# Use of multifrequency bioelectrical impedance analysis for estimation of total body water and extracellular and intracellular fluid volumes in horses

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**Objective**—To evaluate the use of multifrequency bioelectrical impedance analysis (MF-BIA) for estimating total body water (TBW), extracellular fluid volume (ECFV), and intracellular fluid volume (ICFV) in horses.

**Animals**—9 healthy mares.

**Procedure**—TBW and ECFV were measured by use of deuterium oxide and sodium bromide dilution techniques, respectively. Intracellular fluid volume was calculated as the difference between TBW and ECFV. Concurrently, MF-BIA recordings were obtained by use of 4 anatomic electrode positions and 3 measurements of length. Models for MF-BIA data were created for all combinations of length and anatomic electrode position. Models were evaluated to determine the position-length configuration that provided the most consistent estimates of TBW, ECFV, and ICFV, compared with values determined by use of the dilution techniques.

**Results**—Positioning electrodes over the ipsilateral carpus and tarsus and use of height at the tuber sacrale for length provided the closest estimate between values for TBW, ECFV, and ICFV predicted by use of MF-BIA and measured values obtained by dilutional techniques. This model had the narrowest 95% limits of agreement.

**Conclusions and Clinical Relevance**—MF-BIA techniques have been used to predict changes in TBW, ECFV, and ICFV in healthy and diseased humans. Results reported in this study provide an equine-specific model to serve as the basis for further evaluation of MF-BIA in horses with altered fluid states. The MF-BIA techniques have a number of potential applications for use in horses, including evaluation of exercise physiology, pharmacologic studies, and critical-care management. (*Am J Vet Res* 2004;65:320–326)

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Estimates of **total body water (TBW)**, **extracellular fluid volume (ECFV)**, and **intracellular fluid volume (ICFV)** are relied on when formulating plans for fluid replacement, determining drug doses, and monitoring patient responses to treatment. Although radioactive tracers have been used to evaluate fluid compartments in research settings, they have not proven practical for use in clinical situations, which are characterized by dynamic fluid balance.[1-13] An accurate, inexpensive, noninvasive, and portable technique that allows for rapid detection of changes in body fluid compartments would provide a valuable diagnostic and therapeutic tool. Bioimpedance analysis has the potential to provide such a technique, with applications in equine medicine and exercise physiology as well as other research investigations.

Monitoring of body weight represents 1 means to evaluate acute changes in TBW in horses. Unfortunately, for longer periods and changing nutritional status, this measure of TBW flux may become less accurate and more difficult to interpret. Total body water has also been measured in horses by use of the distribution spaces of deuterium,[1,2] antipyrine,[3] and tritium.[4,5] Values for TBW in horses range from 0.610 to 0.710 L/kg.[1-5] However, estimating changes in TBW by use of volume markers is complex, requires attainment of steady-state conditions, does not permit sequential repetitive assessments, and may not be accurate for all clinical conditions.

Extracellular fluid volume has also been measured in horses by use of dilution techniques, including those that involve sodium thiocyanate and sodium bromide.[6] Estimates of ECFV range from 0.212 to 0.308 L/kg, depending on experimental circumstances and the technique used.[7-13] The long equilibration time required for tracer studies makes these methods poorly suited for detection of acute changes in ECFV, such as those caused by dehydration, hyperhidrosis, or diarrhea.

To the authors' knowledge, direct measurements of ICFV have not been performed in horses. This variable has been estimated by use of dilution techniques to determine ECFV and TBW and then the calculation of ICFV as the difference between TBW and ECFV. Direct knowledge of ICFV can be important when evaluating nutritional status, dehydration, or response to fluid therapy.

Although tracer studies are currently the criterion-referenced standard for measurement of TBW and ECFV, **bioelectrical impedance analysis (BIA)** is an

alternative technique that can be used to accurately predict these volumes in humans and other animals. Bioelectrical impedance analysis is based on the fact that an electric current is conducted poorly by fat and bone but conducted well by tissues containing primarily electrolytes and water. Bioelectrical impedance analysis has been used in a number of species to evaluate TBW or body composition, including humans,[14-16] cats,[17,18] harbor seals,[19] bears,[20] dogs,[21] pigs,[22] and horses.[2] It is gaining acceptance in humans for use in evaluating athletic potential,[23] monitoring changes in body composition during disease states,[24,25] and estimating ECFV for drug-dosing purposes.[26]

A dual-frequency bioelectrical impedance device has been developed for use in predicting TBW, ECFV, and ICFV in horses.[2] This technique relies on BIA measurements performed at only 2 frequencies (ie, 5 and 200 kHz). When applied to a regression model, it accurately predicts fluid volumes in euhydrated horses. However, dual-frequency techniques may be inadequate to detect changes in these compartments or in critically ill patients in which ECFV and ICFV relationships are perturbed.[27] On the basis of results from research in human subjects, **multifrequency (MF)**-BIA provides more accurate determination of absolute fluid volumes and changes in these volumes, compared with dual-frequency techniques.[27-29] The MF-BIA uses 50 logarithmically spaced frequency measurements ranging from 5 to 1,000 kHz to compute the electrical resistance characteristics of complex, heterogeneous conducting fluids, such as animal tissues. The model requires resistivity coefficients for male and female subjects. These coefficients are unique for each species and specific anatomic electrode configuration. To our knowledge, these coefficients or the ideal anatomic electrode configurations have not been determined in horses.

