat diet) maintained a constant fat mass (Figure 7). Lean mass tended to decrease in mice switched to a high-fat diet, but that difference was not statistically significant ($p = 0.12$).

We also switched mice accustomed to the 40% caloric diet to the 12% caloric fat diet. Those animals consumed fewer kilocalories (57.0 ± 2.9 vs. 77.7 ± 2.9, week 1; 68.1 ± 2.3 vs. 76.4 ± 2.2, week 2) than mice that were maintained on the caloricly dense diet and lost weight (Figure 8) when their dietary counterparts were gaining body mass. Furthermore, the weight loss was fat mass as determined by QMR, whereas mice continuing to consume the high-fat diet gained fat mass (Figure 9). Apparent muscle mass was maintained for the 2 weeks in mice fed either diet.

Experiment 3. A large group of mice of diverse phenotypes was measured using both DXA and QMR to compare the two methods. Overall, both QMR and DXA measure very similar amounts of fat mass in both lean and obese mice (Figure 10). QMR consistently measured more fat mass than did DXA when fat content was low. This was particularly evident in the smaller female mice (Figure 10A). Because DXA partitions nonbone mass between fat and lean when little fat is present, lean mass is increased accordingly. Lean mass of male and female mice measured by QMR was better correlated to body weight ($R^2 = 0.92$ and 0.94, respectively) than lean mass measured by DXA ($R^2 = 0.76$ and 0.71, respectively; Figure 10). Fat values determined by DXA were highly correlated to fat determined by QMR in both males and females ($R^2 = 0.98$ and 0.95, respectively). Lean mass values were also well correlated between the two methods for males and females ($R^2 =$ 0.90 and 0.85, respectively), but lean mass determined by

Table 4. Body composition* of lean mice

	Lean mass	SE	Fat mass	SE
QMR	14.17	0.18	2.45	0.03
DXA	18.35†	0.21	0.40†	0.06

* Values are mean mass (grams).

† Significantly different from QMR ($p < 0.001$).

Twenty (20.2 ± 0.2 g) mice were measured by QMR and DXA.

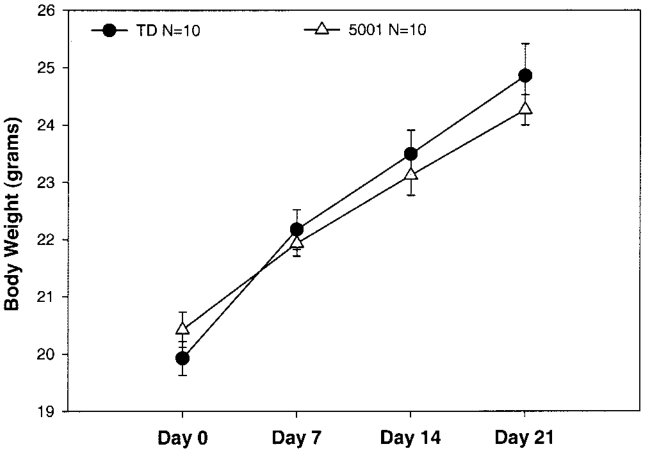

Figure 6: Average body weights of mice fed either a caloricly dense (TD) or lean diet were not different during the 3-week observation period. TD is the Teklad TD95217 diet that is comprised of 40% fat calories, and 5001 is Purina 5001 and is comprised of 12.5% calories from fat. Symbols represent the mean ± SE.

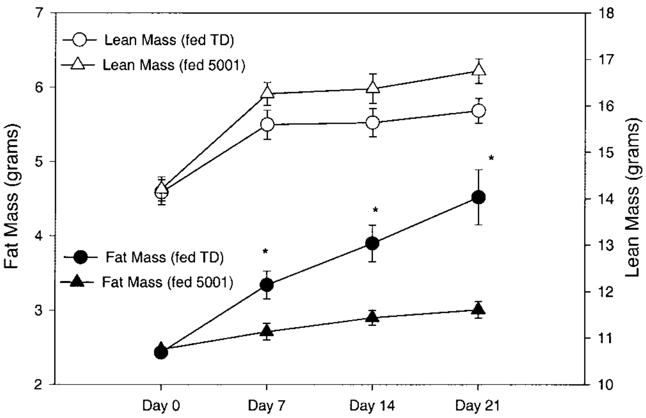

Figure 7: Mice fed a high-fat diet (40%) gain more fat (measured by QMR) than mice fed a caloricly lean diet. Lean mass measured by QMR did not change with diet. *Significantly different from start (Student-Newman-Keuls post hoc test).

DXA was greater than that measured by QMR at any body weight.

