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**VALIDATION OF BIOIMPEDANCE SPECTROSCOPY TO ASSESS ACUTE CHANGES
IN HYDRATION STATUS**

by

Karen Jeanne Higgins

A Dissertation Submitted to the Faculty of the
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ABSTRACT

In this study bioimpedance spectroscopy (BIS) was validated as a field method for measuring short-term, small changes in hydration status by comparing extracellular water change (Δ ECW) estimated by BIS with a criterion method (bromide dilution), dual energy X-ray absorptiometry (DXA) and body weight (BW). A secondary aim was to compare BW to bromide dilution as a method for estimating acute Δ ECW. Finally, BIS was compared to DXA and single frequency bioimpedance analysis (SF-BIA) instruments to assess acute hydration effects on body composition estimates.

During dehydration, no significant differences were found between bromide and BIS measures of Δ ECW. The Δ ECW measured by DXA (DXA- Δ ECW) and BW (BW- Δ ECW) was significantly different from bromide-estimated Δ ECW (Br- Δ ECW), but not from BIS estimates (BIS- Δ ECW). During rehydration, there were no significant differences between Br- Δ ECW and the other methods. When using BW as the reference, results were more consistent in that BW- Δ ECW was significantly correlated with both BIS- Δ ECW and DXA- Δ ECW regardless of hydration status. These findings suggest that bromide may not be an appropriate criterion method for estimating short-term changes in hydration status.

Regardless of hydration status, BIS provided accurate measures of fat-free mass (BIS-FFM) and fat mass (BIS-FM) that were comparable to, or better than, estimates by SF-BIA. At baseline and after dehydration BIS-FFM had the highest correlation with DXA estimates (DXA-FFM), although two SF-BIA instruments (Bio-Resistance Body Composition Analyzer from Valhalla Scientific and The Body Comp Scale from American Weights & Measures) produced good estimates of FFM. Rehydration appeared to affect the accuracy of FFM measurements by BIS and SF-BIA as evidenced by lower, more moderate correlations to DXA-FFM. Phase-

dependent effects on percentage body fat (%BF) estimates were also apparent. In contrast, all methods performed reasonably well for estimates of FM, regardless of hydration status.

In summary, BIS provides accurate estimates of Δ ECW compared to either bromide dilution or BW, especially in the direction of dehydration. BIS also provides accurate estimates of FFM and FM regardless of hydration status. Further study of bromide dilution as a criterion measure is needed to validate its use in measuring Δ ECW during acute shifts in hydration.

CHAPTER 1

INTRODUCTION

Maintenance of hemodynamic stability is critical for health and functional capacity. Hemodynamic status is defined by dehydration, euhydration and overhydration. Acute changes in hydration can have serious and life-threatening consequences. A five-percent acute fluid loss can cause loss of motor control, apathy, nausea, increased heart and respiratory rates (Roth, 1967). Clearly, this has implications for individuals who work and exercise outside in hot climates such as those found in the desert southwest. Reports of death due to dehydration induced by participation in sports or military training are particularly disconcerting since they are entirely preventable. From a clinical perspective, dehydration and over-hydration pose particular challenges. Dehydration in the elderly often goes unnoticed and untreated, resulting in debilitation and potential misdiagnosis. Over-hydration complicates interpretation of laboratory values and stresses biological systems that are already compromised. This potential loss of health, productivity, and physical performance underlies the relevance and importance of establishing noninvasive and inexpensive field methods to monitor acute changes in fluid status.

Single frequency bioimpedance analysis (BIA) was originally proposed as a method to measure total body water (TBW) (Hoffer, Meador, and Simpson, 1969) and fat-free mass (FFM) (Lukaski et al., 1985). BIA later evolved into a measure of extracellular water (ECW) (Lukaski and Bolunchuk, 1988), and body cell mass (BCM) (Kotler et al., 1996; Lukaski, 1996). The application of BIA to body composition measurement in research and clinical settings resulted in extensive studies in a wide variety of populations (Houtkooper et al., 1989, Houtkooper et al., 1992; Stolarczyk et al., 1994). In 1994, the National Institutes of Health (NIH) convened a technology assessment conference to evaluate the appropriate uses and applications of BIA

(Anonymous, 1996). It was concluded that BIA provided a reliable and accurate estimate of TBW under conditions in which body water distribution was not altered. It was not deemed useful for measuring TBW in states of body fluid flux, such as those that occur with acute changes in hydration. Since measures of FM, FFM and BCM are derived from equations based upon the TBW estimated from the measurement of the electrical property of resistance, short-term changes in body composition also could not be accurately measured with BIA. Given these constraints, BIA was considered acceptable for measuring longer-term changes in hydration status and body composition in healthy populations and in patients with chronic conditions such as mild-to moderate obesity and diabetes mellitus, particularly with the use of population-specific equations.