The purpose of the study reported here was to create an MF-BIA model to evaluate TBW, ECFV, and ICFV in horses. Specifically, the ideal anatomic electrode positions and specific tissue resistivity coefficients for horses were determined by comparing estimates of TBW, ECFV, and ICFV obtained by use of MF-BIA to measurements of TBW, ECFV, and ICFV obtained concurrently by use of dilutional techniques.

## Materials and Methods

**Animals**—Nine clinically normal, euvolemic, adult mares were used in the study. Horses ranged from 416 to 572 kg and represented several breeds. The study protocol was approved by the University Animal Use and Care Administrative Advisory Committee.

**Procedure**—Feed was withheld for 12 hours prior to the study. Feed and water were withheld during the first 6 hours of data collection.

Body weight was determined in all horses by use of a digital walk-on scale. Morphometric measurements for length included height at the top of the shoulders (ie, withers), height at the tuber sacrale, and distance from the tail to the point of the shoulder. **Sodium bromide (NaBr)**[a] was diluted in **deuterium oxide ($D_2O$)**[b] to create a 7.9% solution of NaBr. Bromide (25 mg/kg; approx 30 mg of NaBr /kg) and $D_2O$ (0.4 g/kg) were administered IV during a 3-minute period via a catheter inserted in the right jugular vein.

Blood samples (5 mL) were collected into evacuated serum tubes before and 1, 3, 5, and 6 hours after administration of NaBr and $D_2O$. These samples were collected via a catheter inserted in the left jugular vein. All tubes were centrifuged, and serum was separated and stored at –20°C until analyzed for deuterium and bromide concentrations.

**Calculation of TBW by use of $D_2O$ dilution**—Dilution techniques were used to estimate TBW by use of serum deuterium concentrations measured in samples obtained before and 3 and 5 hours after administration. These time points were chosen on the basis of the optimal window (2 to 7 hours after administration) reported[1] for TBW calculation by use of deuterium. Serum samples were extracted, and concentrations of $D_2O$ in condensed water were determined in duplicate by use of Fourier transformation infrared spectroscopy, as described elsewhere.[17,18,30,31] Deuterium measurements were determined as the mean result for the duplicate analyses. Samples in which duplicate measurements differed by > 5% were not used. Deuterium concentrations at 3 and 5 hours were compared to confirm equilibration.

Values for TBW were calculated by use of the following equation:

$$TBW = ([D \bullet APE \bullet 18.02]/[MW \bullet 1{,}000\ \Delta]) \times 0.96$$

where D is the dose (ie, number of grams) of $D_2O$, APE is the atom percentage excess $D_2O$ of the injected dose (ie, 0.999), MW is the molecular weight of $D_2O$ (ie, 20.03 g/mL), $\Delta$ is the difference in $D_2O$ APE before and after administration, and 0.96 represents the correction factor for the binding of deuterium to acidic amino acids and other nonexchangeable sites.[2]

**Calculation of ECFV by use of NaBr dilution**—Bromide concentrations were determined in triplicate by use of high-performance liquid chromatography, as described elsewhere.[6,17,18] Values for ECFV were estimated by use of serum bromide concentrations in samples obtained before and 5 and 6 hours after administration.[6] Concentrations at 5 and 6 hours were compared to confirm equilibration.

The concentration in the sample obtained 5 hours after administration was used to calculate ECFV by use of the following equation:

$$ECFV = (Br\ dose/[Br_{5h} - Br_{0h}]) \times 0.9 \times 0.95$$

where Br dose is the dose of bromide administered, $Br_{5h}$ is the serum bromide concentration at 5 hours, $Br_{0h}$ is the bromide concentration before administration, 0.9 is the correction factor for uptake by erythrocytes, and 0.95 is the correction factor for the Gibbs-Donnan effect.

**Calculation of ICFV**—Values for ICFV were calculated as the difference between TBW and ECFV concentrations. Thus, ICFV = TBW – ECFV.

**Other hematologic and biochemical analyses**—Blood samples (2 mL) were collected before and 1 and 6 hours after administration of NaBr into evacuated tubes containing potassium EDTA. Samples were refrigerated and analyzed to measure PCV and plasma **total protein (TP)** concentration; these measurements were obtained within 12 hours after sample collection. The PCV was determined by the microhematocrit method by use of samples obtained before and 1 and 6 hours after administration. Plasma TP concentration was estimated by use of refractometry on plasma harvested from the same samples.

