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Authors: McGuire, Liam P., and Guglielmo, Christopher G.

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Quantitative magnetic resonance: a rapid, noninvasive body composition analysis technique for live and salvaged bats

Liam P. McGuire* and Christopher G. Guglielmo

Department of Biology, University of Western Ontario, London, Ontario N6A 5B7, Canada

** Correspondent: lmcguir5@uwo.ca*

Quantitative magnetic resonance (QMR) is a new technology for measuring body composition of live, nonanesthetized animals (fat mass, lean mass, and total body water) in 4 min or less. We conducted a validation study to compare QMR body composition analysis of 3 species of bats (mass range 5.77–31.30 g) with traditional chemical extraction. In addition to scans of live animals, we tested the effectiveness of QMR for salvaged specimens (bats killed by wind turbines) and the effects of carcass temperature. Our analysis indicates that QMR body composition analysis is effective for live and salvaged animals. Frozen carcasses could not be analyzed, but results were not dramatically affected for specimens at 4°C and 37°C. QMR analysis eliminates the need to euthanize animals to determine body composition precisely, allows rapid and efficient data collection, and makes it possible to follow individuals longitudinally through time. DOI: 10.1644/10-MAMM-A-051.1.

Key words: bats, body composition, fat stores, lean mass, noninvasive technique, quantitative magnetic resonance

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Body composition analysis is used widely to determine the physiological state of wild animals (Speakman 2001). Fat mass, lean mass, and total body water can vary widely, and bats in particular can exhibit dramatic variations in body composition throughout the year (Krulin and Sealander 1972; O'Farrell and Studier 1976; Weber and Findley 1970) or at specific times of year (Baker et al. 1968; Dodgen and Blood 1956; Ewing et al. 1970; Hood et al. 2006; Kronfeld-Schor et al. 2000; Kunz et al. 1998; O'Shea 1976).

Traditionally, direct measurement of body composition requires chemical extraction of carcasses (Reynolds and Korine 2009; Reynolds and Kunz 2001). Destructive methods, while reliable, are not ideal because they require killing animals (or opportunistically obtaining animals that died from other causes), are labor intensive and time consuming, and generate chemical waste. Alternatively, noninvasive methods based on morphological measurements (total body mass adjusted for body size) are often used. Although some studies find correlations between condition indices and body composition (Pearce et al. 2008; Seewagen 2008), several mathematical and conceptual objections to the technique have been raised (Green 2001; Hayes and Shonkwiler 2001). Condition indices based on morphometric measurements may be state dependent. A female bat with a high mass for her size could either be carrying a large fat store or simply be pregnant. Furthermore, because researchers often use condition indices to approximate fat stores, the implicit assumption is that fat is the only body component that varies in size. Two individuals of the same measured structural size are assumed to have the same lean mass, so the heavier individual must be carrying more fat. However, lean tissue body components can vary substantially, as in migratory birds (Guglielmo and Williams 2003). Consequently, the ideal body composition analysis technique would provide measurements of each tissue type that are independent of state, body mass, structural size, or overall body composition.

Several technologies have been developed to provide noninvasive determination of body composition. Both dual-energy X-ray absorptiometry (Stevenson and van Tets 2008) and total body electrical conductivity (Pearce et al. 2008) have been used to measure body composition of small mammals. However, both technologies are subject to many problems, such as sensitivity to animal position, requirements for anesthesia, and X-ray exposure. Numerous additional indirect measurement techniques (e.g., heavy water dilution) are reviewed in Reynolds and Korine (2009).

We introduce an emerging technology new to field and wildlife biologists, quantitative magnetic resonance analysis (QMR), which provides highly accurate, noninvasive measures of body composition. The capabilities of this technology will allow researchers to address biological questions that were impossible to address with previous techniques. With

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QMR animals are placed into a holding tube and a magnetic resonance scan provides direct measurement of fat mass, lean mass, and total body water in approximately 3 min (although scan parameters can be altered to decrease run time to <1 min). Animals do not need to be anesthetized or restrained. Detailed descriptions of the quantitative methods have been described previously (Taicher et al. 2003; Tinsley et al. 2004). Briefly, QMR uses nuclear magnetic resonance relaxometry to detect different spin relaxation rates in different tissues. The relaxation rate increases in the order free water < fat < water in lean tissue. Calibrations using canola oil and chicken breast standards were developed by the manufacturer and are built into the proprietary software provided with the machine. The software output reports grams of fat, lean, and total body water (free water plus water bound in lean tissues) and does not require any calculation or interpretation by the user.

