Development of two methods to estimate body composition of bears

SEAN D. FARLEY
Department of Zoology, Washington State University, Pullman, WA 99164-4236, U.S.A.

AND

CHARLES T. ROBBINS
Departments of Natural Resource Sciences and Zoology, Washington State University, Pullman, WA 99164-4236, U.S.A.

Received November 10, 1992
Accepted October 14, 1993

FARLEY, S.D., and ROBBINS, C.T. 1994. Development of two methods to estimate body composition of bears. Can. J. Zool. **72**: 220–226.

The measurement of body composition in black bears (*Ursus americanus*), brown bears (*U. arctos*), and polar bears (*U. maritimus*) was investigated by means of isotopic water dilution and bioelectrical impedance analysis (BIA). The basic relationships between body lipid, water, protein, and ash were determined by direct chemical measurement of 13 black and 6 brown bears. Body water and lipid content as a percentage are highly correlated (r^2 = 0.98, standard error of the estimate (SEE) = 1.1%) and inversely proportional. The dry, lipid-free mass averaged 83.5 ± 1.6% protein and 16.5 ± 1.6% ash. Either isotopic water dilution or BIA can be used to estimate body lipid content of healthy, uninjured bears (r^2 = 0.93, SEE = 2.7% and r^2 = 0.96, SEE = 2.2%, respectively). Isotopic water equilibrated with body water by 150 min. Abscesses and recent injuries (i.e., gunshot or snare wounds) produced erroneous body composition estimates when BIA was used, but only when the injury was in the conductor path between the BIA electrodes. Dilution estimates were not affected by injuries. Currently, neither method can be used on dead bears.

FARLEY, S.D., et ROBBINS, C.T. 1994. Development of two methods to estimate body composition of bears. Can. J. Zool. **72** : 220–226.

Des techniques de dilution à l'eau isotopique et d'analyse bioélectrique de l'impédance (BIA) ont servi à évaluer la composition corporelle chez des Ours noirs (*Ursus americanus*), des Ours bruns (*U. arctos*), et des Ours blancs (*U. maritimus*). Les relations de base entre les lipides, l'eau, les protéines, et les cendres ont été déterminées par des mesures chimiques directes chez 13 Ours noirs et 6 Ours bruns. Le contenu en eau et le contenu en lipides (en pourcentage) sont en corrélation très fortes (r^2 = 0,98, estimation de l'erreur standard = 1,1%) et sont inversement proportionnels. La masse sèche sans les graisses est constituée en moyenne de 83,5 ± 1,6% de protéines et de 16,5 ± 1,6% de cendres. Les deux méthodes, la dilution à l'eau isotopique ou l'analyse bioélectrique de l'impédance, peuvent servir à estimer le contenu en lipides du corps chez des ours sains sans blessures (première méthode : r^2 = 0,93, erreur type sur l'estimé =2,7%; deuxième méthode : r^2 = 0,96, erreur type sur l'estimé = 2,2%). L'équilibre entre l'eau isotopique et l'eau du corps met 150 min à s'établir. La présence d'abcès ou de blessures récentes (i.e., coups de feu ou blessures laissées par des pièges) donne lieu à des mesures erronées de la composition corporelle par la méthode BIA, mais seulement lorsque la blessure se trouve dans la voie de transmission du conducteur entre les électrodes. Les estimations par dilution ne sont pas affectées par la présence des blessures. Pour le moment, ni l'une ni l'autre de ces méthodes ne peuvent être utilisées sur des ours morts.

[Traduit par la Rédaction]

Introduction

The development of fast, accurate methods to determine body composition of a living bear has implications for assessing habitat and population quality as well as identifying critical times or events in the animal's life cycle. Classically, bear biologists have used mass, morphometric measurements, visual scores, and various blood chemistries as indirect indicators of body condition (Poelker and Hartwell 1973; Franzmann and Schwartz 1988; Hellgren et al. 1989, 1993). None of these indicators have been validated using the animal's actual body composition, nor do they permit in-depth analysis of energy and matter balances. Cattet (1990) correlated morphometric measures an lipid content of specific sites (marrow and intramuscular) with dissectable fat and nonfat masses of polar bears and black bears. He concluded that such measures were unreliable and that more accurate techniques should be developed to assess the nutritional condition of individual bears. Thus, no method has yet been validated for accurately determining the body composition of living bears.

The two most useful methods currently available to determine body composition of the living bear under field conditions are isotopic water dilution and bioelectrical impedance analysis (BIA). Other available methods are either very inaccurate (e.g., skin-fold thickness) or inappropriate for field use on animals as large as bears (e.g., total body electrical conductivity and densitometry; Coward et al. 1988; Heymsfield and Waki 1991). BIA and isotopic water dilution measure the animal's water content, which then can be used to estimate lipid, protein, and ash contents with equations developed from direct chemical analyses of homogenized animals. The use of water content measurements to predict body composition depends on the close inverse relationship between water and fat and the constancy of the protein and ash contents of the dry, fat-free mass in chemically mature animals (Robbins 1993).

