Noninvasive Estimation of Body Composition in Small Mammals: A Comparison of Conductive and Morphometric Techniques

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ABSTRACT

Body fat stores may serve as an index of condition in mammals. Thus, techniques that measure fat content accurately are important for assessing the ecological correlates of condition in mammal populations. We compared the ability of two conductive techniques, bioelectrical impedance analysis (BIA) and total body electrical conductivity (TOBEC), to predict body composition with that of morphometric methods in three small mammal species: red squirrels ($n = 13$), snowshoe hares ($n = 30$), and yellow-bellied marmots ($n = 4$). Animals were livetrapped in northern Idaho; BIA (all subjects) and TOBEC (squirrels only) measurements were taken following chemical immobilization in the field, and morphometric measurements were taken postmortem. Information provided by BIA and TOBEC failed to improve upon the predictive power of morphometric equations for total body water (TBW) and lean body mass (LBM) in squirrels and hares, which do not store substantial amounts of fat (<5% body mass comprised of fat). Although the same pattern held with respect to LBM in marmots, which accumulate substantial amounts of body fat (>10% body mass), a BIA-based model proved best at estimating TBW, suggesting that the usefulness of conductive techniques may be a function of fat deposition. However, regardless of the technique used to predict body composition, estimates of body fat furnished by our equations failed to approximate actual fat levels accurately in all three test species, probably because these techniques only provide indirect estimates of fat content. These results highlight the limitations inherent in contemporary methods of animal fat estimation and underscore the need for the development of direct and accurate measures of body fat in mammals.

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Introduction

Many mammals rely upon lipids as an important source of endogenous energy, and consequently the accumulation of fat reserves generally leads to improved survival and reproductive success (Robbins 1993). Insofar as body fat stores are related to individual fitness, techniques that measure body composition accurately may allow biologists to assess the relationship between physical condition and survival, productivity, and behavior in mammals (Green 2001; Unangst and Wunder 2001). The body composition of an animal can be quantified accurately using whole body homogenization followed by chemical extraction (Walsberg 1988; Unangst and Wunder 2001). However, this approach is destructive and therefore inappropriate for situations in which repeated measures of condition are required and/or rare or endangered species are involved (Walsberg 1988; Unangst and Wunder 2001). Thus, biologists traditionally have estimated composition using indirect, noninvasive techniques such as morphometric measurements and qualitative visual assessments. However, these methods usually suffer from lack of validation and are often inaccurate, imprecise, and therefore incapable of quantifying an animal's fat reserves (Walsberg 1988; Cattet 1990; Krebs and Singleton 1993; Jakob et al. 1996; Green 2001; Schulte-Hostedde et al. 2001). Accordingly, there exists a need for more reliable means to evaluate animal body composition and its ecological implications.

One available alternative method to morphometric estimates of body composition, bioelectrical impedance analysis (BIA), measures the resistance to conduction (in ohms) of a mild electrical current (800 μA at 50 kHz) through an organism's tissue (Hall et al. 1989; Farley and Robbins 1994). This method relies on the tight inverse relationship between body water and fat content (Robbins 1993; Farley and Robbins 1994). Given that the ease with which an electrical current flows through an animal reflects its water content, BIA provides an estimate of total body water (TBW), lean body mass (LBM), and, by difference, body fat (Hall et al. 1989; Hilderbrand et al. 1998). Another procedure, total body electrical conductivity (TOBEC), measures the extent to which the presence of an animal's body

alters the electromagnetic inductance of a shielded solenoid (Walsberg 1988). This method is based on the principle that the electrical conductivity of an organism is proportional to its fat-free mass; thus, TOBEC signal outputs yield an estimate of LBM that can be converted into fat mass (Walsberg 1988; Fischer et al. 1996).