Sodium, potassium, chloride, and total $CO_2$ concentrations were measured in serum samples collected before and 1 and 6 hours after administration of NaBr. Values were determined by use of a commercial chemistry analyzer.[c] Serum

used for determination of electrolyte concentrations was from the aforementioned samples frozen at –20°C.

**Measurement of MF-BIA**—Following administration of NaBr, measurements of bioelectrical impedance were obtained for all horses. Each horse was restrained in a standing position in a stock; all metal surfaces of the stock were covered with plastic. Precisely determined anatomic areas in the region of the carpus, elbow, stifle, and tarsus were clipped and prepared with alcohol, and subdermal tetrapolar platinum electrodes[d] were placed 2.5 cm apart within those areas. Electrodes were placed parallel to the ground surface at each anatomic location.

The tetrapolar electrodes were placed subdermally in 4 configurations that represented the direction of current flow through tissues. The first configuration (**head-tail [H-T]**) was 1 pair of electrodes positioned over the right side of the cranial border of the first cervical vertebrae and a second pair of electrodes positioned over the caudal aspect of the region of the right tuber ischii (**Fig 1**). The second configuration (ipsilateral elbow-stifle) was 1 pair of electrodes positioned at the most proximal and caudal aspect of the right olecranon and a second pair of electrodes positioned on the dorsal aspect of the right tibia over the tibial tuberosity. The third configuration (contralateral elbow-stifle) was 1 pair of electrodes positioned at the most proximal and caudal aspect of the left olecranon and a second pair of electrodes positioned on the dorsal aspect of the right tibia over the tibial tuberosity. The fourth configuration (**ipsilateral carpus-tarsus [ICT]**) was 1 pair of electrodes positioned on the palmar aspect of the right accessory carpal bone and a second pair of electrodes positioned on the dorsal aspect of the right tarsus at the level of the medial malleolus (**Fig 2**).

A bioimpedance analyzer[e] was used to obtain measurements of **resistance (R)** and **reactance (Xc)** at each of 50 frequencies ranging from 5 to 1,000 kHz. **Impedance (Z)** and **phase angle (θ)** were then computed from the measured values for R and Xc. The MF-BIA measurements were repeated for each of 4 configurations. Data were transmitted from the analyzer to a personal computer and stored until subsequent analysis.

The **R of extracellular water ($R_E$)** and **R of intracellular water ($R_I$)** were computed from the generated Z and θ spectral data for each electrode configuration. The Z and θ data were fitted to an enhanced version of the Cole-Cole model of current conduction through heterogeneous biological tissues by use of iterative nonlinear curve-fitting algorithms[f] derived for use with the bioimpedance analyzer. The enhanced modeling program[f] extended the original Cole-Cole model to allow for frequency invariant time delays caused by the speed at which electrical information was transferred through a conductor.

Predicted extracellular and intracellular fluid volumes were estimated for each electrode configuration from the modeled $R_E$ and $R_I$ values; this was achieved through the use of equations formulated from the Hanai mixture theory, which describes the influence of nonconductive material on the apparent resistivity of surrounding conductive fluid.[32] Extracellular fluid volume was estimated by use of the following equation:

$$V_{ECW} = k_{ECW} \bullet ([L^2 \bullet \sqrt{W}]/R_E)$$

where $V_{ECW}$ is the predicted total extracellular water volume; $k_{ECW}$ is a scaling factor that accounts for the geometry of measurements between a defined electrode array, resistivity of the extracellular fluid, and body density; L is the morphometric length (ie, height at highest point of the dorsal spinous processes of the thoracic vertebrae [ie, withers], height at the tuber sacrale, or distance from the tail to the point of the shoulder); W is body weight in kilograms; and $R_E$ is the resistance of the extracellular water from the model fit. Values for $k_{ECW}$, a constant, were derived by regressing the ECFV predicted by use of the MF-BIA against the ECFV estimated by use of the corrected dilution space for NaBr.

The volume of intracellular water was predicted from further extrapolation of the Hanai theory by use of the following equation[32]:

$$(1 + [V_{ICW}/V_{ECW})^{5/2} = ([R_E + R_I]/R_I) \bullet (1 + [\{k_D \bullet V_{ICW}]/V_{ECW}])$$

where $V_{ICW}$ is the volume of intracellular water, $R_E$ and $R_I$ are the resistance for extracellular and intracellular water from the model fit, respectively, and $k_D$ is the ratio of the apparent resistivity of intracellular water to extracellular water. The value for $k_D$ was derived by the iterative prediction of $V_{ICW}/V_{ECW}$, and adjusting $k_D$ until a minimum mean error between the predicted and measured values was obtained.

