Prediction of hydration status using multi-frequency bioelectrical impedance analysis during exercise and recovery in horses

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Abstract
The present study tested the hypothesis that multi-frequency bioelectrical impedance analysis (MFBIA) can be used to provide reasonable estimates of body mass, total body water (TBW), extracellular fluid volume (ECFV) and plasma volume (PV) at rest, during exercise-induced dehydration and subsequent recovery. Seven exercise-conditioned horses were administered indicators for measurement of resting TBW, ECFV and PV. MFBIA measurements at 24 frequencies between 5 and 280 kHz were obtained at rest, during prolonged submaximal exercise and for up to 13 h of recovery with food and water provided. Impedance–frequency response curves were described by a double-exponential decay equation from which coefficients were used, together with height and length, to generate predictive equations for estimating body mass, TBW, ECFV and PV. Predictive equations for body mass, ECFV and PV provided reasonable estimates of the parameter at rest and during exercise and recovery that were within 6% of absolute values determined using indicators. Despite the inherent error in estimating absolute volumes, the technique allowed accurate (within 1%) determination of the change in compartment volumes within individual horses over time. The number of frequencies at which impedance was measured could be reduced to seven without sacrificing the accuracy of the impedance–frequency relationships or the predictive equations – this enabled a 70% reduction in data-acquisition time (to ~35 s) for each MFBIA measurement series. It is concluded that MFBIA can be used in individual horses to track changes in compartmental hydration status resulting from dehydration and rehydration.

Keywords: indicator dilution technique; total body water; extracellular fluid volume; plasma volume; bioelectrical impedance analysis

Introduction
Exercise\(^1\), transportation\(^2\) and illness\(^3\) without adequate fluid replacement result in dehydration that can impair both performance and health\(^4\). Present techniques used to assess the hydration status of both humans and horses require blood or urine analysis and are invasive, expensive and time-consuming\(^5\). A rapid, non-invasive technique, bioelectrical impedance analysis (BIA) has been used to estimate total body water (TBW) and extracellular fluid volume (ECFV) in humans (for reviews see references 6 and 7) and horses\(^5\). In resting mammals, fluid volumes estimated from BIA models based on the electrical properties of a biological conductor are highly correlated to fluid volumes measured using indicator dilution techniques\(^5,8,9\). However, accurately measuring fluid volumes in individuals undergoing compartmental fluid shifts using BIA is more difficult\(^10\), but has been done with some success in exercising rats\(^11\).

For the purposes of BIA, the body is assumed to be a cylindrical conductor of uniform and constant ionic composition and temperature. A current can be applied to the body at one end, conducted through the body and measured at the other end. The change in voltage between the sending electrode and receiving electrode is measured. The volume of a conductor ($V$; ml) can be calculated as $V = \rho L^2 / Z$, where $\rho$ (Ω cm\(^{-1}\)) is the tissue resistivity, $L$ is the length (cm) of the conductor and $Z$ is the impedance (Ω). Impedance is the opposition of a conductor to the flow of...
an alternating electrical current, and is composed of resistance and reactance. Impedance (Z) is calculated as \( Z^2 = R^2 + X^2 \), where \( R \) is the resistance to current flow and \( X \) is the reactance.

The body, however, is not a uniform cylinder; it has multiple fluid compartments with different ionic properties. Therefore, in practice, BIA is calculated against other measurement techniques, e.g. indicator dilution, and a prediction equation is developed. It has been established that single- and dual-frequency BIA methods cannot be used to measure changes in body fluid compartment volumes\(^9\),\(^12\),\(^15\). Previous work in this laboratory\(^5\) indicated that dual-frequency BIA (DFBIA) can be used to estimate plasma volume (PV) and ECFV in horses at rest, but is not able to track changes in hydration status within individual horses during furosemide-induced dehydration. However, measurements of impedance or reactance at multiple frequencies provide sufficient information with which to create predictive equations\(^14\),\(^16\).