A potential limitation of QMR technology is that temperature can affect results, and therefore the scanner is optimized for measurement of animals at live body temperatures (approximately 37°C). For live, homeothermic animals, temperature variation is not a concern, but it would be advantageous to be able to measure the body composition of torpid or previously killed individuals. A short QMR scan is much faster and easier than chemical extraction, and it leaves a carcass available for further study. This approach also would allow analysis of salvaged specimens (e.g., bats and birds killed by wind turbines).

Effective measurement of body composition by QMR has been demonstrated for laboratory mice (Tinsley et al. 2004); however, laboratory mice are typically much larger than many bats. The objectives of the present study were to determine if QMR technology is effective for the measurement of body composition of living bats (many of which are smaller than the mice used in previous validation); and if the technology can be used to measure the body composition of previously killed specimens, and if so, what effect the temperature of the specimen may have. A final objective of our study is to introduce the QMR body composition analysis technique to biologists working outside of a clinical setting.

Materials and Methods

The QMR scanner we used was the Echo-MRI-B (Echo Medical Systems, Houston, Texas), which was custom-designed for analysis of small birds and bats in consultation with CGG. The system has a 7-cm-diameter antenna positioned between 2 permanent magnets with a field strength of approximately 0.05 tesla. The electronics and computer consume about 500 watts, and full specifications, dimensions, and weight are available from the manufacturer. It can be used in the laboratory or at field sites when transported in a mobile laboratory trailer, which we maintain at 17–24°C because the magnetic properties of the instrument are affected by temperature. However, the instrument has software to correct for temperature variation of the magnets and specimens during calibration and tuning routines. The QMR is calibrated daily using a 94-g canola oil standard, and a small canola oil standard (5 g) is run before and after measurements to ensure performance. Animals are scanned awake and unrestrained in clear plastic holding tubes that vary in internal diameter from 3 to 6.5 cm. Both the closed end of the tube and the stopper behind the animal are perforated to allow air flow. Scan duration depends on the desired precision, and for the present study we scanned bats at the 4 accumulation setting (approximately 220 s).

Live bats.—We collected 28 little brown bats (*Myotis lucifugus*) from a maternity roost near Goderich, Ontario, Canada (43°46′N, 81°43′W) in July 2007. For the QMR scan bats were placed inside a 3-cm–internal-diameter holding tube. The bats readily crawled to the closed end of the tube. We then inserted the stopper behind the bat to prevent the bat from crawling back out again. Following the live scan we euthanized the bats by cervical dislocation, weighed them (±0.001 g), and placed them in individually sealed plastic bags in a −20°C freezer. We repeated the scans with the frozen bats and then placed them in a 4°C refrigerator overnight to thaw. After scanning the bats at 4°C they were heated to 37°C in a water bath and scanned again. The bats were kept in the sealed plastic bags for all scans to prevent desiccation or wetting of the carcass; we determined that the plastic of the bags is not detected by the body composition analyzer.

After completing the QMR scans we measured body composition using chemical extraction. We dried the bats to a constant mass (as determined by repeated measurements) in a 70°C oven. The difference in mass before and after drying was taken as the total body water mass (mass was measured to ±0.001 g). We then homogenized the carcass in a heavy duty blender (model CB15; Waring Commercial, Torrington, Connecticut) and divided the homogenate into 2 preweighed cellulose filter paper envelopes (Whatman #1) for soxhlet extraction with petroleum ether to determine fat mass and fat-free lean mass. The QMR analysis does not provide a value analogous to dry, fat-free lean mass (as typically reported in studies using chemical extraction). Rather, the lean mass measurement of QMR reflects the live mass of nonfat tissues such as muscles and organs. For comparison, we calculated wet lean mass as the total body mass minus the extracted fat mass (equivalent to the fat-free wet mass).

Salvaged bats.—To increase the range of body masses and test the technique in a real-life circumstance we obtained 5 hoary bats (*Lasiurus cinereus*) and 4 silver-haired bats (*Lasionycteris noctivagans*) that had been killed by wind turbines during migration in Alberta, Canada (Baerwald et al. 2008; Barclay et al. 2007). Carcass searches were conducted every morning, ensuring the bats had been killed the previous night. Carcasses were weighed (±0.001 g) and kept frozen at −20°C until analysis. We analyzed the results of the scans of *M. lucifugus* at the different temperatures to choose the best temperature for scanning the bats from the wind turbines. We reheated the hoary and silver-haired bats, in sealed plastic bags, to 37°C in a water bath prior to scanning them in the body composition analyzer. Following scanning, we dried and extracted the bats as described above.