Predicted TBW was calculated as follows:

$$TBW = V_{ECW} + V_{ICW}$$

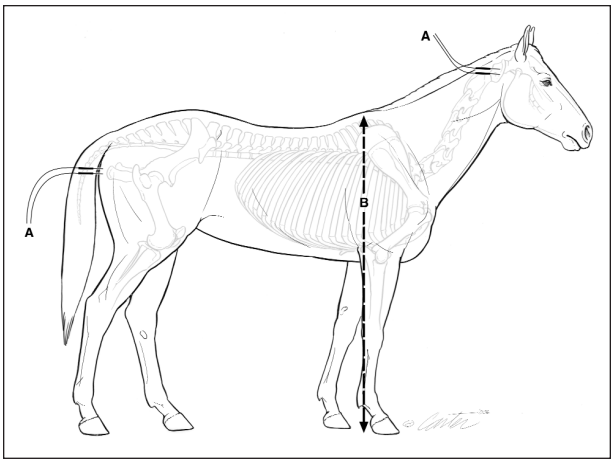


Figure 1—Anatomic position of electrodes and length measurement for the head-tail configuration. Pairs of electrodes were positioned over the right side of the cranial border of the first cervical vertebrae and over the caudal aspect of the right tuber ischii (A). Length measurement used for this configuration was height at the highest point of the dorsal spinous processes of the thoracic vertebrae (ie, withers; B).

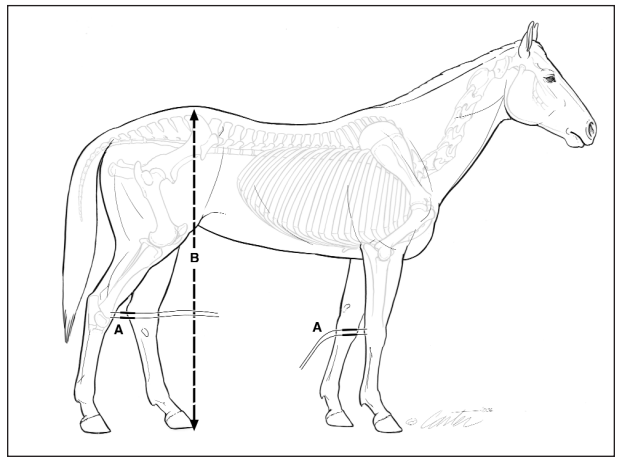


Figure 2—Anatomic position of electrodes and length measurement for the ipsilateral carpus-tarsus (ICT) configuration. Pairs of electrodes were positioned on the palmar aspect of the right accessory carpal bone and a second pair of electrodes positioned on the dorsal aspect of the right tarsus at the level of the medial malleolus (A). Length measurement used for this configuration was height at the top of the tuber sacrale (B).

**Statistical analysis**—All data were expressed as mean ± SD unless otherwise indicated. Comparisons between hematologic and biochemical data were performed by use of a paired Student *t* test. Predicted MF-BIA values for TBW, $V_{ICW}$, and $V_{ECW}$ were compared with values for TBW, ICFV, and ECVF, respectively, which were obtained by use of dilutional methods for each combination of electrode locations and morphometric length measurements. These comparisons were made by use of linear regression, calculation of the mean value of the error between the 2 measurements, Student *t* tests, and the 95% limits of agreement.[33] Bland-Altman plots were used to illustrate differences between mean values obtained by use of the 2 techniques.[34]

## Results

**Dilutional estimates of TBW, ECFV, and ICFV**—Comparison of deuterium concentrations in samples obtained 3 and 5 hours after administration did not reveal a significant ($P = 0.407$) difference in TBW. The deuterium concentration at 3 hours was used for all analyses, except when the duplicate measurements differed by > 5%. In those cases, the serum deuterium concentration in the sample obtained at 5 hours was used. Mean ± SD value for TBW for all horses was 345.8 ± 53.9 L (ie, 0.67 ± 0.06 L/kg).

Comparison of serum bromide concentrations in samples obtained 5 and 6 hours after administration did not reveal a significant ($P = 0.698$) difference in ECFV between these 2 values. The sample obtained at 5 hours for 1 horse was destroyed during transport to the laboratory; thus, the sample obtained at 6 hours for that horse was used for dilutional measurement. Mean ECFV for all horses was 109.6 ± 14.3 L (ie, 0.214 ± 0.01 L/kg). Mean calculated ICFV was 236.3 ± 30.5 L (ie, 0.458 ± 0.06 L/kg).

**Results for MF-BIA**—Two horses would not tolerate placement of electrodes on the tarsus. Data for these 2 horses were excluded only from calculations for the ICT configuration. Placement of electrodes for all other configurations was tolerated well by the horses.