In 1997, another panel was convened to review the progress made on the concerns outlined by the 1994 NIH conference. After review of the most recent evidence, the panel reaffirmed the original conclusion that single frequency BIA body water estimates should be limited to studies in healthy subjects or for disease states in which ECW/ICW ratio was normal (Ellis et al., 1999). This limitation also applied to TBW-derived FFM estimates.

The need to find more accurate methods for determining hydration status and body composition changes, particularly for clinical populations, generated research efforts to investigate a promising new methodology known as bioimpedance spectroscopy (BIS). Preliminary research on BIS in both healthy subjects (van Marken Lichtenbelt et al., 1994) and hemodialysis patients (Ho et al., 1994) indicated that this technology could overcome many of the limitations of BIA. Theoretically, BIS could measure not only TBW, it could also discriminate between the ICW and ECW body compartments. This application would have particular relevance to clinical populations in which states of hydration and ECW/ICW ratios are not static.

The differences between BIS and BIA can be explained by the theoretical underpinnings of the technology. Single frequency BIA measures whole body impedance (Z) of body by introducing a small alternating current (< 1 mA) at a fixed frequency of 50 kHz. Impedance is a function of resistance and reactance where resistance is the opposition of the extracellular body mass to the current flow, and reactance is the additional opposition due to the capacitance of cell membranes and tissue interfaces of intracellular mass. However, Deurenberg and Schouten (1992) demonstrated that a frequency of 50 kHz was too low for full penetration of current through the cell. Thus the assumption that the customary fixed, single frequency of 50 kHz used in BIA devices is large enough to include both resistance and reactance components across the “total body” does not hold true. Additionally, single frequency BIA is based on the assumptions that the conductor has a homogeneous composition, a fixed cross-sectional area, and a uniform density distribution. These assumptions are not met in the human body, particularly in states of altered hydration of the FFM, localized fluid accumulation, and loss or changes in fluid distribution.

In contrast to BIA, BIS (Xitron Technologies, Inc., San Diego, CA) combines a modification of the Hanai mixture theory (Hanai, 1968) for volume estimation and a biophysical model in which a spectrum of frequencies is used to describe resistance and capacitance of the whole body (Cole, 1940). From this range of frequencies (5 kHz to 500 kHz), the resistance of ECW and ICW can be determined and volumes estimated. The resistance and reactance measurements are fit to an electrical circuit model (Cole-Cole) using iterative curve fitting software. The resistance/reactance curves identify the middle or characteristic frequency (f_c) of maximum reactance. In the single frequency BIA methods, the 50 kHz frequency used is considered to be the f_c . In fact, f_c can vary over a wide range of values for healthy individuals, changing with alterations in body water compartments or in cell membranes (Lofgren, 1951).

BIS is the only impedance technology capable of identifying individual f_c . To translate resistance measurements into volume estimates, BIS incorporates the Hanai mixture theory which is based on emulsion sciences. This theory describes the effect of concentration of nonconducting material on the apparent resistivity (ρ) of the surrounding conductor fluid. As such, tissues are considered mixtures of materials with differential resistivities that impact the conductivity. The equation derived for volume estimates is comprised of many elements in which the apparent resistivity of the ECW and ICW, though considered a constant, is modifiable depending upon the population studied.

BIS has been validated against criterion methods in healthy subjects (De Lorenzo et al., 1997; Ellis and Wong, 1998), and in certain clinical populations (van Marken Lichtenbelt et al., 1997; van den Ham et al., 1999). Correlations between BIS-predicted and dilution-determined TBW and ECW in young- to middle-aged subjects have been reported to be $r = 0.89$ to 0.99 (SEE = 1.26L to 2.5 L) for TBW and 0.89 to 0.96 (SEE = 0.88 to 0.97 L) for ECW (Van Loan et al., 1993; Patel et al., 1994; De Lorenzo et al., 1997).

To study the validity of BIS in measuring long-term change in body fluid compartments, Earthman et al. (2000) measured change in intracellular water (Δ ICW) of patients with HIV infection over an average of 20 weeks of anabolic steroid therapy. ICW was determined by BIS and four different SF-BIA equations and compared to ICW pre- and post-therapy as derived from deuterium and bromide dilution (ICW was defined as the difference between TBW and ECW). Only BIS-predicted Δ ICW were not statistically different from criterion derived measures.