Both BIA and TOBEC represent rapid nondestructive and objective means to estimate body composition. Thus far, both methods have been used successfully to quantify body composition in a variety of mammalian, avian, and fish species (e.g., Walsberg 1988; Hall et al. 1989; Roby 1991; Farley and Robbins 1994; Fischer et al. 1996; Pulawa and Florant 2000). However, many previous attempts to evaluate the efficacy of these two procedures have been characterized by a number of limitations: (i) often, BIA and TOBEC have been evaluated in highly controlled settings, where the potential sources of error inherent in the use of these techniques (e.g., sensitivity to the subject animal's body position, level of subject hydration, injuries to the conductor path, moisture due to contact with the ground, weather conditions, and level of anesthesia; Walsberg 1988; Hilderbrand et al. 1998) are minimal (e.g., Walsberg 1988; Hall et al. 1989; Roby 1991; Frawley et al. 1999; Pulawa and Florant 2000; Unangst and Wunder 2001); (ii) most prior studies have involved either species exhibiting considerable variability with respect to fat mass or laboratory animals with fat levels probably exceeding those of free-ranging conspecifics (e.g., Walsberg 1988; Farley and Robbins 1994; Fischer et al. 1996; Bowen et al. 1999; Frawley et al. 1999; Pulawa and Florant 2000); and (iii) in some studies, authors have correlated BIA and TOBEC measurements with TBW and LBM without ascertaining whether the inclusion of conductive information improves significantly on the capacity of morphometric models to predict these values (e.g., Walsberg 1988; Hall et al. 1989; Farley and Robbins 1994; Fischer et al. 1996; Pulawa and Florant 2000; Unangst and Wunder 2001).

The primary objectives of the study presented in this article were (i) to evaluate whether BIA and/or TOBEC, when used in the field, improve significantly upon the explanatory power of body composition models derived solely from morphometric measures and (ii) to determine the extent to which estimates of fat mass (as derived from estimates of TBW or LBM) furnished by the best predictive equations approximate actual lipid levels. Second, we wished to evaluate the extent to which two common morphometric indices of body condition in small mammals, body mass and the residuals from the relationship between body mass and structural size (Gould 1975), as well as a popular postmortem index, bone marrow fat (BMF) content (Warren and Kirkpatrick 1978), approximate actual lipid stores. For this analysis we used two species not known to deposit large quantities of body fat (red squirrel, *Tamiasciurus hudsonicus*; snowshoe hare, *Lepus americanus*) and one species known for fat deposition (yellow-bellied marmot, *Marmota flaviventris*), enabling us to compare the efficacy of morphometric and conductive techniques across a range of lipid levels.

Material and Methods

Field Procedures

We livetrapped 13 red squirrels and 15 snowshoe hares in the Clearwater National Forest, Idaho (46°N, 114°W), as well as an additional 15 hares in the Panhandle National Forest, Idaho (48°N, 116°W), during the fall of 1999, and four marmots along the Clearwater River in Lewiston, Idaho (46°N, 117°W), in the spring of 2000. Animals were handled using techniques in accord with standard guidelines (Canadian Council on Animal Care 1984) and approved by the University of Idaho Animal Care and Use Committee (protocol 9029). All animals were aged (i.e., placed into juvenile or adult categories based upon sexual and/or morphological characteristics), sexed, and weighed on capture and then immobilized using isofluorane (Abbott Laboratories, North Chicago, Ill.).

We prepared all sedated animals for BIA by placing them on a plastic sheet (to control for ground moisture, given that measurements were taken outdoors) in a predetermined position (prone recumbency with head and tail aligned with the body's long axis and legs splayed outward at right angles). Electrodes were attached to both the snout (clamped to upper lip at the level of the incisors) and vent (syringes were inserted subcutaneously on either side of the rectum) of each animal; this placement was chosen because it was easy to repeat, and it minimized error associated with limb position (e.g., Farley and Robbins 1994). The BIA apparatus (model 101, RJL Systems, Detroit) was then used to measure resistance (ohms), and the distance (mm) between electrodes was recorded. This procedure was repeated at least twice, and measures of resistance were averaged to produce a final value. Owing to the inherent measurement error associated with BIA (Hilderbrand et al. 1998), we took additional readings in cases in which the initial two measures differed by >5% until two consecutive measures falling within 5% of each other were obtained. These two readings were averaged to produce a final measure of resistance. The environmental conditions under which BIA measurements were taken varied considerably (temperatures ranged from 0° to 25°C).

For squirrels, TOBEC (EM-SCAN, Springfield, Ill.) was measured subsequent to the previously described BIA procedure. Squirrels were placed in a predetermined supine position with limbs appressed to the body. For proper readings, the entire animal must reside within a defined range of the electromagnetic field; thus, we attached each squirrel's tail to its chest using a small metal book binder clip before insertion into the TOBEC apparatus, a precaution that was unlikely to have affected TOBEC measurements (Castro et al. 1990). Three successive readings were taken per squirrel and were averaged to produce a raw TOBEC score. In cases in which these readings

varied by >5%, additional measures were taken until three consecutive readings varying by <5% were obtained. The environmental conditions under which TOBEC measurements were taken varied considerably (temperatures ranged from 0° to 21°C).

