Total body water and ECFV measured using bioelectrical impedance analysis and indicator dilution in horses

MARIANNE FORRO, SCOTT CIESLAR, GAYLE L. ECKER, ANGELA WALZAK, JAY HAHN, AND MICHAEL I. LINDINGER
Department of Human Biology and Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Received 21 October 1999; accepted in final form 1 April 2000

Multifrequency BIA has been used for the determination of hydration state in humans for a number of years and has been the subject of review (6, 12, 25). The principle of the technique is based on the impedance to the flow of a constant, microampere alternating current passed through conductive fluid cylinders (conductors) that represent trunk and limb segments (13, 20). The electrical conductivity of the body is dependent on the amount of water and electrolytes present in the various body fluid compartments. With the assumption that the conductors have a uniform cross section, the volume (V) of the conductor is proportional to its length (L) squared divided by its impedance (Z), 

\[ V = \rho L^2 / Z \]

where \( \rho \) is a specific resistivity term (6).

Specific resistivity is an electrical characteristic of the conductor that is independent of its volume and shape (6, 35). The resistance to current flow is due to the specific resistivity and the volume of the conducting fluid or the fat-free mass of the animal. Cell membranes act as electrical condensers and are a barrier to current flow at low frequencies (<50 kHz). Hence, bioelectrical impedance measured at 1–5 kHz has been used to estimate ECFV (34, 35). In contrast, at frequencies >50 kHz, cell membranes are not a barrier to current flow, allowing bioelectrical impedance measured at 200 kHz to estimate TBW.

The horse, like humans, may be considered a series of conductive cylinders for the purposes of BIA. As in humans, the impedance of the whole body can be measured by means of a tetrapolar electrode configuration,
by using the forelimb and hindlimb together with the length of the horse to calculate the total and extracellular conductive volumes of the body. Impedance to the flow of electrical current injected into one cylinder and detected in another cylinder can be measured at different frequencies, allowing for the estimation of TBW and ECFV (6, 13, 24, 35).

The primary purposes of this study were 1) to determine the compartmentation of body water in horses via indicator dilution techniques and 2) to simultaneously measure bioelectrical impedance to current flow at impulse frequencies of 5 and 200 kHz. These measurements would allow us to determine whether BIA can be used to reliably estimate TBW and ECFV in horses compared with standard indicator dilution techniques and to determine the practicality of using BIA in horses. Accordingly, we hypothesized that the use of dual-frequency BIA increases the accuracy with which TBW and ECFV can be predicted, compared with using body length or height measures alone without BIA measures.

MATERIALS AND METHODS

Subjects

The experiments consisted of a pilot study that used three horses (two Standardbreds and one Thoroughbred cross) and a subsequent full study that used eight horses (Table 1) from the University of Guelph herd. Horses were fasted overnight but had access to water in their stalls. The horses were healthy but not physically trained to perform exercise. Food and water were withheld during the experiment. The experiments were approved by the Animal Care Committee and the Veterinary Teaching Hospital of the University of Guelph and were performed in accordance with the guidelines of the Canadian Council on Animal Care.

Length was measured with a measuring tape on the left side of the horse as the horizontal distance from the point of the shoulder to the furthest curve of the rump without wrapping the tape around the curve of the rump (Fig. 1). Height of the horse was measured with a height measurement stick for horses as the vertical distance from the ground to the highest point of the withers when the horse was standing squarely on a flat surface (Fig. 1). Body mass was measured with a large animal scale (±0.5 kg, KSL Scales, Kitchener, ON, Canada) during the experiment.

Pilot Study

Pilot studies were conducted on three horses to 1) determine suitable sites for electrode placement, 2) compare stainless steel electrodes with carbon fiber electrodes, and 3) determine the frequencies of current injection required to obtain impedance measures that yielded a high degree of correlation to TBW and ECFV. In the pilot study, ECFV was estimated as 0.222 × body mass (2). On the forelimb, electrodes were placed on clipped and cleaned areas just above and below the right knee, and on the left hindlimb electrodes were placed just above and below the hock. After application of electrode gel to skin sites and electrodes, electrodes were secured on the limbs with wide elastic bands with Velcro attachments. Stainless steel and carbon fiber electrodes were used in sequence at the leg site to compare the two electrode types. On the torso, the carbon fiber electrodes were placed in pairs 15 cm apart (center to center) on the neck and rump and secured to the skin with tape (see Fig. 1 for electrode placement). The stainless steel electrodes were not used on the torso. Impedance measurements were obtained in triplicate by using a Bodystat 5000 multifrequency bioelectrical impedance analyzer (Bodystat, Douglas, Isle of Man, UK), with data collected at frequencies of 5, 50, 200, and 500 kHz.