Results were determined for the 4 anatomic configurations and each of 3 morphometric length measurements (**Table 1**). Values for each of the fluid-compartment volumes were estimated by use of the dilutional and MF-BIA techniques (**Table 2**).

The model with the smallest error for ECFV was determined by use of the ICT configuration with length measured at the height of the tuber sacrale (**Fig 3**). Mean ± SD predicted ECFV determined by use of the MF-BIA for the 7 horses on which we obtained data for this configuration was 112.5 ± 10.8 L, which was in strong agreement with the estimate of 112.5 ± 14.4 L obtained by use of the dilutional technique. Regression analysis of ECFV measured by use of the MF-BIA versus ECFV estimated by use of the dilutional technique yielded a high correlation ($R^2$, 0.86; $P = 0.003$). Mean difference between the 2 techniques was 0.00 ± 5.7 L, and the 95% limits of agreement were –11.4 L to +11.4 L (**Fig 4**). Additionally, regression analysis between the error of the 2 measurements and the mean of the 2 values ($R^2$, 0.44; $P = 0.11$) indicated that size of the error did not correlate with the magnitude of ECFV.

The ICT electrode configuration with length measured at the height of the most dorsal point of the tuber sacrale was also the configuration with the smallest error for TBW (**Fig 5**). Mean TBW predicted by use of the MF-BIA for the 7 horses on which we obtained data with this configuration was 359.1 ± 36.9 L, which was close to the estimated value obtained by use of dilutional techniques (359.1 ± 54.3 L). Regression analysis of TBW measured by use of the MF-BIA versus TBW estimated by use of the dilutional technique yielded a high correlation ($R^2$, 0.82; $P = 0.005$). Mean difference between the 2 techniques was –0.02 ± 26.2 L. The 95% limits of agreement were

Table 1—Mean ± SD of the error and mean absolute error for extracellular fluid volume (ECFV) and total body water (TBW) determined in healthy adult female horses by use of multifrequency bioelectrical impedance analysis (MF-BIA) for 4 electrode position configurations and 3 length measurements

| | Mean ± SD error | | Mean absolute error | |
|---|---|---|---|---|
| **Configuration*** | **ECFV (L)** | **TBW (L)** | **ECFV (L)** | **TBW (L)** |
| $H\text{-}T_{Body}$ | 0.00 ± 11.8 | –0.01 ± 30.3 | 8.9 | 23.4 |
| $H\text{-}T_{Scapula}$ | 0.00 ± 9.6 | 0.01 ± 31.5 | 6.4 | 24.0 |
| $H\text{-}T_{Pelvis}$ | 0.00 ± 10.4 | –0.01 ± 31.3 | 7.9 | 25.6 |
| $IES_{Body}$ | 0.00 ± 11.8 | 0.01 ± 71.1 | 9.1 | 59.6 |
| $IES_{Scapula}$ | 0.00 ± 9.5 | –0.02 ± 68.4 | 6.4 | 52.2 |
| $IES_{Pelvis}$ | 0.00 ± 10.0 | –0.01 ± 64.1 | 6.8 | 50.2 |
| $CES_{Body}$ | 0.00 ± 15.4 | 0.00 ± 87.0 | 11.9 | 71.6 |
| $CES_{Scapula}$ | 0.00 ±12.8 | 0.00 ± 82.9 | 9.3 | 68.5 |
| $CES_{Pelvis}$ | 0.00 ± 13.6 | 0.01 ± 79.1 | 9.9 | 64.0 |
| $ICT_{Body}$ | 0.00 ± 6.5 | 0.01 ± 29.9 | 4.6 | 25.1 |
| $ICT_{Scapula}$ | 0.00 ± 5.8 | 0.01 ± 28.6 | 4.2 | 23.3 |
| $ICT_{Pelvis}$ | 0.00 ± 5.7 | –0.02 ± 26.2 | 3.8 | 20.0 |

*Electrode configurations involved positioning of electrodes at various anatomic regions, including the carpus, elbow, stifle, and tarsus and use of 3 morphometric length measurements. H-T = Head-tail; electrodes positioned at cranial aspect of the right side of the first cervical vertebrae and over the caudal aspect of the right tuber ischii. IES = Ipsilateral elbow-stifle; electrodes positioned at the most proximal and caudal aspect of the right olecranon and on the dorsal aspect of the right tibia over the tibial tuberosity. CES = Contralateral elbow-stifle; electrodes positioned at the most proximal and caudal aspect of the left olecranon and on the dorsal aspect of the right tibia over the tibial tuberosity.ICT = Ipsilateral carpus-tarsus; electrodes positioned on the palmar aspect of the right accessory carpal bone and on the dorsal aspect of the right tarsus at the level of the medial malleolus. Body = Distance from the point of the shoulder to the tuber ischii. Scapula = Distance from the ground to the highest point of the dorsal spinous processes of the thoracic vertebrae (ie, withers). Pelvis = Distance from the ground to the top of the tuber sacrale.

