# Bioelectrical impedance analysis as a means of estimating total body water in grey seals

**W. Don Bowen, Carrie A. Beck, and Sara J. Iverson**

**Abstract**: Estimates of total body water (TBW) play an important role in studies of body composition and energetics in mammals. We evaluated bioelectrical impedance analysis (BIA) as a means of rapidly and inexpensively estimating TBW in 38 grey seals (*Halichoerus grypus*). Twenty-two males and 16 females, representing the range of body sizes in the population, were studied at Sable Island, Nova Scotia. Seals were chemically immobilized with Telazol during BIA measurement. TBW was determined by dilution of tritiated water. The mean difference in duplicate BIA measurements did not differ significantly from zero. BIA-measured resistance accounted for 83% of the variation in TBW over a range of body masses from 38.5 to 294 kg. Bioelectrical conductor volume (length$^2$/resistance) accounted for 97% of the variation in TBW. Average error in predicting TBW was +0.10% for a validation set of nine animals, but errors in predicting TBW of individual seals were up to 25%. Our results indicate that BIA measurements can be a valuable adjunct to the use of isotope dilution for estimating TBW in chemically immobilized grey seals; however, individual estimates may be associated with varying degrees of error.

**Résumé** : L'estimation du contenu hydrique total (TBW) joue un rôle important dans l'étude de la composition corporelle et la dynamique énergétique des mammifères. Nous avons procédé à l'analyse de l'impédance bioélectrique (BIA) comme moyen d'estimation rapide et économique du contenu TBW chez 38 Phoques gris (*Halichoerus grypus*). Vingt-deux mâles et 16 femelles, représentant tout l'éventail des tailles au sein de la population, ont été étudiés à l'île des Sables, Nouvelle-Écosse. Les phoques ont été immobilisés chimiquement au Telazol durant la mesure de l'impédance. Le contenu TWB a été déterminé par dilution d'eau tritiée. La différence moyenne des mesures BIA (au cours de tests répétés) ne diffère pas significativement de zéro. La résistance BIA mesurée explique 83% de la variation du contenu TBW dans une étendue de masses corporelles de 38,5 à 294 kg. Le volume bioélectrique conducteur (longueur$^2$/résistance) explique 97% de la variation du contenu TBW. L'erreur moyenne reliée à la prédiction du contenu TBW est de +0,10% pour un ensemble de validation de neuf animaux, mais les erreurs de prédiction du contenu TBW chez des individus peuvent atteindre 25%. Nos résultats indiquent que la mesure de l'impédance, BIA, constitue un complément précieux à la méthode de dilution des isotopes lors de l'estimation du contenu TBW chez des Phoques gris immobilisés chimiquement, mais les estimations chez des individus en particulier peuvent être associées à des degrés variables d'erreur.

[Traduit par la Rédaction]

## Introduction

Indirect methods of body-composition analysis rely on estimates of total body water (TBW) of an animal, and relationships between TBW, lean body mass, fat, and protein. Changes in body composition can be used to assess the physiological and nutritional status of an individual and to determine energy and material fluxes associated with reproduction and other events in the annual life cycle of mammals (e.g., Costa 1991; Bowen et al. 1992; Worthy et al. 1992; Oftedal et al. 1993; Boness and Bowen 1996). In pinnipeds (seals, fur seals, and sea lions) hydrogen-isotope dilution has gained wide use as an accurate method of estimating TBW (Costa 1987; Bowen and Iverson 1998). In recent years, however, bioelectrical impedance analysis (BIA) has been proposed as an alternative means of estimating TBW that is fast, non-invasive, inexpensive, and reliable for humans (Lukaski et al. 1986; Kushner et al. 1990), bears (Farley and Robbins 1994), and swine (Swantek et al. 1992).