To investigate the responsiveness of BIS in measuring short-term change in body fluid compartments, Howell and Gaines (1994) subjected healthy volunteers to a controlled fluid fast/rehydration manipulation designed to induce an approximate change of 3% in total body weight. Change in total body weight and BIS-derived Δ ECW, Δ ICW and Δ TBW were recorded

after an 18-hour fluid fast and again following a 48-hour rehydration period. BIS- Δ TBW and BIS- Δ ECW showed significant shifts in the expected directions over the fluid fast. As expected, volume of BIS- Δ ICW showed no change over the course of the study.

Findings from more recent studies in which acute changes in hydration status were induced by heat or exercise (Koulmann et al., 2000), or infusion of hypertonic saline solution (Berneis and Keller, 2000) suggested that BIA was not able to accurately estimate TBW when acute shifts in body fluid occur. Gudivaka et al. (1999) reported similar findings in reference to measurements of TBW, ECW and ICW. Using isotope dilution (deuterium and sodium bromide) as criterion measures, expansion of the ECW space was induced by a 2-hour infusion of lactated Ringer solution in a group of healthy subjects. A second group received butamide, a potent diuretic. Single- and multi-frequency instruments for BIA were used to measure the fluid shifts in both groups. The authors concluded that only the multi-frequency model (Xitron 4000B) accurately predicted Δ TBW, Δ ECW and Δ ICW under conditions in which body water compartmentalization is altered.

BIS has been shown to be responsive to the short-term, acute changes in hydration status as characterized by certain clinical conditions. BIS was found to accurately predict TBW in pre- and post-hemodialysis patients (Ho et al., 1994) and to be superior to BIA in measuring TBW, ECW and ICW in cardiac surgery patients (Matthie et al., 1998).

All of these studies support the potential for BIS as a versatile method for measuring long- and short-term changes in body water, and by extension, body composition. However, validating instruments to monitor acute disturbances in body fluid distribution is limited by the accuracy of available reference methods such as isotope dilution. Although this important issue was raised in the 1997 NIH Technology Conference follow-up, it has received little attention. Acute changes in hydration are primarily reflected in the ECW space. Bromide dilution is a

standard criterion measure for ECW. The analytical precision of bromide measurements by this technique is approximately 2% (Miller and Cappon, 1984) with the accuracy estimated at 5% (van Kreel, 1994). Investigation into the use of simple, inexpensive, and non-invasive methods with comparable or improved accuracy and precision is needed to guide future efforts to study acute changes in hydration status.

There were three primary aims of this study designed to address the concerns raised at the NIH Technology Conference and its follow-up specific to acute changes in hydration status:

1. Validate BIS as a field method for measuring short-term, small changes (~3% total body weight) in hydration status by assessing and comparing Δ ECW by BIS (Hydra ECF/ICF Bio-Impedance Spectrum Analyzer (model 4200; Xitron Technologies, Inc., San Diego, CA)) with criterion dilution methods (Δ ECW by bromide), dual energy X-ray absorptiometry (DXA), and total body weight change during a fluid fast/rehydration manipulation.
2. Compare three single frequency BIA methodologies (tetrapolar electrode (Bio-Resistance Body Composition Analyzer, model 1990B; Valhalla Scientific, San Diego, CA); bipolar electrode, hand-held (Body Fat Analyzer, model HBF-306; Omron Healthcare, Inc., Vernon Hills, IL); and bipolar electrode scale (The Body Comp Scale, prototype model FS1535 & PAL101; American Weights & Measures, Rancho Santa Fe, CA)) to BIS as field methods to assess the effects of short-term, small changes in hydration status on estimates of FFM and fat-mass (FM).
3. Compare the responsiveness of bromide dilution and measures of total body weight to determine Δ ECW during a fluid fast/rehydration manipulation.

These aims are explored and presented in the form of publishable journal articles in Chapters 3 and 4. A full review of relevant literature follows in Chapter 2.

CHAPTER 2

REVIEW OF LITERATURE

Bioelectrical Impedance Analysis

Introduction and Definition of Terms

Acute changes in hydration status can have deleterious effects on performance, health and functional capacity. Military personnel and athletes in training can lose 2% to 8% of their total body water (TBW) during an acute exercise bout. This acute change in hydration status can result in loss of motor control, confusion, nausea and in some instances, death. In a clinical setting, acute changes in hydration status can result in misinterpretation of laboratory results and misdiagnosis. The development of bioelectrical impedance analysis (BIA) as simple, rapid, and inexpensive method for estimation of TBW has led to its widespread use in clinical and research settings.