For all three species, animals were killed via drug (isofluorane) overdose following analysis, and death was ensured by cervical dislocation. We used an inhalant drug in order to avoid altering the fluid content of our subjects.

Lab Procedures

Following collection in the field, all carcasses were kept frozen prior to laboratory analysis. In order to minimize moisture loss during storage, carcasses were stored separately in sealed plastic bags. For squirrels and hares, morphometric measurements included total body mass (TBM), body length (snout-to-vent length, SVL), body length with tail (squirrels only; total body length, TBL), chest circumference (CC), skull length (SL), skull width (SW; distance between orbitals), and hind-foot length (HFL). Bone marrow was removed from the tibia and femur bones of each carcass and oven-dried (21°C for 24 h), and an index of BMF was expressed as a percentage of dry from fresh mass (Warren and Kirkpatrick 1978; Keith et al. 1984). We were confident that the removal of BMF would not bias our analysis of carcass fat content, given that BMF represents only a small fraction of an animal's overall fat mass (Mech and DelGiudice 1985).

Fifteen hares were paunched (eviscerated); the viscera and paunched carcasses of these individuals were weighed and analyzed separately in order to evaluate the possibility that various levels of gut fill may affect estimates of body fat (Hilderbrand et al. 1998). Among the hares that were paunched, body fat estimates did not differ in the absence of the viscera (paired *t*-test, mean % difference in fat mass = 0.04%, $t_{14} = 1.57$, $P = 0.14$); thus, whole body fat values were used for the purposes of this investigation. Marmots were subjected to body mass and SVL measurements only; BMF was not removed. Given that carcasses for each study species were frozen prior to morphometric analysis, our lab measurements may have been biased due to morphological changes caused by the freezing process. To assess this possibility, we used paired *t*-tests to evaluate the relationship between two measures taken both in the field and in the lab postfreezing: TBM and SVL. These tests revealed that for all three species, field and lab measurements were closely related (squirrels: TBM $P = 0.85$, SVL $P = 0.59$; hares: TBM $P = 0.90$, SVL $P = 0.63$; marmots: TBM $P = 0.87$, SVL $P = 0.72$). Thus, we concluded that we had not biased our analysis by taking measurements from frozen carcasses. All carcasses were freeze-dried, and TBW was determined by subtracting the freeze-dried mass from the total mass for each carcass. Freeze-dried carcasses were then sectioned into small pieces and ground in a Wiley mill to create a homogenate. Crude fat was determined from duplicate samples by using ether extraction (Association of Official Analytical Chemists 1975). For animals with low fat content, such as squirrels and hares, small errors associated with the ether extraction technique may lead to inaccurate estimates of actual fat mass. In order to minimize this type of error, we insured that the difference between the two ether-extracted samples did not exceed 2% (most differences did not exceed 1%). If a difference >2% was observed, a third extraction was undertaken. LBM was determined by subtracting fat mass from TBM.