Table 2—Estimated volume of various fluid compartments determined by use of a dilution technique and predicted volume of various fluid compartments determined by use of an MF-BIA technique in each of 7 horses

| | TBW | | ECFV | | ICFV | |
|---|---|---|---|---|---|---|
| **Horse** | **Dilution*** | **MF-BIA** | **Dilution†** | **MF-BIA** | **Dilution‡** | **MF-BIA‡** |
| 1 | 341.0 | 334 | 103.7 | 103.5 | 236.3 | 230.5 |
| 2 | 365.3 | 359.6 | 115.9 | 116.7 | 249.4 | 242.9 |
| 3 | 340.6 | 366.3 | 116.3 | 113.9 | 224.3 | 252.4 |
| 4 | 410.3 | 403.9 | 126.4 | 128.2 | 283.9 | 275.6 |
| 5 | 423.3 | 404.6 | 126.1 | 123.7 | 297.2 | 280.8 |
| 6 | 374.1 | 342.2 | 114.4 | 106.2 | 259.7 | 236.1 |
| 7 | 259.0 | 303.2 | 84.9 | 95.6 | 174.1 | 207.6 |

Values reported are number of liters.
*Dilution technique used deuterium oxide to estimate TBW. †Dilution technique used sodium bromide to estimate ECFV. ‡Value for ICFV calculated as the difference between TBW and ECFV values.
ICFV = Intracellular fluid volume.

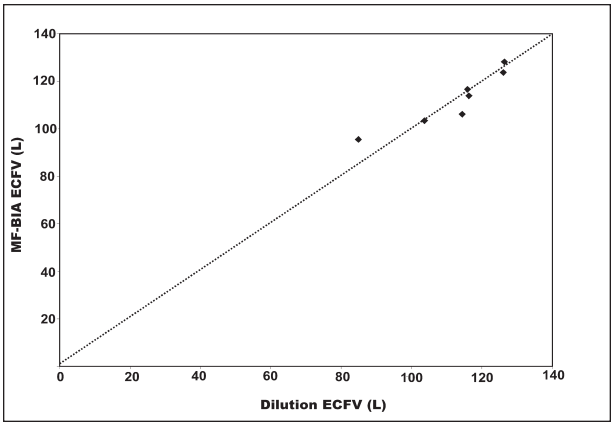


Figure 3—Comparison of extracellular fluid volume (ECFV) in horses calculated by use of a dilution technique and ECFV predicted by use of multifrequency bioelectrical impedance analysis (MF-BIA) for the ICT electrode configuration with length measured at the height at the tuber sacrale. The dashed line represents the line of equality.

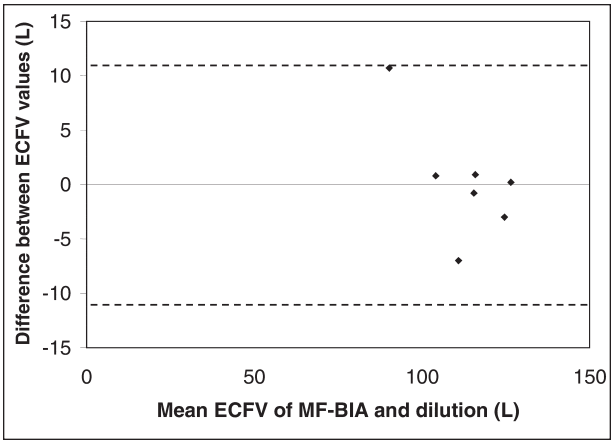


Figure 4—Representative Bland-Altman plot illustrating the difference between ECFV estimated by use of MF-BIA for the ICT electrode configuration with length measured at the height at the tuber sacrale and ECFV estimated by use of a dilution technique plotted against the mean of the 2 methods. The solid line is the mean difference (bias), and dashed lines are the limits of agreement (mean difference ± [1.96•SD]).

–52.4 L to +52.4 L (**Fig 6**). Additionally, linear regression analysis between the error of the 2 measurements and the mean ($R^2$, 0.68; $P$ = 0.10) indicated that the size of the error did not correlate with the magnitude of TBW.

The ICT electrode configuration with the length measured at the height at the point of the tuber sacrale revealed that the mean ICFV, predicted by use of MF-BIA for the 7 horses on which we obtained data with this configuration, was 246.6 ± 25.7 L. The mean ICFV derived by use of the dilution techniques was 246.4 ± 40.8 L. Regression analysis of these 2 estimates of ICFV yielded a high correlation ($R^2$, 0.77; $P$ = 0.009). Mean difference between the 2 techniques was –0.16 ± 21.9 L. The 95% limits of agreement were –44.0 L to +43.6 L. Additionally, linear regression analysis between error of the 2 measurements and the mean ($R^2$, 0.50; $P$ = 0.07) indicated that there was not a significant relationship between the size of the error and the magnitude of ICFV.

