Noninvasive assessment of extracellular and intracellular dehydration in healthy humans using the resistance-reactance–score graph method\(^1,2\)

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**ABSTRACT**

**Background:** Few dehydration assessment measures provide accurate information; most are based on reference change values and very few are diagnostically accurate from a single observation or measure. Bioelectrical impedance may lack the precision to detect common forms of dehydration in healthy individuals. Limitations in bioimpedance may be addressed by a unique resistance-reactance (RXc)–score graph method, which transforms vector components into $z$ scores for use with any impedance analyzer in any population.

**Objective:** We tested whether the RXc-score graph method provides accurate single or serial assessments of dehydration when compared with gold-standard measures of total body water by using stable isotope dilution (deuterium oxide) combined with body-weight changes.

**Design:** We retrospectively analyzed data from a previous study in which 9 healthy young men participated in 3 trials: euhydration (EUH), extracellular dehydration (ED; via a diuretic), and intracellular dehydration (ID; via exercise in the heat).

**Results:** Participants lost 4–5% of their body weight during the dehydration trials; volume loss was similar between trials (ID compared with ED group: $3.5 \pm 0.8$ compared with $3.0 \pm 0.6$ L; $P > 0.05$). Despite significant losses of body water, most RXc vector scores for ED and ID groups were classified as “normal” (within the 75% population tolerance ellipse). However, directional displacement of vectors was consistent with loss of volume in both ED and ID conditions compared with the EUH condition and tended to be longer in ED than in ID conditions ($P = 0.054$).

**Conclusions:** We conclude that, whereas individual RXc-score graph values do not provide accurate detection of dehydration from single measurements, directional changes in vector values from serial measurements are consistent with fluid loss for both ED and ID conditions. The RXc-score graph method may therefore alert clinicians to changes in hydration state, which may bolster the interpretation of other recognized change measures of hydration.

*Received June 5, 2015. Accepted for publication December 14, 2015. First published online February 3, 2016; doi: 10.3945/ajcn.115.115352.

1 Supported by the US Army Medical Research and Materiel Command.

2 The views, opinions, and/or findings contained in this article are those of the authors and should not be construed as an official Department of the Army position, or decision, unless so designated by other official documentation. Any citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement of approval of the products or services of these organizations.

3 Abbreviations used: BIA, bioelectrical impedance analysis; BIVA, Bioelectrical Impedance Vector Analysis; ECF, extracellular fluid; ED, extracellular dehydration; EUH, euhydration; ID, intracellular dehydration; PV, plasma volume; R, resistance; RXc, resistance-reactance; TBW, total body water; Xc, reactance.

**Keywords:** volume depletion, hydration assessment, BIVA, hypohydration, hypovolemia

**INTRODUCTION**

Dehydration is a medical problem associated with morbidity and mortality across the life span (1, 2). However, few dehydration assessment measures provide accurate diagnostic information. Most valid methods require reference change values, and very few provide acceptable diagnostic accuracy from a single observation (3–5). Although clinical laboratory tests (e.g., blood or urine) are typically preferred over signs and symptoms for detecting dehydration (6), these tests have limitations. In particular, laboratory-based tests are not sensitive and specific to all types of dehydration (3), particularly when significant losses of water and solute occur (e.g., volume depletion) (7). One new approach to bioelectrical impedance offers the potential for both noninvasive single and serial monitoring of hydration state, as well as the possibility of distinguishing between intracellular and extracellular dehydration (3, 7).

Traditional bioelectrical impedance analysis (BIA)\(^3\) works by measuring the resistance (R) to the flow of electrical current through body fluids to estimate total body water (TBW) (8). However, given the large variability in both single- and multi-frequency (5–500 kHz) BIA methods, the use of BIA for the detection of body water losses in either healthy or clinical populations is highly limited (9, 10). Piccoli et al. (11) first suggested the possibility of considering R and reactance (Xc) separately from impedance (Z) as a way of improving BIA for hydration assessment. The resistance-reactance (RXc) graph method is related to body hydration on the basis of patterns of the R and Xc graph [bioelectrical impedance vector analysis (BIVA)] (8). The change measurement aspects of the RXc-graph appear to work in some clinical (8, 12) and some nonclinical (13) situations, but this may depend on the type and magnitude of dehydration (3). Because each RXc graph is created unique to the impedance...
The purpose of this study was to test the potential for using the RXc-score graph to detect dehydration from single and serial measurements by using both intracellular and extracellular models of dehydration (3). The 2 types of dehydration were used to produce similarly large TBW losses (>4% body weight) but distinctly different extracellular fluid (ECF) volume losses (L) and ECF concentrations (mmol/kg), thus mimicking the 2 most common types of clinically relevant dehydration. We tested the following RXc-score graph hypotheses: 1) that individual RXc scores would correctly identify subjects as euhydrated and dehydrated, 2) that the directional change in the RXc vector would be consistent with dehydration, and 3) that extracellular dehydration would result in a longer RXc vector due to greater loss of ECF volume.