Isotopic water dilution has been available for at least 30 years and has been used on many species (Richmond et al. 1962; Nagy and Costa 1980; Torbit et al. 1985; Rumpler et al. 1987). BIA is a newer, rapid, noninvasive method originally developed for determining human body composition (Lukaski 1987). BIA measures the resistance to conduction (in ohms) of a low-level alternating current (800 μA at 50 kHz) in an organism. Because the conductivity of body lipids is 4–5%

that of lean tissue, body fluids, and bone, the resistance measured by BIA is an indicator of body water content (Fiorotta et al. 1987; Walsberg 1988; Hall et al. 1989). Thus, in this study the relationships between water, lipid, protein, and ash in bears were determined, and equations necessary for using isotopic water dilution and BIA to predict the body composition of anesthetized black, brown, and polar bears were developed.

Methods

Validation by chemical assessment of body composition

Nineteen wild, problem bears scheduled for euthanasia were shipped to the Bear Research Facility at Washington State University for direct chemical determination of water, lipid, protein, and ash. These bears were of both sexes and included cubs and adults. All animals were held in captivity with food and water supplied ad libitum until normal intake could be ensured. Two animals were allowed to enter hibernation to determine if any major physiological changes would prevent the use of either BIA or isotopic water dilution on hibernating bears.

Food was withheld from all bears for 24 h before they were anesthetized (Telazol, 5.0 mg/kg). Total body water was measured by isotopic water dilution and resistance measurements were made with a bioelectrical impedance analyzer (see sections on water dilution and impedance below for specific details). Each animal was then euthanized with an overdose of pentobarbital (Beuthanasia-D). After euthanasia all animals were weighed to ±200 g with an electronic load cell and shaved, and all ingesta within the gastrointestinal tract were removed. All bears were carefully examined for injury. All hair from each bear was collected and weighed, and a subsample was dried at 100°C to determine dry mass. The ingesta-free bear and the viscera were then triple-wrapped in plastic and stored at −20°C.

The viscera and the frozen, shaved, ingesta-free carcass were homogenized in a commercial whole-body grinder. Complete recovery of all tissue was ensured by thoroughly cleaning the grinder between each homogenization. Subsamples from each animal were retained for chemical analysis. Total body water was determined by freeze-drying subsamples of the homogenate (0.5–1.0 kg) and correcting for mass changes during freezer storage. The dried homogenates were finely ground with dry ice in a Wiley Mill and used for all subsequent chemical analyses. Crude protein content was determined by the macro-Kjeldahl technique, total lipids were extracted for ≥4 h in a Goldfisch apparatus with a 1:2 chloroform:methanol mixture (Folch et al. 1957; Spence and Wolfe 1967), and ash or mineral content was determined by combustion in a muffle furnace at 440°C for 24 h. Chloroform–methanol was chosen over ether as the lipid solvent in order to extract both polar and nonpolar lipids. All assays were carried out in duplicate and means were used in all calculations. Hair subsamples (0.5–5.0 g) were analyzed for crude protein, ash, and lipid contents by the same methods used for whole-body homogenate. Although hair was analyzed separately, all body composition data include hair.

Total body water determination by isotopic water dilution

Body water pool size was determined in vivo by isotopic water dilution (Pace et al. 1947). We performed 57 dilutions on 25 captive black bears, 35 dilutions on 15 captive brown bears, and 62 dilutions on 62 free-ranging polar bears. We considered the multiple measures on black and brown bears to be independent because they were taken either across periods of very active growth in young bears or during major seasonal mass changes (e.g., before and after hibernation) in older bears. Either tritiated (10 μCi/kg; 1 Ci = 37 GBq) or deuterated water (0.5 g/kg; 99.8%; Aldrich Chemicals) was injected intravenously into anesthetized (Telazol, 5.0 mg/kg) bears. Animals were weighed (±200 g) and blood samples collected at 30-min intervals for 4 h. Blood samples were centrifuged (IEC clinical centrifuge, 15 min at top speed) and serum was harvested. Tritiated serum samples were counted on a Packard Tri-Carb liquid scintillation counter. A quench correction was applied (sample channels ratio) and tritium activity was expressed in disintegrations per minute (1 dpm = 0.167 Bq) per millilitre of water. All deuterated samples were vacuum sublimated and deuterium concentration was determined by infrared spectrophotometry (Byers 1979) using a Miran 1FF fixed-filter deuterium analyzer (±0.05% photometric accuracy; Foxboro Co., Foxboro, Mass.). Body water pool size was estimated as the ratio of the amount of isotope injected to the isotope concentration measured in the animal when the injected isotope was completely mixed throughout the total body water pool (Pace et al. 1947; Lukaski 1987).

