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REVIEW

Simple, rapid, and non-invasive measurement of fat, lean, and total water masses of live birds using quantitative magnetic resonance

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Abstract An ideal technology for non-invasive analysis of body composition should provide highly precise and accurate direct measurements of fat, lean mass and total water of non-anaesthetized subjects within minutes. We validate a quantitative magnetic resonance (QMR) body composition analyzer for birds using House Sparrows (*Passer domesticus*), European Starlings (*Sturnus vulgaris*), and Zebra Finches (*Taeniopygia guttata*). Subjects were scanned awake for three replicate scans of 1.5–3.5 min, and results were compared to gravimetric chemical analysis. Coefficients of variation were ≤3% for dry fat, wet lean mass and total water. Accuracy of the raw QMR data for fat and total water were high (relative errors ≤±12.5 and ≤±4%, respectively), but wet lean mass was significantly biased because QMR does not detect structural tissues. Calibration against gravimetric chemical analysis removed bias and improved accuracy; relative errors were ±6–11% for fat, ±1–2% for wet lean mass, and ±2–4% for total water. QMR is field-portable when transported in a temperature-controlled trailer, and can be used to study fuel storage and body composition dynamics during migration, reproduction, nestling growth, or wintering. In the laboratory, QMR can be used for longitudinal studies of birds under photoperiod, endocrine or other manipulations. Measurements taken before and after metabolic challenges, such as flight in a wind tunnel, make it possible to calculate energy costs, fuel selection and changes in hydration. QMR should find wide application in field and laboratory studies.

Keywords Body composition · Condition · Energetics · Lipid · Magnetic resonance · Validation

Zusammenfassung Methoden für nichtinvasive Körperzusammensetzungs-Analysen sollen im Idealfall präzise und direkte Bestimmungen von Fett- und fett-freier Masse sowie dem totalen Wasseranteil von nichtanästisierten Tieren innerhalb von Minuten erlauben. Wir validieren eine quantitative Magnet-Resonanz (QMR) Körperzusammensetzungs-Analyse für Vögel anhand von Haussperling (Passer domesticus), Star (Sturnus vulgaris), und Zebrafink (Taeniopygia guttatai). Die Vögel wurden im wachen Zustand dreimal 1.5 bis 3.5 Minuten lang gemessen, und die Ergebnisse wurden mit einer chemischen Analyse verglichen. Variationskoeffizienten waren ≤ 3% für Fett (ohne Wasser), fett-freie Masse (mit Wasser) und für den gesamten Wasseranteil. Die Präzision der rohen QMR Daten für Fett und Wasseranteil war hoch (relative Messfehler: ≤±12.5% und ≤±4%), aber die Messung von fett-freier Masse (ohne Wasser) war beeinträchtigt, weil QMR keine strukturellen Gewebe erfasst. Die Kalibrierung mit chemischer Analyse behob dieses Problem und erhöhte die Genauigkeit; die relativen Messfehler waren ±6–11% für Fett, ±1–2% für fett-freie Masse (mit Wasser), and ±2–4% für den gesamten Wasseranteil. QMR findet Anwendung bei der Feldarbeit, um Fettdeposition während des Zuges, der Reproduktion, des Nestlingswachstum oder beim Überwintern zu bestimmen. QMR kann auch im Labor angewandt werden, wenn z.B. die Photoperiode oder das Hormonsystem übere längere Zeiträume manipuliert werden. Messungen, die vor und nach Manipulation des

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Stoffwechels stattfinden, wie z.B. nach einem Flug in einem Windtunnel, ermöglichen es, Energiekosten, Brennstoffzusammensetzung und Veränderungen im Hydrationszustand zu berechnen.

Introduction

The centenary celebration of Vogelwarte Helgoland in 2010 was an opportunity to reflect on the tremendous progress that has been made in avian biology over the past century. Reflecting on the past should inspire today's scientists to look toward the future where new technologies will allow us to re-examine long-standing questions, and bring new questions to the fore. In this contribution, we provide a validation of quantitative magnetic resonance (QMR) as a new, non-invasive method for body composition analysis of birds, and provide examples of its application in the field and laboratory.

Many investigations into the biology of birds bring forth the question: "what is going on underneath those feathers?" Whether one works in the field or laboratory, information on the chemical composition of the body (fat, lean mass, water, or specific micronutrients) can provide great insight into physiology, behavior, and ecology. For example, one might be interested in how body fatness, lean mass, or hydration state affect metabolism or behavior (Scott et al. 1996; Bäckman et al. 1997; Schaub et al. 2008), or conversely how various experimental manipulations affect body composition (Wingfield et al. 1997; Fransson et al. 2001; Vézina et al. 2006). In a more general sense, researchers are often interested in how physiological "condition" (usually a surrogate measure of body composition) affects performance during key life history stages such as reproduction or migration. Condition indices are often used as indicators of the quality or health of birds, the habitats they occupy, or the general environment. Clearly, information about body composition is relevant to a wide variety of research questions.

Obtaining accurate body composition data has often required the collection of birds (by killing or salvage) for chemical analysis of carcasses, and this remains an important and valid experimental approach in some circumstances, particularly for detailed analysis of organ and muscle sizes (Piersma et al. 1999; Guglielmo and Williams 2003; Bauchinger et al. 2005), or contaminant loads (McFarland et al. 2002; Mora et al. 2003). However, if tissue analysis is not necessary, ethical considerations demand that we develop and use techniques to measure body composition without killing or harming animals. The major added benefit of non-invasive methods is that they allow for much more powerful longitudinal studies of the effects of experimental treatments on individuals, and of how specific aspects of body composition influence subsequent behavior.

