Indirect prediction of total body water content in healthy adult Beagles by single-frequency bioelectrical impedance analysis

Laurence Yaguiyan-Colliard PhD

Caroline Daumas DVM

Sana Bousbiat PhD

Michel Jaffrin PhD

Philippe Cardot PhD

Dominique Grandjean PhD

Nathalie Priymenko PhD

Patrick Nguyen PhD

Françoise Roux PhD

Received July 24, 2014.
Accepted December 1, 2014.

From the Breeding and Sport Medicine Unit, École Nationale Vétérinaire d'Alfort, Université Paris-Est, Maisons-Alfort, F-94704, France (Yaguiyan-Colliard, Grandjean); the Nutrition and Endocrinology Unit (Daumas, Nguyen) and Intensive Care Medicine Unit (Roux), École Nationale Vétérinaire de Nantes (ONIRIS), Atlanpole La Chantrerie, CS 40706, 44307 Nantes Cedex 3, France; UMR CNRS 7338; Biological Engineering Department, Université de technologie de Compiègne, BP 20529, 60205 Compiègne Cedex, France (Bousbiat, Jaffrin); the Experimental and Clinical Respiratory Neurophysiology Unit, Faculté de médecine Pierre and Marie Curie, Université Pierre et Marie Curie, UMRS-1158, 75634 Paris cedex 13 (Cardot); and the Nutrition Unit, École Nationale Vétérinaire de Toulouse, 31300 Toulouse, France (Priymenko).

Address correspondence to Dr. Yaguiyan-Colliard (nutrition@colliard.fr).

OBJECTIVE
To develop equations for prediction of total body water (TBW) content in unsedated dogs by combining impedance (resistance and reactance) and morphological variables and to compare the results of those equations with TBW content determined by deuterium dilution (TBW_d).

ANIMALS
26 healthy adult Beagles.

PROCEDURES
TBW content was determined directly by deuterium dilution and indirectly with equations developed from measurements obtained by use of a portable bioelectric impedance device and morphological variables including body length, height, weight, and thoracic and abdominal circumferences.

RESULTS
Impedance and morphological data from 16 of the 26 dogs were used to determine coefficients for the following 2 equations: TBW_1 = –0.019 (BL^2/R) + –0.199 (RC + AC) + 0.996W + 0.081H + 12.31; and TBW_2 = 0.048 (BL^2/R) + –0.144 (RC + AC) + 0.777W + 0.066H + 0.031X + 7.47, where AC is abdominal circumference, H is height, BL is body length, R is resistance, RC is rib cage circumference, W is body weight, and X is reactance. Results for TBW_1 (R^2_1 = 0.843) and TBW_2 (R^2_2 = 0.816) were highly correlated with the TBW_d. When the equations were validated with data from the remaining 10 dogs, the respective mean differences between TBW_d and TBW_1 and TBW_2 were 0.17 and 0.11 L, which equated to a nonsignificant underestimation of TBW content by 2.4% and 1.6%, respectively.

CONCLUSIONS AND CLINICAL RELEVANCE
Results indicated that impedance and morphological data can be used to accurately estimate TBW content in adult Beagles. This method of estimating TBW content is less expensive and easier to perform than is measurement of TBW_d, making it appealing for daily use in veterinary practice. (*Am J Vet Res* 2015;76:547–553)

Body composition analysis is essential for meaningful medical follow-up of individual patients,[1] but measurement of body weight alone is not a reliable method of evaluation. Therefore, simple and effective techniques for evaluation of body composition are highly desirable.

Traditionally, the body is divided into 2 compartments (the water compartment and tissue compartment). The water compartment, or TBW, is divided into extracellular and intracellular compartments. The extracellular compartment consists of plasma and interstitial fluids in which cells are found. The tissue compartment consists of the FM and FFM. The FM is regarded as an energy reserve, whereas the FFM represents the metabolically active fraction of a living body. Thus, FFM is a key component in a variety of physiological states (eg, growth, aging, and physical activity) as well as in various disease conditions (eg, metabolic diseases, cancer, and obesity). Because the FM does not contain any water, all the body water within the tissue compartment is contained within the FFM. Therefore, the FFM can be estimated by dividing the TBW mass by the mean hydration coefficient of the body. Subtraction of the TBW mass from the body weight yields the mass of the tissue compartment, and the tissue compartment mass minus the FFM yields the FM. Consequently, evaluation of TBW enables determination of the body composition[2] because the mean hydration rate is constant and is approximately 73.2% in humans[3] and 74.4% in dogs.[4]

Isotopic dilutions are generally used as reference techniques for evaluation of body fluid content. Deuterium dilution is frequently used to estimate TBW content because deuterium is a stable nontoxic com-