METHODS

Subjects

Nine healthy young men gave their written, informed consent to participate in these experiments after the purpose, procedures, and known risks of the tests had been explained. The test protocol was approved by the Scientific Review Committee and Human Use Review Committee at the US Army Research Institute of Environmental Medicine. All of the volunteers were medically screened before participation.

Experimental tests

The present article represents a retrospective analysis of data from a previously reported protocol (14); data collection occurred between 1994 and 1996. Specific methods are detailed elsewhere (14). Briefly, each subject completed 3 tests, spaced ~1 wk apart. A baseline average nude body weight was established during a 10-d preliminary period. This weight was used to calculate target body weight for the dehydration tests.

Experimental procedures

Subjects’ activity and food and fluid intakes the day before the euhydration (EUH) trial were ad libitum before conducting fluid volume measurements. There was no specific exercise associated with the EUH trial. For trials involving dehydration, subjects completed a dehydration procedure to decrease their body weight by 4–5%. Subjects then remained dehydrated overnight and reported the next morning for the fluid volume measurements. For the intracellular dehydration (ID) trial, a standardized exercise-heat exposure was used to induce sweat losses (14). Briefly, the subject reported to the laboratory and performed 3–4 h of intermittent light-intensity exercise in the heat (40°C, 20% relative humidity) to induce sweating. Fluid intake was prohibited. On a separate day, for the extracellular dehydration (ED) trial, an oral diuretic (furosemide) was given to induce fluid losses (14–16). Furosemide is a loop diuretic that inhibits renal reabsorption of sodium and chloride, thereby reducing water reabsorption by the kidney and increasing urine formation. The loss of appreciable solute and water results in dehydration with relatively little change in ECF osmolality. This model to induce isotonic dehydration is a fair representation of the dehydration resulting from diarrhea and vomiting (3).

On the morning after the respective euhydration/dehydration procedures, flexible gel electrode strips (Xitron IS4000; Xitron Technologies Corporation) were placed in the standard tetrapolar arrangement to obtain the required impedance measurement. R and Xc measurements were obtained from a single 50-kHz current (14, 17).

During the EUH condition, TBW was also determined by stable isotope dilution (TBW D2O) (18). Briefly, a 30-g dose of deuterium oxide (D2O) was given to each subject, followed by 100 mL tap water. For each dehydration test, TBW D2O was calculated by adjusting the euhydration TBW D2O for the fluid loss due to dehydration, which was reliably determined from acute body-weight change (3).

Blood samples were analyzed for hematocrit (%) and hemoglobin (g/L) concentrations and plasma osmolality (mmol/kg). Plasma volume (PV) was predicted for euhydration tests (19). Estimates of the percentage change in PV (from euhydration to dehydration) were calculated from the corresponding changes in hemoglobin and hematocrit values (20). Absolute PV was calculated for the dehydration tests from the appropriate predicted and corresponding percentage change values.

Data analysis and statistics

The RXc-score graph method uses a single, low-frequency current (50 kHz) to measure whole-body R and Xc, which were then standardized for height and compared with a bivariate distribution of the Z vector in a reference population (11). RXc-score graph analysis was completed by using custom software written in Excel (Microsoft Corporation) (21, 22) (free software available by request from A Piccoli; e-mail: apiccoli@unipd.it). Individual vector plots were created for each subject in euhydrated and dehydrated conditions in the context of 50%, 75%, and 95% CI ellipses in comparison to the appropriate standard reference interval provided by Piccoli et al. (21) [non-Hispanic white men 20–29 y old with a BMI (in kg/m2) of 19–25]. Directionality for each set of paired vectors was determined from EUH to ID or ED conditions for each subject.

