Evaluation of bioelectrical impedance analysis as an estimator of moose body composition

Kris J. Hundertmark and Charles C. Schwartz

Abstract Estimation of body composition of wild ungulates yields important information regarding nutritional status of individuals and populations; yet, there are few suitable field techniques that are nondestructive, unbiased, precise, and quick to perform. We tested the suitability of bioelectrical impedance analysis (BIA) as an estimator of body composition of moose (*Alces alces*) for use in the field. A derived BIA variable, impedance volume, was a significant predictor of body fat (mass and percentage) and body water (mass and percentage) when sex was added to models as an indicator variable but explained only 48–57% of variation in composition. Best predictive models included impedance volume, sex, body mass, and a body mass × sex interaction. Due to difficulty measuring body mass of moose in the field, we also generated predictive models when body mass was replaced with a proxy ($\text{length} \times \text{girth}^2$). Predictive equations for body water were more precise than were those for body fat. Impedance estimates decreased as the subject's hind leg was straightened, indicating that animal positioning must be standardized to minimize bias. Lack of precision made BIA unsuitable for estimating moose body fat in the field. BIA was a precise and quick estimator of body water in moose, but its limitations make it more suitable for the laboratory than the field.

Key words *Alces alces*, BIA, bioelectrical impedance analysis, body composition, body fat, body water, moose

Body composition, particularly percent body fat, is an important indicator of an animal's physiological status and provides insight into ecological factors such as carrying capacity and environmental stress. The role of fat stores in northern cervids is particularly important because of the seasonal nature of fat accumulation and depletion (Mautz 1978). Estimates of size of fat stores at a given time of year can be used to infer an animal's physical condition, its prospects for survival or reproduction, and the quality of its habitat relative to population density. Accurate and precise estimation of body condition also is necessary for validating models of nutritional dynamics of populations and estimation of carrying capacity (Hubbert 1987, Hobbs 1989).

Nondestructive methods for estimating body composition under field conditions have been developed, but no method has been accepted universally. Isotope (tritium or deuterium) dilution procedures, albeit precise, require extended periods while waiting for equilibration (>2 hr when sampling blood [Farley and Robbins 1994] and >15 hr when sampling urine [Torbit et al. 1985]). Assessment of nutritional status by assigning condition indices or classes (Franzmann 1977, Gerhart et al. 1996) can be informative but ultimately is subjective and can vary among observers. Determina-

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Authors' address: Kenai Moose Research Center, Alaska Department of Fish and Game, 43961 Kalifornsky Beach Road, Suite B, Soldotna, AK 99669, USA; e-mail for Hundertmark: kris_hundertmark@fishgame.state.ak.us; present address for Schwartz: United States Geological Survey, Biological Resources Division, Northern Rocky Mountain Science Center, Montana State University, Bozeman, MT 59717, USA.

tion of subcutaneous fat depth with ultrasound is a quick, viable technique over a wide range of nutritional conditions, but is ineffective after rump fat disappears (Stephenson et al. 1998). Although useful in many circumstances, these techniques are not always suitable for precise determination of body composition in the field.

Bioelectrical impedance analysis (BIA) is a precise and unbiased predictor of human body composition (Lukaski 1987) and has been investigated for potential use in the livestock industry (Jenkins et al. 1988, Swantek et al. 1991). This technique works on the principle of measuring the impedance (resistance to alternating current) of hydrated body tissues to an alternating current of known frequency. Nyboer et al. (1943) demonstrated that

$$V = \frac{\rho L^2}{Z},$$

where V=body water volume, ρ=volume resistivity and is constant for a given conductor, L=conductor length, and Z=impedance. Impedance is computed by $(R_S^2+Xc^2)^{0.5}$ where R_S=resistance and Xc=reactance. Generation of current and measurement of R_S and Xc are accomplished with a plethysmograph.