ABBREVIATIONS

BCS	Body condition score
BIA	Bioimpedance analysis
CV	Coefficient of variation
DEXA	Dual-energy x-ray absorptiometry
FFM	Fat-free mass
FM	Fat mass
TBW	Total body water

pound that can be accurately analyzed in biologic fluids and its distribution in the body is the same as that of water. In humans and dogs, deuterium concentrations are commonly measured by mass spectrometry[5,6] or infrared spectrometry.[7,8] Deuterium concentrations in dogs have also been measured by Fourier-transform infrared spectroscopy.[9] Results of multiple studies[10–12] indicate that the deuterium dilution technique for estimation of TBW content in dogs is accurate to within 1% to 2% of the actual TBW content. However, the deuterium dilution technique is a lengthy and costly process that requires animals to be anesthetized, the collection of several blood samples, and expensive equipment that is rarely found in general veterinary practices, which makes it impractical in most situations. Isotopic dilution is most useful for validation of alternative techniques for evaluation of body composition, such as DEXA[13,14] or quantitative MRI.[14]

A 9-point scale has been proposed for assessment of the body condition of dogs that involves observation and palpation of the animal to evaluate the percentage of body fat.[15] This semiquantitative technique provides an estimate of FM. Each point in the scale differs by approximately 5% from the contiguous points. A score of 5 on this scale corresponds to an ideal FM of 17.5 ± 10% for male dogs and 19.9 ± 10% for female dogs.[15] An algorithm-based system called S.H.A.P.E. that also uses observation and palpation has likewise been validated for estimation of the body composition of dogs.[16] Both techniques are easy, repeatable, and dependable when used by trained staff, and the results in terms of percentage of body weight can be correlated with those obtained by DEXA. However, only superficial muscles and fat volume are evaluated by those 2 methods, and the margin of error associated with the incomplete evaluation of body composition makes those techniques unsuitable for precise monitoring of changes in body composition over time.

Bioelectrical impedance is an alternative to dilution techniques and DEXA for evaluation of body composition. This technique has been validated and is routinely used to estimate the body composition of healthy and ill humans.[17–19] It is quick and noninvasive and uses a low-intensity electrical current that is undetectable by the subject being evaluated. The equipment required for bioelectrical impedance is inexpensive. Bioelectrical impedance has been successfully used in unanesthetized dogs[a] and appears to be a viable method for the measurement of body composition in that species.

The principles of bioelectrical impedance have been described in detail.[17,20,21] Briefly, impedance is a measure of the opposition to an alternating electrical current and is composed of tissue resistance and tissue reactance. It is directly proportional to tissue length and inversely proportional to tissue diameter.[20] A tissue has a certain degree of resistance to the passage of a current depending on its electrolyte and water content. Fat mass has a high degree of resistance to current transmission (ie, high impedance), and cellular membranes behave like electrical conductors and generate tissue reactance. Currents with low frequencies (< 5 kHz) do not penetrate through cell membranes and remain in the extracellular space, whereas currents with high frequencies (≥ 50 kHz) pass through both the extracellular and intracellular spaces.[22]

Single-frequency BIA with a current frequency of 50 kHz has been used and validated for evaluation of TBW content in healthy men with a mean error range of −3.3% to 1.1%.[23–27] In the earliest study,[28] 2 electrodes were used to perform single-frequency BIA, but the technique evolved and is now commonly performed with 4 electrodes.[29] The use of single-frequency BIA for evaluation of TBW content has been validated with various types of equipment for several species including dogs,[30,31,b] which resulted in the derivation of linear regression equations for estimation of TBW content. Unfortunately, those equations can no longer be used because the equipment used to derive them is no longer available or the results have been found to be inconsistent and unreliable,[32] most likely because some measurements were obtained from anesthetized animals and others were obtained from unanesthetized animals. Because the regression equations traditionally used to estimate TBW content from single-frequency BIA were developed on the basis of results obtained from protocols and equipment that are now outdated, new equations associated with currently available protocols and equipment need to be developed.

Single-frequency BIA is an easy method for assessment of body composition that could be implemented in daily veterinary practice. The objectives of the study reported here were to develop equations for prediction of TBW content in unsedated healthy adult dogs by the use of single-frequency BIA with currently available equipment and to compare the results of those equations with the TBW content determined by deuterium dilution (TBW_d).

Materials and Methods

Animals

Twenty-six adult Beagles (2 sexually intact females, 9 neutered females, and 15 sexually intact males) with a mean ± SEM age of 5.9 ± 2.7 years and weight of 13.81 ± 4.27 kg and a mean BCS of 7 (range, 5 to 9) were used in the study. All dogs were housed in accordance with the regulations for animal welfare established by the French Ministry of Agriculture and Fisheries. All experimental protocols adhered to European Union guidelines and were approved by the Animal Use and Care Advisory Committee of the University of Nantes.