Other statistical analyses of data were conducted by using commercial software (Statistica version 7.1; StatSoft, Inc.). A 1-factor repeated-measures ANOVA was used to make comparisons between group means. Tukey’s honestly significant difference post hoc test was applied when main effects were found to be significant (P < 0.05). A study sample size of 9 volunteers provided the necessary statistical power (0.80) to see an effect of equal or greater magnitude to the typical measurement error for TBW measured by deuterium (i.e., effect size >1.0) according to the repeated-measures ANOVA sample-size tables published by Tran (23). Vector lengths were calculated by using the distance formula \[d = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}\] and compared by paired t test. The relation between TBW change and vector length was examined by using Pearson’s correlation. All data are reported as means ± SDs.

RESULTS

Subjects’ demographic characteristics

Subjects’ (n = 9) physical characteristics (shown in Table 1) were typical for healthy young men in the United States and
In the ED condition, 2 subjects showed values dehydrated when viewed from a single RXc-graph vector score, indicating that just 1 of 9 subjects were identified correctly as tolerance ellipse. In the ID condition, 2 subjects showed values within the “normal” range for hydration (0.8 L; ED: –3.0 L) and ED conditions (8 of 9 subjects). TBW losses were significant (P < 0.05) from the EUH value (284 ± 3 mmol/kg; P < 0.05) during the ED condition (2.7 ± 0.6 L) than during the EUH or ID conditions. Plasma osmolality was substantially higher during the ID condition (292 ± 7.3 L) and ED (45.3 L) in nature. By design, the present study involved similar, significant losses of TBW (>3.0 L) in the 2 groups, with larger PV losses in the ID trials and greater osmotic stress in the ID trials. The RXc-score graph correctly classified 18 of 18 subjects as euhydrated but only 4 of 18 subjects as dehydrated (ID and ED conditions) when using a single-vector score referenced against population norms. In contrast, vector displacement was directionally sound in 18 of 18 cases, suggesting that changes in vector displacement correctly capture losses of body water when change measurements are made. The length of the displaced vector was also larger in ED than in ID conditions (8 of 9 observations) but was not significant (P = 0.054).

The RXc graph was developed as an alternative to single, low-frequency BIA (e.g., 50 kHz), which estimates ECF volume to predict TBW on the basis of the strong linear relation between TBW and ECF (24). Good agreement has been reported between single-frequency impedance measures and TBW by dilution in well-hydrated individuals. However, despite small mean differences and large correlation coefficients, the error variance is large (8) and can be in excess of 2 L for TBW (~5% of TBW). The RXc-score graph (21) is thought to control for such variance by placing vectors within population reference ellipses.

In RXc analysis, clinical patterns of the displaced Z vector are proposed to inform on hydration status (parallel vectors; up/down) and cell mass (peripheral vectors; left/right) (8). The RXc graph has therefore been proposed as a noninvasive way to detect clinically relevant dehydration by using either single (relative to tolerance ellipses) or serial (vector displacement) measurements (3). Because each RXc graph is created unique to the impedance analyzer used, an RXc-score graph was created to further simplify the use of BIVA by normalizing vectors to z scores (i.e., difference in individual R and Xc values from the mean/SD) (21). This allows dimensionless Z(R) and Z(Xc) scores to be plotted with tolerance ellipses preserved.

In the present study, the inability of the RXc-score graph approach to correctly identify meaningful dehydration from the RXc-score graph category selected for analysis (21). All of the volunteers completed every aspect of the study.

### Hydration and fluids

TBW losses were significant (P < 0.05) from the EUH condition (48.8 ± 7.5 L) to both the ID (45.4 ± 7.3 L) and ED (45.8 ± 7.5 L) conditions, but there were no differences between ID and ED conditions. TBW losses exceeded the desired –2.0-L threshold in both dehydration trials (ID: –3.5 ± 0.8 L; ED: –3.0 ± 0.6 L). The corresponding levels of dehydration, commonly expressed relative to body mass, were 4.9% ± 0.6% (ID) and 4.3% ± 0.6% (ED). PV did not differ between EUH (3.3 ± 0.3 L) and ID (3.1 ± 0.3 L) conditions but was lower (P < 0.05) during the ED condition (2.7 ± 0.6 L) than during the EUH or ID conditions. Plasma osmolality was substantially higher during the ID condition (292 ± 3 mmol/kg) than during the EUH condition (280 ± 3 mmol/kg; P < 0.05). The ED value was slightly, but significantly, higher than during the EUH or ID conditions. Plasma osmolality was substantial change in hydration status (dehydration by ~4% of body weight) in healthy young men as measured by gold-standard (stable isotope and Δ body weight) analysis. This was true whether dehydration was intracellular (ID) or extracellular (ED) in nature. By design, the present study involved similar, significant losses of TBW (>3.0 L) in the 2 groups, with larger PV losses in the ID trials and greater osmotic stress in the ID trials. The RXc-score graph correctly classified 18 of 18 subjects as euhydrated but only 4 of 18 subjects as dehydrated (ID and ED conditions) when using a single-vector score referenced against population norms. In contrast, vector displacement was directionally sound in 18 of 18 cases, suggesting that changes in vector displacement correctly capture losses of body water when change measurements are made. The length of the displaced vector was also larger in ED than in ID conditions (8 of 9 observations) but was not significant (P = 0.054).