As the relationship between obesity and chronic disease states became more apparent, BIA held promise as a noninvasive technique to measure body composition. Based upon the assumption that the water content of the fat-free mass (FFM) remains constant and that water is primarily associated with the FFM, Lukaski et al. (1985) suggested the use of TBW measurements by BIA for estimation of body composition. In the classic two-compartment model of body composition, body weight can be divided into its FFM and fat mass (FM). Once FFM is known, FM can be determined by the difference between body weight and FFM. BIA-derived TBW values are translated into estimates of FFM through the use of equations based on regression analysis. BIA equations must be validated using a direct measure of TBW (generally by isotope dilution) and FM (usually by hydrodensitometry or DXA). Most of the early published equations were validated using the two-compartment model of body composition

known to have significant limitations due to the inherent assumptions (Lohman, 1996). In the clinical setting, measurement of body composition has been used as an indicator of nutritional status. The parameter of interest, body cell mass (BCM), is defined as ‘the component of body composition that is metabolically active; it is responsible for all the oxygen consumption carbon dioxide production and performs all of the work (Shizgal, 1990) and is most closely associated with ICW (Moore and Boyden, 1963). Based on the initial observations of Thomasset (1963), Lukaski and Bolunchuk (1988) pioneered the use of BIA to measure BCM by employing two fixed, single frequency measurements to estimate ECW and TBW. ICW is then calculated by the difference between TBW and ECW and that value used as an estimate of BCM.

As the field has progressed, it has become divided along three major lines of inquiry. The primary application BIA has been to use single frequency devices in which impedance measurements are made at 50 kHz, and is referred to as single frequency BIA (SF-BIA). The second line of inquiry, termed “multifrequency BIA”, involves the use of impedance data measured at two fixed frequencies, one low (usually 5 kHz) and one high (50, 100, 200 or 500 kHz) (Segal et al., 1991; Deurenberg and Shouten, 1992; Van Loan and Mayclin, 1992), to predict ECW and TBW respectively. For the purposes of this paper, this approach will be referred to as either dual-frequency BIA (DF-BIA) or MF-BIA. This is in contrast to bioimpedance spectroscopy (BIS) which utilizes all of the impedance data measured over an entire spectrum of frequencies, from 5 kHz to 1,000 kHz (Lukaski, 1996).

In the next section, the basic principles of BIA are presented followed by a discussion of SF-BIA, MF-BIA and BIS in relation to measurements of body water compartments. A separate section will address the use of these devices in the determination of FFM and FM.

General Principles of Bioimpedance Analysis

BIA measures impedance (Z) of the body by introducing a small alternating current (< 1 mA) into the body and measuring the potential difference that results. Impedance (Z) is the opposition of a conductor to the flow of alternating current. This opposition has two components or vectors, termed resistance (R) and reactance (X) (Lukaski, 1996). Whereas R is equal to the pure opposition to the flow of alternating current, reactance is the opposition to the flow of electrical current caused by capacitance produced by tissue interfaces and cell membranes (Kushner, 1992). The relationship among Z, R and X can be expressed mathematically as:

$$Z^2 = R^2 + X^2 \text{ (Lukaski et al. 1985).}$$

BIA exploits the inherent differences in the ability of fat, bone and muscle to conduct electricity. Muscle, and other biological tissues high in water and electrolytes, conducts electrical currents readily because the current flows through physiologic fluids by the movement of ions. Tissues containing little water and electrolytes, such as fat and bone, are poor conductors and have a high resistance to the flow of current.

To translate impedance measurements into volume estimates of TBW, the body is viewed as a cylinder that is assumed to have a uniform cross-sectional area filled with a homogeneous conducting material of specific resistivity. The underlying principle of BIA is that impedance of this geometric whole isotropic conductor is related to its length and configuration, cross-sectional area, and applied signal frequency which is expressed mathematically as:

$$V = \rho L^2 / Z$$

Where V = volume; ρ = specific resistivity (assumed to be a constant); L = conductor length (equivalent to the measured height); Z = measured impedance (Kushner, 1992). Thus, the volume of TBW is directly related to body length, or height, and inversely related to impedance. Being that the contribution of resistance to impedance is much greater than that of reactance, and

that the specific resistivity is assumed to be constant, the above equation can be rewritten as $V = L^2/R$, where R is whole body resistance. In biological systems, height (H_t) replaces length (L) and this parameter, H^2/R , is referred to as the impedance index. The impedance index is entered into a regression equation to derive population-specific prediction models to estimate TBW and body composition. Therefore, BIA does not directly measure any biological component, such as fat, on the basis of the physical or biophysical model or reasoning.