Few studies have investigated BIA as a means of estimating TBW in pinnipeds. Based on a small number of measurements on captive harp seals (*Phoca groenlandica*) and ringed (*Phoca hispida*) seals, Gales et al. (1994) concluded that BIA showed great promise. However, Arnould (1995) found BIA to be of limited value in predicting TBW of adult female Antarctic fur seals (*Arctocephalus gazella*). Similarly, Bowen et al. (1998) found that BIA was a poor predictor of TBW in both adult female and male harbour seals (*Phoca vitulina*) but showed some promise for suckling pups. Bowen et al. (1998) also reported that movement of the seal produced considerable variability in the measured BIA resistance and that despite sedation with diazepam, harbour seals typically reacted to the placement of the electrodes by tensing the body musculature. As promising results were reported in chemically immobilized bears (Farley and Robbins

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**Table 1.** Body mass, dorsal standard length (DSL), TBW, and percent TBW (from isotope dilution) for the grey seals used in the study.

| | No. of males | No. of females | Body mass (kg) | DSL (cm) | TBW (kg) | % TBW |
|---|---|---|---|---|---|---|
| Adults | 13 | 12 | 184.0±10.43 (87.0–294.0) | 195±4.2 (152–229) | 111.2±5.96 (53.7–163.2) | 60.8±1.12 (50.5–68.7) |
| Juveniles | 6 | 2 | 74.9±2.89 (66.0–90.0) | 145±3.2 (134–160) | 45.2±2.69 (35.7–58.0) | 60.2±2.05 (53.1–67.4) |
| Pups | 3 | 2 | 43.3±1.62 (38.5–47.0) | 121±2.1 (116–128) | 26.3±0.81 (24.1–28.3) | 60.9±1.00 (59.1–64.8) |

**Note**: Values are given as the mean ± SE, with the range in parentheses.

1994), we conducted a study to evaluate BIA as a means of estimating TBW in chemically anesthetized grey seals (*Halichoerus grypus*).

## Materials and methods

The study was conducted during October 1997 and February and June 1998 on Sable Island, a 40 km long, crescent-shaped vegetated sandbar located about 300 km east of Nova Scotia, Canada (43°90′N, 60°00′W). We captured grey seals using hand-held nets (Bowen et al. 1992). Adults were weighed to the nearest 0.5 kg, whereas juveniles and pups were weighed to the nearest 0.1 kg on Salter spring balances. Procedures used on the grey seals in this study were in accordance with the principles and guidelines of the Canadian Council on Animal Care.

TBW was measured from the dilution of tritiated water (HTO). Immediately after capture the animals were administered a precisely weighed dose of HTO (0.5 mCi/mL (1 Ci = 37 GBq); approximately 0.02 g/kg body mass). The isotope was injected intramuscularly (i.m.) and the syringe and needle were rinsed with unlabeled water (also injected) using a three-way stopcock design to ensure complete HTO delivery. Seals were held in the capture net to permit the isotope to equilibrate with body fluids and bled twice from the extradural vein to provide a basis for establishing that equilibration had occurred. The initial blood sample was taken 75–90 min post administration and the second sample was taken 15–30 min later. Previous studies on grey seals showed that tritiated water injected i.m. usually equilibrates with body water in ≤75 min (C.A. Beck, W.D. Bowen, and S.J. Iverson, unpublished data).

Blood samples (about 8 mL) were collected in Vacutainers without additives. Serum from centrifuged samples was transferred to cryovials and stored at –20°C. Total water was recovered by distilling 50-μL aliquots of serum directly into preweighed scintillation vials. These distillations were performed using the evaporated-freeze-capture method described by Ortiz et al. (1978). The vials were then reweighed to obtain the mass of the distillate to the nearest 0.1 mg. A measured amount of Scintiverse II (10 mL) was added to each vial and each sample was counted for 5 min (2% error in counts) in a Beckman scintillation counter. All samples were analyzed in triplicate and specific activity expressed in counts per minute per gram of distillate (cpm/g). The specific activity of the injectant was determined at the same time as that of the serum samples. HTO dilution space ($D_{HTO}$) was calculated using the following equation:

$$D_{HTO} = (SA_i \times HTO_{admin}/SA_s)/1000$$

where $SA_i$ is the specific activity of the injectant (cpm/g), $HTO_{admin}$ is the amount of tritiated water administered (g), and $SA_s$ is the specific activity of the serum (cpm/g). TBW was estimated from a regression of HTO dilution space on TBW (Bowen and Iverson 1998).