Statistical Analysis

We employed forward stepwise regression to generate models predicting TBW and LBM in squirrels, hares, and marmots. For squirrels and hares, variables made available for inclusion in the models included sex (squirrels: 6 females, 7 males; hares: 18 females, 12 males), age (juvenile vs. adult), TBM (g), HFL (mm), SVL (cm), TBL (cm, squirrels only), SW (mm), SL (mm), and CC (cm). Furthermore, because linear and volumetric measurements may scale allometrically (Cone 1989), TBM values divided by the best linear metric of structural size raised to the first, second, and third power also were made available for inclusion. SVL was found to be the strongest correlate of LBM in both squirrels ($r^2 = 0.66$; $P < 0.001$) and hares ($r^2 = 0.82$; $P < 0.001$), so it was selected as the best metric of structural size (i.e., TBM/SVL, TBM/SVL^2, and TBM/SVL^3 were made available for inclusion in the models). An additional index of condition derived from the residuals from the line generated by regressing TBM against HFL for each sex (termed the residual index; Gould 1975) was calculated for each individual and was included in the analysis. Finally, we tested values furnished by BIA, expressed as the squared distance between the electrodes (SVN at the time of resistance measurement) divided by resistance (shown hereafter as L^{2}RES; Lukaski et al. 1986). For squirrels, the TOBEC-provided index of TBW/LBM was included as a potential variable in the models (Walsberg 1988). For the marmot models, independent variables included sex (two females, two males), TBM, SVL, TBM/SVL, TBM/SVL^2, and TBM/SVL^3, and L^{2}RES. Age effects could not be assessed in squirrels or marmots because all trapped individuals from these species apparently were adults; 10 adult and 20 juvenile hares were trapped. The level of significance for inclusion in the models was set at $\alpha = 0.05$. For each model generated, we tested whether nonlinearity in the independent variable(s) improved the model's fit significantly when we used the corrected Akaike Information Criterion, which accounts for small sample size (AIC$_c$; Burnham et al. 1998); ΔAIC$_c$ values ≥ 2 were deemed to be significant. We also tested for interactions between main effects when more than one variable qualified for inclusion in a model.

Final model equations for LBM (chosen over TBW because fewer steps are involved in the conversion to fat mass) were

used to generate fat mass estimates, which were then compared to actual fat mass values (calculated from ether extraction) using simple linear regression. In squirrels and hares, we also correlated percent femur fat (%FF), percent tibia fat (%TF), average percent bone marrow fat (%BMF), TBM, and the residual of the mass/structural size relationship with the observed percent fat for each animal using simple linear regression.

Results

Red Squirrels

The independent variable that best explained TBW in squirrels was TBM ($r^2 = 0.97$; Table 1). Other parameters also explained a significant amount of variability in squirrel TBW (Table 1). A test for nonlinearity indicated that squaring the body mass term significantly improved the fit of the model ($r^2 = 0.98$, $\Delta AIC_c = 3.42$). The addition of TBL to the model as a second variable also significantly improved the fit of the model to the data ($r^2 = 0.99$, $P = 0.03$). No other variables improved the fit of the model at either the second step (TOBEC improved the model fit marginally at $P = 0.06$; for all other variables, $P > 0.09$) or the third step in the model-building process (all $P > 0.16$). The interaction term between TBL and TBM was not significant ($P = 0.36$). Thus, the final model for TBW in squirrels was

$$\text{TBW} = 22.36 + 0.002 \times \text{TBM}^2 + 1.20 \times \text{TBL}. \quad (1)$$

The independent variable that best explained LBM in squirrels also was TBM ($r^2 = 0.995$; Table 1). Although a number of alternative variables were strongly correlated with LBM (Table 1), none could improve significantly the fit of the model after accounting for the effects of TBM (all $P > 0.25$). Furthermore, no nonlinear formulations of TBM significantly improved the model (all $\Delta AIC_c < 0.04$). Thus, the final model for LBM in squirrels was

$$\text{LBM} = -9.66 + 1.003 \times \text{TBM}. \quad (2)$$

The slope associated with this equation indicated that TBM and LBM scaled proportionally in these animals.

Body fat in squirrels ranged from 6.5 to 11.4 g, while percent body fat ranged from 2.5% to 5.0%. Estimates of body fat furnished by the equation for LBM were not correlated with fat mass values derived from ether extraction (Fig. 1; $P = 0.88$). However, %TF ($r^2 = 0.41$, $P = 0.018$), %FF ($r^2 = 0.64$, $P < 0.0001$), and average %BMF ($r^2 = 0.59$; $P = 0.002$) all were positively correlated with observed percent fat content. TBM ($r^2 = 0.26$, $P = 0.08$) and the mass/structural size residual ($r^2 = 0.26$, $P = 0.08$) also tended to predict observed percent fat. However, both measures were negatively correlated with percent fat (coefficient estimate for TBM = −0.018; coefficient estimate for residual index = −0.020).