Multi-frequency BIA (MFBIA) is based on the principle that fluids and tissues have different electrical conducting properties that affect the path of an electrical current applied to the body at different frequencies. At low frequencies (<55 kHz), the current does not penetrate cell membranes (which act as an imperfect capacitor) and is conducted through the extracellular fluids. At high frequencies (>140 kHz) the current penetrates cell membranes and, as a result, the current is conducted through all tissues of the body and thus reflects TBW\(^8\).\(^15\). MFBIA measures impedance over a range of frequencies and impedance–frequency response curves can be developed and analysed to assess changes in body fluid compartment volumes\(^8\). MFBIA has been used to quantify fluid shifts between extra- and intracellular fluid compartments by several investigators\(^9\),\(^11\),\(^15\),\(^17\).

It is hypothesized that changes in fluid compartment volumes will cause a change in MFBIA impedance–frequency plots of 24 frequencies between 5 and 280 kHz that can be used to develop predictive equations. It is further hypothesized that these predictive equations can be used to estimate the fluid compartment volumes accurately (as they change) in horses during prolonged exercise and recovery.

**Materials and methods**

**Animals**

Seven horses from the University of Guelph herd were used in the study. The characteristics, feeding, training of these horses, indicator dilution, exercise and recovery procedures are described in the companion paper\(^18\), and relevant parameters are given in Table 1. Body length (\( L \)) was measured on the left side of the horse with a measuring tape 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. Height (\( H \)) 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. The coefficients of variation for \( L \) and \( H \) for duplicate measurements performed independently by two different individuals were 0.78%. Body mass was measured with a large animal scale (0.5 kg; Kitchener Scales Ltd, Kitchener, ON, Canada).

**MFBIA**

MFBIA measurements were taken as described by Forro et al.\(^5\). The hair coat was clipped short (to about 2 mm hair length) on the lateral surfaces of the left forelimb and hind limb above the knee and hock respectively, where the electrodes were attached. These sites were cleaned with water to remove dirt and then dried using gauze pads. Conductive paste was rubbed into the area where the electrodes would sit to ensure good conduction. Pairs of 10 cm\(^2\) carbon fibre electrodes (Equistat Ltd, Douglas, Isle of Man, UK) were placed on each of the prepared legs. On the forelimb, the electrode pair (10 cm between centres) was situated below the elbow and above the knee joint on the lateral portion of the radius directly over the common digital extensor, ulnaris lateralis, and radial carpal extensor muscles. On the hind limb, the electrode pair (10 cm between centres) was placed on the tibia directly over the long digital extensor and lateral digital extensor muscles. The electrodes were held in place using a cuff secured by Velcro straps. Shielded leads connected the electrodes to

<table>
<thead>
<tr>
<th>Horse</th>
<th>Breed</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Length (cm)</th>
<th>Body mass (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Thoroughbred</td>
<td>12</td>
<td>155.5</td>
<td>148.6</td>
<td>472.1</td>
</tr>
<tr>
<td>2</td>
<td>Percheron cross</td>
<td>4</td>
<td>153.7</td>
<td>156.2</td>
<td>513.1</td>
</tr>
<tr>
<td>3</td>
<td>Standardbred</td>
<td>12</td>
<td>152.4</td>
<td>153.0</td>
<td>511.4</td>
</tr>
<tr>
<td>4</td>
<td>Standardbred</td>
<td>13</td>
<td>148.0</td>
<td>147.3</td>
<td>443.2</td>
</tr>
<tr>
<td>5</td>
<td>Appaloosa</td>
<td>6</td>
<td>153.7</td>
<td>154.9</td>
<td>501.0</td>
</tr>
<tr>
<td>6</td>
<td>Standardbred</td>
<td>14</td>
<td>156.2</td>
<td>150.5</td>
<td>492.1</td>
</tr>
<tr>
<td>7</td>
<td>Standardbred</td>
<td>10</td>
<td>147.3</td>
<td>148.6</td>
<td>438.9</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>10</td>
<td>152.4</td>
<td>151.3</td>
<td>481.7</td>
</tr>
<tr>
<td>Standard error</td>
<td></td>
<td>1.4</td>
<td>1.3</td>
<td>1.3</td>
<td>11.7</td>
</tr>
</tbody>
</table>
Development of a model for predicting fluid volumes using MFBIA