Equilibration determination

Isotopic equilibration occurs when the injected isotope is distributed evenly throughout the body water pool, including blood and urine. We determined equilibration time in a subsample of bears (14 black and 9 brown bears) that were simultaneously used to total body dilutions and BIA. In addition to the blood samples taken every 30 min, these individuals were placed in metabolism crates (Pritchard and Robbins 1990) and allowed to recover. All bears were provided with water ad libitum and fed a maintenance diet of dog food. Each bear was observed at least once a day for 6 days until urination occurred. The time of urination was recorded and a sample immediately collected. Urine samples were decolorized with activated charcoal and centrifuged (IEC clinical centrifuge, 15 min at top speed), and the supernatant was counted for isotope concentration. Channels ratio quench correction was applied and activity was expressed in disintegrations per minute per millilitre of water. For each animal we regressed the isotope concentrations in urine water against times of urination, then extrapolated to the concentration at time 0. Because some isotope is lost via urination and respiration during the equilibration period, this value at time 0 represents the concentration expected if equilibration had occurred instantaneously after injection. Blood water isotope concentrations during the first 4 h were then expressed as ratios of the extrapolated time 0 concentration. The time of equilibration was determined as the minimum time necessary for ratios to become relatively constant and to show variation that was not significantly reduced in later samples.

Bioelectrical impedance analysis

BIA measurements were taken simultaneously with all isotope dilutions on all bears (25 captive black bears, 15 captive brown bears, and 62 free-ranging polar bears). BIA resistance readings were made with the Model 101A (RJL Systems, Detroit, Mich.) which reads 0–1000 ohms with a resolution of 1 ohm and an accuracy of ±0.5%. Bears were placed sternally recumbent in a standard position on dry ground, concrete, or a plastic sheet. The plastic sheet was used with wet bears to prevent loss of electrical current to the ground. Needle electrodes (21 g, 3.8 cm Vacutainer® needles) were used with the short end inserted subcutaneously into the animal and the long end clamped to the impedance meter's cable.

Because BIA readings are strongly affected by limb position and electrode distance (Kushner 1992), we used a consistent positioning of the bear and the electrodes. Most previous workers have measured whole-body resistance between limbs (Kushner 1992). Thus, we initially measured whole-body resistance between forelimbs and hind limbs for each bear (both contralaterally and isolaterally). When measuring bear forelimb to hind limb resistance, we mimicked the hand and foot electrode placements used by previous researchers working with humans. We minimized interobserver variance in electrode placement by defining anatomically distinct locations for the electrodes. Thus, during hind limb for forelimb measurements the anterior electrode pair was placed on the dorsal aspect of the forepaw, with the current-carrying electrode between the 3rd and 4th digits at a level between the metacarpals and proximal phalanges, while the current-detecting electrode was inserted proximal to the radial carpal and ulnar carpal bones and midway between the radius and ulna of the same animal. The current-carrying electrode of the posterior pair was on the lateral surface of the foot and just dorsal to the most posterior point of the foot pad. The current-detecting electrode was also on the lateral surface of the foot, but dorsal to the calcaneum.

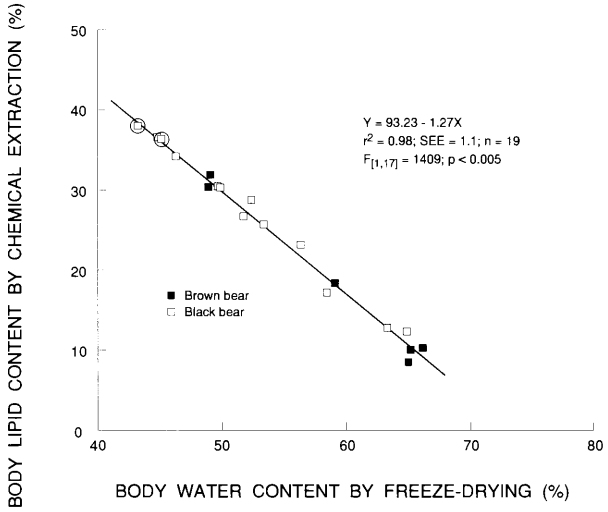

FIG. 1. Relationship between body water and lipid content from direct chemical analyses of 6 brown and 13 black bears. The two circled points were hibernating black bears.