It is not sufficient for a body composition analysis method to just be non-invasive; it must also meet other criteria. To maximize data collection and minimize stress on birds, an ideal method should also be rapid (e.g., $<$5 min), and require no heavy restraint or anaesthesia. Most importantly, it should provide highly precise and accurate, direct measurements of all three major body components (fat, lean mass, and water). Excluding the magnetic resonance methods described below, dual-energy X-ray absorptiometry (DXA or DEXA) has come closest to this ideal by directly measuring fat and wet lean mass of live small mammals and birds with high precision and accuracy (Nagy and Clair 2000; Korine et al. 2004). However, DXA requires the subject to lie completely still which may require anesthesia (Nagy and Clair 2000; but see Korine et al. 2004), and scan time may be relatively long (5–25 min; Nagy 2001). DXA has the potential complication of repeated X-ray exposure to users. Total body electrical conductivity (TOBEC) can directly and accurately measure lean mass, but fat mass is measured indirectly by accounting for total body mass, often resulting in unacceptably poor accuracy, particularly for small birds (Scott et al. 2001). Heavy water dilution provides highly accurate measurement of total body water and lean mass, but again measures fat indirectly, although often with superior accuracy to TOBEC (Karasov and Pinshow 1998; Speakman et al. 2001; S.R. McWilliams and M. Whitman, personal communication). Morphometric analysis is used widely; however, it is indirect, accuracy is generally low, and there is debate about the methods and assumptions used in data analysis (Green 2001; Hayes and Shonkwiler 2001; Seewagen 2008).

The potential for using magnetic resonance to measure body composition has been well known for decades (Ganssen 1989). Fat and non-fat tissues can be readily visualized and quantified in virtual slices made with T1-weighted magnetic resonance imaging (MRI), and then summed across slices to calculate body composition (Wirestam et al. 2008). This method has been used successfully in lightly-restrained, awake birds using scan times of 20 min for White Storks (*Ciconia ciconia*; Berthold et al. 2001) and 2–4 min for small birds (Wirestam et al. 2008; Hedenström et al. 2009; R. Wirestam and A. Hedenström, personal communication). Magnetic resonance spectroscopy (MRS), which relies on the chemical shifts of resonance frequencies of hydrogen nuclei in different types of molecules, has been used to measure the fat content of bird muscle (Berthold et al. 2001). MRI and MRS can thus provide information on the amount and distribution of fat, and the size and chemical composition

of muscles and organs. However, their use for birds (particularly in the field) is limited due to the cost, size, and energy consumption of the instruments, and their availability is generally restricted to medical facilities where bird researchers have little access or priority (Berthold et al. 2001; Hedenström et al. 2009).

Recently, instruments for analysis of body composition by quantitative magnetic resonance (QMR) have been developed which have comparable accuracy and superior precision to DXA for measuring fat and wet lean mass of mice (Taicher et al. 2003). As with other magnetic resonance methods, QMR uses a magnetic field to align the spins of hydrogen nuclei in a sample, which resonate at particular frequencies. Alternating magnetic fields are then applied by a radio antenna at a characteristic resonant frequency to perturb the spins of the hydrogen nuclei. When the transmitter is turned off, the nuclei return to the aligned spin, releasing radio energy. The antenna measures the amplitude of energy released and characteristics of the T1 and T2 relaxation curves, which differ between fat, wet lean tissue, and water (Taicher et al. 2003). In MRI, these and other differences in the way hydrogen nuclei behave in different tissues are used to generate contrast for imaging. In QMR, fat, wet lean mass, and total water are quantified within a scanned volume without considering spatial distribution (Taicher et al. 2003).

Any new method of non-invasive measurement of body composition requires validation and calibration against gravimetric chemical analysis, which is considered the "gold standard" to which all methods are compared (Reynolds and Kunz 2001). Our objectives were: (1) to customize a QMR system for birds and validate it against gravimetric chemical analysis, (2) to measure the precision and accuracy of raw QMR data and calibrated QMR models across a range of bird body sizes, (3) to determine the QMR instrument scan parameters that minimized scan duration without affecting precision and accuracy, and (4) to determine if species-specific calibration was required, and if so the necessary sample size.

Methods

QMR measurements were made using an instrument and accessories that were designed for field and laboratory studies of birds and bats by Echo-Medical Systems (Houston, TX, USA) according to specifications made by CGG. The QMR scanner (model Echo-MRI-B) has two permanent magnets producing field strength of approximately 0.05 Tesla and an antenna of ~7 cm internal diameter. This antenna size was chosen to allow measurement of a wide variety of birds (approximately 10–200 g), while maintaining acceptable precision and accuracy for small birds. The length of the "homogeneous volume" within which measurements are relatively uniform was maximized (approximately 14 cm) to accommodate the long body profile of birds. The clear plastic holding tubes (delimiters) which center subjects in the antenna were altered by removing a small section of the rear plunger to allow the tail, which is not detected by QMR, to fit comfortably. The cap at the forward end of the holding tube was made to be removable so that following a scan a bird can be easily pushed with the rear plunger into the hand or a bag. In addition, the magnet (with antenna) was separated from the electronics and computer so that the magnet can be loaded into and bolted to the frame of a mobile laboratory trailer (Glendale Recreational Vehicles, Strathroy, ON, Canada) for use in the field (Fig. 1). The electronics and computer were mounted together in a shock-, dust- and water-resistant housing. Although the QMR scanner can correct for variability in magnet and specimen temperatures through software calibration and tuning routines, it functions optimally when temperature is maintained between 17 and 24°C in the trailer or laboratory. Power consumption is approximately 500 W, and can be supplied either through a 120 V, 15 A utility connection or by a generator on-board the trailer. An uninterruptable power supply (American Power Conversion, model Back-UPS RS 900) is used to eliminate power fluctuations, and is sufficient to complete a scan and save data in the case of a power failure. Additional specifications of weight, dimensions and performance may be obtained from the manufacturer.