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Table 1.—Regression parameters for body composition components of *Myotis lucifugus* before (live) and after (reheated, refrigerated, or frozen) euthanasia. $n = 28$, NS = not significant ($P > 0.05$).

	Intercept	Slope	Slope 95% *CI*	r^2	*F*	*P*
Fat						
Live	NS	1.14	(1.06, 1.23)	0.965	$F_{1,27} = 749.70$	<0.001
Reheated	−0.23	0.86	(0.80, 0.92)	0.971	$F_{1,26} = 882.14$	<0.001
Refrigerated	−0.36	1.07	(0.92, 1.22)	0.892	$F_{1,26} = 214.14$	<0.001
Frozen	0.50	1.28	(0.28, 2.28)	0.211	$F_{1,26} = 6.94$	0.014
Wet lean						
Live	1.48	0.91	(0.67, 1.15)	0.694	$F_{1,26} = 58.95$	<0.001
Reheated	NS	1.19	(1.17, 1.20)	0.999	$F_{1,27} = 35769$	<0.001
Refrigerated	1.00	0.95	(0.82, 1.09)	0.889	$F_{1,26} = 208.30$	<0.001
Frozen	NS	NS			$F_{1,26} = 0.45$	0.507
Water						
Live	3.31	0.23	(0.13, 0.32)	0.517	$F_{1,24} = 25.65$	<0.001
Reheated	3.33	0.28	(0.11, 0.45)	0.298	$F_{1,26} = 11.02$	0.003
Refrigerated	3.68	0.21	(0.10, 0.32)	0.355	$F_{1,26} = 14.33$	<0.001
Frozen	NS	NS			$F_{1,26} = 2.91$	0.118

Statistical analysis.—We used linear regressions in R (version 2.9.2—R Development Core Team 2009) to measure the relationships between fat, lean, and water masses from soxhlet extraction with the values provided by QMR. Nonsignificant intercepts (not different from 0) were removed from the regression. We considered linear regression models with slopes closest to 1 to be the most accurate (QMR measured values equaling chemical extraction measured values), with high r^2 values indicating high precision (small proportions of variation not accounted for). Other validation studies have reported precision as the measurement repeatability for a single sample. We regularly include a check sample (oil standard) among our samples and find a coefficient of variation of approximately 0.7% (data not shown).

For scans of *M. lucifugus* at different temperatures we compared the 95% confidence intervals (95% *CI*s) for the estimates of the slopes to determine if the slopes were statistically different at different temperatures. If the 95% *CI*s overlapped, we concluded they were not statistically different. In addition, if the *CI* contained 1, we concluded that the slope was not different from 1.

We calculated the absolute error of the body composition analyzer by calculating the absolute value of the difference between the raw QMR output and the value determined by proximate analysis for each component of body composition. We also calculated the relative error by dividing the absolute error by the proximate analysis value and converting to percent. Following analysis of the raw data, we reanalyzed the data using a cross-validation approach. We created a program in SAS version 9.1.3 (SAS Institute, Cary, North Carolina) to select 15 samples randomly and perform a linear regression to obtain a calibration equation. The calibration equation then was applied to the remaining 22 samples to predict the true values from the raw QMR output. We repeated this simulation 1,000 times and each time calculated the error and relative error. The mean of the error from each simulation was taken as the mean that could be expected to occur if using a calibration equation in future studies.

All methods were approved by the University of Western Ontario Animal Use Subcomittee (protocol 2004-027-03) and conducted under a Scientific Collection Permit from the Ontario Ministry of Natural Resources (permit 1039395). All procedures complied with guidelines of the American Society of Mammalogists for use of wild mammals in research (Gannon et al. 2007).

Results

The mean total body mass of *M. lucifugus* was 7.44 ± 0.20 g ($\bar{X} \pm SE$; range 5.77–9.64 g). The values from QMR analyses for living bats were highly correlated with the values obtained by chemical extraction (Table 1). The slope of the regression equation for fat mass was near 1 with a high r^2 value. The slope of the regression for wet lean mass (fat-free wet mass) was also near 1. The total body water estimate was neither accurate (slope not close to 1) nor precise (low r^2). Two of the live bats yielded negative body water measurements by QMR; these values were excluded from our analysis.