Discussion

We describe for the first time a new system for noninvasive measurement and calculation of total adipose mass and apparent skeletal muscle mass of an unanesthetized mouse. The method is not only more precise than other available methods of assessing body composition, but the measurement is more rapid (~1 minute). The system and its method for quantification of body composition are based on NMR.

Whereas conventional X-ray radiographic and computed tomography images depend on electron density, NMR/MRI depends on the density of hydrogen nuclei and the physical state of the tissue as reflected in the T1 and T2 relaxation times. In general, NMR/MRI instruments create contrast between soft tissues by taking advantage of the differences in relaxation times of the hydrogen spins and (or) the hydrogen density in these tissues. Tissue contrast is high among fat, body free fluid, and muscle, based on their NMR signal amplitudes and relaxation times, and can be further enhanced by application of certain RF sequences. Water can be easily distinguished from oils and fats based on NMR high-resolution spectra. Low-resolution NMR techniques will exhibit a spectrum of NMR signals containing one single "wide line" at the weighted mean proton resonance frequency for a mixture of water and oil. This technique is referred to in the literature as "wide-line" NMR (22). NMR signals also depend on the diffusion properties and temperature of the material that is being investigated. Reviews of NMR in general and NMR relaxation phenomenon in particular, which is used in the QMR method, can be found elsewhere (17,23–25).

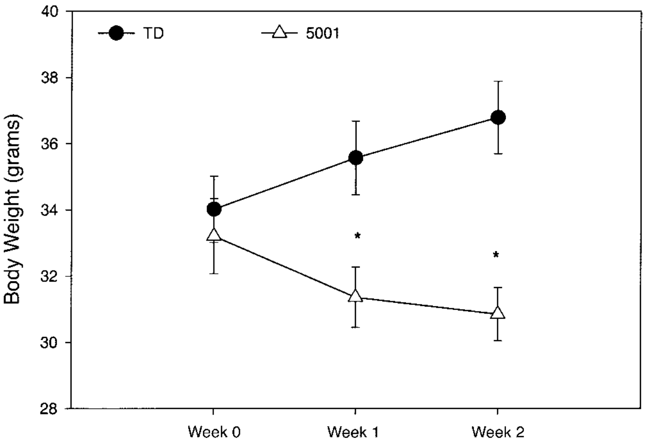

Figure 8: Mice switched from a caloricly dense diet (TD) to a caloricly lean diet (5001) lost body mass during the first 2 weeks after dietary change. *Significantly different compared with mice continued on high-fat diet ($p < 0.03$).

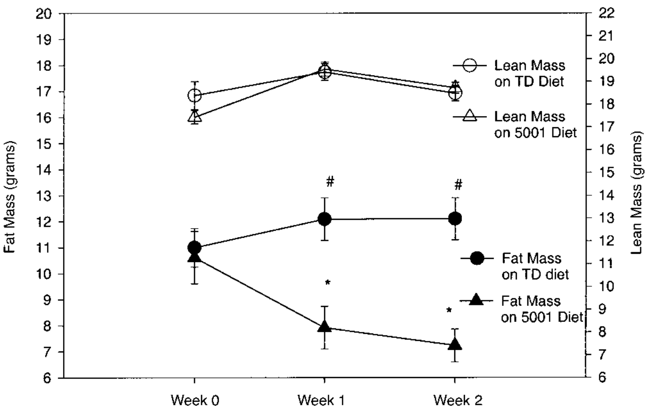

Figure 9: Mice switched from a high-fat diet to a low-fat diet lost fat mass, whereas mice maintained on the high-fat diet gained fat mass. *Significantly different compared with mice switched to low-fat diet and significantly different from week 0 fat value ($p <$ 0.03). #Significant increase in fat mass compared with week 0 ($p < 0.05$).

Differences in NMR properties of water and fat suggest the possibility of using spectroscopic or relaxometric NMR methods for noninvasive whole body composition (WBC) analysis. Mitchell et al. (26) and Kamba et al. (27) demonstrated these techniques both in vivo and in vitro. The spectroscopic method is based on the fact that the two major peaks on the NMR absorption spectrum, representing hydrogen in water and hydrogen in CH_2 groups of fat, are separated by 3.5 parts per million. Relaxometric methods are dependent on differences in T1 relaxation of water protons in muscle and methylene protons in fat. Although the spectroscopic method is more direct and precise, relaxometric methods allow for imaging of very large bodies and