The QMR scanner reported values for fat, lean (wet), free water, and total water masses (±0.001 g). Software allows the user to change the number of "accumulations"

Fig. 1 Mobile laboratory trailer used to transport and operate the Echo-MRI-B quantitative magnetic resonance body composition analyzer. The magnet unit of the QMR analyzer can be loaded into the trailer through an exterior hatch (*1*) and bolted to the frame of the trailer. *Inset* the interior of the laboratory with the QMR magnet under counter and electronics/computer above

or replicate scans that are used to measure fat and lean mass. Measurements made with fewer accumulations are faster, but may be less precise and accurate. A one-accumulation scan takes approximately 90 s and consists of two replicate scans for fat and lean followed by a single scan for body water. A two-accumulation scan (4 fat/lean scans plus 1 water scan) takes approximately 128 s, and a four accumulation scan (8 fat/lean scans plus 1 water scan) takes approximately 220 s. The scanner has settings for "small" (approximately 50 g or less) or "large" birds, which slightly change the scan parameters to better optimize performance. The QMR scanner was calibrated daily using a 94-g canola oil standard, and performance was checked by periodically scanning a 5-g canola oil sample. QMR was not affected by aluminum leg bands.

QMR analysis of live birds

All animal procedures were approved by the University of Western Ontario Animal Use Sub-committee (Protocol # 2005-060-08), and the species chosen for study were not regulated by Canadian Federal or Provincial agencies. House Sparrows (hereafter "Sparrows", *Passer domesticus*; $n = 24$; body mass 26.01 ± 2.01 g mean ± SD) and European Starlings (hereafter "Starlings", *Sturnus vulgaris*; $n = 18$; body mass 77.68 ± 6.57 g) were captured locally near London, Ontario using mist nets and baited traps. We used five Sparrows immediately on the day of capture, and the rest we held in captivity for 2–5 weeks before use. All Starlings had been in captivity for behavioral studies for over 6 months before use. We purchased Zebra Finches (hereafter "Finches", *Taeniopygia guttata*; $n = 10$; body mass 16.12 ± 2.14 g) locally and used them the same day. We did not intentionally fast birds before analysis, but due to experimental conditions, the time since feeding varied from about 0.5 to 4 h as may also be expected in field studies.

We first weighed each bird (±0.1 g, Ohaus CS200; Pine Brook, NJ, USA). We injected birds in the pectoralis major muscle with 99.9 atom % deuterium oxide (Sigma–Aldrich Chemicals; Sparrows 78.3 ± 10.2 mg, mean ± SD, Starlings 113.0 ± 2.5 mg, Finches 41.3 ± 5.8 mg) using 29-ga, 0.5-inch (c.12.5-mm), 0.5-ml insulin syringes for a separate validation of the heavy water dilution method (Karasov and Pinshow 1998; Speakman et al. 2001). The QMR does not detect deuterium oxide (C.G.G., unpublished data), and so we later subtracted the mass of deuterium oxide injected from total body water measured gravimetrically (see below). We do not report the results of the heavy water dilution validation here, but they have been used elsewhere (Rae et al. 2009; Boyle et al. 2010).

We carefully loaded birds head-first, ventral side down into appropriately-sized ventilated holding tubes (Finches, 3 cm I.D.; Sparrows, 4.5 cm I.D.; Starlings, 5.8 cm I.D.). We scanned Sparrows and Finches using the "small bird" setting, and Starlings using the "large bird" setting of the Echo-MRI software. Each bird was scanned a total of six times; three times on the four-accumulation setting, and three times on either the one- (Sparrows) or two- (Starlings and Finches) accumulation settings. The order of scans was randomized by coin toss. Following the final scan, we held birds in a light cotton bag until 60 min had elapsed since the deuterium oxide injection. To measure deuterium oxide dilution, we took a blood sample from the brachial vein of the wing using a 26-ga needle and heparinized capillary tubes. We estimated the mass of blood removed from the number and fill of capillary tubes taken (60 mg per full tube; C.G.G., unpublished data), and later added it to wet lean mass measured gravimetrically. We estimated water content of the blood to be 76.5% assuming a haematocrit of 50% (Ohira et al. 1977), and added it to the total water mass measured gravimetrically.