The relationship between impedance and frequency was described using a second-order polynomial in the form of a double-exponential decay, generating equations of the form:

\[ y = ae^{-bx} + ce^{-dx}, \]

where \( y \) is impedance, \( x \) is frequency, \( a \) and \( c \) are the amplitude of the first and second exponential, respectively, and \( b \) and \( d \) are the rate constant of the first and second exponential, respectively. These relationships were determined for each horse at each time point, and the coefficients \( (a, b, c, d) \) used as parameters together with morphometric measurements \( (L^2, H^2) \) and measured or calculated body fluid volumes (see Lindinger et al.\(^{18}\)) to predict ECFV, TBW, PV and body mass using multiple linear regression analysis. While the shape of the human ‘cylinder’ is adequately described using stature \( (H^2) \), this is not the case for horses and inclusion of the second term, \( L^2 \), better described the shape of the horse and improved predictability. Pearson correlations and backward stepwise regression were used to ensure that these variables contributed to the equations used to predict TBW, ECFV, PV and body mass.

A limitation of the 24-frequency MFBIA approach was that the horse had to remain motionless for 2 min, which was difficult at times, in order to acquire the impedance data. Small adjustments of posture could disrupt the electrode–skin interface, compromising the data. We therefore searched for and selected seven frequencies that yielded double-exponential regression coefficients that were not significantly different from those obtained using 24 frequencies. Using seven frequencies reduced the data collection period to \( \sim 35 \) s. The four lowest frequencies (5, 16, 24 and 50 kHz) represent the steep (second-order) portion of the curve and extracellular conductance (defined primarily by coefficients \( c \) and \( d \)); the three higher frequencies (140, 200 and 280 kHz) represent the shallow (first-order) portion of the curve and whole-body conductance (defined primarily by coefficients \( a \) and \( b \)).

Statistics

Data are presented as mean ± standard error. Changes over time were assessed by one-way repeated-measures analysis of variance (ANOVA). When a significant \( F \)-ratio was obtained, means were compared using the all-pairwise multiple comparison procedure of Holm-Sidak. Comparisons of coefficients using the 24-frequency (full) and seven-frequency (reduced) models were compared using two-way repeated-measures ANOVA for model and time. Statistical significance was accepted when \( P \leq 0.05 \) at a power of 0.8.

Results

Impedance versus frequency relationships

Double-exponential regression analysis of the impedance–frequency response curves yielded equations having regression coefficient \( (r^2) \) that ranged from 0.996 to 1.000 with a mean of 1.000. At all time points, this relationship was very well described by a double-exponential decay equation when expressed as means for the group (Fig. 1a) or for individuals (Fig. 1b). The data for 60 and 240 min of recovery are not shown in Fig. 1a for the purpose of providing a clear presentation of the data; the positions of these two relationships relative to the others are represented by the data provided in Fig. 1b. Exercise resulted in a significant upward shift of the impedance–frequency relationship, and this upward shift was still evident 120 min post-exercise. At 1350 min (13 h) of recovery (18 h time point), the relationship had shifted downwards, significantly below the pre-exercise curve. Accordingly, all coefficients of the double-exponential decay equations changed significantly with time (Fig. 2). At each time point there were no differences between coefficients determined using the 24- and the seven-frequency models.

Predictive equations

Multiple linear regression analysis was used to generate a series of predictive equations. Predictive
equations using the morphometric parameters height squared \((H^2)\) and length squared \((L^2)\) only had poorer correlation coefficient and larger standard error of the estimate (SEE) than those generated using all of the four impedance–frequency curve coefficients. Also, the use of only pre-exercise data yielded poorer correlations than when exercise and recovery data were included. Resistance extrapolated to 0 frequency (termed \(R_2\) by Cole and Cole\(^{14}\)) was computed using both second- and third-order polynomial equations and these were also used to calculate ECFV. Using \(R_2\) with \(H^2\) and \(L^2\) also yielded poorer correlations than when the four impedance–frequency curve coefficients were used. Therefore, the form of the final predictive equation was:

\[
V = \chi + \eta H^2 + \lambda L^2 + \alpha a + \beta b + \gamma c + \delta d
\]

where \(V\) is compartment volume (l) or body mass (kg) and the Greek letter symbols are constants. The equations developed using the 24-frequency (full) model and seven-frequency (reduced) model were not significantly different, with very similar regression parameters (Table 2). The source of variability was animals, as within-animal responses were highly consistent as typified in Fig. 1b. Within-animal estimates of repeatability (test–retest reliability) were obtained by calculating predicted compartment volumes using each of the two to four impedance–frequency relationships obtained for each animal at each time point. Values at each time point, for each individual, were within 1% of each other.