Full Study

Infusion and sampling. The deuterium oxide (D₂O) dilution volume has recently been shown to be useful for the measurement of TBW in horses (1). Similarly, sodium thiocyanate (NaSCN) and Evans blue indicator dilution techniques are well established for the measurement of ECFV (3, 10, 22) and plasma volume (PV; Refs. 17, 21, 22).

Table 1. Characteristics of the horses participating in the study

<table>
<thead>
<tr>
<th>Horse</th>
<th>Breed</th>
<th>Sex</th>
<th>Age, yr</th>
<th>Length, cm</th>
<th>Height, cm</th>
<th>Mass, kg</th>
<th>TBW&lt;sub&gt;MS&lt;/sub&gt;, liters</th>
<th>TBW&lt;sub&gt;A&lt;/sub&gt;, liters</th>
<th>ECFV, liters</th>
<th>PV, liters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pansy</td>
<td>Shetland Pony</td>
<td>Mare</td>
<td>10</td>
<td>178.0</td>
<td>170.0</td>
<td>636.0</td>
<td>419.2</td>
<td>419.7</td>
<td>160.2</td>
<td>25.1</td>
</tr>
<tr>
<td>Neena</td>
<td>Welsh Pony</td>
<td>Mare</td>
<td>10</td>
<td>173.0</td>
<td>168.0</td>
<td>590.0</td>
<td>424.4</td>
<td>421.8</td>
<td>145.0</td>
<td>25.7</td>
</tr>
<tr>
<td>Karen</td>
<td>Standardbred</td>
<td>Mare</td>
<td>16</td>
<td>154.0</td>
<td>151.0</td>
<td>435.0</td>
<td>303.1</td>
<td>310.3</td>
<td>110.4</td>
<td>20.2</td>
</tr>
<tr>
<td>April</td>
<td>Thoroughbred</td>
<td>Mare</td>
<td>10</td>
<td>154.5</td>
<td>155.5</td>
<td>449.0</td>
<td>313.8</td>
<td>315.3</td>
<td>119.9</td>
<td>20.8</td>
</tr>
<tr>
<td>Red</td>
<td>Thoroughbred</td>
<td>Mare</td>
<td>&gt;10</td>
<td>156.5</td>
<td>150.5</td>
<td>465.0</td>
<td>333.7</td>
<td>334.1</td>
<td>123.1</td>
<td>16.5</td>
</tr>
<tr>
<td>Prince</td>
<td>Thoroughbred</td>
<td>Gelding</td>
<td>9</td>
<td>164.0</td>
<td>164.0</td>
<td>536.0</td>
<td>379.8</td>
<td>380.3</td>
<td>143.9</td>
<td>22.8</td>
</tr>
<tr>
<td>Daisy</td>
<td>Percheron</td>
<td>Mare</td>
<td>10</td>
<td>173.0</td>
<td>168.0</td>
<td>590.0</td>
<td>424.4</td>
<td>421.8</td>
<td>145.0</td>
<td>25.7</td>
</tr>
<tr>
<td>Bud</td>
<td>Thoroughbred</td>
<td>Gelding</td>
<td>7</td>
<td>178.0</td>
<td>170.0</td>
<td>636.0</td>
<td>419.2</td>
<td>419.7</td>
<td>160.2</td>
<td>25.1</td>
</tr>
</tbody>
</table>

TBW<sub>MS</sub>, total body water calculated using the equation from Metabolic Solutions; TBW<sub>A</sub>, total body water calculated using the equation from Andrews et al. (1); ECFV, extracellular fluid volume; PV, plasma volume.
The hair coat over the jugular vein, 10–20 cm below the mandible, was clipped short to the skin on both sides of the neck. Each jugular vein catheterization site was aseptically prepared for insertion of catheters. A topical analgesic (EMLA cream, 2.5% lidocaine and 2.5% prilocaine, Astra Pharma, Mississauga, ON, Canada) was applied 30–45 min before insertion of catheters to desensitize the skin. Local anesthetic (2% Xylocaine, Astra Pharma) was injected subcutaneously to complete the analgesia. A catheter (14-gauge, 5.25-in. Angiocath, Becton-Dickinson, Mississauga, ON, Canada) was inserted anterograde into the left and right veins, secured with tape, and stitched to the skin. Four-way stopcocks with 20-in. extensions (Medex-Hilliard) were attached to the catheters for ease of infusion and blood sampling. Patency of the catheters was maintained with sterile, heparinized 0.9% NaCl (2,000 IU/l NaCl).