Deuterium dilution protocol

Total body water content was measured directly by use of an isotopic dilution of deuterium (TBW_d) as described.[9] Food was withheld from each dog for 12 hours prior to injection of the deuterium tracer. Each dog was individually housed in a cage without access to water to allow for body water equilibration beginning 2 hours before and lasting until 4 hours after injection of the deuterium tracer. Prior to administration of the deuterium, a blood sample (2 mL) was collected into an evacuated EDTA-containing tube by jugular venipuncture for determination of Hct, and blood glucose, total protein, albumin, sodium, potassium, and chloride concentrations. The deuterium tracer was prepared with deuterium[c] (99.9% H/H^2; 500 mg/kg) and saline (0.9% NaCl) solution and administered by SC injection at the dorsal aspect of the cervical region. Syringes were weighed before and after tracer injection to determine the dose administered to within 0.1 g. Another blood sample (5 mL) was collected into an evacuated EDTA-containing tube by jugular venipuncture immediately before and at 4 hours after the tracer injection. Blood samples were centrifuged at 2,000 X *g* for 10 minutes; then the plasma was harvested from each sample and stored in sealed vials at –20°C until analysis. For each sample, plasma deuterium concentration was assayed in duplicate by use of Fourier-transform infrared spectroscopy as described.[9]

Morphological measurements

Each dog was weighed and measured after injection of the deuterium tracer. One trained veterinarian (LYC) assigned a BCS to each dog in accordance with the 9-point scale developed by Laflamme.[15] The body length from the external occipital protuberance to the base of the tail was measured with a flexible tape measure. A self-rewinding tape measure[d] was used to measure the circumference of the rib cage at the level of the xiphoid process and the circumference of the abdomen at the level of the umbilicus with the dog at full expiration. The height of each dog from the ground to the dorsal-most aspect of the scapula was measured with a stadiometer.[e] Weight was measured to the nearest 0.01 kg, and all other measurements were measured to the nearest 0.1 cm.

Single-frequency BIA protocol

For single-frequency BIA, each dog was placed in a nonelectrically conductive harness[f] that was suspended from a metal framework so that all 4 of its feet were touching an electrical insulating mat, but its movement was otherwise restricted. Care was taken to ensure that the suspended dog could not contact any electrically conductive object.

Ultrasound transmission gel[g] was applied to the hair dorsal to the elbow joint of the right forelimb and dorsal to the patella of the right hind limb, and 4 standard metal crocodile clips[h] (total length, 50 mm) were used to attach the electrodes to the dog. Two gel-coated clips, 1 for emission and 1 for reception, were clamped on the gel-moistened hair of each right leg in accordance with the cylinder theory for bioelectric impedance recommended for use in humans **(Figure 1)**.[i] Voltage electrodes were positioned 3.5 cm dorsal to the current electrodes. The indentations created by the jaws of the crocodile clips were carefully smoothed to avoid pain induction without altering electrical conductivity. A bioelectrical device[j] was used to apply a 77-µA current with a frequency of 50 kHz to the electrodes, and 5 sequential impedance measurements were obtained for each dog. Data were transmitted directly from the analyzer to a computer and stored in a spreadsheet program.[k] Each dog was restrained in the harness for approximately 5 minutes for the single-frequency BIA.

Statistical analysis

The 5 sequential single-frequency BIA measurements obtained for each of the 26 study dogs were used to evaluate the repeatability of the measurement process by calculation of the CV. For both resistance and reactance variables, the CV was calculated as the SD for the variable divided by the mean for that variable.

Following completion of data collection, the dogs were separated into 2 groups in a random manner by means of a random number generator. For a group of

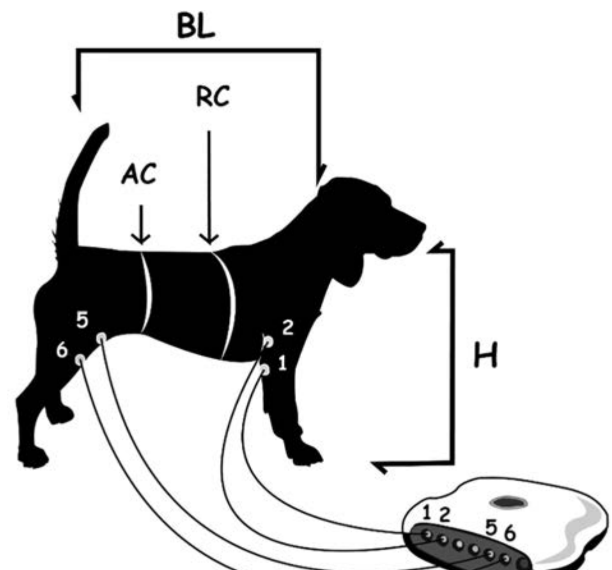

Figure 1—Diagram illustrating the morphological measurements and electrode configuration for single-frequency BIA that were used to predict the TBW content of 26 healthy adult Beagles. Body length (BL) was measured from the external occipital protuberance to the base of the tail, body height (H) was measured from the ground to the dorsal-most aspect of the scapula, and the rib cage circumference (RC) was measured at the level of the xiphoid process and the abdominal circumference (AC) was measured at the level of the umbilicus with the dog at full expiration. For single-frequency BIA, each dog was placed in a nonelectrically conductive harness that was suspended from a metal framework so that all 4 of its feet were touching an electrical insulating mat, but its movement was otherwise restricted. Care was taken to ensure that the suspended dog could not contact any electrically conductive object. The voltage electrodes (2 and 5) were attached to the hair 3.5 cm dorsal to the current electrodes (1 and 6) on the right forelimb and hind limb. All electrodes were attached to the hair with standard metal crocodile clips that were moistened with ultrasound transmission gel. A bioelectrical device was used to apply a 77-µA current with a frequency of 50 kHz to the electrodes, and 5 sequential impedance measurements were obtained for each dog.