Linear regression analysis was used to compare measured versus predicted volumes and body mass using both the full and reduced models (Figs 3 and 4, Table 3). The slopes of the regression lines were all less than 1, indicating that the prediction equations, on average, underestimated fluid compartment volumes and body mass. The poorest correlation was for TBW, for which data were limited to four time points: pre-exercise (PE), exercise (ER and R20) and 13 h of recovery (18 h time point). Despite statistical agreement, a physiologically meaningful estimate of TBW could not be obtained during early recovery when horses were eating and drinking. The correlations for PV, ECFV and body mass were similarly strong, indicating that the equations could be used to predict changes over time.

The ability of the predictive equations to estimate body mass ECFV and PV is demonstrated in Fig. 5. Values for individual horses were calculated using the predictive equations, averaged for each time point, and plotted alongside the ‘measured’ values. Only the predicted values calculated using the reduced model are shown for clarity - values computed using full and reduced models were nearly identical. Two-way, repeated-measures ANOVA was used to compare ‘measured’ and reduced model data with respect to treatment and time. The reduced model data were not different from those measured at any time point and there were significant changes only over time. The time courses of PV and ECFV during exercise and recovery were very well determined using data generated using the predictive equations (Fig. 5a and 5b). The time course of response for body mass shows that the predictive model underestimated the decrease in body mass during the period of exercise, and underestimated the increase in body mass during early recovery (Fig. 5c).
Discussion

In the present study, double-exponential regression analysis of the impedance–frequency response curve showed a significant change in impedance, at all frequencies, with changing fluid volumes and body mass. Therefore, using MFBIA and morphometric data, predictive equations were developed to estimate ECFV, PV, TBW and body mass in individual horses at rest and during exercise and recovery. Importantly, the time course of changes in fluid volumes and body mass within individuals was quantified. Using MFBIA, estimates of absolute compartment volumes may be in error by up to 6%. However, within-animal changes in compartmental hydration status were estimated with an accuracy of less than 1% at each time point.

Table 2. Summary of multiple linear regression results for equations used to generate predictive equations that can be used to estimate body mass, total body water (TBW), extracellular fluid volume (ECFV) and plasma volume (PV). Full model refers to the equation generated using multi-frequency bioelectrical impedance analysis (MFBIA) data from 24 frequencies; reduced model refers to the equations generated using MFBIA data from seven frequencies. See text for the form of the equation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Model</th>
<th>$r^2$</th>
<th>P-value</th>
<th>SEE (kg)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (kg)</td>
<td>Full</td>
<td>0.900</td>
<td>$&lt;0.001$</td>
<td>10.70</td>
<td>2.2</td>
</tr>
<tr>
<td>Reduced</td>
<td>0.902</td>
<td>$&lt;0.001$</td>
<td>10.05</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>TBW (l)</td>
<td>Full</td>
<td>0.511</td>
<td>$&lt;0.001$</td>
<td>14.80</td>
<td>4.7</td>
</tr>
<tr>
<td>Reduced</td>
<td>0.478</td>
<td>$&lt;0.001$</td>
<td>14.96</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td>ECFV (l)</td>
<td>Full</td>
<td>0.801</td>
<td>$&lt;0.001$</td>
<td>6.652</td>
<td>5.6</td>
</tr>
<tr>
<td>Reduced</td>
<td>0.895</td>
<td>$&lt;0.001$</td>
<td>6.378</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>PV (l)</td>
<td>Full</td>
<td>0.700</td>
<td>$&lt;0.001$</td>
<td>1.223</td>
<td>5.6</td>
</tr>
<tr>
<td>Reduced</td>
<td>0.701</td>
<td>$&lt;0.001$</td>
<td>1.261</td>
<td>5.7</td>
<td></td>
</tr>
</tbody>
</table>

$r^2$ – regression coefficient; SEE – standard error of the estimate; % – SEE expressed as a percentage of the initial parameter.