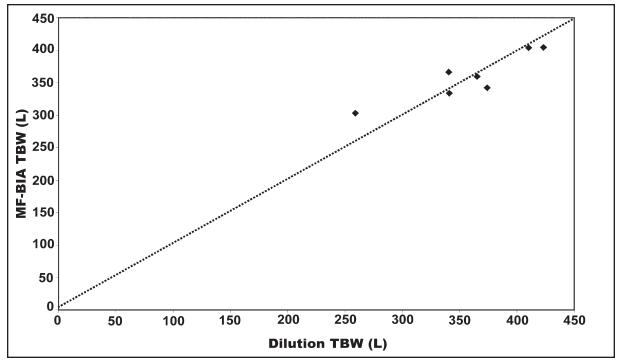


Figure 5—Comparison of total body water (TBW) in horses calculated by use of a dilution technique and TBW predicted by use of MF-BIA for the ICT electrode configuration with length measured at the height at the tuber sacrale. The dashed line represents the line of equality.

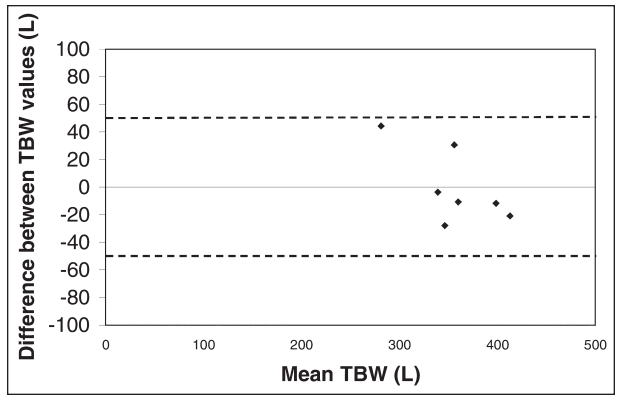


Figure 6—Representative Bland-Altman plot illustrating the difference between TBW estimated by use of MF-BIA for the ICT electrode configuration with length measured at the height at the tuber sacrale and TBW estimated by use of a dilution technique plotted against the mean of the 2 methods. The solid line is the mean difference (bias), and dashed lines are the limits of agreement (mean difference ± [1.96 • SD]).

**Hematologic and biochemical results**—Mean ± SD PCV before administration of NaBr (40.6 ± 5.3%) was significantly ($P < 0.001$) higher than the PCV in samples obtained 1 (29.9 ± 3.2%) and 6 (32.0 ± 2.8%) hours after administration. This decrease in PCV after bromide administration was similar to that reported in another study[6] that used 6 of the same mares. The TP concentration before administration was not significantly different from the TP concentration in samples obtained 1 or 6 hours after administration. Sodium, potassium, and total $CO_2$ concentrations did not differ significantly between samples obtained before and 1 or 6 hours after bromide administration. There was a significant increase in the mean chloride concentration from the sample obtained before administration (96.7 ± 1.4 mmol/L) to concentrations in the samples obtained 1 (97.8 ± 1.1 mmol/L; $P$ = 0.012) and 6 (98.2 ± 1.1 mmol/L; $P$ = 0.034) hours after administration. This represented the expected increase in measured chloride concentration attributable to the bromide anions. On the basis of the manufacturer's evaluation,[8] the ion-selective electrode in the analyzer reportedly records 0.7 mmol of bromide/L for 1 mmol of chloride/L, which is similar to the magnitude of the increase in the chloride concentration for the study reported here.

## Discussion

Analysis of results of the study reported here revealed excellent agreement between MF-BIA and conventional (dilution) methods for detection of ECFV and TBW and calculation of ICFV. Therefore, MF-BIA can be used to evaluate ECFV, ICFV, and TBW in standing, awake, healthy horses.

The optimal anatomic electrode configuration identified in the study (ICT) is similar to the optimal configuration used in the dual-frequency BIA model described for horses.[2] However, this was not the ideal configuration identified in cats by use of the same MF-BIA model.[18] Reasons for this difference are most likely attributable to the standing position of horses as well as other anatomic differences between species. The ICT electrode configuration accommodates the extensive tissue mass of the proximal limb segments of horses. A head-to-tarsus or neck-to-shoulder configuration may also prove to be accurate by incorporating the cervical musculature. The H-T (cervical region to tuber ischii) configuration was not as accurate as the ICT configuration, and this may have been a result of variability in length measurement attributable to head movement.

The best length measurement identified for the ICT configuration was height at the tuber sacrale. Ideally, the length measurement should represent the distance along the body between the 2 pairs of electrodes. However, obtaining these measurements in standing horses is problematic because of varying limb positions. We decided to evaluate the 3 morphometric measurements described in this study because they were easily repeatable and represented a constant relationship to the length between pairs of electrodes. A combination of all 3 length measurements was also evaluated, but this did not improve the accuracy of the model.