After the live scans had been performed, but before any further scans, we discovered a hardware defect (a visibly damaged capacitor on an electronics board) in the body composition analyzer that might have resulted in increased scatter in the data. The problem was resolved and some of the software analysis parameters also were altered to improve the performance of the analyzer. Consequently the scans of the live animals are not directly comparable to the scans of the dead animals (Fig. 1). When specimens were frozen, relationships with total body water and wet lean mass were not significant, and estimates of fat mass were very scattered (Table 1). However, thawing the samples to just 4°C in the refrigerator substantially improved the accuracy (Table 1;

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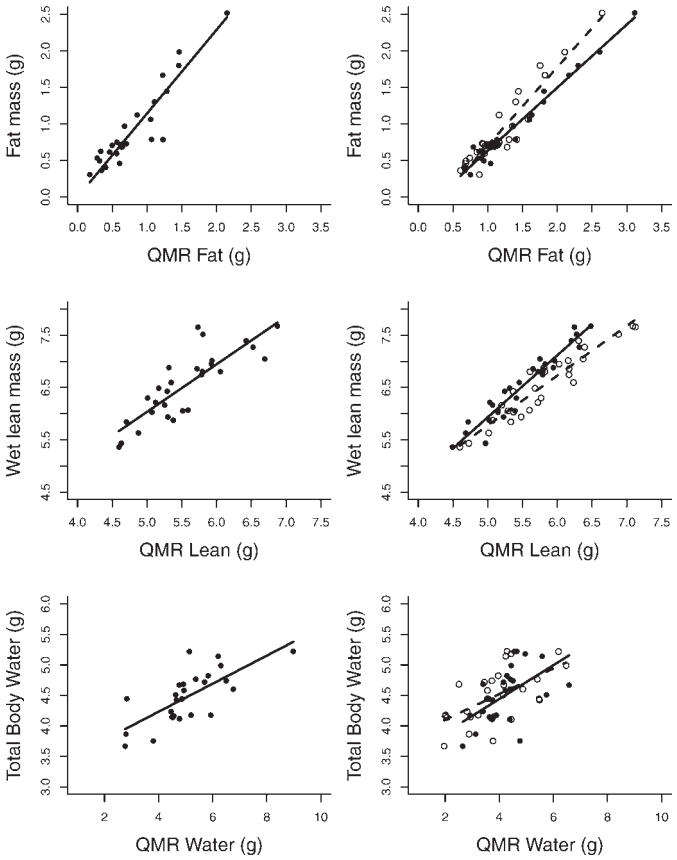


FIG. 1.—Regressions of soxhlet body composition analyses on quantitative magnetic resonance (QMR) for *Myotis lucifugus*. Plots on the left are from scans of live animals. Plots on the right are from the same animals after euthanasia, either refrigerated (○, dashed line) or reheated (●, solid line).

Fig. 1). The slopes of the regression equations predicting fat mass and wet lean mass were not significantly different from 1.

When samples were warmed to 37°C, the estimates of the slopes for fat mass, total body water, and wet lean mass did not differ significantly from those obtained at 4°C. The precision of all estimates except water was very high at the higher temperature ($r^2 > 0.97$).

Relationships remained strong when the range of body mass was expanded to include *L. noctivagans* and *L. cinereus* (Table 2; Fig. 2). Body mass of *L. noctivagans* ranged from 9.00 to 15.10 g and the body mass of *L. cinereus* ranged from 21.00 to 31.30 g. Values of r^2 were >0.98 for all components of body composition. Fat mass was slightly overestimated (slope < 1), and wet lean mass was slightly underestimated

TABLE 2.—Regression parameters for body composition analysis of 3 species of bats by soxhlet extraction and quantitative magnetic resonance (QMR) analysis. $n = 37$, NS = not significant ($P > 0.05$).