BIA has proven to be a useful tool for determination of body composition for bears (*Ursus* spp.; Farley and Robbins 1994, Hilderbrand et al. 1998), wombats (*Lasiorhinus latifrons*; Woolnough et al. 1997), and seals (*Phoca* spp.; Gales et al. 1994). Gerhart et al. (1992), however, reported that body mass was superior to BIA as a predictor of total body water in caribou (*Rangifer tarandus*) and reindeer. Body mass often is used in conjunction with BIA measurements in multiple regression models to predict body composition (Lukaski et al. 1986, Farley and Robbins 1994, Woolnough 1997), but mass is a difficult parameter to measure in the field for large mammals. Measurements of body length and girth, which can be obtained in field situations, can be used as proxies of body mass for moose (Haigh et al. 1980, Hundertmark and Schwartz 1998), but their values as predictors of body composition are not known.

We tested BIA as a potential estimator of body composition in moose (*Alces alces*). We determined whether BIA measurements were significant predictors of body water and fat (expressed as mass and as percentage of body mass) as measured by proximate analysis. We also determined the best predictive model for each of those 4 measures of body composition from a suite of variables including body mass (or a proxy thereof), BIA measurements, and sex.

Methods

Experimental animals were kept in captivity at the Kenai Moose Research Center (MRC), Alaska. Animals were allowed to forage naturally during the summer and were fed a formulated ration (Schwartz et al. 1985) in winter. We selected subjects opportunistically for inclusion in the trial, with a goal of balancing the sex ratio of the sample and obtaining a representative range of body condition. We used 10 females and 9 males. All research activities followed an animal welfare protocol approved by the Alaska Department of Fish and Game.

We used a portable plethysmograph (Model BIA-101, RJL Systems, Inc. Detroit, Mich.) to estimate electrical impedance of moose. The plethysmograph was suitable for use in the field because it was battery-powered, small in size (16.3 × 24.6 × 6.6 cm), and lightweight (1.7 kg). We immobilized individual moose with a mixture of carfentanil HCl and xylazine hydrochloride (Schmitt and Dalton 1987). Immobilized animals were allowed to assume a position of sternal recumbency; any variation in positioning of animals was eliminated so that all animals were tested in similar positions. We constructed electrodes from trocars removed from 18-ga spinal needles and bent them to an angle of 90°, 13 mm from the tip. We inserted the bent end of an electrode subcutaneously at the carpal joint on the foreleg and at the joint between the metatarsus and the hoof on the hind leg on the side of the body most exposed while the moose was recumbent. We placed a second electrode 7.5 cm proximal to the original electrode at each position. We tested a second set of positions on some animals wherein the electrodes were placed on the peak of the spinal column between the shoulders (hump) and at the base of the tail (rump), thereby removing the legs from the analysis. The tips of the electrodes always were oriented distally under the skin. We connected electrodes to the plethysmograph via alligator clips on the end of 10-ft cables. An alternating current of 800 μA at 50 kHz was introduced through the source (distal) electrodes, and R_S and Xc were recorded to the nearest ohm across the detector (proximal) electrodes. We also measured total body length (*TL*) and chest girth (*CG*) to the nearest cm. Total length was measured along the dorsal surface of the body from the top of the naso-labial pad to the tip of the tail, and *CG* was the distance from the peak of the spinal hump to the midpoint of the sternum multiplied by 2.

We evaluated the effect of animal position on BIA parameters for a subset of 9 animals by taking readings with the hind leg in various positions. In our experience, position of the hind leg varies greatly among immobilized moose. We quantified changes in position by measuring the straight-line distance between knee and tarsal joint of the bent leg to the nearest cm. That distance increased as the leg was straightened, and we noted changes in R_S and Xc at various distances.