Late in the isotope-equilibration period, seals were administered Telazol (a chemical anesthetic consisting of equal parts of tiletamine and zolazepam) and placed on a dry plastic sheet prior to measurement of body length and BIA. Dorsal standard length (McLaren 1993) was measured as the straight-line distance from the nose to the tip of the tail with the seal lying on its ventral surface. Resistance ($R_s$) and reactance ($X_c$) were measured using a tetrapolar impedance plethysmograph (BIA; Model 101A, RJL Systems, Detroit, Mich.). An 800-μA current was applied at 50 kHz through the two outer electrodes. We used similar electrode construction, configuration, and placement on the seal as described by Gales et al. (1994) and Bowen et al. (1998). Briefly, the electrodes were 22-gauge 1.5-in. vacutainer needles attached to banana plugs on the RJL leads. Posterior electrodes were inserted into muscle on the lateral midline, 4 cm anterior to the ankle joint. Anterior electrodes were inserted into muscle along the middorsal line, 4 cm behind the ears. This distance was reduced to 2 cm for the five pups that were studied. Electrodes were oriented anterior–posterior. Electrodes remained in place until readings of $R_s$ and $X_c$ stabilized (usually <5 s).

The conduction of an electric current is proportional to the water and electrolyte distribution within an animal. Biological impedance ($Z$), $R_s$, and $X_c$ vary inversely with the volume and water composition of the body (Nyboer et al. 1943; Lukaski et al. 1986). Impedance is defined as

$$Z\ (\Omega) = (R_s^2 + X_c^2)^{0.5}$$

With a constant electrical signal frequency and relatively constant conductor configuration, impedance is related to bioelectrical conductor volume as follows:

$$\mathrm{Vol}\left(\frac{\mathrm{cm}^2}{\Omega}\right) = \frac{L^2}{Z}$$

where $L$ is body length (cm). Since the magnitude of $X_c$ is small relative to $R_s$, and $R_s$ is a better predictor of impedance than is $X_c$ (Lukaski et al. 1986), $R_s$ can be used in place of $Z$ and the above equation becomes

$$\mathrm{Vol}\left(\frac{\mathrm{cm}^2}{\Omega}\right) = \left(\frac{L^2}{R_s}\right)$$

Standard error is reported as the measure of variability about the mean.

## Results

Isotope estimates of TBW were obtained from a total of 38 grey seals (25 adults, 8 juveniles, and 5 pups that were 6–9 months old; Table 1). Similar numbers of males and fe-

**Table 2.** Doses of chemical anesthesia given to grey seals.

| | | Dose (mg/kg body mass) | |
|---|---|---|---|
| Age-class | *n* | Mean | SE |
| Adult males | 13 | 0.47 | 0.02 |
| Adult females | 12 | 0.92 | 0.02 |
| Juveniles | 8 | 1.14 | 0.10 |
| Pups | 5 | 1.03 | 0.07 |

males were studied in the adult and pup age-classes, but more juvenile males were studied than females. Among the three age-classes, TBW ranged from 24.1 to 163.2 kg and percent TBW from 50.5 to 68.7 (Table 1), providing a wide range of body compositions against which BIA could be evaluated. All but 3 of the 38 seals had a dry pelage during the BIA measurements. Two of the juveniles were damp as a result of light rain, whereas the hind flippers and head of one adult male had been wetted to ensure that the animal did not suffer hyperthermia.

The dose of Telazol administered to achieve the desired level of anesthesia differed with age–sex class (Table 2). Adult males required about half the dose given to adult females and immature seals of both sexes. The doses given to juveniles and pups did not differ significantly (Bonferroni multiple comparison test, $p = 0.86$; combined mean = 1.10 ± 0.06 mg/kg, $n = 13$); however, juveniles required a larger dose than adult females (Bonferroni multiple comparison test, $p = 0.034$).