	Intercept	Slope	Slope 95% *CI*	r^2	*F*	*P*
Fat	NS	0.72	(0.70, 0.74)	0.991	$F_{1,36} = 4{,}102.8$	<0.001
Wet lean	NS	1.18	(1.17, 1.19)	0.999	$F_{1,36} = 3{,}020.6$	<0.001
Water	NS	1.07	(1.04, 1.11)	0.991	$F_{1,36} = 4{,}053.1$	<0.001

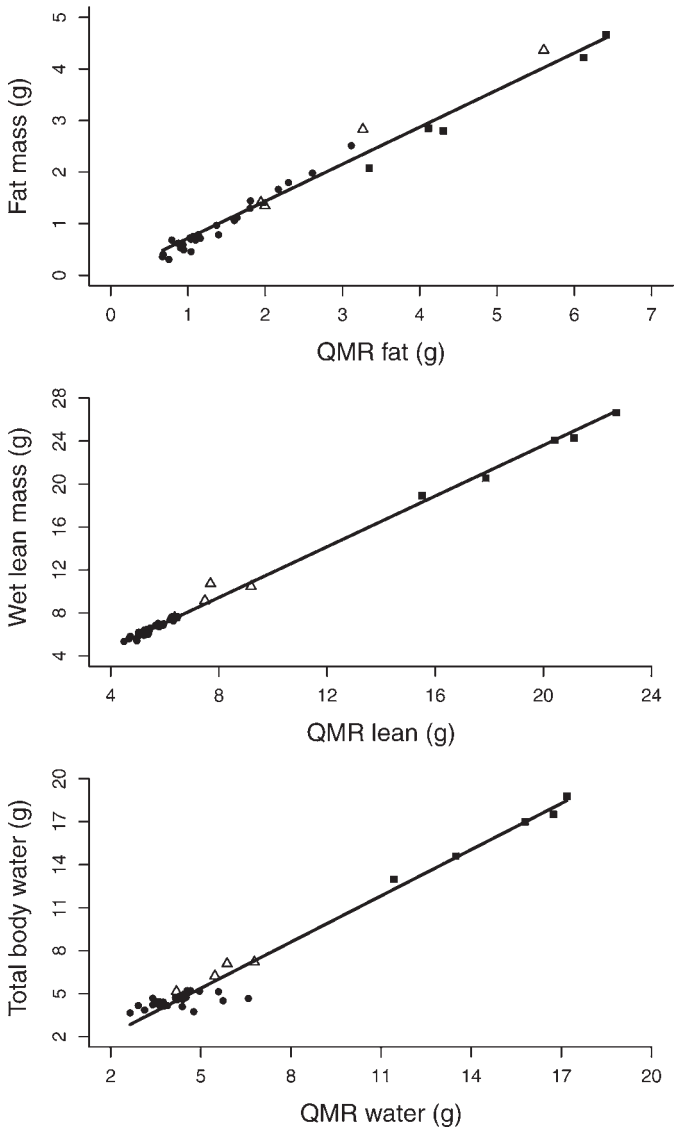


FIG. 2.—Body composition regressions from reheated carcasses of 3 bat species—*Myotis lucifugus* (●), *Lasionycteris noctivagans* (△), and *Lasiurus cinereus* (■).

(slope > 1). Over the larger mass range the predictive equation for total body water was greatly improved. The slope was very near to 1, and the r^2 value increased to 0.99.

Over the entire range of body mass errors obtained from the raw output of the body composition analyzer were reasonable for fat, wet lean, and water masses, but applying the cross-validation equations greatly reduced the predicted errors (Table 3). When using the cross-validated equations, fat mass is estimated within 130 mg and lean mass is estimated within 280 mg.

DISCUSSION

Previous studies (Jones et al. 2009; Nixon et al. 2010; Taicher et al. 2003; Tinsley et al. 2004) have demonstrated the effectiveness of QMR for measuring body composition of rodents in clinical settings. However, the subjects used in these studies were all substantially larger than the majority of

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Table 3.—Absolute and relative error of quantitative magnetic resonance (QMR) measurements of body composition components. Results are presented for raw values (direct comparison of QMR output to proximate analysis) and cross-validated values (from 1,000 iteration simulation—see text). All data are shown as mean ± *SD*.

	Raw		Cross-validated	
	Absolute error (g)	Relative error (%)	Absolute error (g)	Relative error (%)
Fat	0.60 ± 0.43	51.56 ± 26.70	0.13 ± 0.03	11.59 ± 2.38
Wet lean	1.42 ± 0.88	15.84 ± 3.21	0.28 ± 0.15	3.00 ± 0.81
Water	0.76 ± 0.43	13.94 ± 8.69	0.58 ± 0.27	10.51 ± 2.01

the bats used in our study. In some cases in previous studies the fat mass alone was greater than the total body mass of the bats used in our study. Our body composition analyzer was custom-designed for animals with body masses of ≥10 g. However, despite the small body masses of the *M. lucifugus*, the body composition analyzer was effective for measuring fat mass and wet lean mass. The QMR analysis did not provide accurate estimates of total body water for *M. lucifugus*, which could represent a limitation of the equipment; some of the *M. lucifugus* used in this study weighed only 6 g, far below the design specification. For studies concerned with the accuracy of body water measurements, more tests should be performed. Effective body water measurements can be made, at least at larger body masses (Fig. 2).