Animals were euthanized within 24 hr after we obtained BIA measurements. We weighed animals whole, eviscerated and skinned (leaving as much subcutaneous fat as possible on the carcass). We bisected empty carcasses along the spinal column, with half of the carcass frozen for analysis. After removal of ingesta, the viscera were frozen, as were samples of shaved hide. We cut the frozen side of the carcass and the visceral mass into 51- and 25-mm slices, respectively, on a commercial band saw. We collected sawdust that accumulated at the base of the saw blade for each component, which we thoroughly mixed and refroze prior to proximate analysis (Huot and Picard 1988). We freeze-dried hide samples and ground them in a Wiley mill before subjecting them to proximate analysis. We determined crude fat by ether extraction, crude protein content by the Kjeldahl procedure (Association of Official Analytical Chemists 1975), ash content by burning in a muffle furnace at 550°C for 2 hr, and percent organic dry matter (1.00–moisture content) by drying samples in a 100°C oven for 12–16 hr and subtracting ash content. We analyzed three replicates of each sample and analyzed additional samples if coefficients of variation for the original 3 replicates exceeded 5%. We estimated body composition from weighted estimates of body components from carcass, viscera, and hide. References to body composition in this report refer to the ingesta-free body, which we defined as the entire body less contents of the gastrointestinal tract.

We determined whether the derived BIA variable TL^2/Z, hereafter referred to as impedance volume, was related to chemically determined estimates of body composition by linear regression. We also included sex as an indicator variable in those models (male = 0, female = 1) because sex-related differences in predictive models were noted in studies of BIA for humans (Lukaski 1987). When sex-related differences existed, we tested for significant interactions between sex and impedance volume with analysis of covariance (ANCOVA). We searched for the best predictive models by examining all possible regressions for each dependent variable. The full models contained sex, impedance volume, body mass, and significant interaction effects as variables. We repeated that analysis with $TL \times CG^2$ substituted as a proxy for body mass to derive models for situations in which estimates of mass were not available. Haigh et al. (1980) and Hundertmark and Schwartz (1998) demonstrated that $TL \times CG^2$, expressed in meters to avoid scaling problems, was a good predictor of body mass in moose. We evaluated regression models by examining adjusted coefficients of determination (R^2_{adj}), standard errors of estimates ($S_{Y \times X}$), and *Cp* (Mallows 1973). *Cp* evaluated the fit of a model relative to number of predictors and was analogous to Akaike's Information Criterion (Atilgan 1996). Within a set of models, the most suitable was defined by the minimum value of *Cp*, calculated as

$$Cp = \frac{\sum d^2_{y \times p}}{s^2} + 2p - n,$$

where $\sum d^2_{y \times p}$ was residual sum of squares for the model under consideration, s^2 was residual mean square for the full model (all predictors) and was assumed to be an unbiased estimate of σ^2, n = number of observations, and p = number of predictors (including the constant) in the model.

Results

Body mass of moose in our trial ranged from 180–535 kg. Percent body fat, as determined by proximate analysis, ranged from 0.3–19.4%, which likely included the extremes of body condition found in the wild.

Electrode placement on the rump and hump yielded mean R_S and Xc values that were 8.6% and 10.8%, respectively, of mean values of electrode placement on legs. Limited sensitivity of rump–hump measurements resulted in their rejection as a potential indicator of composition of the entire body. Only impedance values obtained from electrode positions on legs were used to predict body composition.

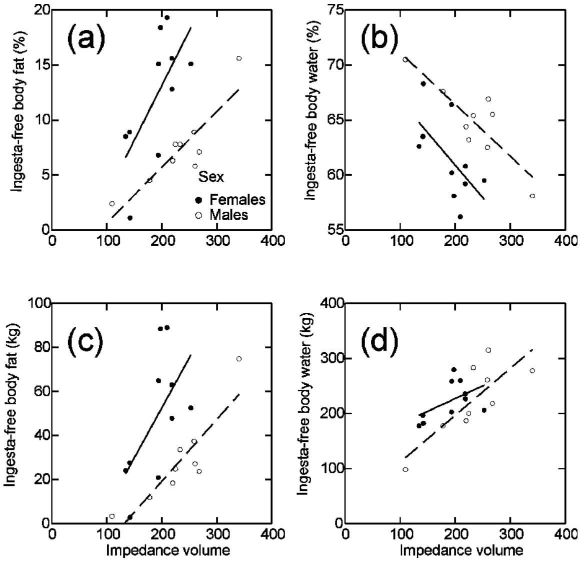

Figure 1. Relationships between impedance volume (TL^2 / Z) and body composition estimates in moose. Sex-related differences are apparent, and sex-specific responses share common slopes. Plots represent sex-specific regression models for *a*) percent body fat, *b*) percent body water, *c*) fat mass, and *d*) water mass.