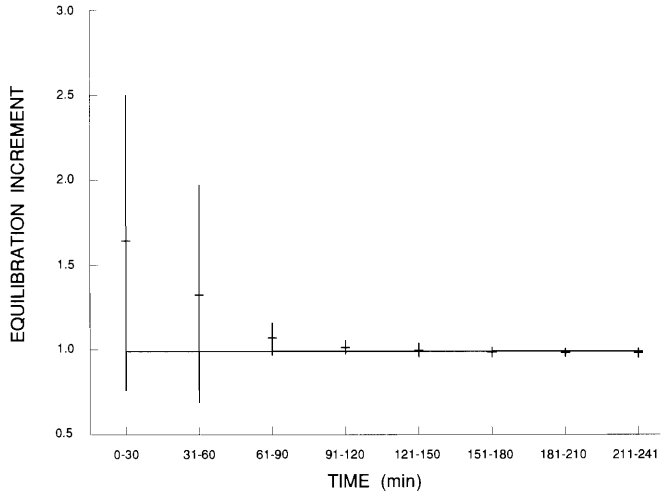

FIG. 2. Isotope concentration in blood water at 30-min intervals after injection relative to the extrapolated time 0 concentration in urine water samples from 14 brown and 9 black bears. Increment values are the mean ± 1 SD. An equilibration increment of 1 means that the isotope concentration in blood water and the extrapolated time 0 value are equal.

Finally, to determine the importance of standardizing limb position, the limbs were moved either closer to or farther from the body and new resistances and distances from the standard position were recorded.

Another resistance measure that would use more easily identified electrode positions and would not require the rigid standardization necessary when using the limbs was sought. However, bears were always positioned sternally as previously described. The most promising of several measures that were tried was snout to tail resistance. This was measured with the anterior electrode pair clamped to the upper lip (needles were not used) at the level of the canines. Good electrical contact was ensured by wetting the lips with water. The posterior electrodes were placed 3 cm on either side of the junction of the sacrum and the first caudal vertebrae (e.g., base of the tail). The current-carrying electrodes were always on the animal's right side. Because this resistance measure was developed midway through the study, snout to tail resistance was measured on only 12 of the 19 euthanized bears.

Because BIA resistance is a function of conductor configuration (Kushner 1992; Kushner and Schoeller 1986; Nyboer 1972), we measured the linear and circumferential dimensions of the head, ears, neck, trunk, and legs necessary to estimate total surface area and total body volume (Moen 1973). In addition, snout to vent length and snout to tip of tail length were measured following the dorsal contours.

The change in BIA measurements from death to 5 h later was also determined on four bears. At the time of death, two of the animals were placed in a −20°C freezer and two were kept at room temperature (22°C).

Simple linear and multiple linear regressions were used to develop predictive equations. Standard errors of the estimates (SEE) and coefficients of determination were used to compare predictive equations. ANOVA was used to test for species differences.

Results

Chemical assessment

The methods used to measure water, protein, lipid, and ash accounted for 100.7 ± 0.6% ($\bar{x}$ ± SD) of the bear's mass. Lipid and water content (%) were inversely correlated, with no significant differences between black and brown bears ($p = 0.33$) or between hibernating and nonhibernating bears ($p = 0.89$) (Fig. 1). The fat-free or lean mass averaged 73.4% water (i.e., the x intercept of Fig. 1 at 0% fat). The dry, lipid-free residue contained 83.5 ± 1.6% protein and 16.5 ± 1.6% ash. The oven-dry mass of hair (Y, g) increased curvilinearly with body mass (X, kg) and can be estimated by the equation $Y = 47.9(X^{0.67})$ ($r^2 = 0.78$; SEE = 221). The dry matter of hair contained 94.5 ± 2.0% protein, 3.2 ± 0.4% ash, and 2.3 ± 1.2% lipid.

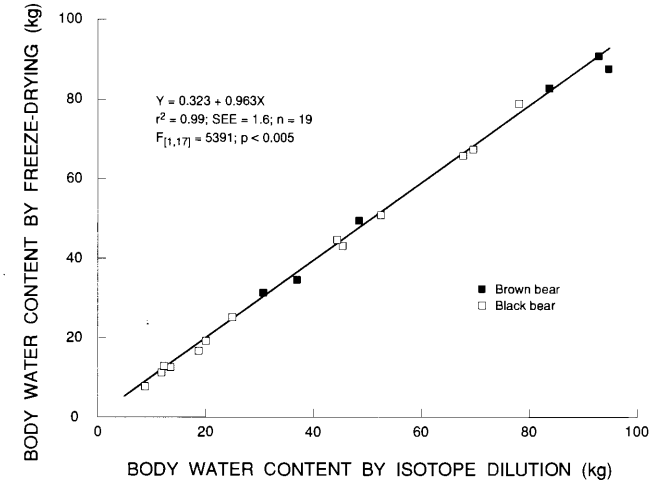

FIG. 3. Comparison of body water content determined by isotope dilution and whole-body grinding–sublimation in bears.