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### Serial measurements

For all of the subjects, the directional change in the RXc vector (from EUH to ID or ED conditions) was consistent with loss of body water (i.e., all of the values were displaced upward and to the right) (8). As can be seen from visual inspection of Figures 1 and 2, the lengths of the change vectors for the ED trial appear longer than those for the ID trial. The average calculated lengths were 2.5 ± 1.0 arbitrary units (ED) and 1.9 ± 0.5 arbitrary units (ID) (P = 0.054). Although not significant, vector lengths were greater in 8 of 9 ED compared with ID pairs and were 40% longer on average. The relation between changes in TBW and vector length was weak for both ID (r = 0.36) and ED (r = 0.41) conditions.

### DISCUSSION

The major finding of the present study was that paired vector values based on RXc-score graph analysis consistently identified a substantial change in hydration status (dehydration by ~4% of body weight) in healthy young men as measured by gold-standard (stable isotope and Δ body weight) analysis. This was true whether dehydration was intracellular (ID) or extracellular (ED) in nature. By design, the present study involved similar, significant losses of TBW (>3.0 L) in the 2 groups, with larger PV losses in the ID trials and greater osmotic stress in the ID trials. The RXc-score graph correctly classified 18 of 18 subjects as euhydrated but only 4 of 18 subjects as dehydrated (ID and ED conditions) when using a single-vector score referenced against population norms. In contrast, vector displacement was directionally sound in 18 of 18 cases, suggesting that changes in vector displacement correctly capture losses of body water when change measurements are made. The length of the displaced vector was also larger in ED than in ID conditions (8 of 9 observations) but was not significant (P = 0.054).

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### TABLE 1

Subject demographic characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ± SD</th>
<th>Range</th>
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<tr>
<td>Age, y</td>
<td>23.6 ± 5.1</td>
<td>18–35</td>
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<tr>
<td>Height, m</td>
<td>1.78 ± 0.11</td>
<td>1.71–1.89</td>
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<tr>
<td>Weight, kg</td>
<td>76.8 ± 11.2</td>
<td>63.8–100.1</td>
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<tr>
<td>Body fat, %</td>
<td>14.6 ± 2.4</td>
<td>8.5–16.8</td>
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<tr>
<td>BMI, kg/m²</td>
<td>24.1 ± 2.1</td>
<td>21.3–27.7</td>
</tr>
<tr>
<td>TBW, L</td>
<td>48.8 ± 7.5</td>
<td>39.4–61.8</td>
</tr>
<tr>
<td>VO₂ peak, mL · kg⁻¹ · min⁻¹</td>
<td>54.8 ± 3.8</td>
<td>49.0–62.2</td>
</tr>
<tr>
<td>Body surface area, m²</td>
<td>1.94 ± 0.17</td>
<td>1.74–2.28</td>
</tr>
</tbody>
</table>

1. n = 9. TBW, total body water; VO₂ peak, peak oxygen uptake.
2. Measured by deuterium oxide (D₂O).