Gravimetric analysis of carcasses

Immediately following blood sampling, we anaesthetized birds using inhaled isoflurane (Abbott Laboratories, St. Laurent, QC, Canada), and after they were unresponsive, killed them by cervical dislocation. Sparrow and Finch carcasses were either processed fresh or frozen at −20°C for up to 3 days. Starling carcasses were frozen for 1 month before analysis. Fresh or thawed carcasses were weighed (±0.001 g), plucked, and reweighed. We then cut them into smaller pieces and dried them at 70°C in a gravity convection oven for a minimum of 1 week, which was sufficient to reach constant mass. Dried carcasses were reweighed for calculation of total water (correcting for deuterium oxide injected), and then ground to a powder using a 75-g capacity pulverizer on a 3.75-hp heavy duty blender (model LBC15, 20,800 max rpm; Waring Commercial, Torrington, CT, USA). Portions of the homogenate were measured into two pre-weighed cellulose filter paper envelopes (Whatman #1), reweighed and extracted for 6–8 h with petroleum ether (b.p. 30–60°C) in a soxhlet apparatus. Following extraction, envelopes were re-dried to constant mass at 70°C, and weighed to calculate dry fat and dry lean masses. Since the QMR scanner measures wet lean tissue, gravimetric wet lean mass was calculated as the sum of plucked carcass dry lean mass, water lost during drying (minus deuterium oxide injected), and wet blood sample mass (see above); effectively the fat-free plucked bird.

Statistical analysis

For each body component (dry fat, wet lean, and total water), we determined the precision of one-, two- and

four-accumulation QMR scans by calculating the coefficient of variation (SD/mean × 100) for the three replicate scans of each bird made on the same accumulation setting. We used four accumulation data for all further calibration because every bird had been measured on the four-accumulation setting, and we expected it to be the most precise and accurate. We determined the quality of the raw QMR data for predicting body composition by calculating bias, absolute error (g) and relative error (%). We calculated bias for each body component of each bird as the difference (±) between the mean QMR value and the value from gravimetric analysis (Bias = QMR value − Gravimetric value). To determine accuracy, we calculated absolute error for each individual by taking the absolute value of the bias (Abs Error = |QMR value − Gravimetric value|), and relative error by dividing the absolute error by the value from gravimetric analysis and converting to percent [Rel Error = 100 × (|QMR value − Gravimetric value|)/Gravimetric value].

We used analysis of covariance to test for differences among species in the relationships between QMR values and values derived from gravimetric analysis. We first evaluated species-specific differences in the slopes of these relationships by testing for significant interaction between the fixed factor (species) and the covariate (QMR value). If the interaction was not significant, it was dropped from the model to test for the main effect of species, and homogeneous groups of species were determined by testing for differences in least squares means among species using the *pdiff* option in SAS (version 9.1.3; SAS Institute, Cary, NC, USA). Since there was little (fat) or no (lean) overlap in the data between Sparrows/Finches and Starlings we decided to develop completely separate calibration equations in cases where ANCOVA identified these groupings (see below). Calibration equations relating QMR values for body components to values from gravimetric analysis were made using OLS regression, and intercepts that were not significantly different from zero were dropped from regressions.

We assessed the quality of calibration equations, and the sample sizes needed to derive reliable calibrations using a cross-validation approach. To determine if calibrations derived from one species could be applied to other similar species, we used calibration equations for Sparrows ($n = 24$) to predict gravimetric dry fat, wet lean and total water masses of Finches ($n = 10$). We then calculated bias, absolute error, and relative error for Finches as described above, except that masses predicted by the calibration equations were used in place of raw QMR values. For Sparrows and Starlings, we wrote simulation macros in SAS that randomly selected samples ($n = 10$ or 15 for Sparrows, $n = 10$ for Starlings), and calculated OLS calibration equations with or without intercepts as determined previously from the analysis of the entire dataset for each body component of each species. The calibration equations were then used to predict the gravimetric body component values of the remaining samples ($n = 14$ or 9 for Sparrows, $n = 8$ for Starlings). Simulations were repeated 1,000 times, and for each run we calculated bias, absolute error and relative error using the predicted masses and actual masses as described previously. The mean biases, absolute errors and relative errors from these simulations represent those expected from the average calibration exercise for these or similar species.

Results

The precision of the Echo-MRI-B was very high, and was generally better for the two- and four-accumulation settings than the one-accumulation setting (Table 1). Average coefficients of variation for three replicate scans were less than 1% for lean mass, and approximately 2–3% for dry fat and total water masses on the two- or four-accumulation settings.

Uncalibrated QMR analysis

The raw QMR data for dry fat mass were significantly biased for all three bird species: overestimating fat of Sparrows and Finches, and underestimating fat of Starlings (Table 2). Without correction for bias, absolute errors ranged from approximately ±150 mg for Sparrows and Finches to ±500 mg for Starlings, and relative errors ranged from ±9.5 to 12.5%.

Gravimetric wet lean mass was underestimated by raw QMR lean mass in all three bird species (Table 1) because QMR lean mass does not include the skeleton, keratinized structures, and other body components that are included in gravimetric lean mass. As a result, both absolute and relative error was high for wet lean mass.

Total water mass measured gravimetrically was overestimated by QMR total water mass in Finches and Starlings, but not in Sparrows (Table 1). Absolute error ranged from approximately ±300 mg in Sparrows and Finches to about ±900 mg in Starlings, and relative error was about ±2–4%.

