Hydration of exercised Standardbred racehorses assessed noninvasively using multi-frequency bioelectrical impedance analysis

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Summary

Reasons for performing study: In human and animal clinical practice, multi-frequency bioelectrical impedance analysis (MF-BIA) is increasingly used as a diagnostic tool to assess hydration of intra-and extracellular fluid compartments. Accurate determination of changes in hydration status within individuals over time has remained problematic due to the requirement for complete impedance-frequency relationships at the time points of interest.

Objectives: To use MF-BIA in 13 Standardbred racehorses and 7 ‘endurance’ research horses to determine if MF-BIA could be used to track changes in total body water (TBW), intracellular fluid volume (ICFV) and extracellular fluid volume (ECFV) resulting from exercise.

Methods: Jugular venous blood was sampled at rest and for 2–13 h following exercise. TBW, ECFV and plasma volume (PV) were measured at rest using indicator dilution techniques (D2O, thiocyanate and Evans Blue, respectively). TBW, ECFV, ICFV and PV were correlated to impedance measures and predictive equations used to determine hydration status from MF-BIA measures.

Results: TBW loss continued throughout the recovery period, and was primarily borne by the ECF compartment at 90 mins of recovery.

Conclusions: MF-BIA predictions of compartmental hydration status were significantly correlated to measured/calculated decreases in these compartments.

Potential relevance: Practical applications for MF-BIA in horses include monitoring of hydration status during transport and competition, assessment of body composition, clinical health assessment and critical care management.

Introduction

Exercise (Naylor et al. 1993), transportation (Friend 2000) and illness (Carlson 1997) can result in dehydration detrimental to equine health and performance (Maughan and Lindinger 1995). Current assessment of hydration remains difficult due to the need for indirect determination of compartment fluid volumes using indicator dilution techniques. These ‘gold standard’ techniques for evaluating fluid and hydration status are invasive, expensive and time-consuming (Forro et al. 2000). There is a need for a rapid, accurate, portable technique for noninvasively assessing changes in body fluid compartments in field and clinical situations. Bioelectrical impedance analysis (BIA) has the potential to provide such a technique, and has been used to estimate total body water (TBW) and extracellular fluid volume (ECFV) in man (Buchholz et al. 2004; Kyle et al. 2004) and horses (Forro et al. 2000; McKeen and Lindinger 2004; Fielding et al. 2004).

Multi-frequency BIA (MF-BIA) 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 (<5 kHz) the current does not penetrate cell membranes and therefore conducts through the extracellular fluids. At high frequencies (>140 kHz) the current is conducted through all tissues and therefore reflects TBW. MF-BIA measures electrical impedance over a range of logarithmically spaced frequencies, and predictive equations for compartmental fluid volumes can be developed based on the resulting impedance-frequency response curves.

MF-BIA has been shown to accurately quantify volume changes in fluid compartments in man (Gudivaka et al. 1999; Pialoux et al. 2004) and rats (Cornish et al. 2001). However, studies of acute changes in compartmental fluid volumes using horses are rare (McKeen and Lindinger 2004). We have previously shown that MF-BIA can be used to estimate body fluid volumes in individual horses, both at rest and during prolonged submaximal exercise (McKeen and Lindinger 2004). However, there remains a need to determine the practical utility of the MF-BIA approach in the long term, using horses exercising at different intensities and durations, with consideration of training state.

Standardbred racehorses in training undergo substantial fluid shifts and dehydration (Waller and Lindinger 2005) and therefore could potentially benefit from rapid monitoring of hydration status by MF-BIA. The purposes of the present study were: (a) to apply MF-BIA to Standardbred racehorses in training, to assess the ability of MF-BIA to predict the TBW, PV, ECFV and ICFV losses of high intensity exercise; (b) to further develop the ability of MF-BIA to predict fluid volumes in resting horses and in horses recovering from exercise-induced dehydration. The impedance-frequency responses will be compared to those seen with endurance-type exercise (McKeen and Lindinger 2004). It is hypothesised that the predictive equations developed by McKeen...

Materials and methods

Animals

Thirteen Standardbred racehorses in racing condition were used (Waller and Lindinger 2005). Horses were fed a diet consisting of mixed grass hay twice daily, and grain 3 times daily (Phase Three pellets), with free access to water. Horses were housed in individual box stalls, and trained 5–6 days/week with occasional turnout in grassy paddocks. The animal care and use procedures were approved by the University of Guelph Animal Care Committee and performed in accordance with the guidelines of the Canadian Council on Animal Care.