Volume estimates based on BIA measurements are also frequency dependent (Cole, 1940). At low frequencies, the impedance of cell membranes and tissue interfaces is too large for conduction of current within the cells to occur. Consequently, the current is conducted only through the extracellular fluid. This measured impedance is considered resistive with no reactive component (Lukaski, 1996). As frequency increases, reactance increases to a critical threshold at which the opposition to current flow due to the capacitance is overcome. This critical threshold is termed the characteristic frequency (f_c), the point of maximal reactance. At frequencies exceeding this critical threshold, the current penetrates the cell walls and flows through the intracellular mass. Thus, at very low and very high frequencies the overall impedance is essentially independent of the capacitance. Measurements made with low frequencies, where virtually no conduction occurs, reflect ECW (De Lorenzo et al., 1997). In contrast, conduction is absolute at high frequencies and thus reflects total body water (TBW). The difference between TBW and ECW is ICW. As previously described, ICW approximates body cell mass (BCM), an important indicator of nutritional status often used in clinical settings.

The theory underlying BIS diverges from that of SF-BIA in two critical ways. The first difference is that extracellular resistance (R_e) and intracellular resistance (R_i) are determined by a model proposed by Cole (1940) in which Z varies as a function of frequency. At zero frequency ($f_0 = 0$), the current does not penetrate the cell membranes; thus Z is purely resistive and equal to

that of the extracellular compartment ($Z_0 = R_e$). At infinite frequencies (f_∞) the cells become transparent to the current such that Z is equal to R for a parallel circuit ($Z_\infty = R_e R_i / R_e + R_i$). Because measurements are not technically possible at zero or infinity frequency, the Z_0 and Z_∞ values have to be derived mathematically by fitting the shape of the X vs. R curve. The X vs. R values plotted as a function of frequency characteristically form a semicircular arc (Foster and Lukaski, 1996) describing changes in X relative to R as the frequency of the current is varied. In the simplest model, the Z_0 and Z_∞ are assumed to be resistance values for the ECW and TBW space respectively (Ellis, 2000).

The second distinguishing characteristic of BIS is that the body water volume predictions are based upon emulsion science theory as developed by Hanai (1968), rather than a statistical analysis of the impedance index and population-specific variables. This theory describes the effect of concentration of nonconducting material on the apparent resistivity (ρ) of the surrounding conductor fluid. As such, tissues are considered mixtures of materials with differential resistivities that impact the conductivity.

ECW and ICW volumes are predicted from the modeled R_e and R_i using the following equations derived from the Hanai mixture theory:

$$(1) \quad V_{ECW} = k_{ECW} / (Ht^2 \times Wt^{0.5} / R_e)^{2/3} \text{ and,}$$

$$(2) \quad (1 + V_{ICW} / V_{ECW})^{5/2} = [(R_e + R_i) / R_i] \times [1 + (k_p \times V_{ICW} / V_{ECW})]$$

where Ht = height; Wt = weight; and the R values are resistance values for the intra- and extracellular compartments. The k_{ECW} and k_p values are assumed constant and can be defined in terms of resistivity of extracellular and intracellular tissues, body density, and body volume (De Lorenzo et al., 1997) as follows:

$$(3) \quad k_{ECW} = 1 / 1,000 [K_B \times \rho_{ECW}^2 / D_B]^{1/3}$$

where $K_B = 4.3$ and is a factor correcting for whole body measurement between wrist and ankle, relating the relative proportions of the leg, arm, trunk and height to remove the geometry effects on resistivity; D_B is body density (kg/L) assumed constant at 1.05 kg/L and,

$$(4) \quad k_p = \rho_{ICW} / \rho_{ECW}$$

where ρ_{ECW} and ρ_{ICW} are the resistivities of extracellular and intracellular fluid ($\Omega \cdot \text{cm}$), respectively. These resistivity constants have been validated and appear to give the most physically plausible sized ECW and ICW spaces (De Lorenzo et al., 1997).

The application of the Hanai mixture theory used in BIS to predict body water volumes is supported in studies published by Patel et al. (1994) and De Lorenzo et al. (1997). Predicting volumes based on these scientific principles directly address the error sources and provide a more direct assessment of body water compartments as compared to BIA.

Single Frequency Bioimpedance Analysis (SF-BIA)

Single frequency analyzers were the first commercially available applications of the BIA methodology, and compose the majority of analyzers used in the research setting (Chumlea and Guo, 1994). After the convening of the 1994 NIH conference on BIA, a call for standardization of the method was made by the expert panel. It had become clear that BIA measurements were affected by changes in body position (Roos et al., 1992; Shirrefs and Maughan, 1994), consumption of food or beverages (Deurenberg et al., 1988; Rising et al., 1991), ambient air and skin temperature (Caton et al., 1988), recent physical activity (Liang and Norris, 1993), menstrual cycle (Gleichauf and Roe, 1989) and electrode placement (Lukaski et al., 1985). Although the report of the 1997 update on the NIH Technology Conference (Ellis, 1999) indicated that standardization continued to be a problem, standard measurement conditions have been adopted by the majority of researchers.