The dilution indicator used for measuring TBW was D₂O (110 mg/kg; Refs. 1, 13), infused as a 50% vol/vol solution with 20% wt/vol solution in 0.9% sterile saline for a total volume of 60–100 ml. ECFV was measured using NaSCN (22 mg/kg; Refs. 3, 10), infused as a 50% vol/vol solution in 0.9% sterile saline for a total volume of 40–60 ml. PW was measured using Evans blue (0.11 mg/kg; Refs. 17, 23, 24) infused as a 0.5% wt/vol solution in 0.9% sterile saline for a total volume of 40–60 ml. D₂O was purchased from Sigma Chemical (St. Louis, MO) or from Acros (Fisher Scientific, Nepean, ON, Canada), NaSCN from Sigma Chemical, and Evans blue from Fisher Scientific.

The indicators were injected in sequence (Evans blue, 9 ml; D₂O, 25–50 ml; NaSCN, 25–50 ml) using the right catheter over a period of 5 min. Sterility of the infusates was ensured by using a nonpyrogenic, sterile 0.22-μm nylon filter (Millex-Ap/GS Filter, Millipore S.A. 67, Mosheim, France) placed on the syringe. Immediately after the final infusion, the catheter was flushed with 50 ml of sterile 0.9% saline. Blood was sampled from the left catheter with 7.5-ml lithium-heparin syringes (Monovette-Sarstedt, Sarstedt, Germany) before infusion of indicators and 10, 20, 30, 45, 60, 90, and 120 min after infusion. Each blood sample was transferred to five 1.5-ml Eppendorf centrifuge tubes. Four blood samples were centrifuged for 3 min at 15,000 g, and the other was placed on ice for subsequent whole blood analysis. Plasma (2–3 ml) was stored in 1.5-ml Eppendorf tubes and kept on ice until analyzed for Evans blue and NaSCN. Remaining plasma was stored in 1.8-ml screw-cap cryovials at −20°C until analyzed for D₂O.

BIA measurements. On the basis of the pilot study results, it was decided that the electrodes should be situated on the legs above the knee and hock, with bioelectrical impedance measured at 5 and 200 kHz. The hair coat was clipped short on the lateral surfaces of the left forelimb and hindlimb above the knee and hock (about 2-mm hair length). These sites were cleaned well with water and were dried with gauze or towels. BIA measurements were obtained by using a prototype Equistat 2005 (Equistat, Douglas, Isle of Man, UK) with 20-in. extensions (Medex-Hilliard) placed on prepared areas. Before electrode placement, some conductive gel was rubbed into the hair coat directly where the electrode would sit. A small amount of gel was also applied to the surface of each electrode. On the forelimb, the two electrodes (10 cm between centers) were situated below the elbow on the lateral portion of the radius directly over the common digital extensor, ulnaris lateralis, and radial carpal extensor muscles. On the hindlimb, the electrode pair (10 cm between centers) was placed on the tibia directly over the long digital extensor and lateral digital extensor muscles. The distal electrode was used for current injection (800 μA emitted at frequencies of 5 kHz and 200 kHz), and the proximal electrode was used for current detection from the other limb. The instrument reported impedance in ohms at each frequency. Measurements were repeated a minimum of three times to ensure repeatability and consistency.

Analyses. The whole blood sample was analyzed, in duplicate, for hematocrit and plasma ion and metabolite concentrations (Nova Stat Profile 9+, NOVA Biomedical, Waltham, MA). Plasma protein concentration was determined by refractometry (clinical refractometer model SPR-T2, Atago, Tokyo, Japan). Plasma was analyzed for Evans blue concentration via the dual-wavelength method (17) by use of a spectrophotometer (DU-70, Beckman, Mississauga, ON, Canada). Plasma NaSCN concentration was measured spectrophotometrically by a microvolume modification of the method described by Chatterjee et al. (5). Analysis of plasma D₂O concentration was performed by Metabolic Solutions (Nashua, NH), as described previously (1). The D₂O in plasma and water samples was reduced at 490°C to produce deuterium gas that was measured with an isotope-ratio mass spectrometer. The data are expressed in delta D/ml (6) relative to Vienna standard mean ocean water (VSMOW).