Table 1—Mean ± SD values for single-frequency BIA and morphological variables and TBW content for 16 healthy adult Beagles used to determine the regression coefficients for 2 linear regression equations developed for prediction of TBW content and 10 healthy adult Beagles used to validate those regression equations.

Variable	Dogs used to determine regression coefficients for predictive equations	Dogs used to validate predictive equations
Resistance (Ω)	152.04 ± 24.32	139.37 ± 14.16
Reactance (Ω)	37.26 ± 11.80	32.11 ± 4.68
Body weight (kg)	13.78 ± 4.28	13.85 ± 4.48
Body length (cm)	53.16 ± 5.11	51.13 ± 3.96
Body height (cm)	38.17 ± 3.33	37.94 ± 2.02
Rib cage circumference (cm)	56.99 ± 5.33	58.55 ± 7.27
Abdominal circumference (cm)	51.69 ± 8.06	52.67 ± 9.56
TBW_d (L)	7.13 ± 2.13	6.86 ± 1.46
TBW_1 (L)	7.18 ± 2.08	6.69 ± 1.46
$TBW_d - TBW_1$ (L)	–0.05 ± .047	0.17 ± 0.59
TBW_2 (L)	7.14 ± 2.10	6.75 ± 1.61
$TBW_d - TBW_2$ (L)	–0.01 ± 0.39	0.11 ± 0.69

For all dogs, body length was measured from the external occipital protuberance to the base of the tail, body height was measured from the ground to the dorsal-most aspect of the scapula, and the rib cage circumference was measured at the level of the xiphoid process and the abdominal circumference was measured at the level of the umbilicus with the dog at full expiration. Total body water content was determined directly by deuterium dilution (TBW_d), which was used as the outcome variable for 2 previously described linear regression models to determine the associated regression coefficients that best predicted TBW content. The first equation (TBW_1) was the same as the second equation (TBW_2) except it did not include reactance. The resulting equations were as follows: $TBW_1 = –0.019 (BL^2/R) + –0.199 (RC + AC) + 0.996W + 0.081H + 12.31$; and $TBW_2 = 0.048 (BL^2/R) + –0.144 (RC + AC) + 0.777W + 0.066H + 0.031X + 7.47$, where AC is the abdominal circumference, BL is the body length, H is the height, R is resistance, RC is rib-cage circumference, W is body weight, and X is reactance. The respective differences between TBW_d and TBW_1 and TBW_2 were not significantly ($P > 0.05$) different from 0 for either group of dogs.

16 dogs, the TBW_d was used as the outcome, or dependent variable, and the morphological and single-frequency BIA data were fit into 2 linear regression models previously described[33] to determine the associated regression coefficients that best estimated the TBW content. The first equation (TBW_1) was the same as the second equation (TBW_2), except it did not include reactance. Briefly, the equations were defined as follows: $TBW_1 = a(BL^2/R) + b(RC + AC) + cW + dH + f$, and $TBW_2 = a(BL^2/R) + b(RC + AC) + cW + dH eX + f$, where a, b, c, d, e, and f are regression coefficients; AC is the abdominal circumference; BL is the body length; H is the height; R is resistance; RC is rib-cage circumference; W is body weight; and X is reactance.

Data from the remaining 10 dogs were used to validate TBW_1 and TBW_2 by the use of a paired *t* test to compare the TBW content estimated by each of the 2 regression equations with the TBW_d. Linear regression was then used to determine the correlation and coefficient of determination (R^2) between the TBW content predicted by each of the regression equations and the TBW_d. The Bland-Altman method[34] was used to assess the agreement between the TBW content predicted by each of the regression equations and TBW_d. For all analyses, values of $P < 0.05$ were considered significant.

Results

Dogs

Results of blood samples obtained prior to injection of the deuterium tracer indicated that the Hct and blood glucose, total protein, albumin, sodium, potassium, and chloride concentrations were within the respective reference ranges for all dogs (data not shown). All dogs remained fairly quiet in the restraining device without investigator interference and tolerated the electrodes well throughout the duration of the single-frequency BIA.