Recall that BIA measures impedance (Z) that is transformed into estimates of TBW through the use of the impedance index (H^2/R). Using height as a measure of conductor length, Hoffer et al. (1969) showed that the H^2/Z was a better predictor of tritium-derived estimates of TBW than was the weight alone in 20 normal volunteers ($r = 0.92$ vs. $r = 0.74$). Subsequent validation studies demonstrated that H^2/R was the most significant independent predictor of TBW, accounting for 94–96% of the total variability (Lukaski et al., 1985; Kushner and Schoeller, 1986), with an SEE between 1.4 and 3.25 L (Kushner, 1992). Including variables such as weight, age, and gender further reduced the standard error estimate (Segal et al., 1985) improving accuracy. This generated a multitude of age- and population-specific equations (Lukaski et al., 1985; Kushner and Schoeller, 1986; Davies et al., 1988; Heitmann, 1990; Kushner, 1992) which became characteristic of the field. In general, the reported prediction errors for equations developed in various studies are 1.5–2.5 kg for TBW and 1.0–1.5 kg for ECW (Baumgartner, 1996).

Whole body BIA is based on the assumption that the human body is a conductor of uniform length and cross-sectional area. In reality, the geometrical shape of the human body more closely approximates a series of five cylinders: two arms, two legs, and the trunk. Since R is inversely proportional to cross-sectional area, the upper and lower extremities (which have the smallest cross-sectional area) have the most influence on whole body R measurements. Thus, BIA disproportionately considers the extremities, with the arm contributing to 45% of whole body R and the trunk 5–12% (Zhu et al., 1998). Challenges to the original assumptions of BIA led investigators to experiment with segmental analysis, particularly as it related to body composition and fluid shifts in clinical populations. Although some guidelines for the method to have been published (Organ et al., 1994; Cornish et al., 1999), no standards for the placement of the electrodes for segmental analysis have been universally adopted. Compared to traditional SF-

BIA, segmental BIA has been shown to be potentially advantageous for the assessment of whole body composition in children (Fuller et al., 2002) and for measuring body water compartments in clinical populations (Song et al., 1999; Bracco et al., 2000), but not for TBW estimation in healthy adults (Wotton et al., 2000).

Single Frequency Bioimpedance Measurement of Acute Changes in Hydration

There are few studies that specifically address the use of traditional SF-BIA for measuring acute changes in hydration status in healthy individuals. Such studies are complicated by the fact that any artificially-induced changes in hydration status may also impact the ionic concentration of the body water compartments. Impedance (Z) of body fluids and tissues are dependent on the electrolyte concentrations. Moreover, the specific resistivity of the compartments is altered by changing concentrations of sodium, potassium, chloride, and bicarbonate ions (Kushner, Gudivaka, and Schoeller, 1996). Thus, the underlying assumptions of BIA, that tissue are electrically isotropic and of constant specific resistivity, do not hold true. Nevertheless, clinical conditions characterized by acute fluctuations of body water compartments provided the only early in vivo model to study the accuracy of BIA for determining acute changes in TBW.

Clinical researchers measured alterations in fluid status in patients undergoing dialysis for renal disease (Bohm et al., 1990; Kushner, deVries, and Gudivaka, 1996), surgery (Schroeder, Christie, and Hill, 1990; Fearon et al., 1992), and paracentesis for ascites in end stage liver disease (Schloerb et al., 1996). Significant intra- and interindividual variability was consistently reported, leading investigators to question its precision and utility for use in individual patients (Jacobs, 1996). Nonetheless, small studies revealed that BIA estimates of TBW change were highly correlated with volume of fluid removed during dialysis (Bohm et al., 1990), but less

strongly with changes in total body weight before and after dialysis (Kurtin et al., 1990). Resistance, as a predictor variable, was moderately correlated with fluid balance in cardiac patients (Meguid et al., 1992), but not in the critically ill experiencing a weight change of less than 3 kg (Roos et al., 1993). Given that small, short-term weight shifts generally reflect changes in the ECW compartment, these findings indicated that traditional SF-BIA was not accurate enough to measure body water in states of fluid flux; a point duly noted in the NIH technology assessment conference statement (1996).

Dual Frequency (DF-BIA)/Multifrequency BIA (MF- BIA)

DF-BIA evolved from the idea that frequency-specific measurements would provide a more precise index of particular biological variables, specifically ECW and TBW, than a single frequency measurement. The basis for this hypothesis was observations from cell suspension experiments in which Thomasset (1963) demonstrated that current applied at 5 kHz was conducted around the cells. At higher frequencies, the electrical current penetrated the cell. It was proposed that, in biological systems, ECW would be predicted at 5 kHz and TBW at 1 MHz.