Calculations. TBW was calculated from plasma D₂O concentrations by two different sets of equations: Eqs. M1 to M3 by Metabolic Solutions (Nashua, NH) and equation Eq. M4 from Andrews et al. (1).

\[
TBW = \frac{(W - A)}{D_2O} \times \frac{(\delta_{dose} - \delta_{tap})}{(\delta_{post} - \delta_{pre})} \times \frac{(MWdose)}{1000} \times 1.04 \times 18.02 \text{ g/mol} \times \frac{1}{\text{kg}} \times \frac{\text{water}}{\text{TBW}} \times 0.001 \times \frac{\text{VSMOW}}{\text{D}_2O} \times 100 \times (\delta_p - \delta_i) \times R_{ad} \quad \text{(M1)}
\]

where W is grams of water used to dilute the dose, A is grams of dose administered to the subject, is grams of dose diluted for analysis, \( \delta_{pre} \) and \( \delta_{post} \) are the delta deuterium values determined for the predose and postdose samples, \( \delta_{dose} \) is the measured deuterium content of the diluted dose, and \( \delta_{tap} \) is the measured deuterium content of local (tap) water. To convert TBW to kilograms

\[
TBW (kg) = \frac{(TBW) \times 18.02 \text{ g/mol}}{1000 \text{ g/kg}} \quad \text{(M2)}
\]

The D₂O dilution technique overestimates TBW by 4% because of binding of deuterium to acidic amino acids and other nonexchangeable sites. To correct for the nonexchange of deuterium in the body, a corrected TBW (TBWMS) was obtained by dividing TBW from Eq. M2 by 1.04

\[
TBW_{MS} = \frac{TBW}{1.04} \quad \text{(M3)}
\]

The value calculated from the equation of Andrews et al. (1) was also divided by 1.04 and is reported as TBW

\[
TBW_A = \frac{Dose \times \text{APE}_{dose} \times 18.02 \text{ g/mol}}{1.04 \times \text{MW}_{dose} \times 100 \times (\delta_p - \delta_i) \times R_{ad}} \quad \text{(M4)}
\]

where Dose is dose in grams, APEdose is atom percent excess of dose (99.9%), MWdose is molecular weight of D₂O = 20.02 g/mol, \( \delta_p \) is \( \Delta D_2O \) vs. VSMOW for plateau sample, \( \delta_i \) is \( \Delta D_2O \) of baseline sample (time 0), and \( R_{ad} \) is ratio of deuterium to hydrogen in VSMOW (standard = 0.00015576).

ECFV was calculated from plasma NaSCN concentration (5) and PV from plasma Evans blue concentration (16). Intracellular fluid volume (ICFV) was calculated as the difference between TBW and ECFV.

Statistics. Single and multiple stepwise linear regression analyses were used to determine relationships among measured variables. Statistical significance was accepted at \( P < 0.05 \) at a power of 80%.
Table 2. Regression equations correlating total body water to mass, height, and length

<table>
<thead>
<tr>
<th>Equation Number</th>
<th>Regression Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBW = -36.7 + (0.768 × M); r² = 0.976, P &lt; 0.001, SEE = 17.5 liters</td>
<td>1</td>
</tr>
<tr>
<td>TBW = -493.2 + (5.225 × D); r² = 0.984, P &lt; 0.001, SEE = 14.4 liters</td>
<td>2</td>
</tr>
<tr>
<td>TBW = b + (m × D) + (n × 200 kHz); r² = 0.984, P &lt; 0.001, SEE = 15.6 liters</td>
<td>2 with BIA</td>
</tr>
<tr>
<td>TBW = -331.6 + (4.350 × H); r² = 0.969, P &lt; 0.001, SEE = 20.1 liters</td>
<td>3</td>
</tr>
<tr>
<td>TBW = -449.6 + (3.637 × D) + (1.360 × H); r² = 0.988, P &lt; 0.001, SEE = 13.7 liters</td>
<td>4</td>
</tr>
<tr>
<td>TBW = -1187.8 + (0.519 × M) + (0.858 × D) + (2.211 × H); r² = 0.991, P &lt; 0.001, SEE = 13.0 liters</td>
<td>5</td>
</tr>
</tbody>
</table>

TBW, total body water (liters); M, mass (kg); H, height (cm); D, length (cm); SEE, standard error of the estimate; BIA, bioelectrical impedance analysis. (n = 8 horses ranging in mass from 212 to 631 kg). The letters b, m, and n represent coefficients used in the regression equations that cannot be released.