Horses were fed 3 flakes of hay and 2 kg of grain on the evening prior to the start of the experiment, and fed 2 kg of grain the morning of the experiment. Blood sampling commenced approximately 2 h after the morning meal, and no further food or water provided until the end of the sampling period (approximately 5 h). Body mass was measured with a large-animal scale (0.5 kg KSL Scales). The catheterisation, indicator infusion and blood sampling procedures are detailed in a prior paper reporting on the fluid shift and electrolyte responses of these horses (Waller and Lindinger 2005).

Experimental protocol

After fitting the horse with bilateral jugular vein catheters and infusion of fluid volume indicators, bioelectrical impedance readings were obtained while the horse was standing at rest in stocks. Two resting time points were obtained approximately 1 h apart, each in duplicate or triplicate such that very similar impedance values were obtained with subsequent readings. After the end of the indicator sampling period, the horse was fitted with tack, the training buggy attached, and was then exercised on a 0.5 mile, groomed, stone dust track. Two bouts of exercise separated by 45 mins were performed. The first consisted of a warm-up (1 mile of walking then 2.5 miles of jogging at submaximal intensity), 1 mile of maximum intensity trotting/pacing and a brief cool-down (1 mile of submaximal jogging). The horse was then rested in its stall (no access to food or water at any time) for ~45 mins before the second bout. The second bout consisted of a warm-up (1 mile walk and a 1 mile jog), 1 mile maximum intensity trotting/pacing and a brief cool-down (5–10 mins walk on the track). Horses were hosed liberally with cool water and hair coat scraped ~30 mins after exercise.

Post exercise BIA readings were obtained in the stocks approximately 100 mins after completion of exercise, at a time when horses were no longer sweating. Ambient temperature for the exercise and recovery periods ranged from 9–11°C for the first 9 horses studied, and 4–5°C for the last 4 horses studied.

Bioelectrical impedance analysis

MF-BIA measurements were taken as described previously (Forro et al. 2000; McKeen and Lindinger 2004). The hair coat was clipped short (to about 2 mm hair length) on the lateral surfaces of the left fore- and hindlimb 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 cm² carbon fibre electrodes 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 hindlimb, 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 the MF-BIA instrument. MF-BIA was performed using a tetra-polar arrangement in which an 800 µA alternating current was applied along the length of the body using the 2 distal electrodes, and voltage drop measured by the 2 proximal electrodes. There is negligible effect of changes in skin temperature and sweating when using a tetra-polar electrode configuration for acquisition of BIA data (Cornish et al. 1998). The instrument recorded impedance (Z) in ohms at 7 frequencies: 5, 16, 24, 50, 140, 200 and 280 kHz. Measurements were repeated, without removing the electrodes, until identical impedance values were obtained at least twice in sequence (coefficients a, b, c and d (see below) had coefficients of variation (CVs) of less than 0.05%); each sequence required approximately 2 minsto obtain. Occasionally, when a horse moved a limb, one or more electrodes had to be adjusted in order to re-establish good contact between skin and electrodes and obtain a repeatable measurement series.

Blood analysis

Analysis of blood for the plasma concentrations of fluid volume indicators for these horses has been previously described (Waller and Lindinger 2005). Briefly, plasma was analysed for Evans Blue concentration (CV 1.3%) via the dual-wavelength method (Flodager and Blomqvist 1991) by use of a spectrophotometer. Plasma NaSCN concentration was measured (CV 2.1%) spectrophotometrically by a microvolume modification (Forro et al. 2000) of the method described by Chatterjee et al. (1988). Analysis of plasma D₂O concentration (CV 1.1%) was performed by Metabolic Solutions.

Calculations

TBW at rest was calculated from plasma D₂O concentrations as described previously (Forro et al. 2000; McKeen and Lindinger 2004). TBW at the end of exercise and at 90 mins of recovery

