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**Bioelectrical Impedance Analysis Does Not Detect an Increase in Total Body
Water Following Isotonic Fluid Consumption**

Evan L. Matthews & Peter A. Hosick

Department of Exercise Science and Physical Education, Montclair State University,
Montclair, NJ, USA

Running Head: Isotonic Fluid and BIA

Corresponding Author:

Evan L. Matthews, PhD
Montclair State University
1 Normal Avenue
Montclair, NJ 07043 USA
Phone: (973) 655-3948
Fax: (973) 655-5461
Email: matthewse@montclair.edu

ABSTRACT

PURPOSE: To determine if single frequency foot-to-foot bioelectrical impedance analysis (BIA) can detect acute changes in total body water (TBW) following consumption of isotonic saline. **METHODS:** All participants ate a sodium free meal 4hr prior to the data collection visit and had euhydration confirmed using urine specific gravity at the beginning of the experimental visit. Subjects drank 466mL of isotonic saline (Na^+ $140\text{mmol}\cdot\text{L}^{-1}$) following baseline measures. Blood sampling and BIA were performed at baseline and every 30min for 3hr post saline consumption. **RESULTS:** Ten healthy participants completed this study. Plasma volume ($5\%\Delta$, $p<0.001$) and serum sodium concentration ($1\%\Delta$, $p<0.001$) increased by 60min and 90min, respectively. Body mass ($p<0.001$) displayed a biphasic response increasing to a peak at 30min ($+0.38\Delta\text{kg}$) and then decreasing to its minimum at 180min ($-0.35\Delta\text{kg}$). BIA impedance ($p=0.678$) was unaffected by the saline administration. BIA derived TBW ($p=0.039$) decreased from baseline starting at 150min ($0.21\Delta\text{kg}$). **CONCLUSION:**

- Athletes and coaches wishing to achieve hyperhydration can do so through the consumption of isotonic fluid.
- 50kHz foot-to-foot bioelectrical impedance analysis derived total body water is inadequate for measuring hyperhydration.
- Future studies should examine the physiological and performance effects of such a hyperhydration protocol.

Key words: Body Composition, Exercise Nutrition, Hydration, Sports Nutrition, Physiology, Athlete Performance

INTRODUCTION

Hydration is important for thermoregulation during exercise in a hot environment (Sawka et al. 2001). Poor hydration practices can lead to poor athletic performance (Casa et al. 2005; Sawka et al. 2007) or life threatening heat illness (Bohm et al. 2013). To protect against dehydration during exercise, an athlete can hyperhydrate prior to exercise (Latzka et al. 1998). Hyperhydration protocols and the definition of hyperhydration vary between studies (Greenleaf and Castle 1971; Latzka et al. 1998; Morris et al. 2015), but can be generally defined as an increase in bodily fluids following prior confirmation of euhydration [euhydration: urine specific gravity $\leq 1.020 \text{ g}\cdot\text{mL}^{-1}$ (ACSM et al. 2007; NCAA 2017)]. The benefits of hyperhydration are an increase in plasma and total blood volume (Savoie et al. 2015) and an increase in the amount of fluid loss required to become dehydrated (Latzka et al. 1998). However, done so improperly and hyperhydration can lead to excessive weight gain prior to exercise, and in extreme cases a life-threatening condition called hyponatremia (Sawka et al. 2007). Athletes and coaches attempting to achieve safe and effective hyperhydration would benefit by having access to a non-invasive method for tracking body water content.

Bioelectrical impedance analysis (BIA) is a quick, simple, and non-invasive method for assessing total body water (TBW) (O'Brien et al. 2002). Acute changes in TBW often lead to changes in body electrolyte concentration (Food and Nutrition Board 2005) which can decrease the accuracy of BIA estimates (Berbeis and Keller 2000; O'Brien et al. 2002). This is especially true for single frequency 50kHz BIA, the most common and affordable version of this technology (Mialich et al. 2014). However, it may be possible to hyperhydrate in such a way as to increase plasma volume, while

minimally altering the concentration of important electrolytes like sodium. Theoretically, BIA would still be capable of accurately assessing TBW in such a state. Therefore, there are two purposes for the current study. 1), to determine the acute effects of orally consumed isotonic saline on plasma volume and blood sodium concentration. In this study hyperhydration was confirmed by an increase in plasma volume following prior confirmation of euhydration at baseline. 2), to determine the acute effects of increased plasma volume with minimal changes in serum sodium (sNa^+) concentration on 50kHz foot-to-foot BIA derived TBW estimates.