Table 3. Regression equations correlating extracellular fluid volume to mass, height, length, and bioelectrical impedance measured at 5 kHz

<table>
<thead>
<tr>
<th>Equation Number</th>
<th>Regression Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECFV = -7.49 + (0.272 × M); r² = 0.973, P &lt; 0.001, SEE = 6.7 liters</td>
<td>6</td>
</tr>
<tr>
<td>ECFV = -169.0 + (1.850 × D); r² = 0.979, P &lt; 0.001, SEE = 5.92 liters</td>
<td>7</td>
</tr>
<tr>
<td>ECFV = b + (m × D) + (n × 5 kHz); r² = 0.942, P &lt; 0.001, SEE = 1.90 liters</td>
<td>7 with BIA</td>
</tr>
<tr>
<td>ECFV = -112.4 + (1.545 × H); r² = 0.969, P &lt; 0.001, SEE = 7.16 liters</td>
<td>8</td>
</tr>
<tr>
<td>ECFV = b + (m × H) + (n × 5 kHz); r² = 0.975, P &lt; 0.001, SEE = 1.25 liters</td>
<td>8 with BIA</td>
</tr>
<tr>
<td>ECFV = -150.3 + (1.166 × D) + (0.586 × H); r² = 0.984, P &lt; 0.001, SEE = 5.58 liters</td>
<td>9</td>
</tr>
<tr>
<td>ECFV = 3.266 + (0.241 × M) + (0.919 × H) + (0.981 × H); r² = 0.990, P &lt; 0.001, SEE = 4.98 liters</td>
<td>10</td>
</tr>
<tr>
<td>ECFV = b + (m × M) + (n × D) + (q × H) − (r × 5 kHz); r² = 0.999, P &lt; 0.001, SEE = 1.78 liters</td>
<td>10 with BIA</td>
</tr>
</tbody>
</table>

ECFV, extracellular fluid volume. 5 kHz is the bioelectrical impedance measured at 5 kHz frequency (Ω); n = 8 horses ranging in mass from 212 to 631 kg. The letters b, m, n, q, and v represent coefficients used in the regression equations that cannot be released.

Table 4. Regression equations correlating plasma volume to mass, height, length, and bioelectrical impedance measured at 5 kHz

<table>
<thead>
<tr>
<th>Equation Number</th>
<th>Regression Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PV = -2.623 + (0.047 × M); r² = 0.921, P &lt; 0.001, SEE = 2.03 liters</td>
<td>11</td>
</tr>
<tr>
<td>PV = -30.95 + (0.323 × D); r² = 0.941, P &lt; 0.001, SEE = 1.75 liters</td>
<td>12</td>
</tr>
<tr>
<td>PV = b + (m × D) + (n × 5 kHz); r² = 0.942, P &lt; 0.001, SEE = 1.90 liters</td>
<td>12 with BIA</td>
</tr>
<tr>
<td>PV = -21.70 + (0.274 × H); r² = 0.961; P &lt; 0.001, SEE = 1.43 liters</td>
<td>13</td>
</tr>
<tr>
<td>PV = b + (m × H) + (n × 5 kHz); r² = 0.975, P &lt; 0.001, SEE = 1.25 liters</td>
<td>13 with BIA</td>
</tr>
<tr>
<td>PV = -24.30 + (0.080 × D) + (0.208 × H); r² = 0.963, P &lt; 0.001, SEE = 1.52 liters</td>
<td>14</td>
</tr>
<tr>
<td>PV = -9.875 + (0.0236 × M) + (0.0116 × D) + (0.245 × H); r² = 0.964, P &lt; 0.001, SEE = 1.66 liters</td>
<td>15</td>
</tr>
<tr>
<td>PV = b + (m × M) + (n × D) + (q × H) − (r × 5 kHz); r² = 0.994, P &lt; 0.001, SEE = 1.31 liters</td>
<td>15 with BIA</td>
</tr>
</tbody>
</table>

PV, plasma volume; n = 8 horses ranging in mass from 212 to 631 kg. The letters b, m, n, q, and v represent coefficients used in the regression equations that cannot be released.

RESULTS

Pilot Study

The range in body mass spanned 119 kg (406.5–525.5 kg), length ranged from 152 to 160 cm, and height ranged from 146.5 to 156.5 cm.

There was no difference in the bioelectrical impedance measurements obtained using stainless steel vs. carbon fiber electrodes. At 5 kHz, impedance was 221 ± 1 Ω and 219 ± 1 Ω for stainless steel and carbon fiber electrodes, respectively. At 50 kHz, impedance values were 184 ± 1 and 183 ± 1 Ω; at 200 kHz, 157 ± 1 and 156 ± 1 Ω; and at 500 kHz, 145 ± 2 and 142 ± 1 Ω.