### TABLE 1: Characteristics of horses used in the study.

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>Bwt (kg)</th>
<th>TBW (l)</th>
<th>ICFV (l)</th>
<th>ECFV (l)</th>
<th>PV (l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>3.3</td>
<td>481.7</td>
<td>311.9</td>
<td>189.4</td>
<td>122.5</td>
</tr>
<tr>
<td>s.d.</td>
<td>0.9</td>
<td>47.0</td>
<td>30.2</td>
<td>21.5</td>
<td>11.5</td>
</tr>
<tr>
<td>s.e.</td>
<td>0.2</td>
<td>13.0</td>
<td>8.4</td>
<td>6.0</td>
<td>3.2</td>
</tr>
<tr>
<td>Median</td>
<td>3.0</td>
<td>148.6</td>
<td>92.1</td>
<td>65.8</td>
<td>35.0</td>
</tr>
<tr>
<td>Range</td>
<td>2.0</td>
<td>148.6</td>
<td>92.1</td>
<td>65.8</td>
<td>35.0</td>
</tr>
</tbody>
</table>

Values are for n = 13 Standardbred horses; 8 geldings, 4 mares, 1 stallion.
was calculated from the decrease in bodyweight after fecal and urine losses were accounted for, (by subsequent re-weighing), such that:

\[ \text{TBW}_t = \text{TBW}_i - (\text{bwit} - \text{bwtt} + \text{M}_{\text{urine}} + \text{M}_{\text{faeces}}) \]

where TBW_i and TBW_t are initial total body water, and total body water at time t, respectively, and bwit = initial bodyweight, bwtt = bodyweight at time t, M_{urine} = mass of urine lost, and M_{faeces} = mass of faeces lost. M_{faeces} was determined by collection and weighing of all faecal output during the course of the study. M_{urine} was determined by weighing the horses immediately before and after they were prompted to void in their stalls.

PV and ECFV before exercise were calculated using plasma Evans Blue and thiocyanate, respectively, dilution curves as described previously (Forro et al. 2000; Lindinger et al. 2005). The change in PV was calculated from plasma protein concentration as:

\[ \Delta \text{PV} = (P_f - P_i)/P_i \]

where P_i and P_f are initial and final plasma [protein], respectively. The final (postexercise) PV was calculated as:

\[ \text{PV}_f = \text{PV}_i + (\Delta \text{PV} \times \text{PV}_i) \]

ECFV at time t was calculated from the changes in PV, such that:

\[ \text{ECFV}_t = \text{ECFV}_0 + (\Delta \text{PV} \times \text{ECFV}_0) \]

ICFV was calculated as the difference between TBW and ECFV.

**Development of a model for predicting fluid volumes using MF-BIA**

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:

\[ y = a \cdot e^{bx} + c \cdot e^{dx} \]

where y = impedance, x is frequency, a and c are the amplitudes of the first and second exponentials, respectively, and b and d are the rate constants of the first and second exponentials, 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 (length - L, height - H) and measured or calculated body fluid volumes to predict ECFV, TBW, PV and bodyweight using multiple linear regression analysis (McKeen and Lindinger 2004). While the shape of the human ‘cylinder’ is adequately described using stature (H^2), the inclusion of L 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 bodyweight.

Using 7 frequencies reduces the data collection period to approximately 35 secs. The 4 lowest frequencies (5, 16, 24, and 330) were used for modeling. The 4 highest frequencies (170, 180, 190, and 200) provide more information on how the different body fluid volumes change with exercise and were used to validate the model.

**Fig 1:** Total body water (TBW), extracellular fluid volume (ECFV), calculated intracellular fluid volume (ICFV), plasma volume and bodyweight in 13 Standardbred racehorses prior to and ~100 mins after a 2-bout exercise training session. Solid bars: measured; hatched bars: predicted using 7-frequency bioelectrical impedance analysis. There were no differences between measured and predicted values pre- or post exercise. Values are mean ± s.e. * significantly different than pre-exercise.
50 kHz) represent the steep (second order) portion of the curve and extracellular conductance (defined primarily by coefficients c and d); the 3 higher frequencies (140, 200, 280 kHz) represent the shallow (first order) portion of the curve and whole body conductance (defined primarily by coefficients a and b).

Effects of electrode type and floor surfaces on BIA readings

A separate group of 8 horses was studied to determine the effect of two electrode types and difference in floor surface on the BIA readings and calculated fluid volumes/bodyweight. The 2 electrode types were the Equistat carbon fibre electrodes and a 50 mm diameter self-adhesive electrode having an 18 mm diameter central gel core beneath the connector pin (model FS-50C). Using the carbon fibre electrodes, the effect of rotating the electrode pair forward or backwards 5 cm on the leg on BIA readings was also determined. Each horse was sequentially stood on dry rubber flooring, dry concrete floor or wet concrete flooring. BIA readings were obtained at least in duplicate for each situation.