Snowshoe Hares

The independent variable that best explained TBW in hares was TBM ($r^2 = 0.93$; Table 2). Many of the remaining variables

Table 1: Variables available for inclusion in predictive model of total body water (TBW) and lean body mass (LBM) in red squirrels

		TBW		LBM	
Variable	Min–Max	Partial Correlation	*P*	Partial Correlation	*P*
TBM (g)	195–266	.986	<.001	.997	<.001
TBM/SVL (g/cm)	10.0–13.30	.960	<.001	.967	<.001
Residual	−41.23 to 38.77	.970	<.001	.987	<.001
TBM/SVL^2 (g/cm^2)	.51–.67	.797	.001	.796	.001
SVL (cm)	18.5–21.0	.789	.001	.809	.001
L^{2}RES (cm^2/Ω)	.57–1.02	.751	.003	.772	.002
SL (mm)	51.0–56.0	.523	.067	.543	.06
Sex		.491	.088	.529	.06
CC (cm)	11.0–12.5	.478	.099	.522	.07
TBM/SVL^3 (g/cm^3)	.026–.033	.396	.18	.387	.19
HFL (mm)	44.0–47.0	.401	.17	.363	.22
TOBEC	610–1,038	.329	.27	.269	.37
SW (mm)	19.0–25.0	−.002	.99	.053	.86
TBL (cm)	29.0–33.5	.107	.73	.037	.91

Note. Relationships generated using linear regression on variables measured in 13 wild-caught red squirrels. Variables are presented in descending order of significance in the models. Variable key: total body mass (TBM); snout-to-vent length (SVL); bioelectrical impedance analysis (BIA) score (L^{2}RES); skull length (SL); chest circumference (CC); hind-foot length (HFL); total body electrical conductivity (TOBEC) score; skull width (SW); total body length (TBL).

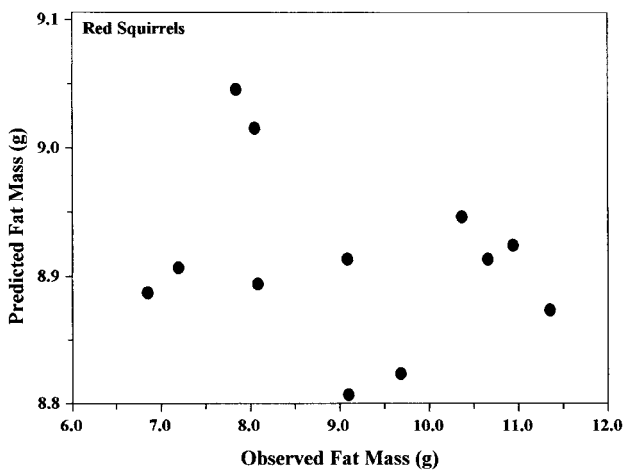

Figure 1. Relationship between body fat estimates produced by the best predictive equation for red squirrels (LBM = −9.66 + 1.0033 × TBM; fat mass = TBM − LBM) and fat mass values estimated via ether extraction (r^2 = 0.03, P = 0.88).

also were related significantly to TBW (Table 2). However, the incorporation of additional independent variables failed to improve the ability of the mass-based model to predict TBW (all $P > 0.17$). Similarly, no nonlinear formulations of TBM yielded an improved fit (all $\Delta AIC_c < 0.16$). Thus, the final model for TBW in hares was

$$TBW = 38.50 + 0.66 \times TBM. \quad (3)$$

TBM also was best able to explain variation in hare LBM (r^2 = 0.99; Table 2). Although most of the remaining parameters were related significantly to LBM (Table 2), their inclusion failed to improve the fit of the model after accounting for the effects of body mass (all $P > 0.16$). Similarly, no nonlinear formulation of TBM significantly enhanced the explanatory ability of the model (all $\Delta AIC_c < 0.11$). Therefore, the model that best explained LBM in hares was

$$LBM = 6.19 + 0.98 \times TBM. \quad (4)$$

As in squirrels, the slope associated with this equation for hares indicated that TBM and LBM scaled proportionally in hares.

Fat mass in hares ranged from 6.7 to 47.6 g (percent body fat ranged from 0.9% to 3.9%). Estimates of fat mass furnished by the equations for LBM were related significantly and positively to fat mass values derived from ether extraction (Fig. 2; r^2 = 0.39, $P < 0.001$). Similarly, %TF (r^2 = 0.25, P = 0.007), %FF (r^2 = 0.20, P = 0.01), %BMF (r^2 = 0.22, P = 0.008), TBM (r^2 = 0.22, P = 0.01), and the residual index (r^2 = 0.47, $P < 0.001$) each showed a significant positive relationship with observed percent body fat.