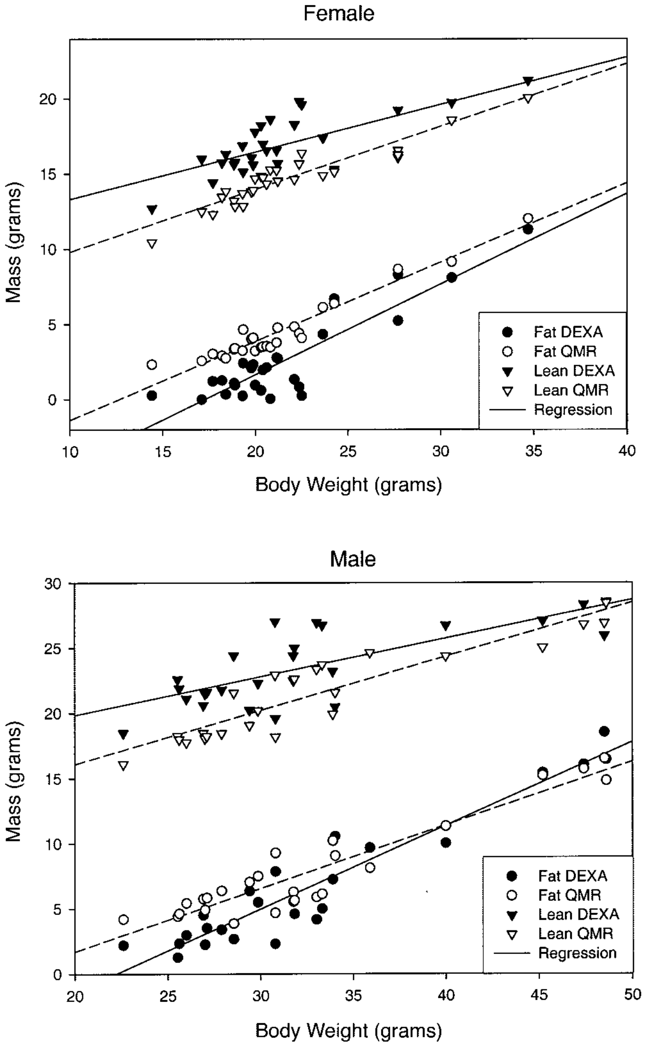

Figure 10: Correlation of body weight to fat mass and lean mass of female mice and male mice using QMR and DXA. Apparent muscle mass of male mice is more consistent when fat is measured by QMR compared with DXA.

determining distribution of fat deposits in the body; they also allow for measuring size and shape of specific organs.

There are limitations for application of NMR to WBC analysis. Separating hydrogen peaks of water and CH_2 groups in spectroscopy is limited by the strength and homogeneity of the magnetic field; thus, whole body spectroscopy is applicable only to very small animals. NMR imaging of the whole body is less precise and more time-consuming. The equipment is quite expensive to acquire, operate, and maintain. There are no good "gold standards" of WBC to calibrate or compute accuracy. Furthermore, "pure fat" or "pure muscle" tissue would possess different properties for different parts of the body and could even differ among different samples from the same body site. In such situations, a multivariate statistical analysis has substantial advantage over univariate calibration because absolute accuracy is less important than reliability and precision.

The accuracy of QMR measurement can be addressed by comparison with DXA and chemical methods. Values for fat mass are the easiest to compare. Chemical analysis was based on petroleum ether extraction that efficiently extracts fats in the form of triglycerides and cholesterol esters but fails to measure another 17% present as structural lipids such as phospholipids (28). DXA has been thoroughly described and classified as a property-based type II method (29), based on the fact that it measures two variables using assumed constants. The planar area of the animal not containing bone is analyzed by pixels for the contribution of lower attenuation fat mass and higher attenuation lean mass. Tissue lying above or below bone is estimated using assumptions about the nature of tissue in the proximity of bone. Because the ratio of attenuation at low energy to attenuation at high energy for fat is slightly lower compared with components of nonfat tissue, it is possible to mathematically estimate the relative contribution of fat and lean to body mass. This requires the adoption of constant values for the average contributions of the various components of nonfat tissue such as water, protein, and minerals, thereby introducing a certain amount of error. Because the variance associated with estimates of fat and nonfat tissue are interdependent, it is not surprising that estimates of fat in very lean animals are associated with high variability. QMR is a measurement based on calibration with a set of standards containing known amounts of a standard fat (canola oil), lean chicken breast muscle, and saline. Thus, QMR and DXA should give comparable values for fat mass, whereas chemical analysis may be expected to give a lower value. Indeed, in this set of experiments, QMR and DXA gave virtually the same average value for fat mass (Table 2), and chemical analysis yielded ~15% lower average values.