Statistics

Data are presented as mean ± s.e. Changes over time were assessed by one-way repeated measures analysis of variance. When a significant F ratio was obtained, means were compared using the all pairwise multiple comparison procedure of Holm-Sidak. Statistical significance was accepted when P<0.05 at a power of 0.8.

Results

Fluid volumes by indicator dilution and MF-BIA before and after exercise

Pre-exercise means for PV, ECFV, TBW and bodyweight were similar when measured directly or when calculated using predictive equations developed from MF-BIA data (Fig 1). With the exception of predicted PV (which showed no change), the predicted decreases in mean fluid volumes and bodyweight were similar to the measured values. The best predictive equations for PV, ECFV, TBW and bodyweight included the terms height, length, the 4 curve coefficients and the impedance at the following frequencies: the PV equation used impedance at 5, 16 and 24 kHz; the ECFV equation used impedance at 5, 16, 24 and 50 kHz; and the TBW and bodyweight equations used impedance measured at 200 and 280 kHz. There was highly significant linear correlation between measured and predicted fluid volumes and bodyweight, both at rest and after exercise (Fig 2).

The predictive equations developed previously using exercise conditioned research horses (n = 7; McKeen and Lindinger 2004) were not capable of predicting reasonable values with these Standardbred racehorses. The pre- and approximately 100 mins post exercise data from the present and previous studies were combined to generate a new set of predictive equations. These equations yielded statistically significant agreement between measured and predicted volumes and bodyweight for the 13 horses of the present study; however, the 7 horses of the previous study remained outliers.

![Fig 2: Linear regression relationships (solid lines) and 95% confidence interval (dashed lines) between measured and predicted plasma volume (PV), extracellular fluid volume (ECFV), total body water (TBW) and bodyweight in 13 Standardbred racehorses before and ~100 mins after a 2-bout exercise training session.](image-url)
Effects of electrode type and surfaces on MF-BIA responses

The self-adhesive electrodes consistently resulted in underestimated values for PV, ECFV, TBW and bodyweight compared to carbon fibre electrodes. Offsetting the electrode by 5 cm resulted in significantly different volumes (some increased, some decreased). There was no difference in predicted volumes and bodyweight whether the horse was standing on dry concrete, wet concrete or dry rubber.

Discussion

The present study is the first to describe the simultaneous changes in body fluid compartment volumes using MF-BIA in exercised, Standardbred racehorses. MF-BIA equations were developed that accurately predicted fluid volumes at rest and after exercise, and therefore accurately determined decreases in TBW, ECFV, ICFV but not PV. Because of the small numbers of animals used in this, and previous (McKeen and Lindinger 2004) studies there remains difficulties in applying the equation to the general equine population.

Comparison with previous studies

There are only a handful of studies that have examined the use of bioelectrical impedance analysis for assessing hydration state in horses. What is consistent amongst these is that MF-BIA can be used for the reasonable accurate determination of fluid volumes and bodyweight in resting horses prior to exercise. The use of dual frequency BIA does not produce sufficient data to enable prediction of change in fluid volumes within a given horse over time (Forro et al. 2000). This subsequently led to the collection and analysis of impedance measured at 24 (McKeen and Lindinger 2004) or more (Fielding et al. 2004) frequencies. McKeen and Lindinger (2004) demonstrated that the quality of the data obtained using a 24 frequency sample is similar to that obtained with a 7 frequency sample, which greatly reduces (to less than 30 secs) the time required to obtain each set of readings.

Predicted PV showed no change between pre- and post exercise in the present study; however, MF-BIA accurately predicted changes in PV in the study by McKeen and Lindinger (2004). It has been demonstrated in rats that large (>10%) increases in plasma [Na⁺] and [Cl⁻] result in decreased impedance values (Rees et al. 1999). In the present study, changes in plasma ion concentrations (Waller and Lindinger 2005) were not sufficiently large at the time of BIA readings to have produced significant changes in measured impedance, based on the findings of Rees et al. (1999). Nevertheless, smaller changes in plasma ion concentrations or ion fluxes between body compartments may have contributed to the inability of BIA to predict PV.