Equilibration times

Equilibration of injected isotopic water occurred by 150 min postinjection (Fig. 2). Slight variation in the timing of samples collected shortly before or after 150 min had a negligible effect on the measured isotope concentration. The 150-min isotope concentrations were slightly higher than the time 0 value (1.044 ± 0.008). Thus, samples taken at 150 min

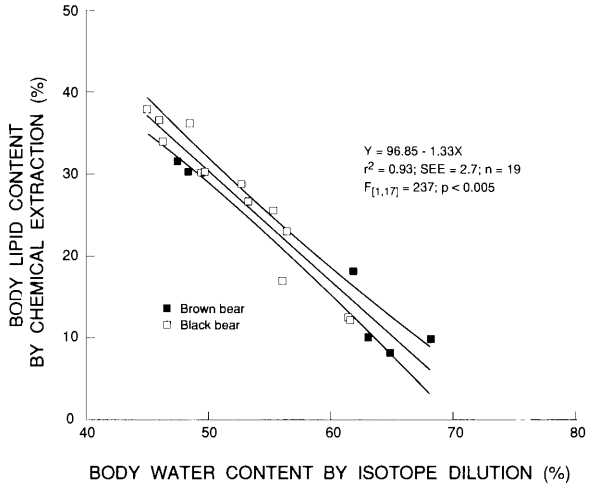

FIG. 4. Relationship between body lipid content determined by grinding–chemical extraction and body water content measured by isotope dilution. The regression line is bracketed by 95% confidence intervals.

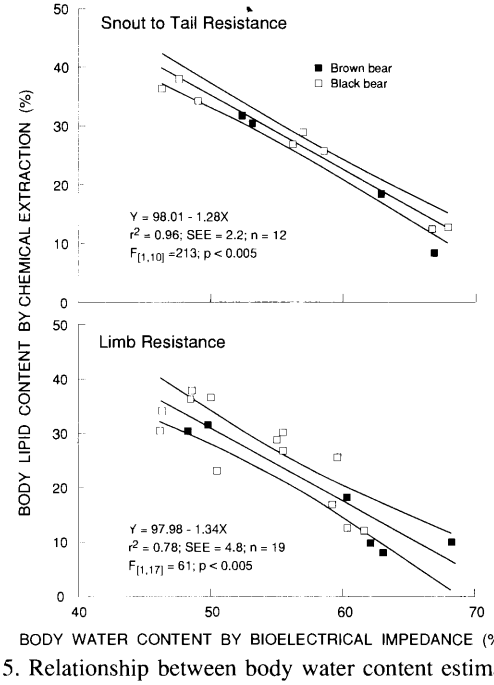

FIG. 5. Relationship between body water content estimated by the multiple linear regressions from Table 2 and body lipid content determined by whole-body grinding–chemical extraction.

were considered to represent equilibrated values and were corrected to time 0 by dividing by 1.044.

Isotopic dilution and BIA

Water dilution using the above correction, 1.044, overestimated body water determined by freeze-drying by 3.7% (i.e., 1 minus the slope of the regression in Fig. 3). Because this correction contributes to the overestimate of 3.7% from using serum samples collected at 150 min, we did not apply either correction to subsequent measurements of body water content, as the systematic error is reduced to less than 1%. Isotopically determined body water content provided

TABLE 1. Coefficients of determination and standard error of the estimate for simple linear regression between total body water (isotope dilution) and three morphometric measures and three resistance measures

Species and predictive variable[a]	r^2	SEE
Morphometric measures		
Black bear		
BM	0.96	5.4
TSA	0.88	8.8
TBV	0.86	9.4
Brown bear		
BM	0.89	12.8
TSA	0.62	23.0
TBV	0.66	21.9
Polar bear		
BM	0.94	15.9
TSA	0.89	21.9
TBV	0.87	23.4
Resistance measures		
Black bear		
SVL^2/STAILR	0.97	4.3
SVL^2/MEANLIMBR	0.97	4.6
XED^2/MEANLIMBR	0.95	5.9
Brown bear		
SVL^2/STAILR	0.95	9.3
SVL^2/MEANLIMBR	0.96	7.5
XED^2/MEANLIMBR	0.83	16.0
Polar bear		
SVL^2/STAILR	0.99	6.6
SVL^2/MEANLIMBR	0.97	10.4
XED^2/MEANLIMBR	0.96	12.6

[a]BM, body mass (kg); TSA, total surface area (m^2); TBV, total body volume (m^3); SVL, snout–vent length; STAILR, snout to tail resistance; MEANLIMBR, mean limb resistance; XED, mean distance between forelimb and hind-limb electrodes.

an estimate of body lipid content with an increase in error of 1.6 units relative to direct chemical determination (i.e., SEEs from Fig. 4 minus Fig. 1).