Calibration and cross validation

There were no differences among species in the slopes of relationships between QMR values and gravimetric analysis values for any body component (species by QMR interactions; dry fat mass $F_{2,46} = 0.01$, $P = 0.99$; wet lean mass $F_{2,46} = 1.03$, $P = 0.37$; total water mass $F_{2,46} = 0.72$, $P = 0.49$; Fig. 1). After removing interaction terms, the

Table 1 Precision of quantitative magnetic resonance analysis for fat, wet lean mass, and total body water of House Sparrows (*Passer domesticus*) ($n = 24$), European Starlings (*Sturnus vulgaris*) ($n = 18$) and Zebra Finches (*Taeniopygia guttata*) ($n = 10$)

Component	Average coefficient of variation (%)		
	1 accumulation	2 accumulations	4 accumulations
Fat	6.7 (4.3)	2.4 (1.7)	3.2 (2.4)
Wet lean	0.7 (0.4)	0.7 (2.0)	0.4 (0.4)
Water	4.9 (3.6)	1.9 (1.6)	3.1 (2.8)

Each bird was scanned a total of six times; three times on two alternate settings (Sparrows = 1 and 4 accumulations, Starlings and Finches = 2 and 4 accumulations). Values presented are the average coefficients of variation (SD/mean × 100) for all data collected on each accumulation setting (±SD)

Table 2 Bias, absolute error and relative error (means ± SD) of raw, uncalibrated quantitative magnetic resonance (QMR) body composition data compared to gravimetric chemical analysis of House Sparrows (*HOSP*, $n = 24$), European Starlings (*EUST*, $n = 18$) and Zebra Finches (*ZEFI*, $n = 10$)

Component	Species	Raw		
		Bias (g)	Absolute error (±g)	Relative error (±%)
Fat	HOSP	0.08 (0.15)*	0.14 (0.10)*	12.5 (15.7)*
	EUST	−0.33 (0.59)*	0.53 (0.41)*	10.6 (8.7)*
	ZEFI	0.15 (0.11)*	0.16 (0.08)*	9.5 (4.8)*
Wet lean	HOSP	−1.79 (0.27)*	1.79 (0.27)*	8.4 (1.3)*
	EUST	−13.18 (1.14)*	13.18 (1.14)*	18.8 (1.5)*
	ZEFI	−1.55 (0.32)*	1.55 (0.32)*	11.2 (2.6)*
Water	HOSP	N.S.	0.29 (0.25)*	1.9 (1.7)*
	EUST	0.69 (0.86)*	0.92 (0.58)*	2.1 (1.3)*
	ZEFI	0.36 (0.30)*	0.37 (0.30)*	3.7 (2.9)*

N.S. Not significant

* Significantly different from zero using a Student's t test ($P < 0.05$)

main effect of species was significant for dry fat mass ($F_{2,48} = 9.20$, $P < 0.001$; Fig. 2a) and wet lean mass ($F_{2,48} = 54.02$, $P < 0.001$; Fig. 2b), but not for total water mass ($F_{2,48} = 2.34$, $P = 0.11$; Fig. 2c). Analysis of least squares means indicated that calibration relationships for both dry fat and wet lean masses were the same for Sparrows and Finches ($P > 0.70$), but both were different for Starlings ($P < 0.001$).

We developed predictive calibration equations for homogeneous groups using OLS regression (Table 3). Only the slopes of equations predicting fat mass of Sparrows/Finches and total water for all species were significantly different from 1.00, showing close correspondence between QMR and gravimetric data. The intercept for predicting dry fat mass of Starlings was marginally non-significant ($P = 0.051$), but we retained it in the calibration equation because ANCOVA had indicated a difference in intercept between Starlings and Sparrows/Finches, and doing so slightly reduced the residual sum of squares and relative error of the model (data not shown). Calibration equations for wet lean mass had significantly positive intercepts, as expected from the negative bias of QMR raw data (Table 2). To account for the effect of skeletal mass on the misfit of QMR to gravimetric analysis data, we obtained measurements of dry skeletons of House Sparrows and European Starlings (Cornell University Laboratory of Ornithology collections, D. Cerasale, personal communication). Skeleton mass was unrelated to tarsus length in either species (Sparrows, $F_{1,9} = 2.6$, $P = 0.14$; Starlings, $F_{1,9} < 0.01$ $P = 0.96$), and so we did not try to correct for body size variation in skeletal mass. We subtracted the mean dry skeleton mass for each species (Sparrows = 1.417 g, Starlings = 3.968 g) from the gravimetric wet lean mass of Sparrows and Starlings in our study and then recalculated the calibration equations. In both species, the intercept for predicting gravimetric wet lean mass from QMR lean mass was no longer significant after correction for dry skeleton (Sparrows, $t = 0.14$, $P = 0.89$, Starlings, $t = 1.60$, $P = 0.13$). Total water mass was measured very accurately by QMR across all species tested.

Correction of the QMR data using calibration equations removed or greatly reduced bias, and improved accuracy, particularly for wet lean mass (Table 4). When calibration