Relationships between impedance volume and components of body composition were sex-specific (Figure 1), but tests of significance for interaction terms in ANCOVA indicated that sex-specific relationships shared common slopes (% fat: $F_{1,15}=1.91$, $P=0.19$; kg fat: $F_{1,15}=0.80$, $P=0.39$; % water: $F_{1,15}=0.20$, $P=0.66$; kg water: $F_{1,15}=1.06$, $P=0.32$). Pooled regression models indicated that impedance volume was a significant predictor of body water and fat (mass and percentage), adjusted for sex (Table 1).

Based on *Cp* values among all possible regression models, a model incorporating impedance volume, sex, and a body mass×sex interaction was selected as best for predicting fat mass, fat percentage, and water percentage in the body (Table 2). Substituting $TL \times CG^2$ for body mass resulted in selection of the same form of model for those dependent variables; values of regression diagnostics were either similar (percentage water) or inferior (fat mass and percentage) to values for models incorporating body mass (Table 2). The full model was selected as best for predicting water mass when an estimate of body mass was available and had the best fit of all models, explaining 97% of variation in water mass in the ingesta-free body. When the process was repeated with a proxy for body mass, a model containing only $TL \times CG^2$ and an interaction term as independent variables was selected as the best predictor of water mass. The best model for water mass that contained impedance volume (kg water= $28.4+0.064[TL^2/Z]+17.4[TL \times CG^2]-2.4[TL \times CG^2 \times \text{Sex}]$) had regression diagnostics ($R^2_{\text{adj}}=0.80$, $S_{Y \times X}=23.0$) similar to the best model and was of the same form as models chosen for prediction of other components (Table 2).

The precision of estimates varied, dependent primarily on the component of body composition being predicted. Standard errors of estimates, expressed as percentages of means of dependent variables, ranged from 3.5–28.4% when body mass was known and from 3.5–36.4% when body mass was not known. The largest relative standard errors were associated with estimates of fat mass and percentage (Table 2). The lowest values were associated with models for percent body water, followed closely by models for water mass that included body mass as a predictor.

As the distance between the knee and tarsal joint of a moose increased, BIA values decreased. To determine the significance of this effect, we standardized BIA values among animals by dividing the observations for each animal by the adjusted mean (from ANOVA) for that animal. We confirmed homogeneity of slopes among animals by a nonsignificant interaction between distance and animal in ANCOVA ($F_{8,16}=0.22$, $P=0.98$). A regression analysis of standardized impedance volume (dependent variable) and distance (independent variable) indicated that leg position had an effect on resistance and reactance measurements ($t_{32}=-12.2$, $P<0.001$), and the regression model explained 82% of the variance in BIA measurements within individuals (Figure 2).

Table 1. Regression models for predicting fat and water mass in the ingesta-free body of moose (n = 19) with sex and impedance volume (TL^2 / Z). Sex is an indicator variable (male = 0, female = 1).

Dependent variable	Model	R^2_{adj}	$S_{Y \times X}$	$S_{Y \times X}$ (as % of $\bar{Y}$)
Fat (%)	$-7.78 + 0.065(TL^2 / Z) + 7.56(\text{Sex})$	0.55	3.6	36.4
Fat (kg)	$76.65 - 0.051(TL^2 / Z) - 5.56(\text{Sex})$	0.49	19.0	49.1
Water (%)	$-49.03 + 0.33(TL^2 / Z) + 33.87(\text{Sex})$	0.57	2.6	4.1
Water (kg)	$53.09 + 0.74(TL^2 / Z) + 29.38(\text{Sex})$	0.48	37.1	16.6

Table 2. Regression models for predicting fat and water (mass and percentage) in the ingesta-free body of moose (n = 19). Each model represents the best model based on values of Cp from all possible regressions of impedance volume (TL^2 / Z), sex, body mass and a body mass × sex interaction. Two models are given for each dependent variable for cases where either body mass is known or a proxy for body mass ($TL \times CG^2$) is used. $TL \times CG^2$ is expressed in meters to avoid scaling problems. Sex is an indicator variable (male = 0, female = 1).