Five morphometric and 10 resistance measures were compared by simple linear regression to total body water measured from isotope dilution. The three best morphometric predictors were body mass (BM), total surface area, and total body volume (Table 1). Because surface area and total volume are time-consuming measures and are not better predictors than body mass, only body mass was used in multiple linear regressions. Snout to vent length combined with either snout to tail or mean limb resistance was the best resistance measure (Table 1).

Multiple linear regressions combining BM and snout to tail or mean limb resistances improved the predictive capability relative to simple linear regressions. Snout to tail resistances and BM were equal or better predictors than mean limb resistances and BM in all three species (Table 2). Error was not decreased when data for each species were grouped by sex. The multiple linear regressions incorporating body resistances and masses estimated body lipid content with an increased error of 1.1–3.7 units relative to direct chemical determination (i.e., SEEs from Fig. 5 minus Fig. 1).

TABLE 2. Multiple linear regressions for estimating total body water (TBW; kg) by dilution from body mass and snout to tail or limb resistance

	Equation	r^2	SEE
Black bear	TBW = −0.224 + 0.197 (SVL^2/STAILR) + 0.137 (BM)	0.98	4.0
	TBW = 1.588 + 0.249 (SVL^2/MEANLIMBR) + 0.203 (BM)	0.98	3.7
Brown bear	TBW = 2.785 + 0.175 (SVL^2/STAILR) + 0.186 (BM)	0.98	6.0
	TBW = 7.341 + 0.297 (SVL^2/MEANLIMBR) + 0.146 (BM)	0.98	6.0
Polar bear	TBW = −1.860 + 0.231 (SVL^2/STAILR) + 0.074 (BM)	0.99	6.6
	TBW = 6.643 + 0.256 (SVL^2/MEANLIMBR) + 0.097 (BM)	0.97	10.2

NOTE: For explanation of abbreviations see Table 1.

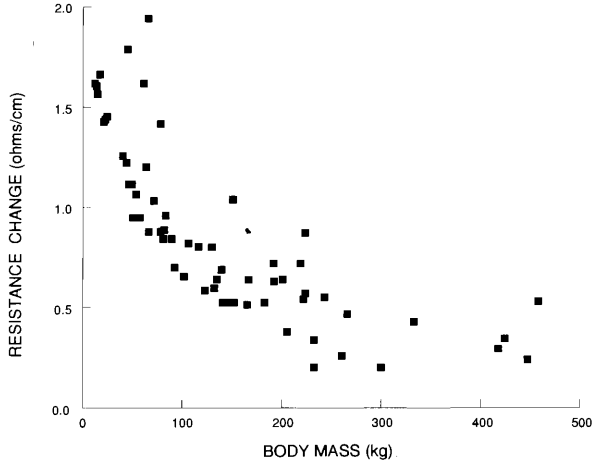

FIG. 6. Relationship between body mass and the change in resistance with various limb positions.

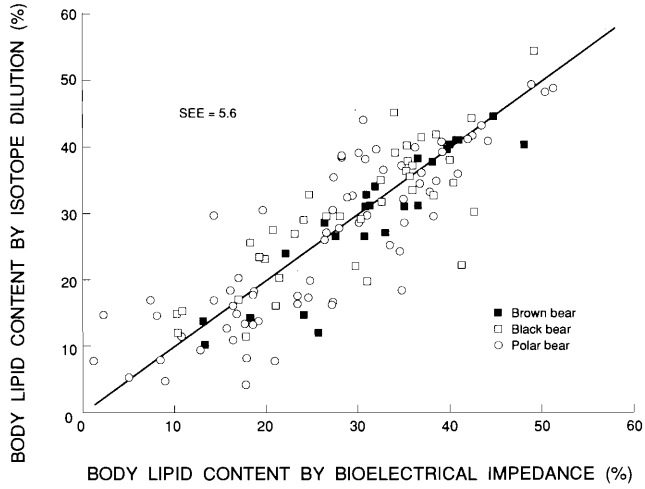

FIG. 7. Comparison of body lipid content in brown, black, and polar bears determined by isotope dilution (Fig. 4) and snout to tail resistance equations (Table 2 and Fig. 5). The line shows the 1:1 relationship, and the standard error of the estimate is calculated relative to that line.

Bioelectrical impedance analyses using the limbs were affected by any variation in limb or electrode placement (Fig. 6). The lack of rigid standardization in positioning the limbs affected resistance readings curvilinearly with bear size and had the greatest effect on small bears. Limb resistances were noticeably lower (i.e., by as much as 18%) on bears with abscesses or wounds (such as snare and gunshot wounds) when the injury was in the conductor path. For these bears, the resistance measurements on the injured limb were not used in calculating the mean, whole-body resistance. The gunshot wounds were from small caliber bullets and generally were not apparent from visual inspection of the unshaved bear. Lipid contents estimated by water dilution and BIA are highly correlated (Fig. 7), but when the two are directly compared the variance is larger than in previous equations because of the combination of error from both methods (Figs. 4 and 5, Table 2). The residuals of Fig. 7 are normally distributed and display no systematic trend associated with size.