MATERIALS AND METHODS

All procedures and protocols in this investigation conform to the Declaration of Helsinki and were approved by the Montclair State University Institutional Review Board. All participants signed a written informed consent prior to participation¹.

Baseline Hydration

Upon arrival to the laboratory participants were asked to provide a urine sample and to completely empty their bladder. This sample was immediately tested for urine specific gravity using a refractometer (Cambridge Instruments Inc., NY, USA). The participant was considered dehydrated if they had a urine specific gravity $>1.020g \cdot mL^{-1}$ (ACSM et al. 2007; NCAA 2017).

¹ Inclusion and exclusion criterion available in the supplement.

Hyperhydration Protocol

Participants were asked to drink 466mL of deionized water with 3.85g of table salt (1500mg sodium) in <1min. The sodium load was set to 1500mg because this is the tolerable upper intake level for sodium according to the American Heart Association (Horn et al. 2016). The sodium concentration was set to $140\text{mmol}\cdot\text{L}^{-1}$ because it is approximately equal to the average sNa^+ concentration of an adult (Mayo Clinic 2018); thus requiring 466mL of deionized water to reach this concentration. Equilibration of water tracers between the gastrointestinal contents and the blood happens in $\approx 45\text{min}$ (Edelman 1952) using volumes similar to that given. However, providing salt within the water will increase the osmolality and tonicity of the solution which may slow gastric emptying rates by approximately half (Leiper 2015). No food or drink of any kind was allowed after the hyperhydration drink was consumed until the study was complete.

Time Series Measurements

All time series measurements were assessed before and every 30min after the participant consumed the isotonic saline solution for 3hr (seven total time points). Hemoglobin, hematocrit, and serum electrolytes were assessed². Changes in hemoglobin and hematocrit were used to calculate changes in plasma volume, total blood volume, and red blood cell volume (Dill and Costill 1974). BIA measurements of TBW, impedance, and body mass were performed using proprietary equations and a single frequency 50kHz foot-to-foot scale (TBF-300A, Tanita Corporation of America, Inc., IL, USA). Single frequency BIA is known to primarily assess extracellular water

² Blood sampling and analysis information available in the supplement.

such as blood plasma (Ellis et al. 1999). It is the most common and affordable version of this technology (Ellis et al. 1999; Mialich et al. 2014), and the version most likely to be available to coaches and athletes³.

Statistical Analysis

Time series data are reported as change scores, [post time point] – [baseline], or percent change scores, ([post time point] – [baseline]) / [baseline] * 100. Normality was tested using the Shapiro-Wilk test. Normally distributed data was analyzed using a one-way ANOVA and Dunnett's multiple comparisons post-hoc to compare vs baseline. Red blood cell volume and body mass were not normally distributed and were therefore analyzed with a Friedman non-parametric test and the Dunnett's multiple comparisons post-hoc. Alpha level of significance was set at $p < 0.05$ for all statistical tests. Values expressed as means \pm SEM.

RESULTS

Table 1 shows participant characteristics⁴. Hemodilution occurred with hematocrit ($p < 0.001$, $r^2 = 0.463$) and hemoglobin ($p = 0.001$, $r^2 = 0.322$) decreasing from baseline starting at 60min and 90min, respectively (Figure 1). Serum sodium concentration ($p < 0.001$, $r^2 = 0.575$) increased starting at 90min while plasma volume ($p < 0.001$, $r^2 = 0.528$) increased beginning at 60min (Figure 1). There was no change in red blood

³ Renal excretion methods and results, as well as, the coefficient of variation for key laboratory measurements are available in the supplement.

⁴ Supplementary figure S1 containing cardiovascular responses is available in the supplement.

cell volume ($p=0.138$) (Figure 1). Total blood volume ($p=0.001$, $r^2=0.320$) increased starting at 90min (Figure 1).

Body mass ($p<0.001$) displayed a biphasic response increasing to a peak at 30min ($+0.38\Delta\text{kg}$) and then decreasing to its minimum at 180min ($-0.35\Delta\text{kg}$) (Figure 2). BIA impedance ($p=0.678$, $r^2=0.069$) was unaffected by the saline administration (Figure 2). BIA derived TBW ($p=0.039$, $r^2=0.212$) decreased from baseline starting at 150min (Figure 2).