	Body mass				$TL \times CG^2$			
Dependent variable	Model	R^2_{adj}	$S_{Y \times X}$	$S_{Y \times X}$ (as % of $\bar{Y}$)	Model	R^2_{adj}	$S_{Y \times X}$	$S_{Y \times X}$ (as % of $\bar{Y}$)
Fat (%)	$-3.9 + 0.048(TL^2/Z) - 14.3(\text{Sex}) + 0.050(\text{Mass} \times \text{Sex})$	0.81	2.3	23.6	$-4.9 + 0.053(TL^2/Z) - 12.8(\text{Sex}) + 1.6(TL \times CG^2 \times \text{Sex})$	0.78	2.5	25.0
Fat (kg)	$-27.0 + 0.24(TL^2/Z) - 90.7(\text{Sex}) + 0.29(\text{Mass} \times \text{Sex})$	0.83	11.0	28.4	$-34.8 + 0.27(TL^2/Z) - 67.7(\text{Sex}) + 8.2(TL \times CG^2 \times \text{Sex})$	0.72	14.1	36.4
Water (%)	$2.1 - 0.19(TL^2/Z) + 43.4(\text{Sex}) + 0.68(\text{Mass}) - 0.17(\text{Mass} \times \text{Sex})$	0.70	2.2	3.5	$32.3 + 18.41(TL \times CG^2) - 2.8(TL \times CG^2 \times \text{Sex})$	0.70	2.2	3.5
Water (kg)	$2.1 - 0.19(TL^2/Z) + 43.4(\text{Sex}) + 0.68(\text{Mass}) - 0.17(\text{Mass} \times \text{Sex})$	0.97	9.4	4.2	$32.3 + 18.41(TL \times CG^2) - 2.8(TL \times CG^2 \times \text{Sex})$	0.81	22.4	10.1

Discussion

Techniques useful for estimation of body composition in field situations should be quick and easy to perform and, with large animals such as moose, should not require an estimate of body mass. In the absence of estimates of body mass, BIA was a significant predictor of water and fat composition of the body, but those models explained only 48–57% of variation in dependent variables, which we believe is low for a potential predictive tool. When mass estimates were made available to the regression model, their inclusion with a BIA parameter produced the best models for predicting fat and water mass. Estimating mass of adult moose in the field, however, is problematic (Hundertmark and Schwartz 1998).

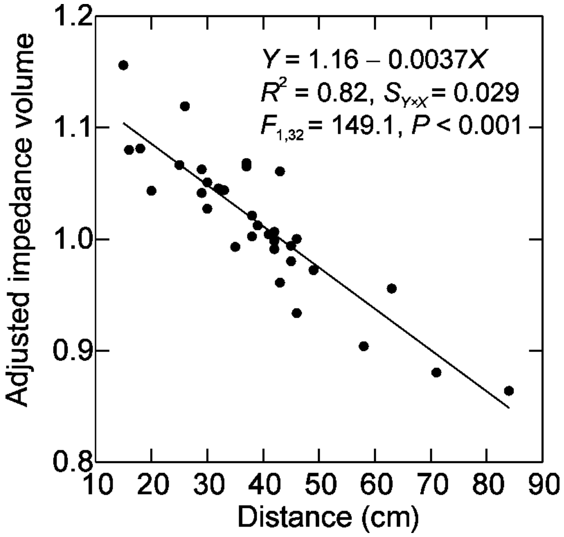

Figure 2. Relationship of distance between the knee and tarsal joint of moose and concomitant estimates of impedance volume (TL^2 / Z). Variation among individuals in impedance volume estimates required standardization of those estimates prior to assessing the correlation with extent of leg position.