Development of the present model

Researchers have used various mathematical approaches for using MFBIA data to estimate body fluid volumes (for review see De Lorenzo et al.6). One of the first developed and most widely used methods is the Cole–Cole model14, which uses resistance and reactance measured at different frequencies to determine ECFV and TBW. The Cole–Cole model predicts ECFV using the resistance extrapolated to 0 frequency (termed R2), and TBW is predicted using
resistance extrapolated to infinite frequency ($R_\infty$) or the impedance measured at maximal reactance. According to Schoeller, who performed a detailed comparison of several models, the Cole–Cole model ‘measures’ ECFV and intracellular fluid volume (ICFV), where the high-frequency resistance is a function of both ECFV and ICFV. The more recently proposed Siconolfi model fits impedance measured at specific frequencies to a third-order polynomial equation. These authors utilized the total circuit resistance (RT), defined as the resistance at the point where impedance changes by 1% for a 25 kHz change in frequency (the impedance plateau of an impedance–frequency response curve), to calculate TBW. The point at which the third-order polynomial intersects the resistance axis at 0 frequency is $R_2$ and is used to calculate ECFV. However, impedance–frequency plots are not third-order polynomials even though a third-order polynomial equation can be used to describe the data (Fig. 6; Ward et al.). Depending on the instrumentation used by the researcher, a disadvantage of the Cole–Cole model is that measurements of resistance and phase angle are required; however, some BIA instruments do not measure phase angle. In the present study we used an MFBIA instrument that measures resistance and

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**Figure 3** Linear regression graphs of measured versus predicted plasma volume (PV) (a, b) and measured versus predicted extracellular fluid volume (ECFV) (c, d). The data obtained using the full 24-frequency model are shown in (a, c), the reduced seven-frequency model in (b, d). The solid regression line has been forced through 0; the dashed lines show the 95% confidence interval. Regression parameters are provided in Table 3.
FIG. 4 Linear regression graphs of measured versus predicted total body water (TBW) (a, b) and measured versus predicted body mass (c, d). The data obtained using the full 24-frequency model are shown in (a, c), the reduced seven-frequency model in (b, d). The solid regression line has been forced through 0; the dashed lines show the 95% confidence interval. Regression parameters are provided in Table 3.

Table 3 Summary of results of linear regression equations comparing 'measured' versus predicted body mass, total body water (TBW), extracellular fluid volume (ECFV) and plasma volume (PV). Full model refers to the equation generated using multi-frequency bioelectrical impedance analysis (MFBIA) data from 24 frequencies; reduced model refers to the equations generated using MFBIA data from seven frequencies.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Model</th>
<th>Slope</th>
<th>( r^2 )</th>
<th>( P )-value</th>
<th>SEE</th>
<th>( % )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (kg)</td>
<td>Full</td>
<td>0.900</td>
<td>0.900</td>
<td>&lt;0.001</td>
<td>9.122</td>
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<tr>
<td></td>
<td>Reduced</td>
<td>0.982</td>
<td>0.870</td>
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<td>11.60</td>
<td>2.4</td>
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<tr>
<td>TBW (l)</td>
<td>Full</td>
<td>0.506</td>
<td>0.507</td>
<td>&lt;0.001</td>
<td>9.645</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>Reduced</td>
<td>0.415</td>
<td>0.319</td>
<td>&lt;0.001</td>
<td>11.71</td>
<td>3.8</td>
</tr>
<tr>
<td>ECFV (l)</td>
<td>Full</td>
<td>0.801</td>
<td>0.801</td>
<td>&lt;0.001</td>
<td>5.613</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>Reduced</td>
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<td>0.798</td>
<td>&lt;0.001</td>
<td>5.709</td>
<td>4.8</td>
</tr>
<tr>
<td>PV (l)</td>
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<td>0.703</td>
<td>&lt;0.001</td>
<td>0.962</td>
<td>4.4</td>
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<tr>
<td></td>
<td>Reduced</td>
<td>0.861</td>
<td>0.705</td>
<td>&lt;0.001</td>
<td>0.871</td>
<td>4.4</td>
</tr>
</tbody>
</table>

\( r^2 \) – regression coefficient; SEE – standard error of the estimate; \( \% \) – SEE expressed as a percentage of the initial parameter. * Not forced through 0.
calculates impedance at 24 discrete frequencies between 1 and 280 kHz. The present study proposes an alternative approach, based on the Siconolfi and Cole–Cole models, that does not require measurement of phase angle.