It was noted, but not quantified, that bears under light anesthesia had resistance readings that fluctuated with head and limb movements. Bears resting in standing water gave erroneously low readings. Resistance in dead bears increased within minutes of death, and 5 h after death it was 36% higher at −20°C and 14% higher at 22°C. Resistance had not stabilized by 5 h.

Discussion

The relationship between water and lipid contents of bears, the concentration of protein and ash in the dry, lipid-free mass, the water content of the lean mass, and the composition of the hair are very similar to values determined on many species (Sheng and Huggins 1979; Robbins 1993). Similarly, the initial overestimate of the body water pool by isotope dilution, 3.7%, is within the range of overestimates commonly occurring in other studies, 2–5% (Sheng and Huggins 1979; Nagy and Costa 1980; Schoeller et al. 1980; Reilly and Fedak 1990).

Isotopic water dilution and BIA estimate in vivo body composition with equal accuracy under carefully controlled and standardized conditions. However, both methods have constraints or limitations. Free-ranging bears that have recently consumed a large meal may have a correspondingly large ingesta volume, which could produce erroneous estimates of body composition when either method is used. Water dilution requires injections of sterile labeled water, anesthesia of 150 min duration, blood samples, and sophis-

ticated laboratory analyses. The dilution method is not affected by depth of anesthesia, ambient temperature, or rainfall. Conversely, bioelectrical impedance analysis requires no injections or blood sampling and can be accomplished by experienced personnel in approximately 15 min. However, bioelectrical impedance analyses are greatly affected by depth of anesthesia, limb and electrode placement, and operator experience. The animal must be still and relaxed during BIA measurement. Because BIA readings taken with electrodes placed on the limbs are greatly influenced by limb and electrode position, we recommend using the snout to tail resistance method. Extremes of temperature and rainfall can affect BIA measurements and require precautions (e.g., warming the BIA meter in extreme cold and using ground-insulating plastic sheets). Researchers using BIA for quick field determinations of body lipid content should immediately estimate body water and lipid composition in order to check for illogical values (e.g., negative percent body fat) arising from improper application of the technique.

Although both methods will be affected by an animal's hydration status (Brodie et al. 1991), rarely can this be judged under field conditions. While small abscesses, such as those commonly found on bears, will not influence water dilution values, even minor tissue trauma can affect BIA readings if the injury is located along the conductor path. Hence, every bear should be thoroughly checked for wounds and general condition prior to taking BIA readings.

Accurate body mass measurements are critical because both isotopic dilution and BIA provide a measure of absolute water content which is then transformed to percent body fat. Any error in mass will be incorporated into the conversion from water to fat content. Thus, these methods require the use of accurate, field-portable, electronic load cells when the bear's mass is measured.

Neither method will estimate body composition post mortem, though BIA may be useful if equations specific to dead bears are developed (Raphael et al. 1991). Any additional work to develop these equations will necessitate controlling carcass temperature and time since death.

Acknowledgements

This research was supported with funds provided by the Intermountain Research Station, United States Forest Service (J. Lyon), the National Fish and Wildlife Foundation, and two branches of the United States Fish and Wildlife Service: the Alaska Fish and Wildlife Research Center (G. Garner and S. Armstrup) and the Interagency Grizzly Bear Recovery Coordinator (C. Servheen). Special assistance with polar bear work was provided by Dr. M. Ramsay and pilots S. Miller and R. Anderson. Logistical arrangements were carried out by the Churchill Northern Studies Centre in Manitoba, Canada, and the Polar Continental Shelf Project in Resolute, N.W.T., Canada. Bioelectrical impedance equipment was provided by Whitmire Medical. We also thank the numerous students who have assisted with bear care and bear handling over the course of this work.

Brodie, D.A., Eston, R.G., Coxon, A.Y., Kreitzman, S.N., Stockdale, H.R., and Howard, A.N. 1991. Effect of changes of water and electrolytes on the validity of conventional methods of measuring fat-free mass. Ann. Nutr. Metab. **35**: 89–97.

Byers, F.M. 1979. Extraction and measurement of deuterium oxide at tracer levels in biological fluids. Anal. Chem. **98**: 208–213.

Cattet, M. 1990. Predicting nutritional condition of black bears and polar bears on the basis of morphological and physiological measurements. Can. J. Zool. **68**: 32–39.

Coward, W.A., Parkinson, S.A., and Murgatroyd, P.R. 1988. Body composition measurements for nutrition research. Nutr. Res. Rev. **1**: 115–124.

Fiorotto, M., Cochran, W.J., Funk, R.C., Sheng, H.P., and Klish, W.J. 1987. Total body electrical conductivity measurements: effects of body composition and geometry. Am. J. Physiol. **252**: R794–R800.