Single-frequency BIA and morphological measurements

The single-frequency BIA and morphological variables for all dogs were summarized **(Table 1)**. The repeatability of the single-frequency BIA measurements was good; the median CV was 1.60% (range, 0.22% to 3.15%) for resistance and 2.20% (range, 0.42% to 6.17%) for reactance.

Correlation between TBW_d and the predictive equations

The regression equations that provided the best fit of the single-frequency BIA and morphological measurement data for prediction of TBW content were as follows: $TBW_1 = -0.019 (BL^2/R) + -0.199 (RC + AC) + 0.996W + 0.081H + 12.31$ and $TBW_2 = 0.048 (BL^2/R) + -0.144 (RC + AC) + 0.777W + 0.066H + 0.031X + 7.47$. When those 2 equations were used to predict the TBW content for the group of 16 dogs that provided the data used to determine the regression coefficients, the TBW contents predicted by TBW_1 and TBW_2 were 0.7% ($P = 0.674$) and 0.14% ($P = 0.919$), respectively, higher than the

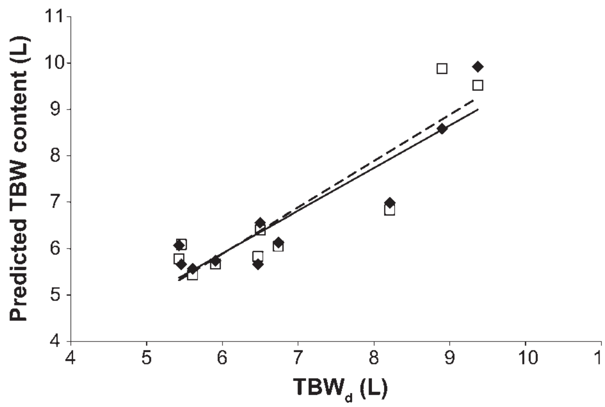

Figure 2—Scatterplot of TBW content predicted by each of 2 linear regression equations (TBW_1 [black diamonds] and TBW_2 [white squares]) versus the TBW content measured by deuterium dilution (TBW_d) for 10 healthy adult Beagles. The first equation (TBW_1) was the same as the second equation (TBW_2), except it did not include reactance. The equations were as follows: $TBW_1 = -0.019\ (BL^2/R) + -0.199\ (RC + AC) + 0.996W + 0.087H + 12.31$; and $TBW_2 = 0.048\ (BL^2/R) + -0.144\ (RC + AC) + 0.777W + 0.066H + 0.031X + 7.47$, where AC is the abdominal circumference, BL is the body length, H is the height, R is resistance, RC is the rib-cage circumference, W is body weight, and X is reactance. The line of best fit for the data for TBW_1 (solid black line) has an equation of $0.919x + 0.385$ and $R^2_1 = 0.843$ and that for TBW_2 (dashed line) has an equation of $0.997x - 0.095$ and $R^2_2 = 0.816$. Because $R^2_1 > R^2_2$, these findings suggested that TBW_1 fit the data slightly better than did TBW_2.

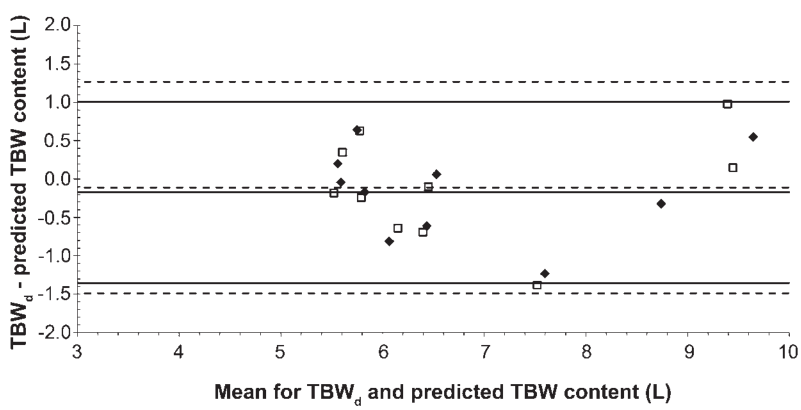

Figure 3—Bland-Altman plot of the respective differences between TBW_d and TBW_1 (black diamonds) and TBW_2 (white squares) versus the respective means for TBW_d and TBW_1 and TBW_2 for the dogs of Figure 2. The middle solid line represents the mean difference between TBW_d and TBW_1, and the 2 outer solid lines delimit the 95% limits of agreement (mean ± 2 SD) for that mean. The middle dashed line represents the mean difference between TBW_d and TBW_2, and the 2 outer dashed lines delimit the 95% limits of agreement for that mean. ***See*** Figure 2 for remainder of key.

TBW_d (Table 1), but neither of those values was significantly different from 0.