Bioelectrical impedance measured at each frequency was considerably less at the torso site than at the legs. The variability, as represented by the standard error of triplicate measures on each horse, was also less with torso impedance measurements than with leg impedance measurements. There was also less intersubject variability with torso site measures compared with leg site measures. At 5 kHz, impedance was 44.1 ± 1.8 Ω and 211 ± 16 Ω for torso and leg sites, respectively. At 50 kHz, impedance was 33.4 ± 1.3 and 175 ± 12 Ω; at 200 kHz, 23.0 ± 1.0 and 148 ± 12 Ω; and at 500 kHz, 24.6 ± 2.8 and 132 ± 12 Ω.

During the course of our observations and measurements (total of 83 data sets) on seven horses, it was found that the stance of the horse had no effect on bioelectrical impedance measured at the leg or torso sites.

Blood and Plasma Characteristics

All measured plasma and plasma characteristics were normal: hematocrit 36 ± 2%, plasma protein concentration 61 ± 3 g/l, Na⁺ concentration 136 ± 1
mmol/l, K⁺ concentration 4.1 ± 0.2 mmol/l, Ca²⁺ concentration 1.26 ± 0.02 mmol/l, Cl⁻ concentration 99 ± 2 mmol/l, and glucose concentration 5.9 ± 0.4 mmol/l.

**Fluid Compartment Volumes**

Measured values for TBW, ECFV, and PV are presented in Table 1. When normalized for body mass, TBW MS was 0.677 ± 0.022 l/kg, ECFV was 0.253 ± 0.006 l/kg, ICFV was 0.356 ± 0.013, and PV was 0.040 ± 0.002 l/kg. There was no difference between TBW MS compared with TBW A (0.676 ± 0.023 l/kg).

**Linear Regression Analysis**

TBW was highly correlated to mass, height, and length (Table 2). Length was the single most powerful predictor of TBW. Using length and height together provided an excellent estimate of TBW, reducing the standard error of the estimate (SEE) by 25% compared with using mass alone. Using mass in addition to length and height provided no additional predictive power. Inclusion of bioelectrical impedance measured at 200 kHz in the regression analysis had no effect on the SEE of these euhydrated horses and did not increase the predictive power of the equations. Figure 2A shows that measured TBW MS agreed closely with calculated TBW (Eq. 2 with BIA, Table 2).

ECFV was also highly correlated to mass, height, and length (Table 3). As with TBW, length was the single most powerful predictor of ECFV. Using bioelectrical impedance measured at 5 kHz with length (Eq. 7, Table 3) resulted in a threefold decrease in the SEE. Similar results were obtained when using height and bioelectrical impedance measured at 5 kHz, with this correlation yielding the lowest SEE of 1.25 liters (Eq. 8, Table 3). There was no benefit from including mass into the regression analysis, nor from using a combination of length and height together. Calculated ECFV (Eq. 7 with BIA, Table 3) agreed closely with measured ECFV (Fig. 2B).

PV, a component of ECFV, was also highly correlated to mass, height, length, and bioelectrical impedance measured at 5 kHz (Table 4). Height was a stronger predictor of PV than was length, and the combination of height and bioelectrical impedance measured at 5 kHz yielded the lowest SEE (Eq. 13, Table 4). Calculated PV using height with impedance at 5 kHz (Eq. 13 with BIA, Table 4) closely matched measured PV (Fig. 2C). PV was also highly correlated to ECFV according to the relationship

\[
PV = -1.004 + (0.170 \times ECFV); \ n = 8, \ (16)
\]

\[r^2 = 0.917, \ P < 0.001, \ SEE = 2.08 \text{ liters}
\]

Body mass (kg) was highly correlated to length (in cm; Eq. 17), and slightly more so to the combination of length and height (in cm; Eq. 19). There was no improvement in the correlation with the addition of impedance measurements.

\[
\text{Mass} = -584.6 + (6.741 \times \text{liters}); \ n = 8, \ (17)
\]

\[r^2 = 0.989, \ P < 0.001, \ SEE = 15.3 \text{ kg}
\]

\[
\text{Mass} = -356.2 + (5.478 \times H); \ n = 8, \ (18)
\]

\[r^2 = 0.928, \ P < 0.001, \ SEE = 39.5 \text{ kg}
\]

\[
\text{Mass} = -637.0 - (1.638 \times H) + (8.654 \times \text{liters}); \ n = 8, \ r^2 = 0.992, \ SEE = 14.0 \text{ kg} \ (19)
\]
DISCUSSION

The present study simultaneously measured TBW, ECFV, and PV in a group of horses ranging in mass from 212 to 636 kg. Compartment volumes, normalized to the mass of the animal, were remarkably consistent over the range of body mass. Furthermore, the size of these volume compartments could be estimated with reasonably good accuracy by using dual-frequency BIA when horse length or height was used in the predictive equation. For ECFV and PV, using bioelectrical impedance measured at 5 kHz greatly improved the predictive estimates of these volumes compared with not using BIA measures.