DISCUSSION

In this investigation we gave a bolus oral dose of saline with a sodium concentration equivalent to $s\text{Na}^+$. The first aim sought to evaluate its impact on blood plasma volume and $s\text{Na}^+$. We hypothesized that plasma volume would increase with minimal to no change in $s\text{Na}^+$. While $s\text{Na}^+$ did increase, it did so to a far lesser extent than plasma volume ($s\text{Na}^+ \approx 1\%\Delta$; plasma volume $\approx 5\%\Delta$). Importantly, this occurred with no change in red blood cell volume indicating little to no fluid shift between the intracellular and extracellular space.

The second and primary aim of the current investigation was to assess the effects of an isotonic oral hyperhydration protocol on TBW. We hypothesized that 50kHz foot-to-foot BIA derived TBW would increase as a result of an increase in plasma volume. The reason for this is 50kHz BIA is known to primarily assess extracellular water such as blood plasma (Ellis et al. 1999) because it cannot penetrate cell walls (Jaffrin and Morel 2008). Despite a sizeable increase in plasma volume in the current investigation, BIA derived TBW actually decreased. However, the decrease in TBW did

not occur until late in the trial and appears to be the result of a decrease in body mass from urination rather than an alteration in impedance.

A number of studies have been conducted to evaluate the effects of fluid and electrolyte alterations and the effects they have on BIA (O'Brien et al. 2002). However, only a small number of studies have looked at hyperhydration in euhydrated participants. Those studies suggest hyperhydration resulting from the consumption of hypotonic beverages does not impact 50kHz impedance (Gomez et al. 1993; Berbeis and Keller 2000). Conversely, hyperhydrating with a beverage containing higher sodium content than blood will result in decreased BIA derived impedance (Deurenberg et al. 1988; Gomez et al. 1993). Similarly, Scheltinga et al. (Scheltinga et al. 1991) infused normal saline, which has greater sodium concentration (normal saline Na^+ $154\text{mmol}\cdot\text{L}^{-1}$) than normally found in blood (blood Na^+ $\approx 140\text{mmol}\cdot\text{L}^{-1}$), and saw a decrease in BIA derived impedance in all body segments. The current investigation sought to minimize changes in blood sodium concentration ($+2.1\Delta\text{mmol}\cdot\text{L}^{-1}$) while hyperhydrating participants by giving an oral saline load matching average blood sodium concentration. Our BIA impedance results did not change following saline consumption, and therefore more closely aligned with protocols that gave hypotonic saline loads. However, it is worth noting that the current investigation utilized a foot-to-foot BIA device, whereas previous studies utilized hand-to-foot devices. This altered conducting pathway may affect the ability to directly compare these studies.

Two previous studies utilized 50kHz foot-to-foot BIA to assess its ability to detect changes in TBW and or impedance following sports drink consumption with mixed results. Dixon et al. (Dixon et al. 2006) found no change in BIA impedance or TBW

following consumption of 591mL of water or sports drink. However, the participants were dehydrated [urine specific gravity $>1.020\text{g}\cdot\text{mL}^{-1}$] (ACSM et al. 2007; NCAA 2017) at baseline (water visit $1.024\text{g}\cdot\text{mL}^{-1}$; sports drink visit $1.022\text{g}\cdot\text{mL}^{-1}$) which may have affected the results. It should be noted that some have recommended different urine specific gravity thresholds for euhydration ($1.013\text{-}1.029\text{g}\cdot\text{mL}^{-1}$ (Armstrong et al. 1994)). Androutsos et al. (Androutsos et al. 2015) gave 750mL of water or sports drink and found that both led to an immediate increase in impedance. Unfortunately, these studies only gave oral fluids with a lower concentration of sodium than human blood making it difficult to directly compare the results to the current investigation.

There are some limitations to the current investigation. The Tanita TBF-300A uses proprietary equations, and another machine or equations, may find different results. In addition, this machine only reports impedance, while resistance and reactance are not assessed. In the 4hr between the controlled meal and the start of the data collection visit participants were allowed to drink plain water on an ad libitum basis which may have impacted results. However, it has been found that approximately 400mL or less of plain water will equilibrate between the gastrointestinal contents and the blood in $\approx 45\text{min}$ (Edelman 1952). Also, the volume of fluid given during the current investigation may not be enough to elicit athletic or thermoregulatory benefits from hyperhydration. However, previous research suggests that 403mL infused intravenously increased stroke volume during exercise by 8-11% (Hopper et al. 1988) with no further improvements when infusing a larger volume (706mL).