Marmots

The independent variable that best explained TBW in marmots was L^2RES (Table 3; r^2 = 0.99, P = 0.001). A number of other variables also were related significantly to TBW (Table 3); however, the incorporation of these variables failed to improve significantly the ability of the BIA-based model to predict TBW (all $P > 0.33$). Thus, the model that best explained TBW in marmots was

$$TBW = -2{,}191.81 + 537.18 \times L^2RES. \quad (5)$$

TBM was best able to explain variation in LBM (r^2 = 0.99; Table 3). Although several other variables were able to explain a significant amount of variation in marmot LBM (Table 3), the inclusion of these variables failed to improve the fit of the TBM-based model (all $P > 0.24$). Nonlinearity in the independent variable (TBM) did not yield a significantly improved fit ($\Delta AIC_c < 0.23$). Thus, the model that best explained LBM in marmots was

$$LBM = -273.28 + 1.04 \times TBM. \quad (6)$$

The coefficient associated with TBM in this equation indicated that TBM and LBM scaled proportionally in marmots.

Fat mass in marmots ranged from 202.8 to 243.6 g (percent body fat ranged from 10% to 21%). Estimates of fat mass furnished by the equation for LBM were not related significantly to observed fat mass derived from ether extraction (Fig. 3; r^2 = 0.57, P = 0.24).

Discussion

For red squirrels, snowshoe hares, and yellow-bellied marmots, most of the morphometric variables included in this study were closely related to TBW/LBM, suggesting that a variety of measurements may serve as viable ways to estimate these two values in some small mammal species. Among the morphometric measures analyzed, TBM always provided the best estimate of both TBW and LBM (Tables 1–3). The residual index, scaled mass values (i.e., TBM/SVL, TBM/SVL^2, TBM/SVL^3), and structural size (i.e., HFL, SVL, TBL) tended to provide slightly less accurate yet reliable estimates of TBW and LBM, while the remaining measures exhibited no specific pattern with respect to their predictive ability (Tables 1–3). Sex and age (hares only) variables never were retained in the predictive models for TBW and LBM (Tables 1–3), which indicates that the relationship between morphology and TBW/LBM in the species tested is robust to changes in these demographic factors.

Values furnished by TOBEC were not related to either TBW or LBM in squirrels (Table 1). Given that other researchers have used this technique in mammals successfully in controlled settings (e.g., Walsberg 1988; Unangst and Wunder 2001), this result suggests that the efficacy of TOBEC analysis as a means

Table 2: Variables available for inclusion in predictive model of total body water (TBW) and lean body mass (LBM) in snowshoe hares

		TBW		LBM	
Variable	Min–Max	Partial Correlation	*P*	Partial Correlation	*P*
TBM (g)	560–1410	.933	<.001	.997	<.001
TBM/SVL (g/cm)	16.50–32.02	.928	<.001	.963	<.001
L^2RES (cm^2/Ω)	1.01–3.30	.862	<.001	.911	<.001
SVL (cm)	34.0–46.0	.709	<.001	.820	<.001
HFL (mm)	101–143	.665	<.001	.770	<.001
TBM/SVL^2 (g/cm^2)	.46–.73	.757	<.001	.734	<.001
SL (mm)	58.0–86.0	.508	<.001	.592	<.001
Age		.514	<.001	.574	<.001
CC (cm)	18.8–27.3	.457	<.001	.528	<.001
Residual	.95–1.02	.456	<.001	.428	.001
SW (mm)	28–36	.329	.001	.368	.001
TBM/SVL^3 (g/cm^3)	.011–.023	.171	.02	.121	.060
Sex		.021	.44	.014	.53

Note. Relationships generated using linear regression on variables measured in 30 wild-caught snowshoe hares. Variables are presented in descending order of significance in the models. Variable key: total body mass (TBM); snout-to-vent length (SVL); bioelectrical impedance analysis (BIA) score (L^2RES); hind-foot length (HFL); skull length (SL); chest circumference (CC); skull width (SW).

to evaluate small mammal body condition in the field is much reduced, at least in species that lack notable fat accumulation. A number of factors may underlie the apparent difference in the performance of TOBEC under field and lab conditions, including increased environmental variability in the field, the possibility that lab animals accumulate abnormally high and constant levels of fat relative to their free-ranging counterparts, and the fact that previous lab workers have evaluated TOBEC in conjunction with TBM without testing the predictive capacity of TOBEC scores alone. Unlike the values furnished by TOBEC, BIA scores were related significantly to TBW and LBM in each of the study species, which indicates that this procedure can be employed successfully in the field to measure these two attributes irrespective of fat accumulation levels. However, BIA scores were not as strongly correlated with body composition as morphometric measures, and the addition of L^2RES values failed to improve the fit of predictive models based solely on morphometric measures (TBM) in both squirrels and hares.