Errors inherent in using the third-order polynomial model of Siconolfi are that the high-frequency portion of the curve falls increasingly below measured impedance, and the slope of the low-frequency portion of the curve may be too steep, yielding overestimates of R2 compared with other methods. Also, using Siconolfi’s definition of RT, RT could not be computed with the present data without extrapolating to very high frequencies – this may be a function of the large body mass of the horse. Impedance–frequency plots used by us22 (and present study) and others16 are better fitted by a second-order polynomial in the form of a double-exponential decay (present study) or by a sixth-order polynomial16. Ward et al.16, using non-linear regression data-fitting software, fitted impedance data collected at 496 discrete frequencies to progressively higher-order polynomials to find the best fit. A best fit was obtained using a sixth-order inverse polynomial, with no improvement using higher-order polynomials. When we tried this approach with the present data (see Fig. 6 for example), there was no improvement in curve description using polynomials greater than a second order, when utilized in the form of a double-exponential decay (adjusted $r^2 = 0.999$), than with a sixth-order polynomial (adjusted $r^2 = 0.999$). The adjusted $r^2$ obtained with a third-order polynomial was 0.997.

In the present study, impedance–frequency response curves (at 24 frequencies) obtained for each horse at all time points were well fitted (mean $r^2 = 1.000$, range 0.996–1.000) by a second-order exponential in the form of a double-exponential decay over the frequency range 5–280 kHz (Fig. 1b). According to terms of the double-exponential

![Figure 5](image_url)  
**Fig. 5** The time course of 'measured' (■) and predicted (●) body mass (a), extracellular fluid volume (ECFV) (b) and plasma volume (PV) (c). Data are mean ± standard error, n = 7. There are no differences at any time point between measured and predicted values. *Significantly different from 120 min (pre-exercise)*

![Figure 6](image_url)  
**Fig. 6** Impedance–frequency response curves for one horse pre-exercise (●) showing fits obtained using a second-order polynomial in the form of a double-exponential decay (dashed line), a third-order polynomial (dotted line) and a sixth-order polynomial (solid line). Note how the third-order polynomial line deviates most from the data. 

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equation, the portion of the equation containing coefficients $c$ and $d$ contributes little to the overall equation after about 140 kHz and is approximately 0 at frequencies > 280 kHz. The quality of the fit was maintained over time despite upward or downward shifts of the curve attributable to changes in fluid compartment volumes (Fig. 1), and this was maintained (mean $r^2 = 1.000$) when the seven frequencies for the reduced model (5, 16, 24, 50, 140, 200 and 280 kHz) were used.

When analysing MFBIA data obtained in pilot studies, we observed that after a furosemide-induced dehydration a given horse could have similar impedances at 5 and 280 kHz before and after dehydration, but the shape of the impedance–frequency response curves was different. This is exemplified in Fig. 7, where 30% decreases in parameters $b$ and $d$ (the rate constant of the first and second exponential, respectively) are imposed with $a$ and $c$ (the amplitude of the first and second exponential, respectively) held constant. This observation led us to utilize the parameters that described the curve, as opposed to just $R^2$ and impedance at 280 kHz.

**Ability of the present model to predict changes in fluid volumes**

Despite the ability to describe the impedance–frequency response curves precisely, and the fact that these curves shifted with changes in fluid volumes within horses over time, the volumes of fluid compartments were not predicted as accurately as expected. Plasma volume, ECFV and body mass were predicted accurately ($r^2 > 0.7$, $P < 0.001$), while TBW was not (Table 3). An important limitation for TBW was that TBW could not be estimated during the recovery period because gains in body mass due to simultaneous intake of food and water during this period are not indicative of gains in TBW. While the pre-exercise time point for TBW was measured, those obtained during the exercise period and at the 18th time point were estimated from changes in body mass. The present results point to the need to obtain improved estimates of TBW following the initial direct measurement using indicator dilution techniques.