The ICT configuration was the configuration least tolerated by the horses in the study. Specifically, 2 horses reacted strongly to electrode placement over the tarsus and would not stand quietly while the MF-BIA measurements were made. A less sensitive area proximal to the tarsus may be a reasonable alternative site that would allow similar electrode positioning with improved tolerance. The H-T configuration that used height at the withers also had good accuracy and may represent a more practical alternative to be used in clinical settings (Fig 1). All horses tolerated electrode placement for the H-T configuration well, and height at the highest point of the dorsal spinous processes of the thoracic vertebrae (ie, withers) is a common value known by most horse owners.

Our regression model was created for healthy, nonpregnant, euvolemic mares. Studies[15,16] with human subjects suggest that different models may be needed for males and females, and sex-specific equations are often used. Differing ethnic populations and body types may also require that adjustments be made to standard models; this variability may also apply to differences in sexes and breeds of horses.[35] Additionally, increases in fat-free mass may contribute to errors in measurements, particularly in terms of underestimation when predicting TBW.[14] These factors require further evaluation in horses. Evaluation of Bland-Altman plots suggests that the model may become less accurate at lower values of TBW and ECFV (Fig 4 and 6). However, regression equations for these data did not reveal a significant relationship between the mean value and size of the error. It is possible that a larger number of data points would change this finding. The horse with the lowest body weight in the study consistently had the largest error in terms of difference between values predicted by use of MF-BIA and values estimated by use of dilution techniques for TBW and ECFV. Analysis of the data with exclusion of values for this horse improved the accuracy of the model but did not change the results. It is unknown whether this horse represented decreased predictability of the model at smaller fluid volumes or whether it represented an unrecognized error during data collection or analysis or some unique finding particular to that horse. Testing of the model with a wider range of body weights is needed to investigate this observation.

A large number of potential applications exist for the use of MF-BIA in horses. Responses to fluid therapy could be monitored frequently, allowing for rapid alterations when necessary. For example, horses racing in endurance competitions could be monitored before the race and at subsequent checkpoints, thereby minimizing dehydration that is common during these events. Drug doses could be based on a more accurate estimate of volumes of distribution for various fluid compartments, rather than simply relying on body weight. Nutritional status could be monitored. Thus, research and clinical uses of this technique potentially have wide applications in equine medicine.

The model for anatomic electrode placement identified in the study reported here provides a starting point for additional studies on the use of MF-BIA in horses in research and clinical settings. The next step is validation of the model created in this study on additional horses and for conditions of fluid fluxes, such as dehydration and volume expansion. Once MF-BIA has been validated for these experimental conditions, it can be applied to clinical settings as a means of rapidly evaluating changes in fluid balance in critically ill or exercising horses. The advantages of MF-BIA over dual-frequency techniques include the fact that MF systems use multiple data points, mathematical modeling that allows for partitioning of tissues into component parts, and mixture equations that establish a relationship between R and body fluid compartments. In contrast, dual-frequency systems use multiple regression analyses to establish empirical relationships between Z at 1 frequency and the ratio of TBW to ECFV. For these reasons, MF-BIA is a more precise, less- biased predictor of TBW and ECFV.[35] These advantages may become more important when applying bioimpedance to the altered physiologic state of critically ill horses, where multiple frequencies will allow for development of new models and equations in these populations.

The ICT electrode configuration that used height measured at the tuber sacrale represented the MF-BIA model with the smallest error between predicted (MF-BIA) and measured (dilution technique) values

for TBW, ECFV, and ICFV in horses. On the basis of analysis of results of this study, MF-BIA techniques can be rapidly performed and results are comparable to those of radioactive tracer studies for measuring TBW, ECFV, and ICFV in horses. Thus, MF-BIA may be helpful in measuring acute changes in these fluid compartments in horses.

[a]Sodium bromide, Fisher Scientific Co, Fairlawn, NJ.
[b]Deuterium oxide, Cambridge Isotope Laboratories, Andover, Mass.
[c]Hitachi 717 chemistry analyzer, Boehringer Mannheim, Indianapolis, Ind.
[d]Grass platinum, tetrapolar, subdermal 30-gauge needle electrodes, 122 cm, Astro-Med Inc, West Warwick, RI.
[e]Hydra ECF/ICF bioimpedance analyzer, model 4200, Xitron Technologies, San Diego, Calif.
[f]BIS 4200 utilities software, Xitron Technologies, San Diego, Calif.
[g]Simmons M, Sugahara K, Watanabe M. Development of an improved chloride electrode for the Boehringer Mannheim/Hitachi 700 series analyzers, (poster presentation). Natl Meet Am Assoc Clin Chem New Orleans, 1988.

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