Pilot Study

There was no difference in impedance readings between the stainless steel and carbon fiber electrodes. It was thought that that localized changes in skin temperature (and hence blood flow) may affect whole body bioelectrical impedance measurements; however, this has recently been disproved (9). Nonetheless, it is suggested that carbon fiber electrodes be used because 1) avoiding changes in skin temperature may increase comfort and acceptance with repeated use and 2) the fact that carbon fiber electrodes are soft and compliant makes them less likely to cause compression and discomfort on the legs.

The intrasubject variability of bioelectrical impedance measurements obtained from the torso were considerably reduced when leg measurements. This finding, along with the fact that the torso represents the main conductive cylinder in the body, indicates that the torso may be a preferred site for obtaining measures of bioelectrical impedance in large mammals. However, at this site, the difficulties include the need to shave the hair coat and ensuring good contact of the electrodes to the skin sites, particularly if the horse is moving around a bit. Therefore, the torso is not a preferred site.

On the leg sites, the choice of electrode placement above and below the knee or hock, although yielding reasonably good data, was also deemed not satisfactory for the following reasons. First, the interelectrode distance over the joint was variable from one time to the next and between horses. This variance was due to anatomical differences between horses and to the shape of the leg around the joints, which made it difficult to maintain the electrodes in the same position. Second, equine athletes, as do humans, often experience joint swelling and inflammation; use of a swollen or inflamed joint for BIA measurements could be a source of error. For ease of measurement and for avoidance of the knee and hock joints, it was concluded that placement of both electrodes should be a minimum of 5 cm above the knee or hock joint and that this placement be standardized, as described in MATERIALS AND METHODS.

Bioelectrical and morphometric data were analyzed by using a series of single and stepwise multiple linear regression analyses. Statistical analysis of the pilot study data indicated that length, height, and bioelectrical impedance at both 5 and 200 kHz were required to obtain the best prediction of body mass and estimated ECFV. Impedance measurements made at 50 and 500 kHz provided no additional power or accuracy to the predictive equations.

Full Study

Methodology. In contrast to previous studies that have utilized mass as an independent variable for estimating the volume of body fluid compartments in horses (see Ref. 2), the present study emphasizes the use of length and height for two important reasons. First, body mass is a dependent variable that changes in response to feeding and hydration state, and as such it can change substantially and rapidly with a time course that can be in the order of minutes. Therefore, the use of body mass may result in inaccurate estimates of “normal” compartment volumes, as has been shown in humans with fluid disorders (34). Second, length and height measures are constant, independent variables that can be accurately obtained by careful measurement using a measuring tape (length) and height-measuring stick.

Volume of fluid compartments. The present measures of TBW, ECFV, and PV agree well with those obtained in previous studies using various indicator dilution techniques (Table 5). TBW, ECFV, and PV, when normalized to body mass, were very consistent among horses over the >400-kg mass range of the present study. It is noteworthy that ECFVs obtained in the present study are similar to those reported by Kohn et al. (22), in which the horses were physically fit and not fasted before determination of ECFV. The higher values obtained by Spurlock et al. (36) may be due to the fact that NaSCN was infused in conjunction with antipyrine (to determine TBW), which interferes with the NaSCN determination and must be extracted before NaSCN concentrations can be quantified.

There is evidence in the literature that the volume of water compartments, expressed per kilogram body mass, varies with trained state and breed (Table 5). In longitudinal studies conducted in humans (8) and horses (28), as well as cross-sectional studies performed on horses (relatively untrained: present study and Ref. 23, PV = 39.4 ± 1.4 ml/kg; well trained: Ref. 24, PV = 46.8 ± 2.9 ml/kg), PV increases after several weeks of exercise training. It appears that TBW and PV are greater in hot-blooded horses (Thoroughbreds, Arabians, quarter horses, Standardbreds) compared with draft horses (21, 26). Furthermore, among horses studied by Marcilese et al. (26), Thoroughbred English racehorses had a markedly greater PV than did saddle horses, and both had a PV greater than that measured in the untrained horses in the present study. The PV data of the present study agree well with other studies of untrained horses (7). Some of the discrepancy among studies may also be due to differences in measurement and sampling techniques (17) and fasted state of the horse. In our lab, endurance-trained Thoroughbreds...
had a resting PV of 0.049 ± 0.003 l/kg (24), 10% higher than the horses in the present study.