For the 10 dogs that were used to validate TBW_1 and TBW_2, the respective correlations between the predicted TBW content and the TBW_d were assessed graphically **(Figure 2)**. The coefficients of determination for both TBW_1 ($R^2_1 = 0.843$) and TBW_2 ($R^2_2 = 0.816$) were very high, which indicated that the TBW content predicted by both equations was highly correlated with the TBW_d. However, for this group of dogs, the TBW content predicted by TBW_1 and TBW_2 was 2.4% ($P = 0.378$) and 1.6% ($P = 0.619$), respectively, lower than the TBW_d (Table 1), although neither value was significantly different from 0. Assessment of the Bland-Altman plots for this group of dogs similarly indicated a high level of agreement between the respective TBW contents predicted by TBW_1 and TBW_2 and the TBW_d; the mean difference between the predicted TBW content and the TBW_d did not exceed 2 SD from the mean for any of the 10 dogs **(Figure 3)**.

Discussion

Results of the present study indicated that TBW content measured by deuterium dilution (TBW_d; gold standard) was highly correlated with that predicted by each of 2 linear regression equations that included results of single-frequency BIA and morphological measurements of healthy adult Beagles. The first equation (TBW_1) contained the same predictors as did the second equation (TBW_2), except it did not include reactance. The TBW content predicted by both TBW_1 and TBW_2 was highly correlated with TBW_d. Thus, the use of morphological measurements and results of single-frequency BIA may be an inexpensive and practical method for estimating the TBW content of canine patients in clinical veterinary practice.

In the present study, BIA with a frequency of 50 kHz was successfully performed in unsedated healthy adult dogs with minimal stress by the use of a simple restraint system that restrained the dog in a physiological standing position for the few seconds necessary for the acquisition of BIA measurements. The electrode clips were slightly modified so that they could be used without causing any discomfort. This was important because patient discomfort would hinder the adoption of single-frequency BIA for use in clinical practice.

Investigators of another study[35] estimated the body fat percentage in unsedated dogs by the use of a handheld BIA device. In that study,[35] 4 electrodes were positioned in a longitudinal manner along the dorsal lumbar region in direct contact with the skin, and a 70% isopropyl alcohol solution was used as the conductive agent on the electrodes. To our knowledge, the present study is the first to compare the TBW content of dogs estimated by the use of previously described linear regression equations[a] that involved the results of morphological measurements and single-frequency BIA with the TBW_d.

Although the BIA device used in the present study was capable of inducing currents with varying frequencies, we chose to measure resistance and reactance only at 50 kHz because that is the standard frequency used for estimation of TBW content by BIA.[22] Indeed, the objectives for this study were to standardize the single-frequency BIA protocol for estimating TBW content in healthy dogs and evaluate new equipment that could be used in field or practice conditions.

The median CVs for resistance (1.60%) and reactance (2.20%) for the single-frequency BIA measurements in the present study were low, which indicated that the precision, or repeatability, of the measurements obtained by the BIA device was good. In a study[35] of 46 dogs of various breeds, the median CV was 1.64% (range, 0.00% to 5.53%) for 5 serial measurements obtained by the use of a handheld BIA device. In another study[16] in which the same handheld BIA device was used to obtain 5 serial measurements from 24 dogs with varying body conditions, the median CV was 2.20% (range, 0.00% to 11.2%). Thus, it appears that the repeatability of measurements obtained by BIA is good for dogs regardless of the breed or body condition.

In the present study, we randomly divided the study population into 2 groups. We used the data from the group of 16 dogs to determine the regression coefficients for TBW_1 and TBW_2 and the data from the group of 10 dogs to validate the resulting equations. During the validation phase of the study, TBW_1 underestimated the TBW content by 0.17 L (2.4%) and TBW_2 underestimated TBW content by 0.11 L (1.6%), but neither prediction deviated significantly from TBW_d. In fact, the mean deviations of TBW_1 and TBW_2 from TBW_d in the present study were lower than the mean deviation of TBW content predicted by means of multifrequency BIA from TBW_d (2.59%) for dogs in another study.[1]

Bioelectrical impedance analysis can be affected by many factors including electrode positioning, instrumentation, ambient temperature, conductance of the subject's footing (eg, examination table), and the subject's hydration status, consumption of food and water, recent physical activity, posture, and skin temperature.[25,36–38] In the present study, we tried to standardize and control as many of those variables as possible. Furthermore, results of the biochemical analyses indicated that all variables assessed were within the respective reference ranges for all dogs, which suggested that none of the dogs had biochemical abnormalities that could have altered the conduction of the electric current in the body.

In humans, the use of BIA data to predict body composition requires the development of separate equations on the basis of sex, age, ethnicity, and physical activity.[39] Results of a study[31] conducted to support the licensing of a BIA device indicate a correlation of 0.64 between single-frequency BIA at 50 kHz and a deuterium dilution technique for estimation of body fat percentage in dogs of various sizes. However, when the body fat percentage estimated by the use of results obtained by that equipment was compared with the body fat percentage measured by DEXA for various breeds of dogs, the results obtained by the BIA device were found to be unreliable and inappropriate for clinical practice.[32] Consequently, the equations developed and validated for prediction of TBW content in the present study should not be considered externally valid for breeds other than Beagles.