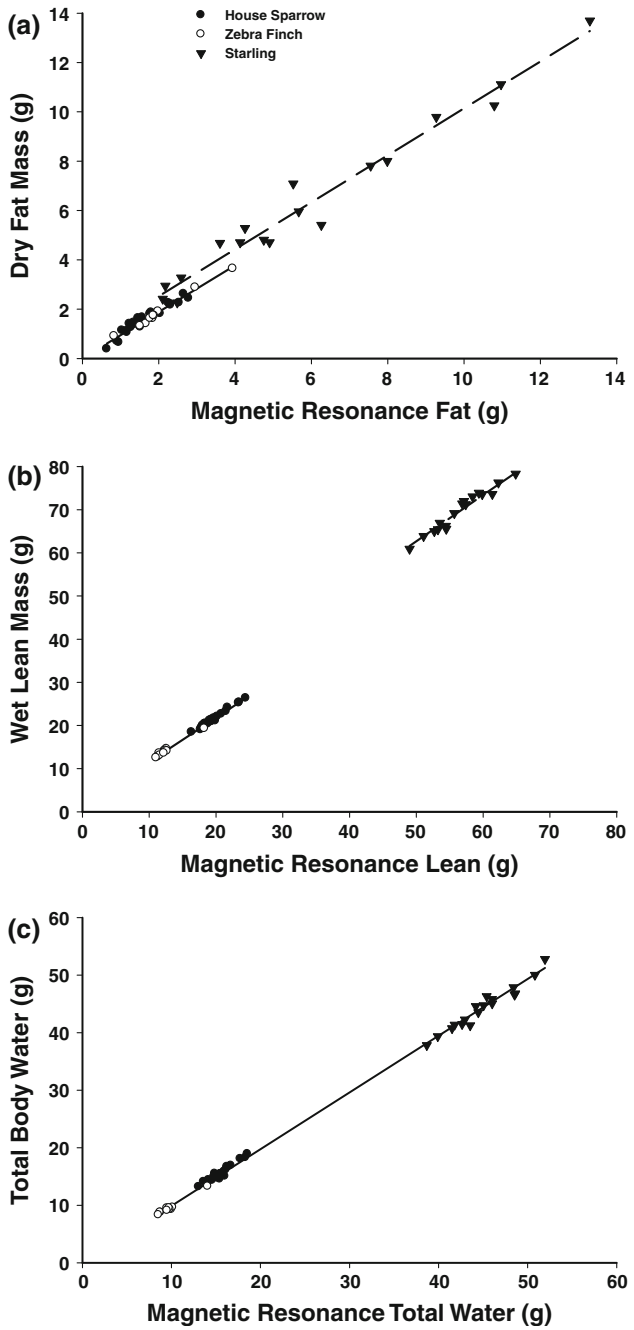

Fig. 2 Relationships between quantitative magnetic resonance (QMR) body composition analysis data and proximate chemical analysis data for dry fat (**a**), wet lean mass (**b**), and total body water (**c**) of House Sparrows (*Passer domesticus*) (*filled circles*, $n = 24$), European Starlings (*Sturnus vulgaris*) (*filled triangles*, $n = 18$), and Zebra Finches (*Taeniopygia guttata*) (*open circles*, $n = 10$). Calibration equations are given in Table 3

equations developed with Sparrows were applied to Finches, we obtained unbiased predictions of gravimetric dry fat and wet lean masses with absolute errors of ±90 and ±290 mg, respectively. Total water mass was still biased for Finches when the Sparrow calibration was used ($t = 3.75$, $P = 0.005$); however, absolute and relative errors remained small. Our simulations indicated that predictions of all three body components could be obtained with very small absolute and relative errors with calibration sample sizes of 10–15 individuals, and in Sparrows, prediction was only marginally reduced by using 10 individuals (data not shown). Overall, once QMR measurements were corrected using calibration equations, they predicted gravimetric dry fat mass within ±6–11%, wet lean mass within ±1–2%, and total water mass within ±2–4%.

For Sparrows, calibration equations made with one-accumulation data were generally poorer than four-accumulation models (lower R^2, lower slopes, and/or significant intercepts; data not shown). However, models developed with two-accumulation data for Starlings and Finches were nearly identical to those developed with four-accumulation data, indicating that two-accumulation scans provide the best quality data for the shortest scan duration.

Discussion

Quantitative magnetic resonance was first developed and validated for body composition analysis of small mammals used in medical research (Taicher et al. 2003), and so our primary objective was to determine the performance characteristics of QMR for live birds. Our comparison with gravimetric chemical analysis shows that QMR can measure dry fat, wet lean mass, and total water of live birds with high precision and accuracy, comparable to, or better than, the best alternative currently available, DXA (Nagy and Clair 2000; Korine et al. 2004). Unlike DXA, QMR also measures total water, does not require anaesthesia or strict immobilization, is not limited by the small scanning area of portable DXA instruments (Korine et al. 2004), and has no X-ray exposure. Furthermore, a scan lasting just over 2 min was sufficient to measure all three body components with high precision and accuracy. QMR measurement is only slightly more complicated and time consuming than weighing a bird, so that users can be easily trained and large numbers of birds can be processed quickly. In a recent field season, over 150 birds were scanned in a single day at a migration stopover site.

It is important to recognize that our error calculations are based on the assumption that gravimetric chemical analysis is performed without error, and therefore all error is due to the QMR instrument. This is clearly not the case, and in fact there are many sources of error during chemical analysis, such as desiccation, balance error, incomplete solvent extraction, and the calculation of total composition from the analysis of subsamples. Interestingly, slopes for predicting gravimetric fat and wet lean mass were slightly less than and greater than unity, respectively. This could be

Table 3 Calibration equations obtained by OLS regression for predicting gravimetric body composition from quantitative magnetic resonance (QMR) body composition data collected using a four accumulation setting with House Sparrows (*HOSP*, $n = 24$), European Starlings (*EUST*, $n = 18$) and Zebra Finches (*ZEFI*, $n = 10$)

Component	Species	*B*	Intercept	*F*	*df*	*P*	R^2
Fat	HOSP/ZEFI	0.94[a]	N.S.	5,905.3	1,33	0.001	0.99
	EUST	0.95	0.62[b]	494.1	1,16	0.001	0.98
Wet lean	HOSP/ZEFI	1.02	1.35	5,976.0	1,33	0.001	0.99
	EUST	1.07	9.06	374.1	1,16	0.001	0.96
Water	All Species	0.99[a]	N.S.	116,465	1,51	0.001	0.99