As DF- and MF-BIA technology became available, many experimented with resistance measurements at various single, fixed frequencies to determine the best frequency at which to perform measurements to predict TBW and ECW. Investigators correlated criterion measures by dilution volumes with a range of impedances in human subjects and developed a new set of prediction equations. Segal et al. (1991) reported significant correlation coefficients for H^2/R at 5 kHz with ECW ($r^2 = 0.93$) when corrected for height and weight; and H^2/R at 100 kHz with TBW ($r^2 = 0.947$) corrected for weight in 36 healthy men. Cross validation analysis on two randomly selected subsets indicated that the prediction equations were reproducible and valid. However, in HIV-positive male subjects (Paton et al., 1998) and surgical patients (Hannan et al., 1994), no

statistically significant differences between isotope-dilution predicted TBW with measurements made at frequencies of 5, 100, 200, 500 and 1,000 kHz compared with SF-BIA were found. Deurenberg, Tagliabue, and Schouten (1995) reported comparable correlation coefficients for estimates of TBW at 50 and 100 kHz ($r^2 = 0.95$), and for ECW measured at 1 and 5 kHz ($r^2 = 0.86$ – 0.87). More recently, Simpson et al. (2001) demonstrated a higher correlation between TBW estimates from measurements made at 50 kHz ($r^2 = 0.99$) compared to 200 kHz ($r^2 = 0.96$). The findings of these studies indicate that, on the basis of correlations between R or Z and ECW, frequencies <10 kHz are good predictors of ECW. However, the best frequency at which to measure TBW is not as clear. Consequently, there is no consensus about the appropriate frequency for use in predicting TBW; therefore, the use of population-specific prediction equations using 50 kHz (Kushner and Schoeller, 1986; Lukaski and Bolunchuk, 1988; Kotler et al., 1996; Paton et al., 1998), 100 kHz (Segal et al., 1991; Deurenberg et al., 1995), 200 kHz (Hannan et al., 1998), 224 kHz (Van Loan and Mayclin, 1992) and 500 kHz (Hannan et al., 1994) persists.

Dual/Multi-Frequency Bioimpedance Measurement of Acute Changes in Hydration

As with SF-BIA, studies examining the use of DF- and MF-BIA to predict change in ECW and TBW are scarce. In a series of early studies (Tedner et al., 1983; Tedner and Lins, 1984), it was demonstrated that acute fluid shifts induced by hemodialysis or intravenous infusion were more accurately predicted by measuring the change in impedance at 1.5 kHz and 150 kHz, than at 50 kHz. Deurenberg and Shouten (1992) validated the use of 50 and 100 kHz frequencies to predict Δ TBW after administration of a diuretic drug in 12 healthy subjects and reported that impedance at 1 kHz was the best predictor of Δ ECW. In contrast, BIA was not able to detect differences in TBW despite measured changes in impedance. Despite measured changes in

impedance, however, BIA was not able to detect differences in TBW under conditions of unknown hydration status and unknown osmolality (Berneis and Keller, 2000). Such conflicting results may be more indicative of the complex nature of measuring acute changes in body water compartments than the precision or accuracy of the instrument. For example, diuretic-induced hypohydration results in natriuresis creating an iso-osmotic state. This not only alters the dynamics of water redistribution but also changes the resistance of the medium (Sawka, 1992). Until experimental designs can be developed in which the underlying assumptions of BIA are not violated simply by the methodology, a definitive answer as to the use of DF-BIA in the measurement of ECW and TBW will be elusive.

Given that all of the assumptions required for the use of SF-BIA also hold true for MF-BIA, the primary advantage of MF-BIA is its versatility in estimating ECW and TBW using several different single, fixed frequencies. To its detriment, MF-BIA estimates of body water compartments are statistically derived, not directly measured. BIS has the potential to overcome these limitations, especially under conditions in which acute changes in hydration status occur. Indeed, Ho et al. (1994) demonstrated that BIS had a reduced percentage error of measurement (6.2%) when compared to linear regression modeling using H^2/R measured at 148 kHz (6.7%) in hemodialysis patients, although both methods could accurately predict TBW as measured by deuterium dilution space. As will be discussed in the next section, research increasingly highlights the potential value of BIS, particularly for use in individuals with altered fluid distribution.