Lean mass cannot be easily compared across the three methods. Chemical analysis provides a value for protein mass, moisture, and ash as a measure of total body mineral content. These can be combined to produce a value that compares with DXA lean mass plus bone mineral content. This avoids the difference between whole body ash by chemical analysis and bone mineral content by DXA. QMR, on the other hand, provides a value for lean tissue that is more accurately described as muscle mass equivalence, whereas no value for bone mass and no independent measure of water bound in tissues is available. Thus, QMR can be expected to provide a measure of lean mass that is lower and not fully equivalent to DXA or chemical analysis. Because QMR is calibrated with lean muscle, it should provide a more representative measure of skeletal muscle mass. Additional insight was provided by the Pearson product moment analysis. Lean mass as measured by DXA was better correlated to lean mass by chemical analysis than lean mass defined by QMR. This is not surprising because lean mass combined with fat mass measured by chemical analysis accounts, on average, for over 98% of the total body mass, and DXA partitions total body mass into fat and lean. Body fat was highly correlated among the three methods, with the exception that determination of low fat mass by DXA was less than that measured by the other two methods. Total body fat was consistently measured by QMR with superb accuracy when compared with gravimetric mass of oil. It also had excellent precision. The accuracy of lean measurement is dependent in part on the association of water to protein and glycogen in muscle tissue and the similarity of that to the association of water molecules to other organs and fluids of the body. Further study will be required to better understand the use of this technology for measuring all lean tissue mass. Nevertheless, the lean measure made by QMR should be a more representative measure of muscle mass than the DXA measurement of lean mass. In nonobese mice, lean mass should be highly correlated to body weight, and values measured by QMR were, indeed, more highly correlated to body weight than those measured by DXA (Figure 10).

The superior precision afforded by the QMR approach to body composition analysis was demonstrated by experiment 1, where the CV ranged from 0.3% to 0.7% for the measurement of fat mass and 0.5% to 0.9% for the measurement of lean mass (Table 1). Precision in terms of CV assigned for chemical analysis of fat provided by periodic replicate analysis of a whole peanut preparation is 3.4%. The CV for fat in animal tissue is expected to be higher (30). Live free-moving mice were measured with less, but still very good, precision (0.8% to 1.6% for fat mass; 2.0% to 3.6% for lean mass). This precision was better than that observed with anesthetized mice using DXA. The error introduced by movement can be largely overcome by making measurements in triplicate because QMR requires only ~1 minute. The advantage of making linear measurements of body composition rapidly and precisely was demonstrated by switching mice from regular chow diet to a high-fat diet. A mean 0.63-gram increase in body fat was measured after 7 days and was statistically significant. Lean mass values were reduced by 21 days after the dietary change, but the difference was not statistically significant. In this case, QMR technology was able to detect changes in body composition that took place without significant differences in body weight or caloric intake. Mice switched from high-fat content diet to a regular chow diet lost a significant amount of fat mass. These results are consistent with the findings of Flatt (31), which indicate that the size of the adipose tissue mass is dependent on fat content of the diet and genetically determined variables that regulate rate of glucose and fatty acid oxidation. Fat mass of organs such as liver and intestines, as well as fat mass of feces, can also be accurately measured with the EchoMRI, but we are not certain of the meaning of lean mass values for these samples.

A new quantitative or wide-line NMR-based system is described. Body composition by QMR was found to correlate well with both DXA and chemical analysis. Results for average fat mass by QMR and DXA were virtually identical. Precision was markedly improved using QMR compared with DXA, particularly in measuring fat mass. QMR was well suited for measuring smaller quantities of fat. Lean mass measured by QMR is less than lean mass defined by DXA or chemical analysis. Values for lean mass measured by QMR exhibit very good precision and were well correlated to both DXA and chemical analysis. In experiments 1 and 3, it was clear that QMR and DXA accurately measured fat in mice above a certain size and adiposity. QMR continued to measure fat in a linear relationship to body weight even in the smallest mice, whereas linearity in relation to body weight was lost as smaller mice were measured by DXA. QMR was able to rapidly detect diet-induced changes in body composition when there was no change in body weight. Moreover, measurements made by QMR were faster (~1 minute) than DXA (15 to 20 minutes), permitting replicate measurements. Movement of the unanesthetized mouse only slightly decreased precision, and the short time required for body composition analysis did not alter feeding patterns. In comparison, DXA analysis requires anesthesia to eliminate movement and allow proper positioning of the subject. Anesthesia may disrupt feeding patterns and other physiological systems on the day of analysis depending on the agent used. The principal advantage of both methods, however, is the ability to make linear measurements of body composition, which allows for detecting when a change occurs in response to interventions in energy homeostasis by diet manipulations or administration of neurotransmitters, hormones, and drugs targeting energy balance.

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