Folch, J., Lees, M., and Sloane Stanley, G.H. 1957. A simple method for isolation and purification of total lipids from animal tissues. J. Biol. Chem. **226**: 497–509.

Franzmann, A.W., and Schwartz, C.C. 1988. Evaluating condition of Alaskan black bears with blood profiles. J. Wildl. Manage. **52**: 63–70.

Hall, C.B., Lukaski, H.C., and Marchello, M.J. 1989. Estimation of rat body composition using tetrapolar bioelectrical impedance analysis. Nutr. Rep. Int. **39**: 627–633.

Hellgren, E.C., Vaughan, M.R., and Kirkpatrick, R.L. 1989. Seasonal patterns in physiology and nutrition of black bears in Great Dismal Swamp, Virginia – North Carolina. Can. J. Zool. **67**: 1837–1850.

Hellgren, E.C., Rogers, L.L., and Seal, U.S. 1993. Serum chemistry and hematology of black bears: physiological indices of habitat quality or seasonal patterns? J. Mammal. **74**: 304–315.

Heymsfield, S.B., and Waki, M. 1991. Bocy composition in humans: advances in the development of multicompartment chemical models. Nutr. Rev. **49**: 97–108.

Kushner, R.F. 1992. Bioelectrical impedance analysis: a review of principles and applications. J. Am. Coll. Nutr. **11**: 199–209.

Kushner, R.F., and Schoeller, D.A. 1986. Estimation of total body water by bioelectrical impedance analysis. Am. J. Clin. Nutr. **44**: 417–424.

Lukaski, H.C. 1987. Methods for the assessment of human body composition: traditional and new. Am. J. Clin. Nutr. **46**: 537–556.

Moen, A.N. 1973. Wildlife ecology. W.H. Freeman and Co., San Francisco.

Nagy, K.A., and Costa, D.P. 1980. Water flux in animals: analysis of potential errors in the tritiated water method. Am. J. Physiol. **238** (Regulatory Integrative Comp. Physiol 7): R454–R465.

Nyboer, J. 1972. Workable volume and flow concepts of biosegments by electrical impedance plethysmograph. T.-I.-T. J. Life Sci. **2**: 1–13.

Pace, N., Kline, L., Schachman, H.K., and Harfenist, M. 1947. Studies on body composition. IV. Use of radioactive hydrogen for measurement in vivo of total body water. J. Biol. Chem. **168**: 459–469.

Poelker, R.J., and Hartwell, H.D. 1973. Black bear of Washington: its biology, natural history and relationship to forest regeneration. Washington State Game Department, Olympia, Biol. Bull. No. 14.

Pritchard, G.T., and Robbins, C.T. 1990. Digestive and metabolic efficiencies of grizzly and black bears. Can. J. Zool. **68**: 1645–1651.

Raphael, M.G., Harlow, H.J., and Buskirk, S.W. 1991. Estimating body fat by using bioelectrical-impedance measurements: a preliminary assessment. U.S. for. Serv. Gen. Tech. Rep. PNW-GTR-279.

Reilly, J.J., and Fedak, M.A. 1990. Measurement of the body composition of living gray seals by hydrogen isotope dilution J. Appl. Physiol. **69**: 885–891.

Richmond, C.R., Langham, W.H., and Trujillo, I.I. 1962. Comparative metabolism of tritiated water by mammals. J. Cell. Comp. Physiol. **59**: 45–53.

Robbins, C.T. 1993. Wildlife feeding and nutrition. Academic Press, San Diego.

Rumpler, W.V., Allen, M.E., Ullrey, D.E., Earle, R.D., Schmitt, S.M., and Cooley, T.M. 1987. Body composition of

white-tailed deer estimated by deuterium oxide dilution. Can. J. Zool. **65**: 204–208.

Schoeller, D.A., van Santen, E., Peterson, D.W., Dietz, W., Jaspan, J., and Klein, P.D. 1980. Total body water measurement in humans with ^{18}O and ^{2}H labeled water. Am. J. Clin. Nutr. **33** 2686–2693.

Sheng, H-P., and Huggins, R.A. 1979. A review of body composition studies with emphasis on total body water and fat. Am. J. Clin. Nutr. **32**: 630–647.

Spence, M.W., and Wolfe, L.S. 1967. Gangliosides in developing rat brain. Isolation and composition of subcellular membranes enriched in gangliosides. Can. J. Biochem. **45**: 671–688.

Torbit, S.C., Carpenter, L.H., Alldredge, A.W., and Swift, D.M. 1985. Mule deer body compositionùa comparison of methods. J. Wildl. Manage. **49**: 86–91.

Walsberg, G.E. 1988. Evaluation of a nondestructive method for determining fat stores in small birds and mammals. Physiol. Zool. **61**: 153–159.