The general calibration equation form is Gravimetric value = *B*(QMR value) + Intercept

N.S. Not significant

[a] Slope significantly different from 1.00, $P < 0.05$

[b] $P = 0.051$, retained in model because it reduced residual sum of squares and relative error

Table 4 Bias, absolute error and relative error (means ± SD) of calibrated quantitative magnetic resonance (QMR) body composition data compared to gravimetric chemical analysis of House Sparrows (*HOSP*), European Starlings (*EUST*) and Zebra Finches (*ZEFI*)

Component	Species	Calibrated		
		Bias (g)	Absolute error (±g)	Relative error (±%)
Fat	HOSP	N.S.	0.13 (0.02)*	11.4 (3.6)*
	EUST	N.S.	0.51 (0.11)*	10.1 (2.8)*
	ZEFI	N.S.	0.09 (0.05)*	5.8 (4.5)*
Wet lean	HOSP	N.S.	0.23 (0.05)*	1.1 (0.3)*
	EUST	0.12 (0.58)*	1.06 (0.23)*	1.5 (0.3)*
	ZEFI	N.S.	0.29 (0.20)*	2.0 (1.0)*
Water	HOSP	N.S.	0.31 (0.07)*	2.0 (0.5)*
	EUST	N.S.	0.68 (0.18)*	1.5 (0.4)*
	ZEFI	0.36 (0.30)*	0.36 (0.30)*	3.7 (2.9)*

See text for calibration procedures

N.S. Not significant

* Significantly different from zero using a Student's *t* test ($P < 0.05$)

caused in part or whole by less efficient solvent extraction of fat as tissue fat content increased, rather than a problem with QMR. Therefore, the error values we report should probably be considered maxima.

As might be expected from the greatly different physical principles underlying QMR and chemical analysis, the raw data collected by QMR were biased relative to the gravimetric measurements, which increased error. This was especially evident for lean mass because gravimetric lean mass includes the skeleton and other structures that are not measured by QMR. Though this bias may at first seem a limitation, QMR lean mass may actually be a more useful representation of functional tissues of interest (muscles and organs), and we found that we could eliminate intercepts from lean mass calibration equations by simply subtracting average skeletal mass from gravimetric wet lean mass. Furthermore, all calibration equations had slopes that were very close to, or not significantly different from, unity. Therefore, in some cases, it may be reasonable to use uncalibrated QMR data, particularly if one is studying changes in body composition within individuals through time, such as in longitudinal studies.

Calibration against gravimetric chemical analysis improved the accuracy of QMR, especially for prediction of wet lean mass. The predictive equations for dry fat differed between Sparrows/Finches and Starlings, and may represent a real body size effect or more likely a difference between the "small bird" and "large bird" settings in the Echo-MRI software. Our subsequent tests with canola oil standards and live birds scanned on both settings indicate that the software setting can slightly affect the resultant fat and lean values (C.G.G., unpublished data). For wet lean mass equations, the difference in intercepts between Sparrows/Finches versus Starlings was certainly due to large differences in mass of the skeleton and other non-detected tissues, but may also have been caused partly by the different scan settings. Prediction of total water was unaffected by species or scan setting.

Species-specific calibration does not appear to be necessary for birds of similar size. We found that there were no differences between Sparrows and Finches, and a calibration made with Sparrows eliminated most bias and greatly improved accuracy for predicting body composition of Finches. Excellent calibration is possible with 10–15 birds, and can be optimized by selecting individuals with a wide range of fat and lean masses. However, separate calibration is necessary for small and large bird settings using appropriately sized birds.

The smallest birds used in our study weighed about 15 g, but we have demonstrated similar performance of the Echo-MRI-B for bats as small as 6 g (McGuire and Guglielmo 2010). Thus, we are confident that QMR can be used for much smaller birds. In principle, precision and accuracy could be further improved for small birds by using a smaller diameter antenna (Echo Medical Systems, personal communication). QMR can also be used for salvaged carcasses if they are equilibrated to room temperature and are not desiccated (McGuire and Guglielmo 2010).

Provided information about internal structure is not necessary, QMR is a better option for body composition analysis than MRI because QMR is simple to use, portable, and much less expensive. Moreover, the precision and accuracy of MRI for body composition analysis of birds have not been thoroughly assessed. In MRI studies, fat is estimated by classifying image pixels as fat-containing based on signal intensity threshold criteria, summing to a total pixel area, and then converting to fat mass by accounting for slice thickness, the distance between slices, and the gravimetric density of adipose tissue (Wirestam et al. 2008). Error may be introduced at any of these steps, and only fat that can be visualized can be measured, potentially omitting fat stored within the cells of muscles and other tissues. MRS can add additional information of tissue fat content (Berthold et al. 2001), but extrapolating MRS to full body composition may be difficult and time consuming. The accuracy of MRI for measuring fat mass has been demonstrated indirectly by comparing fat estimated in MRI-scanned birds to a separate sample of birds that were visually fat scored in an identical way and analyzed by gravimetric chemical analysis (Hedenström et al. 2009).