Andrews et al. (1) appear to have been the first to use and report the D2O dilution space as a measure of TBW in horses, although earlier studies utilized tritiated water (11, 14). In contrast to previous studies that used oral administration of D2O in horses (1), the present study infused the D2O (mixed 50–50 with 0.9% NaCl) into the jugular vein to increase the rate of equilibration by bypassing gastric emptying and intestinal absorption. Accordingly, steady-state values of TBW were obtained within 120 min of infusion, compared with 3 h or more after oral administration. For unknown reasons, TBW values measured using tritiated water appear greater than those measured using D2O. The TBW reported in the present study agrees well with that of Spurlock (36) and Andrews et al. (1), in good agreement with TBW measured by direct analysis of carcass water content of ponies (33).

The predictive equations determined in the present study indicate that the volume of body fluid compartments may not scale simply as a function of body mass. Using the scaling factors for TBW (0.666 × body mass) and ECFV (0.222 × body mass) provided by Carlson (2) will result in an increasing probability of error as the mass of the horse decreases, yet these equations are reasonably accurate in the 350- to 550-kg range.

BIA. The results indicate that TBW and body mass can be accurately predicted in mature, euhydrated horses by using the variables length and height, without the need for bioelectrical impedance measurements. It stands to reason, therefore, that if these equations are used to estimate TBW in a dehydrated horse, then the value obtained will provide an estimate of what TBW should be in that horse in the euhydrated condition. Such an estimate will therefore overestimate TBW in the dehydrated horse. During the course of endurance rides, horses may lose up to 50 liters of water in 4–6 h (4, 15), yielding a magnitude of loss in TBW that is much greater than the SEE obtained with the regression equations of Table 2. Similar losses of TBW occur in response to the loop diuretic furosemide administration (18). It is highly likely, therefore, that such decreases in TBW would be manifest by a change in bioelectrical impedance measured at 200 kHz, suggesting that BIA could be used in conjunction with horse length and height to detect dehydration in horses. BIA may also be useful to assess the magnitude and rate of increase in PV and ECFV that occurs during exercise training (as noted above) and with heat acclimation (24). Although further research on horses needs to be conducted using BIA to determine changes in TBW and ECFV, this technique has been used successfully to assess hydration status in humans who have been administered furosemide (31).

Exercie, training, diuresis, and heat acclimation all result in simultaneous changes in hematocrit, plasma, and extracellular and intracellular fluid ion concentrations and volumes that may need to be considered when using BIA for assessing hydration status. Impedance to current flow is a function of the volume of the various body fluid compartments and the ion concentrations in those compartments; therefore, simultaneous changes in compartment volume and ion concentration may impair the accuracy of the technique. Because red blood cells are a tissue compartment and likely behave as the rest of the body’s cellular mass, it is not likely that changes in hematocrit during exercise or training will have an appreciable effect on BIA measurements.

The “gold standard” technique most used for the determination of TBW in horses has been the dilution...
of tritiated water (see Table 5). Because tritium is radioactive, it has not been used for the clinical determination of hydration status. The use of D₂O produces similar values of TBW as does tritiated water (Table 5), indicating that D₂O could be used in clinical testing; however, there are appreciable time constraints and costs associated with D₂O analysis. In contrast, a rapid and inexpensive determination of hydration status and body water compartmentation could be achieved by using BIA. Length or height, when used in conjunction with bioelectrical impedance measured at 5 kHz, improved the predictive accuracy of estimates of ECFV (Table 3) and PV (Table 4). The use of bioelectrical impedance measured at 5 kHz resulted in a three- to fourfold decrease in SEE for ECFV and PV. Length and height measurements yielded strong correlations because they reflect the distances through which current passes in cylindrical segments of the body. A similar degree of predictive accuracy has been reported in humans when using height squared (with intentional omission of body mass) as the sole morphometric variable (34, 35).

Conclusions

BIA is useful for improving the predictive accuracy for rapid, noninvasive estimation of ECFV and PV in mature, euhydrated, resting horses. For the clinician and researcher, BIA provides many advantages over dilution techniques, which are time consuming and invasive. Because body mass is not required as an input variable, BIA, together with measures of length and height, can be readily used in field and farm situations to assess hydration status. Ongoing research will determine the practical value of the BIA technique for estimating TBW, ECFV, and PV in dehydrated horses.

We acknowledge the assistance of Nelson Cole, Barry Cole, Gerry Finlay, and staff of the Veterinary Teaching Hospital. We thank Dr. Leslie Huber for providing veterinary assistance and consultation. We thank Dr. Andrew FM, Nadeau JA, Saabye L, and Saxton AM. Measurement of total body water content in horses, using deuterium oxide dilution. Am J Vet Res 58: 1060–1064, 1997.


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