With the exception of one juvenile and two pups, all seals attained similar depths of anesthesia, characterized by regular breathing, good corneal reflex, lack of body movement, and inability to hold the head off the ground. These three immature seals were immobile but could move their heads somewhat upon tactile simulation. Under the level of anesthesia achieved, seals did not noticeably react to the placement of the needle electrodes and exhibited no movement during the period of measurement. The three immature seals described above initially reacted mildly to placement of the electrodes but were immobile during measurement of resistance. Duplicate $R_s$ and $X_c$ readings were obtained from 16 individuals. The time interval between these readings ranged from 2 to 4 min. Relative to the initial reading, the mean difference between the 16 duplicate $R_s$ readings was 0.4 Ω or +0.5 ± 1.20%. This difference was not significantly different from zero (one-sample Kolmogorov–Smirnov test, $p = 0.20$).

We also examined the effect of the length of the needle electrodes on measured $R_s$, as we had previously used shorter electrodes in our study of harbour seals. In eight grey seals (four adults and four juveniles), $R_s$ was initially measured using the 1.5-in. needle electrodes and then immediately thereafter, at the same location, with the 1.0-in. electrodes. The short-electrode $R_s$ values averaged +4.3 Ω or +7.0 ± 1.84% higher than the long-electrode readings. This difference was significantly greater than the variation observed in repeated readings with the long electrodes ($t_{22} = 3.2$, $p < 0.01$).

Measured $R_s$ values (range 43–89) were higher than $X_c$ values (range 8–20) in all cases; $X_c$ contributed little to the estimate of impedance and could effectively be ignored. $R_s$ was inversely correlated with TBW (Pearson's $r = -0.91$, $p < 0.001$, $n = 38$; Fig. 1). Bioelectrical conductor volume (i.e., $L^2/R_s$) was positively correlated with TBW (Pearson's $r = 0.97$, $p < 0.001$); however, the variance about the regression increased with conductor volume. To deal with this heteroscedasticity, we log-transformed both variables. The resulting regression (eq. 1) explained 96.9% of the variation in TBW ($p < 0.001$, $n = 38$; Fig. 2).

We assessed the predictive strength of eq. 1 in two ways. First, we cross-validated the equation using a leave-one-out approach. To do this, we ran a series of 38 regressions with one of the values successively removed. We then compared the resulting predictive residual sum of squares (PRESS) with the residual sum of squares of eq. 1. The PRESS value of 0.0831 compared well with the residual sum of squares (0.0748) of eq. 1, indicating that, on average, we should expect accurate prediction from the model. The re-transformed standard error of the predicted values was ±1.0 kg.

In the second approach, we selected 10 random samples of 29 observations, without replacement, to develop a predictive equation and then estimated the predicted values and the residuals of nine cases that were not used to fit the model. The percent error of the re-transformed predictions averaged +0.1 ± 1.30%; however, individual errors ranged from –23.3 to +24.9%.

## Discussion

In contrast to the results of our earlier work on diazepam-sedated harbour seals (Bowen et al. 1998), both $R_s$ and conductor volume were strongly related to TBW in grey seals that were chemically anesthetized. These measures accounted for about 83 and 97% of the variation in TBW, respectively. Since we studied similar numbers of males and females over the range of body sizes found in the grey seal population, it appears that a single equation may provide useful estimates of TBW in grey seals. Both the PRESS statistic and the prediction errors from validation samples indicate that, on average, BIA can accurately estimate TBW in grey seals. However, the results from the validation samples also indicated that errors of as much as 25% may occur in predictions of TBW in individual seals. Without further improvement, this will limit the value of BIA, especially in longitudinal studies of individuals.