### BIVA

**Single measurements**

Figures 1 and 2 show individual RXc-score graph analyses comparing EUH to ID (Figure 1) or ED (Figure 2) conditions in the context of the elliptical population 50%, 75%, and 95% CIs for healthy young individuals (21). Vector scores for the EUH condition were within the clinical pattern of “normal hydration” (n = 6) or slightly overhydrated (n = 3) (Figures 1 and 2). In response to dehydration produced by sweating and fluid restriction (Figure 1) or furosemide (Figure 2), 6 of 9 subjects still showed values within the “normal” range for hydration (<75% tolerance ellipse). In the ID condition, 2 subjects showed values between 50% and 75%, whereas 1 subject had a value >75%, indicating that just 1 of 9 subjects were identified correctly as dehydrated when viewed from a single RXc-graph vector score. In the ED condition, 2 subjects showed values >75%, whereas 1 had a value >95%, which gave 3 of 9 correct classifications of dehydration.
a single measurement (vector score) is consistent with the inability of most hydration assessment measures to do the same (3). Typical reference intervals for hydration measurements of interest are generally wider than the change produced by meaningful dehydration. As a result, single observational measurements that have been meaningfully changed (i.e., postmeasures only) may still appear “normal” in the broader context of the reference interval. In the present study, the average baseline TBW (48.8 L) was reduced, on average, by \( \approx 3.0 \text{ L} \) (6–7% TBW). It is likely that the range of “normal” TBW values among the populations that comprise the ellipses is simply wider than the average within-subject change in TBW caused by our experimental interventions. In other words, with the use of the RXc-score graph approach, a value of 45 L TBW (reduced from 48 L TBW) still appears to be perfectly normal. This is likely true although populations have been stratified by age, ethnicity, and sex (21).

In contrast to single-vector scores, serial RXc-score graph measurements provided consistent information on the hydration changes in the subjects in a given pair of trials (EUH to ID or ID to EUH).
EUH to ED). In the present study, for 18 of 18 observations, vector displacement occurred in accordance with proposed RXc-score graph theory (11, 21). Interestingly, the length of the displaced vector was greater for the ED condition in 8 of 9 pairs ($P = 0.054$). It seems plausible that this may be related to the fact that PV losses were significantly larger in ED ($\sim 0.6$ L) than ID ($\sim 0.2$ L) groups. At the core of the RXc-score graph is a BIA measurement made at 50 kHz where the prediction of TBW remains a linear function of the extracellular volume (24). The RXc-score graph might therefore find use as a serial hydration-monitoring tool (12), particularly in controlled clinical settings. However, it should be remembered that this methodology is far from a straightforward approach and simpler measures, such as changes in body mass, can provide nearly perfect correspondence to fluid loss in a variety of clinical settings in which fluid loss is a concern and change measures can be made (3, 25–27).

The vector length calculations performed in this study represent a unique application toward the RXc-score graph method for examining serial measurements of hydration status. Vector lengths seemed to track the larger PV losses in ED compared with ID conditions, but not TBW losses, which were actually 0.5 L less in the ED than in the ID conditions. Individual TBW changes were poorly correlated with vector lengths; thus, a limitation of the RXc-score graph method is that the magnitude of TBW loss cannot be assessed. In this context, individual PV change data could not be recovered from the data archive, thus limiting a retrospective analysis of ECP volumes against vectors. It is possible that the vector length itself might afford insight into extracellular dehydration. Future work (3) might establish the day-to-day variation in serial vector length measurements in experimentally euhydrated individuals to better understand the typical “noise” in the vector length measurement, against which lengths like those reported herein for experimental dehydration (signal) could be compared to determine the signal to noise ratio. Similarly, vector length comparisons of moderate and severe dehydration could be compared to establish any possible relations between vector lengths and dehydration magnitude (or PV loss). In addition, experiments involving dehydration followed by rehydration would be of interest to evaluate whether vector movement “reverses” back toward its baseline euhydrated value. Importantly, the RXc-score graph approach (21) allows retrospective analysis of historical studies to potentially answer these questions post hoc.

We conclude that, under tightly controlled experimental conditions, single “spot” measurements using the RXc-score graph method detected dehydration correctly only 22% of the time (4 of 18 subjects). However, using serial RXc-score measurements, directional changes in vector values were consistent 100% of the time (18 of 18 subjects), independent of whether dehydration was intracellular or extracellular in character. However, these directional changes require a “true” baseline euhydrated value, which can be a limitation in most clinical settings. The RXc-score graph method may therefore alert clinicians to changes in hydration state (12), which may help strengthen clinical interpretation of other recognized change measures of hydration (28) but will not provide a diagnostic value from a single measurement.

We thank A Piccoli for providing us with the BIVA analysis tools.

The authors’ responsibilities were as follows—all authors: contributed to the study conception, data analysis, interpretation, and writing. The authors had no conflicts of interest to report.

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