Conclusions

The current study found that a saline solution equivalent in sodium concentration to blood will lead to increased plasma volume, but this hyperhydration is not detectable with single frequency 50kHz BIA. Future studies should examine other frequencies, other BIA analyzers, and multiple frequency BIA (Kyle et al. 2004) in an effort to find a fast and non-invasive method for coaches and athletes to assess hyperhydration protocols.

TAKE HOME POINTS

- Consuming a salt water solution (saline) with a sodium concentration equal to that of average blood will result in hyperhydration within ≈ 1 hr.
- 50kHz foot-to-foot bioelectrical impedance analysis cannot be used to track hyperhydration.

DECLARATION OF INTEREST STATEMENT

No conflicts of interest are declared by the authors.

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Table 1. Participant Characteristics

Baseline Demographic Data	
N (M, F)	10 (6, 4)
Age (Years)	27 ± 2
Height (cm)	171 ± 3
Body Mass (kg)	71 ± 4
BIA Impedance (Ω)	139.4 ± 0.5
BIA Total Body Water (kg)	104.3 ± 0.5
BIA Body Fat (%)	22.6 ± 2.5
SBP (mmHg)	118 ± 4
DBP (mmHg)	74 ± 2
Heart Rate (Beats·min ⁻¹)	64 ± 5
Hemoglobin (g·dL ⁻¹)	13.8 ± 0.2
Hematocrit (%)	46 ± 1
Serum Na ⁺ (mmol·L ⁻¹)	139.4 ± 0.5
Urine Specific Gravity (g·mL ⁻¹)	1.007 ± 0.001
Urine Na ⁺ (mmol·L ⁻¹)	28.3 ± 8.7

All values were collected during baseline before saline

was consumed. Values are means±SEM. BIA,

bioelectrical impedance analysis; SBP, systolic blood

pressure; DBP, diastolic blood pressure.

FIGURE LEGENDS

Figure 1. The effect of oral isotonic saline consumption on hematocrit (panel A), hemoglobin (panel B), serum sodium concentration (panel C), plasma volume (panel D), red blood cell volume (panel E), and total blood volume (panel F) over a 3hr follow-up period. * $p < 0.05$ vs baseline. Values are means \pm SEM.

Figure 2. The effect of oral isotonic saline consumption on body mass (panel A), bioelectrical impedance analysis (BIA) impedance (panel B), and BIA total body water (panel C) over a 3hr follow-up period. Single frequency foot-to-foot BIA was used. * $p < 0.05$ vs baseline. Values are means \pm SEM.