For BIA to be useful as a means of estimating body water, the information derived from BIA measurements, namely $R_s$ and $L^2/R_s$, must be the terms that contribute most to the prediction (Kushner et al. 1990). Thus, unlike other studies in which BIA has been used (e.g., Gales et al. 1994), we did not include body mass as a term in the regression model. When body mass was used in a forward, stepwise regression, it was included first, and the inclusion of conductor volume increased the explained variation in TBW by only about 1%.

What could account for the promising performance of BIA in predicting TBW in grey seals, given our earlier results on harbour seals? We made only three changes in our methodology for the grey seal study. The first, and likely most important, was the use of chemical anesthesia to immobilize the seals during the period of $R_s$ and $X_c$ measurements. Unlike the readings from sedated harbour seals, which tended to vary considerably, both $R_s$ and $X_c$ values in

**Fig. 1.** Relationship between resistance ($R_s$) and TBW in 38 grey seals.

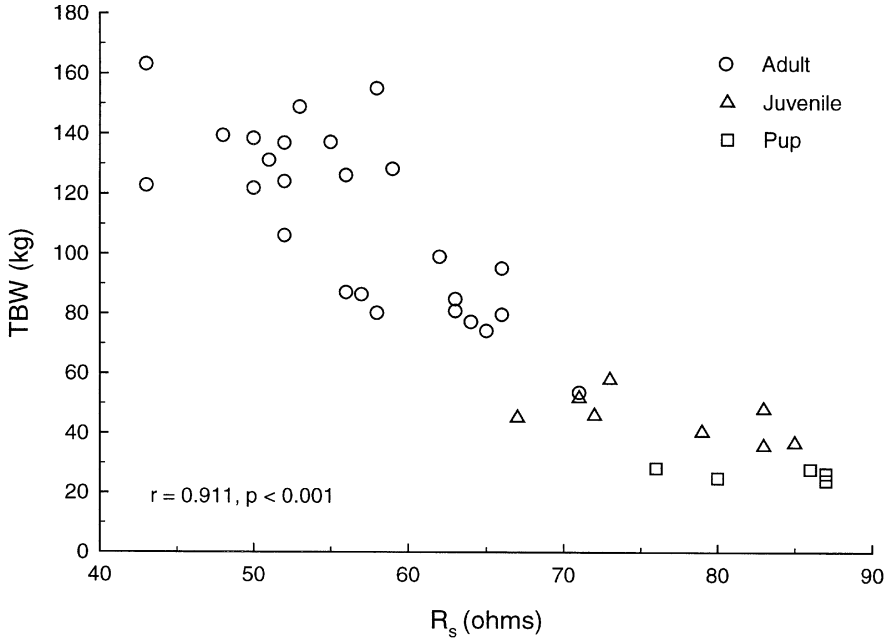
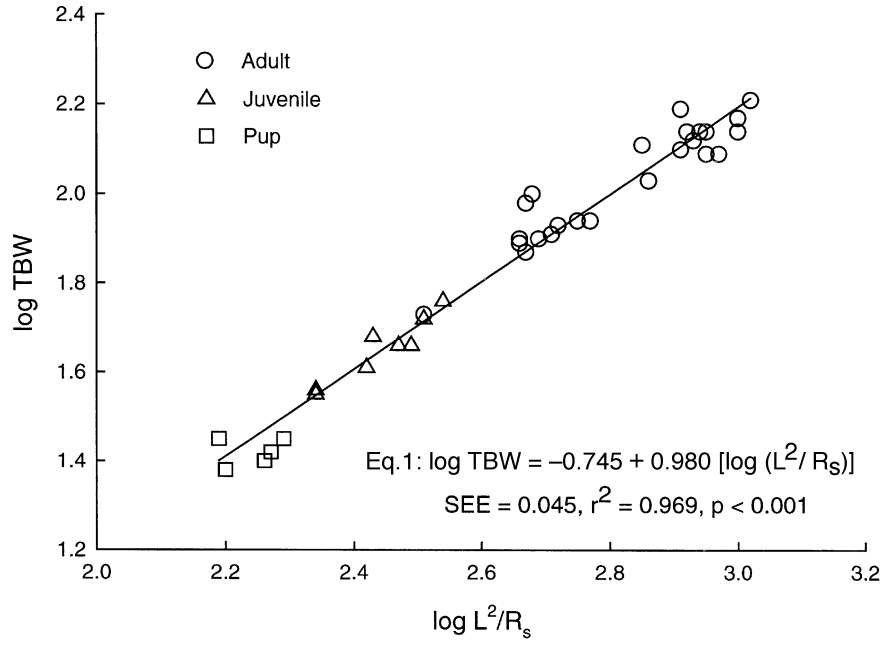