Both BIA and TOBEC machines are expensive and require intensive training prior to effective use. Thus, the failure of conductive information to improve significantly upon the capacity of morphometric equations to predict TBW and LBM in lean small mammals implies that the use of these procedures on such animals in the field may not be justified. Accordingly, lab studies showing a close association between fat content and BIA and TOBEC measurements in small mammals (e.g., Walsberg 1988; Hall et al. 1989) may not apply in many field situations where the potential for fat accumulation among individuals probably is reduced. Yet, in the fat-depositing marmot, BIA scores actually provided the best estimate of TBW (Table 3). This trend was not repeated with respect to LBM; TBM was the best predictor, though L^2RES also furnished reliable estimates. Nevertheless, our results suggest that measurement error with BIA may decrease with increasing fat mass, with estimates that involve fat-accumulating species proving to be more accurate than those that involve relatively lean species. This possibility is supported by the fact that BIA has been used

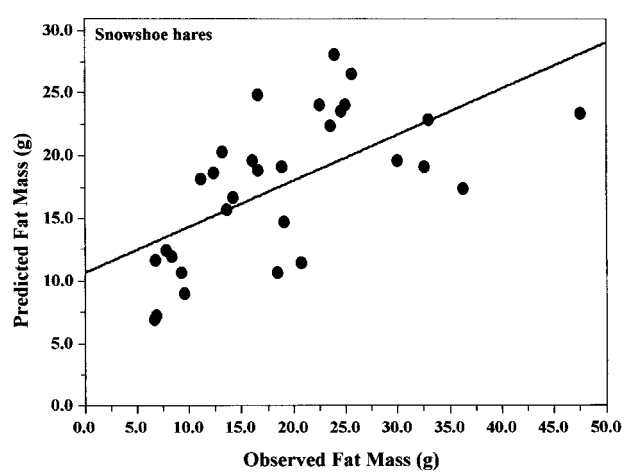

Figure 2. Relationship between body fat estimates produced by the best predictive equation for snowshoe hares (LBM = 6.19 + 0.98 × TBM; fat mass = TBM − LBM) and fat mass values estimated via ether extraction (r^2 = 0.39, $P < 0.001$). Regression line included due to the significance of the relationship.

Table 3: Variables available for inclusion in predictive model of total body water (TBW) and lean body mass (LBM) in yellow-bellied marmots

		TBW		LBM	
Variable	Min–Max	Partial Correlation	*P*	Partial Correlation	*P*
TBM (g)	1,160–2,000	.983	.009	.999	.001
TBM/SVL (g/cm)	25.21–39.23	.964	.018	.998	.001
TBM/SVL^2 (g/cm^2)	.55–.77	.958	.021	.997	.001
TBM/SVL^3 (g/cm^3)	.011–.024	.937	.032	.992	.004
SVL (cm)	46.0–51.0	.966	.017	.989	.005
L^{2}RES (cm^2/Ω)	5.41–6.44	.998	.001	.978	.011
Sex		.076	.73	.189	.57

Note. Relationships generated using linear regression on variables measured in four wild-caught marmots. Variables are presented in descending order of significance in the models. Variable key: total body mass (TBM); snout-to-vent length (SVL); bioelectrical impedance analyasis (BIA) score (L^{2}RES).

with considerable success to predict body fat in large, fat-accumulating mammals (black and brown bears; Farley and Robbins 1994; Hilderbrand et al. 1998), and by similar findings in studies involving use of TOBEC and isotopic dilution (e.g., Schulte-Hostedde et al. 2001; Unangst and Wunder 2001).