Limitations of the present study include a small sample size and the fact that most of the study subjects were healthy overweight dogs of a single breed. Further research is necessary to determine whether TBW content predicted by the equations developed and validated in the present study by the use of morphological data and results of single-frequency BIA is accurate across a large number of dogs of various breeds with varying BCS and health status.

In the present study, results obtained from morphological measurements and single-frequency BIA at 50 kHz were used to develop and validate linear regression equations that accurately predicted TBW content in healthy adult Beagles. All measurements were obtained in unsedated dogs in a natural standing position. The protocol described in this study can be used in clinical practice because it was quick and well tolerated by dogs.

Acknowledgments

This manuscript represents a portion of a dissertation submitted by Dr. Yaguiyan-Colliard to the Université Pierre-et-Marie-Curie L'école doctorale physiologie, physiopathologie et thérapeutique (ED 394) as partial fulfillment of the requirements for a Doctor of Philosophy degree.

Plates for biochemical analyses were provided by IDEXX France.

The authors thank Lauréline Colliard for the creation of Figure 1 and Marc Chodkiewicz for help with manuscript preparation.

The authors declare that they have no competing interests.

Footnotes

a. Okawa M, Ban T, Umeda T, et al. Investigation of percent body fat in dogs visiting veterinary practices in Japan (abstr). *J Vet Intern Med* 2007;21:656.
b. Burkholder WJ. *Body composition of dogs determined by carcass composition analysis, deuterium oxide dilution, subjective and objective morphometry, and bioelectrical impedance*. PhD thesis. Blacksburg, Va: Virginia Polytechnic Institute and State University, 1994;1–357.
c. Eurisotop, Gif-sur-Yvette, France.
d. Holtex +, Aix En Provence, France.
e. Tanita France SA, Neuilly Sur Seine, France.
f. K9Surf Hoist, Droitwich, England.
g. Aquasonic, Parker Laboratories Inc, Fairfield, NJ.
h. Reference No. 431630, Duseaux Bellière (DBELEC), Le Havre, France.
i. Moreno MV. *Etude de la composition corporelle par impédancemétrie sur des adultes et des enfants sains et pathologiques*. PhD thesis. Compiègne, France: Université de Technologie de Compiègne, 2007;1–138.
j. Z-Métrix, BioparHom, Bourget du Lac, France.
k. Microsoft Office Excel, Microsoft France, Issy Les Moulineaux, France.
l. Elliott D. *Multifrequency bioelectrical impedance analysis in healthy cats and dogs*. PhD thesis. Davis, Calif: University of California-Davis, 2001;1–234.

References

1. Thibault R, Pichard C. The evaluation of body composition: a useful tool for clinical practice. *Ann Nutr Metab* 2012;60:6–16.