Values of $S_{Y \times X}$ for models of fat percentage and mass were approximately twice as great for the best models we evaluated compared to analogous models developed using ultrasonic measurement of subcutaneous fat (Stephenson et al. 1998). The relatively greater values of $S_{Y \times X}$ for predictive models for body fatness indicated that BIA in moose might be effective only for measuring body water. Considering that BIA works by measuring characteristics of alternating current passing through hydrated (fat-free) body tissues, this finding was not surprising. The general inverse relationship between percent body water and percent body fat undoubtedly caused fat composition to be related to impedance to some degree, but in our sample that relationship was not strong enough to warrant our recommendation for use of BIA as a precise estimator of body fat.

Sex-related differences in predictive models were noted in studies of BIA for humans (Lukaski 1987) but not for bears (Farley and Robbins 1994) or seals (Gales et al. 1994). The differences noted herein likely were not a function of BIA, however. Sex also was a significant predictor of body composition when body mass was the only other independent variable in the models. That may represent a species-specific phenomenon, and future evaluations of the application of BIA to other species should consider sex-specific models.

Consistent positioning of the body among subjects was essential for generating reliable and repeatable results with BIA; however, this is difficult to achieve with large animals under field conditions. Moose can assume various positions when immobilized, particularly in deep snow, and repositioning animals to achieve consistency can be difficult. Inconsistent positioning among animals changes the geometry of the signal path and introduces bias to estimates of composition (Hall et al. 1989). Our data indicated that an increase in knee–tarsal joint distance of 30 cm caused a decrease of approximately 10% in TL^2/Z. The change in the estimate of body water mass associated with that change in leg position ranged from 1.5–2.5% depending on sex of the animal and the value of TL^2/Z. Although such a bias alone may not cause biologically meaningful changes in composition estimates, potential bias introduced by other positional inconsistencies would be additive. Positioning the electrodes along the torso, as is done for bears (Farley and Robbins 1994), would eliminate concerns about leg positioning. In our trials, however, R_S and Xc values were much lower and varied less with changes in body composition when the rump–hump configuration was tested in moose, which made the technique less sensitive to changes in composition. Another potential confounding factor was that BIA requires that impedance of the subject be measured on a nonconductive surface; this requirement would be difficult to achieve with moose under field conditions. Measuring animals in dry snow does not present a problem, but measurements on conductive surfaces such as wet snow or with wet animals are problematic (Farley and Robbins 1994, Robert et al. 1994).

Management implications

BIA is a viable technique for estimating body composition in the field, but lack of precision in estimating fat composition of moose leads us to not recommend it for that purpose. Existing techniques, such as ultrasound (Stephenson et al. 1998), are more appropriate for estimating fat in moose. BIA is an acceptable method for determining body water of large animals, particularly when an estimate of body mass is available and a quick, inexpensive technique is preferable. Nonetheless, the problems associated with achieving consistent body position with moose, particularly when immobilized in deep snow, and current leakage when conducted on a wet substrate, make this technique more suitable for the controlled conditions of a laboratory setting.

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Kris Hundertmark (photo) has worked for the Alaska Department of Fish and Game since 1982 and has been a research biologist with the Kenai Moose Research Center (MRC) since 1988. He holds degrees from Pennsylvania State University (B.S.), Oregon State University (M.S.), and University of Alaska Fairbanks (Ph.D.). His past work at the MRC has focused on population biology and nutritional ecology of moose, and his current research interests include population and ecological genetics of managed populations. He has twice been a member of the Executive Board of the Alaska Chapter of TWS. During this work, ***Charles (Chuck) Schwartz*** was Director of the MRC, where he conducted research on moose, black bears, and brown bears. He holds degrees from Ohio State University (B.S.) and Colorado State University (M.S., Ph.D.). He is a former president of the Alaska Chapter of TWS, and is a recipient of the Arthur S. Einarsen Award from the Northwest Section of TWS. Currently, he is leader of the Interagency Grizzly Bear Study Team for the Greater Yellowstone Ecosystem and is employed by the U.S. Geological Survey's Biological Resources Division in Bozeman, Montana.

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