**Fig. 2.** Relationship between conductor volume ($L^2/R_s$, $cm^2$/ohm) and TBW (kg) in 38 grey seals.




grey seals were stable within the 10-s measurement period and were highly repeatable. We would have liked to compare the performance of BIA in both diazepam-sedated and chemically immobilized grey seals, but in our experience, diazepam does not sedate grey seals sufficiently to make the comparison meaningful. The second modification was to place the seal on a dry plastic sheet during the measurement of $R_s$ and $X_c$. This was done to prevent loss of electrical current to the ground (Farley and Robbins 1994) from wet seals or from dry seals on the damp sand. The effect of this procedure on our BIA measurements is difficult to evaluate, as all but three of the seals we studied were dry. However, the BIA measurements from the two juveniles and one adult that were damp or partly wet were not exceptional in any way. The final change was the use of 1.5-in. needle electrodes rather than the 1-in. electrodes used on harbour seals. Although the longer electrodes gave significantly lower $R_s$ values, there was still a strong correlation (Pearson's $r = –0.95$, $p < 0.001$, $n = 8$) between $R_s$ and TBW when only the data from the short electrodes were used. Thus, electrode length cannot explain the improved results.

The relationship between $R_s$ and TBW appeared to be somewhat nonlinear (Fig. 1), although a linear model fit the relationship between Vol and TBW quite well (Fig. 2). The wide range of body sizes and ages of seals we studied raises the possibility of differences in volume resistivity, which is

usually assumed to be constant. Grey seals show considerable size dimorphism, males being larger than females (McLaren 1993). Resistivity may vary as a function of changes in the geometry of seals of different sizes and ages, which are related to size dimorphism and the development of secondary sexual characteristics (Cha et al. 1995). Thus, although our data suggest a single equation, it may be possible to improve predictions with BIA by developing separate equations for different age–sex classes of grey seals, as was done in humans (e.g., Lukaski et al. 1986; Suprasongsin et al. 1995).

We conclude that BIA measurements in grey seals may provide a valuable alternative to the more expensive and time-consuming method of hydrogen isotope dilution for estimating mean differences in TBW among groups of grey seals. However, isotope-dilution estimates of TBW in pinnipeds are more precise (Bowen and Iverson 1998) than those obtained from BIA in this study. Thus, investigators will have to weigh the speed of BIA against the cost and precision of isotope dilution. Hilderbrand et al. (1998) reached a similar conclusion about the relative precision of BIA and isotope-dilution methods for estimating fat content (derived from estimates of TBW) in bears. Furthermore, although it is possible to study group differences using BIA, measuring changes in TBW within individuals may be of greatest interest in addressing many research questions. Kushner et al. (1990) concluded that BIA is a useful tool for measuring individual changes in body composition of women. However, predicting TBW in individual grey seals may produce unacceptable errors using eq. 1, and further work will be required to validate BIA for this purpose.

**Note added in proof:** TBW of one adult female that was used to develop the predictive equation was measured again 7 months later using both tritium dilution and BIA. BIA overestimated TBW by 3 and 13% relative to the tritium at first and second measurements, respectively. The change in TBW between the two measurements was 0.1 kg when measured using tritium and 8.6 kg when measured using the predictive equation. The overestimation of TBW by BIA at the second measurement implied a negative fat content. This further supports our conclusion that BIA may not be an appropriate tool for examining longitudinal changes within individuals.

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