2. Wang Z, Deurenberg P, Wang W, et al. Hydration of fat-free body mass: review and critique of a classic body-composition constant. *Am J Clin Nutr* 1999;69:833–841.
3. Pace N, Kline L, Schachman HK, et al. Studies on body composition: IV. Use of radioactive hydrogen for measurement in vivo of total body water. *J Biol Chem* 1947;168:459–469.
4. Harrison HE, Darrow DC, Yannet H. The total electrolyte content of animals and its probable relation to the distribution of water. *J Biol Chem* 1936;113:515–529.
5. Schloerb PR, Friis-Hansen RI, Edelman IS, et al. The measurement of total body water in the human subject by deuterium oxide dilution. *J Clin Invest* 1950;29:1296–1310.
6. Mawby DI, Bartges JW, d'Avignon A, et al. Comparison of various methods for estimating body fat in dogs. *J Am Anim Hosp Assoc* 2004;40:109–114.
7. Lukaski HC, Johnson PE. A simple, inexpensive method of determining total body water using a tracer dose of D20 infrared absorption of biological fluids. *Am J Clin Nutr* 1985;41:363–370.
8. Zweens J, Frankena H, Reicher A, et al. Infrared-spectrometric determination of D_2O in biological fluids. Reappraisal of the method and application to the measurement of total body water and daily water turnover in the dog. *Pflugers Arch* 1980;385:71–77.
9. Ferrier L, Robert P, Dumon H, et al. Evaluation of body composition in dogs by isotopic dilution using a low-cost technique, Fourier-transformed infrared spectroscopy. *J Nutr* 2002;132:1725S–1727S.
10. Painter EE. Total body water in the dog. *Am J Physiol* 1940;129:744–755.
11. Moore FD, Muldowney FP, Haxhe JJ, et al. Body composition in the dog. I. Findings in the normal animal. *J Surg Res* 1962;2:245–253.
12. Burkholder WJ, Thatcher CD. Validation of predictive equations for use of deuterium oxide dilution to determine body composition of dogs. *Am J Vet Res* 1998;59:927–937.
13. Son HR, d'Avignon DA, Laflamme DP. Comparison of dual-energy x-ray absorptiometry and measurement of total body water content by deuterium oxide dilution for estimating body composition in dogs. *Am J Vet Res* 1998;59:529–532.
14. Zanghi BM, Cupp CJ, Yuanlong P, et al. Noninvasive measurements of body composition and body water via quantitative magnetic resonance, deuterium water, and dual-energy x-ray absorptiometry in awake and sedated dogs. *Am J Vet Res* 2013;74:733–743.
15. Laflamme D. Development and validation of a body condition score system for dogs. *Canine Pract* 1997;22(4):10–15.
16. German AJ, Holden SL, Moxham GL, et al. A simple reliable tool for owners to assess the body condition of their dog or cat. *J Nutr* 2006;136:2031S–2033S.
17. Jaffrin MY. Body composition determination by bioimpedance: an update. *Curr Opin Clin Nutr Metab Care* 2009;12:482–486.
18. Casey AF. Measuring body composition in individuals with intellectual disability. *J Obes* [serial online] 2013;2013:628428. Available at: www.hindawi.com/journals/jobe/2013/628428/. Accessed Jul 24, 2014.
19. Böhm A, Heitmann BL. The use of bioelectrical impedance analysis for body composition in epidemiological studies. *Eur J Clin Nutr* 2013;67:S79–S85.
20. Nyboer J. Workable volume and flow concepts of bio-segments by electrical impedance plethysmography. *TIT J Life Sci* 1972;2:1–13.
21. Kushner RF. Bioelectrical impedance analysis: a review of principles and applications. *J Am Coll Nutr* 1992;11:199–209.
22. Patel RV, Matthie JR, Withers PO, et al. Estimation of total body and extracellular water using single- and multiple-frequency bioimpedance. *Ann Pharmacother* 1994;28:565–569.
23. Kushner RF, Schoeller DA. Estimation of total body water by bioelectrical impedance analysis. *Am J Clin Nutr* 1986;44:417–424.
24. van Marken Lichtenbelt WD, Westerterp KR, Wouters L, et al. Validation of bioelectrical-impedance measurements as a method to estimate body-water compartments. *Am J Clin Nutr* 1994;60:159–166.
25. Gudivaka R, Schoeller DA, Kushner RF, et al. Single- and multifrequency models for bioelectrical impedance analysis of body water compartments. *J Appl Physiol* 1999;87:1087–1096.
26. Buchholz AC, Bartok C, Schoeller DA. The validity of bioelectrical impedance models in clinical populations. *Nutr Clin Pract* 2004;19:433–446.
27. Jaffrin MY, Morel H. Extracellular volume measurements using bioimpédance spectroscopy-Hanai method and wrist-ankle resistance at 50 kHz. *Med Biol Eng Comput* 2009;47:77–84.
28. Thomassett A. Proprietes bioelectriques des tissus. Mesure de l'impédance en clinique. *Lyon Med* 1962;207:107–118.
29. Hoffer EC, Meador CK, Simpson DC. A relationship between whole body impedance and total body water volume. *Ann NY Acad Sci* 1970;170:452–461.
30. Sheltinga MR, Helton WS, Rounds J, et al. Impedance electrodes positioned on proximal portions of limbs quantifies fluid compartments in dogs. *J Appl Physiol* 1991;5:2039–2044.
31. Free Patents Online. Pet body fat measuring tool: United States patent application 20090076408. Available at: www.freepatentsonline.com/20090076408.html. Accessed May 7, 2014.
32. German AJ, Holden SL, Morris PJ, et al. Comparison of a bioimpedance monitor with dual-energy x-ray absorptiometry for noninvasive estimation of percentage body fat in dogs. *Am J Vet Res* 2010;71:393–398.
33. Jaffrin MY, Morel H. Body fluid volumes measurements by impedance: a review of bioimpedance spectroscopy (BIS) and bioimpedance analysis (BIA) methods. *Med Eng Phys* 2008;30:1257–1269.
34. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986;1:307–310.
35. Stone R, Berghoff N, Steiner JM, et al. Use of a bioelectric impedance device in obese and lean healthy dogs to estimate body fat percentage. *Vet Ther* 2009;10:59–70.
36. O'Brien C, Baker-Fulco CJ, Young AJ, et al. Bioimpedance assessment of hypohydration. *Med Sci Sports Exerc* 1999;31:1466–1471.
37. Kushner RF, Gudivaka R, Schoeller DA. Clinical characteristics influencing bioelectrical impedance analysis measurements. *Am J Clin Nutr* 1996;64:423S–427S.
38. Scharfetter H, Monif M, László Z, et al. Effect of postural changes on the reliability of volume estimations from bioimpedance spectroscopy data. *Kidney Int* 1997;51:1078–1087.
39. Ward LC. Segmental bioelectrical impedance analysis: an update. *Curr Opin Clin Nutr Metab Care* 2012;15:424–429.