The ability of MFBIA to predict the changes in PV and ECFV was very good on average (Fig. 5a and 5b). However, for individual horses, the predicted value varied from the ‘measured’ value by as much as 121 at some time points (Figs 3 and 4). This variation may be due to variations inherent in both the calculation of the ‘measured’ value (see Lindinger et al. 18) and the parameters that go into development of the predictive equations. This variation lies primarily between animals as described by the SEE terms provided in Table 2. However, the within-animal estimates were in fact highly consistent as exemplified in Fig. 1b. Because it is not practical to develop a series of predictive equations for each individual horse, using predictive equations derived from data using these seven horses produced sufficient variability to put the absolute volume of a fluid compartment in error by up to 6% (Table 2). Nevertheless, and very importantly with respect to the practical application of the technology, the absolute change in compartment volume within horses at each time point can be determined accurately (within 1%; as exemplified in Figs 2 and 5), something that was previously not achievable. Therefore the instrumentation is capable of accurately assessing the time course of changes in hydration status, although the absolute (initial) volume may be in error by up to 6%.

The change in body mass predicted by MFBIA could be used to assess hydrated state; however, the predictability of body mass was not as good as that for ECFV. In particular, the present MFBIA model underestimated by 50% the decrease in body mass that occurred.

![Figure 7](image-url)
during the period of exercise (Fig. 5c). It appears that the MFPIA technique for predicting body mass is not sufficiently sensitive to detect the rapid decreases in PV and ECFV that occur during exercise, but these appear to be detected early in recovery (400 min time point). In early recovery, the initial rapid increase in body mass is poorly predicted, but this is probably due to the fact that this early gain represents gut fill and not increases in body fluid compartment volumes per se. Body mass, when considered as an MFPIA compartment, is more complex than ECFV or TBW because it is a function of dry mass of all of the tissues, the hydrated state of these tissues and their electrical properties (which also change with changes in hydrated state).

Usefulness of the model
The ability of MFPIA to predict shifts of fluid within body fluid compartments in horses over time is consistent with other MFPIA studies in rats and humans. Also, previous studies using single-, dual- or triple-frequency BIA were not able to determine changes in fluid volumes. It therefore appears that MFPIA approaches that measure impedance using at least seven different frequencies are required accurately to determine changes in fluid compartment volumes within individual animals.

The present MFPIA model improves the estimation of fluid compartment volumes in horses compared with attempts using the DFBIA model of Forro et al. The present model can be used to assess the hydrated state of individual horses; however, there remains considerable scope for improving the ability to predict absolute compartmental volumes accurately. In the present study, the accuracy is partially impaired by the limited number of horses used, and perhaps by the quality of the exercise-induced dehydration and subsequent recovery. The reliability and accuracy of the predictive equations based on MFPIA data are dependent on the quality of the measured ECFV, PV, TBW and impedance values. The time course of change of fluid compartment volumes and ionic composition during exercise and recovery is complex. It is possible that, in the present study, the indicators may not have accurately tracked temporal changes in body fluid volumes. Skin temperature and ion concentrations within fluid compartments are factors known to affect the frequency–impedance relationship. A tetra-polar electrode configuration, as used in the present study, negates the effect of changes in skin temperature on impedance.

A fundamental precept of BIA is that electrical conductivity depends on the volume and ionic composition of the conductor. Therefore, accurate prediction of the volume of a fluid compartment requires that electrolyte concentrations, i.e. conductor resistivity, remain constant. Rees et al. found that, in saline-infused rats, substantial increases (>10%) in plasma [Na$^+$] and [Cl$^-$] are required to produce statistically significant decreases in conductor resistivity and impedance. In the present study, changes in plasma ion concentrations were not sufficiently large (data not shown) to have produced statistically meaningful changes in measured impedance, based on the findings of Rees et al. None the less, smaller changes in ion concentrations may have contributed to the observed variability.

Additional studies using different exercise intensities and durations, and furosemide-induced dehydration with subsequent rehydration, with consideration of fitness and reproductive state, are needed to determine the utility of this approach in the long term. The results of the present study show that accurate estimates of changes in compartmental fluid volumes can be obtained rapidly and repeatedly using the present MFPIA approach.

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