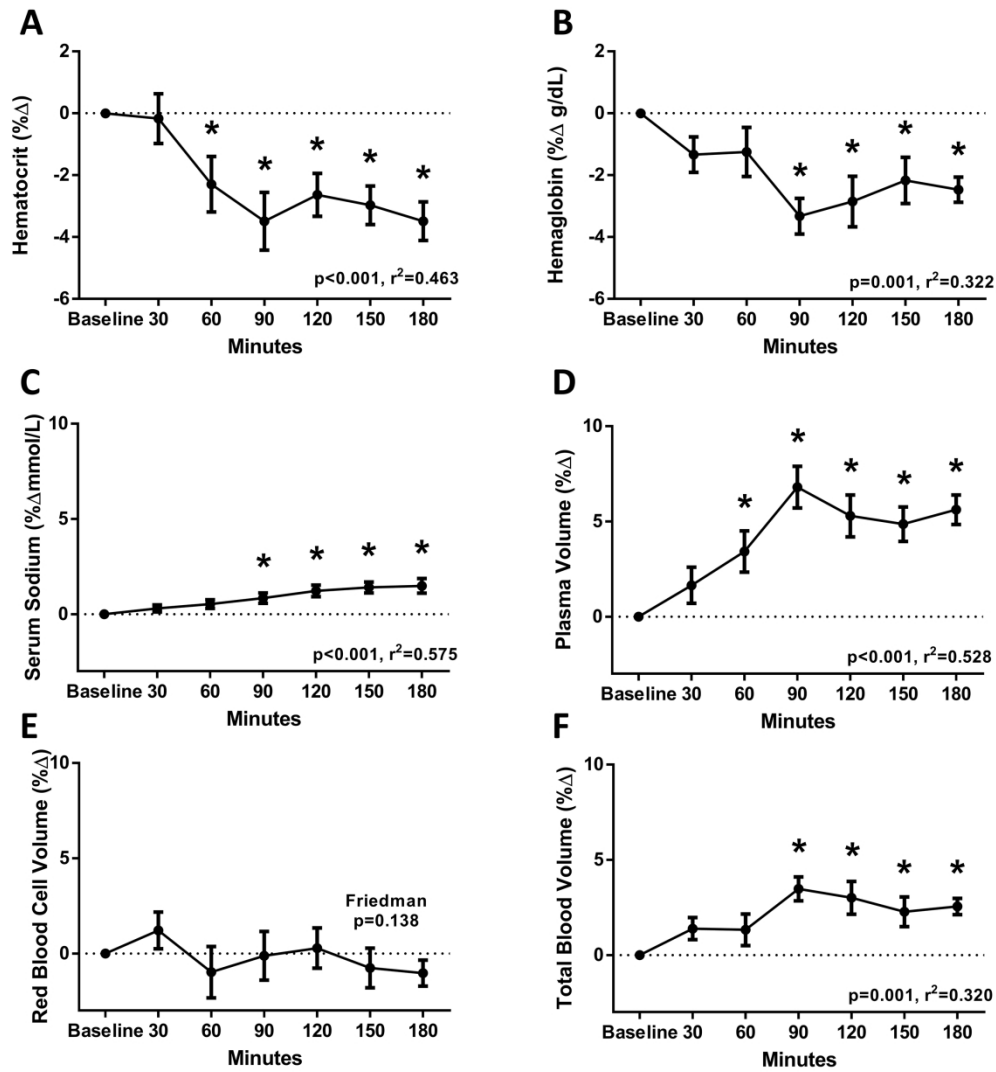


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128x140mm (600 x 600 DPI)

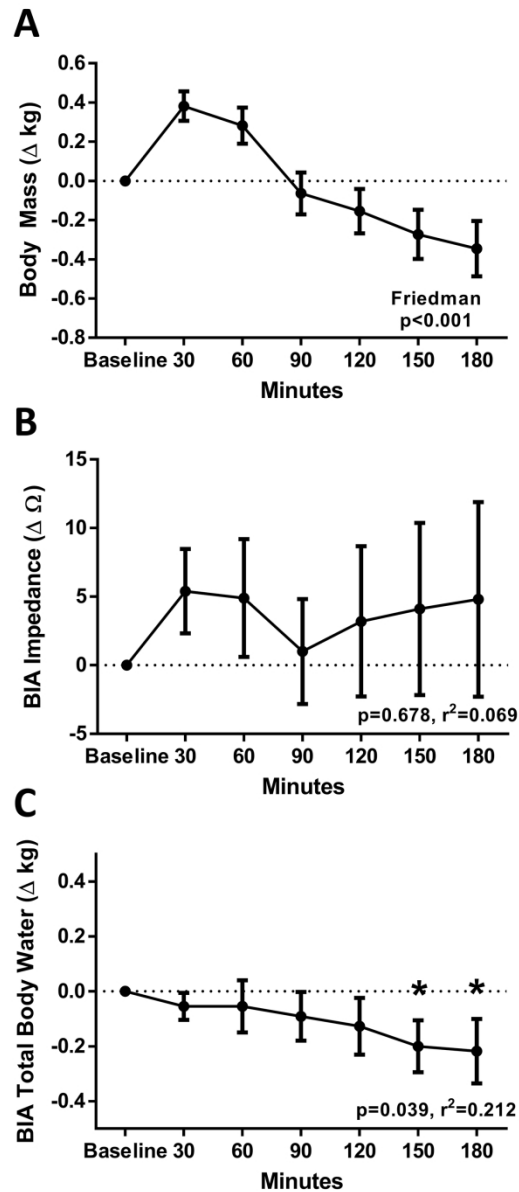


Figure 2. The effect of oral isotonic saline consumption on body mass (panel A), bioelectrical impedance analysis (BIA) impedance (panel B), and BIA total body water (panel C) over a 3hr follow-up period. Single frequency foot-to-foot BIA was used. * $p < 0.05$ vs baseline. Values are means \pm SEM.

64x146mm (600 x 600 DPI)