None of the models of LBM proved particularly good at predicting fat content, despite featuring tight relationships between TBM and LBM (all $r^2 > 0.98$). In squirrels, the relationship between estimated and actual fat values failed to be significant (Fig. 1). A similar trend existed for marmots (Fig. 3), although the partial correlation value indicates that the relationship may have become significant with increased sample size. In hares, the relationship between estimated and observed fat mass was significant (Fig. 2). However, the coefficient of variation (0.39) was too low to allow for accurate characterization of fat content in individual animals. At best, the equation for snowshoe hare (and perhaps marmot) LBM could be used to make rough comparisons between groups of animals (e.g., populations). The inability of the equations to predict body fat accurately in any of the species tested probably stems from the fact that they estimate fat indirectly through a conversion from LBM; because body fat typically represents a small percentage of TBM in small mammals, even slight errors associated with the measurement of TBW or LBM become magnified during the conversion process, a problem that has been noted in previous studies (e.g., Fischer et al. 1996; Schulte-Hostedde et al. 2001; Unangst and Wunder 2001). Indeed, this problem implies that studies of body composition in lean small mammals may benefit by focusing on protein rather than body fat because protein content can be estimated more accurately from LBM and may be a more important energy source for these animals (e.g., Murray 2002).

The traditional methods for evaluating condition in small mammals (TBM, BMF index, and residual index) compared favorably with our LBM-based equations in their ability to predict fat content. Indeed, among squirrels, all BMF measurements were related positively to percent fat, suggesting that these postmortem measures need not be replaced with either more complex morphometric models (e.g., our LBM model) or conductive techniques when squirrel carcasses are available. TBM and the residual index also were related to percent fat, but the relationships were negative, a surprising trend that warrants further scrutiny. Among hares, the residual index proved to be the best traditional estimate of fat content; indeed, it provided a better fit than our LBM equation, validating its use in previous studies of hare condition (e.g., O'Donoghue and Krebs 1992; Hodges et al. 1999; Murray 2002). Furthermore, all relationships were significant and positive, which indicates that both pre- (TBM, residual) and postmortem (BMF) measures can be used to furnish rough estimates of fat content in

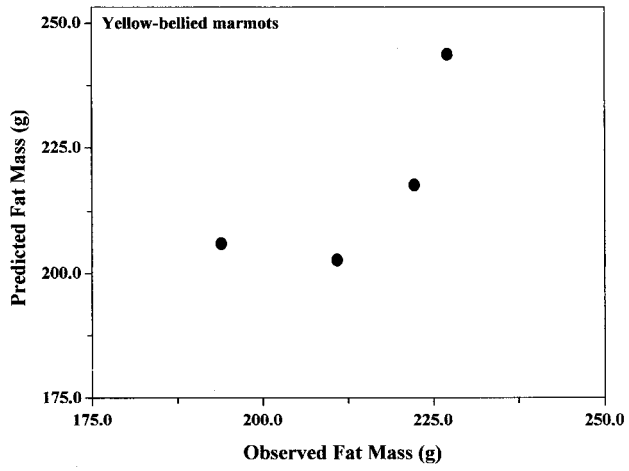

Figure 3. Relationship between body fat estimates produced by the best predictive equation for yellow-bellied marmots (LBM = −273.28 + 1.04 × TBM; fat mass = TBM − LBM) and fat mass values estimated via ether extraction ($r^2 = 0.57$, $P = 0.24$).

hares. Like the LBM models, however, none of the traditional measures explained enough variation in fat content to allow for differentiation between individual squirrels and hares (all $r^2 < 0.64$), again probably because of their indirect nature.

In sum, the results presented here suggest that the efficacy of conductive methods such as BIA and TOBEC may depend on the mean lipid content of the subject species, with these techniques yielding estimates of body composition comparable (or even superior) to those given by morphometric measures in small mammals that deposit appreciable amounts of fat. In general, however, morphometric measures outperformed conductive methods, a trend that also has been observed in ungulates (e.g., Cook et al. 2001). Thus, future field studies of body composition in mammals should perhaps rely on careful morphometric measurement rather than expensive conductive analysis. Even the best morphometric equations we generated were only accurate enough to allow for broad comparisons with respect to body fat (e.g., population means). Even if BIA and TOBEC had improved significantly upon the ability of our morphometric models to predict LBM, the error associated with the conversion from LBM to fat mass probably would have prevented accurate indirect measurement of fat content. Thus, techniques that measure fat directly (e.g., cyclopropane gas; Henen 1991; Gessaman et al. 1998), and presumably more accurately, must be developed for field use if fine-scale differences between fat content in individual small mammals are to be addressed.

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