The impedance-frequency relationship data obtained in these Standardbred racehorses were analysed using the predictive equations developed previously by McKeen and Lindinger (2004) on research horses. These equations did not generate meaningful fluid volumes for the present group of horses. This result points to a current and important limitation of the MF-BIA technique. Data from hundreds to thousands of individuals have been used in the development of predictive equations in human BIA instruments, therefore the equations from these instruments are generalizable to a substantial portion of the appropriate population. In contrast, relatively few horses have been used to develop predictive equations for equine use. It may be surmised, based on literature in man (Kushner and Schoeller 1986; Deurenberg et al. 1995; Armstrong et al. 1997; Siconolfi et al. 1997), that factors contributing to discrepant determination of fluid volumes and bodyweight include breed differences, state of physical conditioning/body composition, and underlying abnormalities and pathologies.

Technical considerations

The self-adhesive electrode yielded underestimated of PV, ECFV, TBW and bodyweight. The erroneously high impedance readings, compared to those obtained using the carbon fibre electrodes, are responsible. This may have been the result of the relatively long hair coat on the electrode sites and an inadequate degree of connection of the electrode with the skin. This is overcome with the carbon fibre electrode configuration because of the strap design used to securely keep the electrodes in position.

There was no difference in predicted fluid volumes and bodyweight whether the horse was standing on dry concrete, wet concrete or dry rubber. This suggested that the hoof provides a measure of insulation from potentially conductive floor surfaces (wet concrete).

Consistent and correct placement of electrodes is very important for obtaining measures of impedance that can be used to generate reliable predictions of fluid volumes and bodyweight. This is particularly important when electrodes are removed after a series of pre-event readings and there is a desire to obtain post event readings. Although a rather large rotational offset of 5 cm was used in this study, it is expected that significantly different volumes (some increased, some decreased) will be obtained if electrode placement is not consistent to within a few cm during subsequent readings. It is therefore important to be very consistent in the use of landmarks for placing the electrodes, and ‘permanently’ marking the electrode placement on the hair coat.

Applications of the technology

A large number of potential applications exist for the use of MF-BIA in equine medicine, transport and exercise physiology. With MF-BIA, dehydration during competition and transportation to and from events could be monitored frequently, allowing for rapid interventions when necessary. For example, Standardbred racehorses are typically shipped at least 1–4 h before arriving at the track 5 h before a race. They are put through a warm-up of 2 short duration high intensity bounces, and it is typical for many trainers to feed the horses minimally, if at all and therefore for the horses to consume very little or no water up to 10 h before a race. It is established that prolonged transport results in dehydration (Friend 2000), and a similar exercise protocol has been shown to result in substantial losses of TBW, ECFV and ICFV (Waller and Lindinger 2005). Although a furosemide-induced dehydration may be beneficial to racing performance (Hinchcliff et al. 1996), more prolonged or substantial dehydration may be detrimental to health and performance (Maughan and Lindinger 1995). The present study suggests that using MF-BIA, pre-race hydration status, as well as responses to fluid therapy could be easily and noninvasively monitored in Standardbreds, thus ensuring adequate hydration and optimal racing performance.

Many clinical applications for MF-BIA also exist, as it allows for determination not only of TBW, but also the ratio of...
ECFV:TBW to determine if dehydration is primarily extracellular, intracellular, or a combination. For example, it has been shown that different types of exercise result in different losses from extracellular and intracellular fluid compartments (Lindinger et al. 2004; Waller and Lindinger 2005). As well, drug doses could be more accurately determined based on estimates of volumes of distribution, rather than simply on bodyweight.

**Further work**

Ongoing applied research needs to be directed at larger groups of horses, different breeds of horses, foals, and horses with varied body condition in order to develop predictive equations suitable for use with these equine populations. While a current limitation is the ability to apply predictive equations developed using one group of horses to the general equine population, the technique still does permit the qualitative determination of volume changes in individual horses. So while accurate values of PV, TBW, ECFV and ICFV may not be obtained, the data provided prior to and after a dehydrating event will provide an indicator of the magnitude and sources (ECF, ICF) of the dehydration.

**Conclusions**

It is concluded that MF-BIA can be used to accurately determine fluid volumes and bodyweight in Standardbred racehorses before and after exercise-induced dehydration.

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**Manufacturer’s addresses**

1Kentucky Equine Research Inc., Versailles, Kentucky, USA.
2Kitchener, Ontario, Canada.
3Equistat Ltd., Douglas, Isle of Man, UK.
4DU-70 Beckman, Missisauga, Ontario, Canada.
5Nashua, New Hampshire, USA.
6Skinact ECG electrode, Leonhard Lang GmbH, Innsbruck, Austria.

**References**