Potential side effects of QMR

The magnetic field used for QMR is approximately 1,000 times stronger than the ambient geomagnetic field. Therefore, QMR could negatively affect free-living birds if it disrupts sensory systems used for geomagnetic orientation and navigation. Recent evidence indicates that birds orient to the inclination angle of the geomagnetic field using light-dependent excitation of photopigments in the eye (Stapput et al. 2008; Zapka et al. 2009), which should not be affected after a QMR scan is complete (H. Mouritsen, personal communication). Birds also have iron-mineral geomagnetic sensors in the beak (Williams and Wild 2001; Fleissner et al. 2003), which could in principle be affected by exposure to a strong magnetic field (H. Mouritsen, personal communication). The beak sensors do not appear to be involved in orientation behavior, but may serve other functions in navigation, such as sensing field strength to convey information on location (Fransson et al. 2001; Stapput et al. 2008; Zapka et al. 2009). There is good evidence that birds can rapidly recalibrate their geomagnetic senses using skylight polarization during sunrise and sunset (Cochran et al. 2004; Muheim et al. 2007, 2009). Thus, any effects of QMR may be transitory, and if birds are scanned early in the day it may be expected that they would recalibrate by the evening or following morning.

Field studies are required to determine if there are any lasting effects of QMR on migratory birds. Ovenbirds (*Seiurus aurocapilla*) that were scanned by QMR (Seewagen and Guglielmo 2010) behaved similarly during stopover refueling to those that had not been scanned (Seewagen et al. 2010). QMR had no effect on stopover duration or departure direction of migrating Silver-haired Bats (*Lasionycteris noctivagans*; L.P.M., S.A. MacKenzie, C.G.G. and P.D. Taylor, unpublished data). We are currently using telemetry and mark–recapture data to test for effects of QMR analysis on stopover duration, movement behavior, and departure direction of several passerine species (L. Kennedy, S.A. MacKenzie, P.D. Taylor and C.G.G., unpublished data).

Applications of QMR in the field and laboratory

Since collecting the validation data reported here, we and others have used QMR to measure body composition of several thousand individual birds and bats for a broad variety of studies. QMR has been used in the field to study the effects of fat and lean mass on stopover duration (Seewagen and Guglielmo 2010), and the mixture of fat and lean mass deposited by passerine landbirds during stopover (Seewagen and Guglielmo 2011). We have used QMR body composition data to estimate potential flight ranges of migrating Silver-haired Bats stopping at Long Point, Ontario, Canada (L.P.M., S.A. MacKenzie, C.G.G. and P.D. Taylor, unpublished data). We have also measured body composition of nearly 3,000 birds of a variety of species as they refueled at Long Point, to study factors that may affect the rates and relative amounts of fat and lean mass deposition during stopover (L. Kennedy and C.G.G., unpublished data). In 2010, QMR was used to study the effects of weather and other factors on the dynamics of body composition during the pre-nesting and

nesting periods of adult Tree Swallows (*Tachycineta bicolor*), and nutrient allocation during growth in their nestlings (A. Boyle, D. Winkler and C.G.G., unpublished data). Thus, with the support of a trailer or field station, QMR can be used effectively for studies of wild birds.

QMR is also extremely useful for laboratory studies. We have used QMR for longitudinal studies of fat and lean mass of White-throated Sparrows (*Zonotrichia albicollis*) in response to photoperiod and leptin treatments (D.J. Cerasale, D.M. Zajac and C.G.G., unpublished data), and of European Starlings in response to diet and exercise treatments (U. Bauchinger, A.R.G., E. Price, C.G.G. and S.R. McWilliams, unpublished data). We use QMR frequently to analyze salvaged bird and bat carcasses, which saves time, money and solvents required for chemical analysis.

The most revolutionary application of QMR could be in studies of energetics, fuel selection and water balance. By simply scanning a bird immediately before and after a metabolic challenge, it is possible to calculate the amounts of fat and lean mass catabolized, energy cost, and changes in hydration state. For example, Gerson and Guglielmo (2011) used QMR to show that water stress increased lean mass catabolism in resting House Sparrows. By scanning Swainson's Thrushes (*Catharus ustulatus*) immediately before and after wind tunnel flights lasting up to 5 h, we have found that ambient humidity affects the rate of lean mass catabolism during flight as a mechanism to provision water (A.R.G. and C.G.G., unpublished data). The energy cost of flight calculated from QMR (4.2 W) was nearly identical to an estimate of flight costs of free flying Swainson's Thrushes measured in the field by the doubly-labeled water technique (Wikelski et al. 2003). QMR could simplify isotopically labeled water experiments by providing rapid measurements of the body water pool. Although further validation with respirometry and/or isotopically labeled water may be necessary, QMR shows great promise for these types of studies.

Validation studies are necessary to determine the precision and accuracy of new non-invasive methods of body composition analysis, but they have sometimes been criticized for killing animals without the techniques then being widely applied to study important questions (Piersma and Klaassen 1999). We have found QMR to be nearly indispensible and have integrated it into most of the studies we undertake. In cases where a field site is too remote for QMR, heavy water dilution can be used; a method that has been known for decades, and is accurate, inexpensive, and reasonably easy (Karasov and Pinshow 1998; Rae et al. 2009; Boyle et al. 2010). We urge physiologists and ecologists to use methods like QMR, DXA, and heavy water dilution to gather accurate and detailed information about the dynamics of fat and lean mass in free-living and captive birds.

Both fat and lean mass can change rapidly and differentially in birds, but we know little about the environmental factors that determine these changes or the physiological mechanisms responsible. Further advancement of our understanding requires that we use non-invasive methods with sufficient sensitivity to detect subtle variations. QMR provides such a technique and promises to yield important insights into the physiology, behavior and ecology of birds.

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