In most cases we were satisfied with the accuracy and precision of the estimates provided by QMR analysis. Raw QMR output estimated fat with ±0.60 g error and wet lean mass with ±1.42 g error. Using a calibration equation (as in our cross-validation procedure) greatly reduced the errors (±0.13 g for fat mass and ±0.28 g for wet lean mass). Although the reduced errors are impressive, some caution is required. Calculation of absolute and relative error assumes that the measurement obtained from chemical extraction is made without error. Chemical extraction is considered the ''gold standard'' for body composition analysis, but any sample loss or measurement errors made during homogenization, transfer, or weighing samples will influence the absolute and relative errors calculated for the QMR measurements. The slope of the regression equations was not always 1 in our analysis, and we cannot exclude the possibility that the error is in the chemical extraction values and not the QMR values. The overestimation of fat mass by QMR may be an example of inaccurate measurement by chemical analysis. The solvent we used (petroleum ether) removes all neutral lipids but does not account for structural lipids (Jones et al. 2009). If structural lipids account for a substantial portion of body mass, rather than concluding that QMR overestimates fat mass, the correct conclusion would be that chemical extraction underestimates fat mass. Future validations could conduct a 2-stage extraction using chloroform–methanol in a 2nd extraction to remove all lipid compounds.

Frozen specimens could not be analyzed by QMR. However, once the samples were thawed, the body composition analyzer was robust to temperature effects. Whether at 4°C or 37°C, QMR estimates closely predicted the values obtained from soxhlet extraction. The precision was greater at 37°C, but we did not observe a substantial effect of having cold samples. Room temperature likely would be sufficient for future analyses. Both reheated and refrigerated samples provided reasonably similar values to the live scans. Unfortunately, due to the changes in hardware and software between the 2 sets of scans, it is not possible to draw direct comparisons. However, the results from the killed specimens were not dramatically different from those of the live scans.

The body composition analyzer provided excellent results across the larger size range when *L. noctivagans* and *L. cinereus* were included. The results obtained from these specimens confirm that it is possible to determine body composition from salvaged samples and show that samples need not be freshly killed. Specimens of *L. noctivagans* and *L. cinereus* used in this analysis were collected from beneath wind turbines. The time from the bats being killed to the time that the bats were frozen was variable and unknown (other than that they were killed some time during the night), and consequently some caution is required when considering these samples as they are all likely to be desiccated to various degrees. Estimates of total body water and wet lean mass, although accurate to the true composition of the sample, might not accurately reflect the actual composition of the animal before it was killed. Estimates of fat mass are likely to be more robust as fat is stored with very little associated water and thus less affected by desiccation.

The combined results of all of the analyses presented here highlight the broad range of conditions for which QMR body composition analysis is effective. Accurate predictions of body composition can be obtained from bats as small as 6 g. The bats can be alive or previously killed, and they can be at normal body temperature or cooler, provided they are not frozen. Salvaged specimens need not be freshly killed; accurate results can be obtained from carcasses collected some time after death.

A further advantage of QMR body composition analysis is the biological relevance of the lean-mass measure. In chemical extraction analysis lean mass is typically measured as fat-free dry mass, effectively anything that is not fat or water. This would include inert tissues such as bones, teeth, claws, and fur. The lean mass provided by QMR analysis is the wet mass of lean tissues such as muscles and organs. Adding QMR fat mass, QMR lean mass, and skeletal mass (not measured by QMR) would approximately equal total wet body mass. For studies that seek to relate body composition to physiological or behavioral correlates, the QMR measurement of lean mass is more biologically relevant than fat-free dry mass.

Overall, QMR analysis provides a highly effective technique for determining the body composition of a wide range of samples under a wide range of conditions. The analysis is fast, clean, and simple and avoids the need for destructive analysis, thus leaving the test subject free for further analysis or release. This noninvasive technology allows for the rapid and efficient collection of large amounts of data and a greater potential to conduct longitudinal studies of individuals.

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