Bioimpedance Spectroscopy (BIS)

In contrast to using fixed, multiple frequencies and linear regression modeling to predict body compartment water volumes, BIS models impedance data derived from scanning a wide

range of frequencies through the application of Cole-Cole curve fitting procedures and equations derived from mixture theory. The first successful validation of ECW, TBW and ICW by using BIS was reported in 1992 (Matthie et al., 1992), with subsequent validation of TBW and ECW against isotope dilution in healthy adults (van Marken Lichtenbelt et al., 1994; Armstrong et al., 1997), hemodialysis patients (Ho et al., 1994) and growth hormone deficient adults (van Marken Lichtenbelt et al., 1997). Correlations between BIS-predicted and dilution-determined TBW and ECW in young- to middle-aged subjects have been reported to be $r = 0.89$ to 0.99 ($SEE = 1.26$ to 2.5 L) for TBW and 0.89 to 0.96 ($SEE = 0.88$ to 0.97 L) for ECW (Van Loan et al., 1993; Patel et al., 1994; De Lorenzo et al., 1997).

Some studies comparing BIS and BIA approaches report no advantage of BIS of predicting volumes of ECW, TBW and ICW (Paton et al., 1998). Using correlation statistics, impedance measured at any frequency will equally predict absolute volumes of each body water compartment (van Marken Lichtenbelt et al., 1994). This is due to the high intercorrelation of these variables given that ECW and ICW are generally tightly regulated and comprise TBW. As such, the ability of a method to measure change in body water compartments is a better indicator of the validity of the instrument.

Earthman et al. (2000) compared BIS to SF-BIA for predicting Δ TBW, Δ ECW and Δ ICW over an average of 20 weeks of anabolic steroid treatment in HIV-positive subjects. The SF-BIA equations tested included Deurenberg et al. at 100 kHz R (1995), Segal et al. at 100 kHz R (1991), Hannan et al. at 200 kHz (1998) and 500 kHz (1994). In addition, 50 kHz equations developed specifically from subjects with HIV were also evaluated (Kotler et al., 1996; Paton et al., 1998). As expected, predictions of absolute ECW, TBW, and ICW volumes were similar for all methods tested. However, only BIS-predicted Δ ICW were not statistically different from criterion-derived measures (TBW by deuterium dilution – ECW by bromide dilution). Van Loan

et al. (1995) were able to successfully track changes in TBW before, during, and after pregnancy with BIS. The accuracy of measurements was improved after adjustment for changes in resistivity due to increases in plasma volume and the corresponding decline in hematocrit, hemoglobin and electrolytes. These studies highlight the potential of BIS to monitoring long-term change in body water compartments.

Two studies of note suggest that BIS is also responsive to short-term, acute changes in hydration of healthy adults. In the first study, a weight loss of approximately 3% was induced by subjecting university students to a controlled fluid fast/rehydration manipulation (Howell and Gaines, 1994). Change in the total body weight and BIS-estimated ECW, ICW and TBW was recorded after an 18-hour fluid fast and again following a 48-hour rehydration period. All parameters showed no significant differences from baseline levels after rehydration. However, BIS-estimated ECW and TBW indicated significant shifts in the predicted directions over the fluid fast. No change occurred in the ICW compartment. These findings were supported by O'Brien et al. (1999) in which BIS was used to assess Δ TBW associated with exercise/heat-induced hypohydration that resulted in a 4–5% weight change in healthy men. Estimated Δ TBW by BIS was significantly correlated with Δ TBW by deuterium dilution as body water became depleted. Hence, BIS appears to be responsive to small changes in hydration status.

Measuring acute fluid changes in clinical populations has yielded less consistent results. BIS has been shown to underestimate TBW (Ho et al., 1994; van den Ham et al., 1999; Cox-Reijven et al., 2001; Cox-Reijven, van Kreel, and Soeters, 2003) and to overestimate ECW in some (Van Loan et al., 1995; van den Ham et al., 1999), but not all (Cox-Reijven and Soeters, 2000) validation studies. Matthie et al. (1998) established that resistivity is a scalar term dependent upon the dilution method used. Different dilution methods produce differently sized ECW and TBW spaces. For example, ECW measured by sulfate space is 20% smaller than ECW

measured by bromide space (De Lorenzo et al., 1997). Thus, by changing the resistivity constant to adjust for such difference, smaller mean differences in volume estimates between BIS and dilution were found. Studies on hemodialysis patients (Jabara and Mehta, 1995; Jaffrin et al., 1996) and the critically ill (Plank and Hill, 2000) suggest that BIS is responsive to acute changes in body water compartments. However, more research is needed to determine population-specific adjustments in the BIS mixture model to improve accuracy of BIS in measuring change in clinical populations (Matthie et al., 1998; Ellis and Wong, 1998; Cox-Reijven et al., 2000).

It appears that the dynamics of acute fluid shifts induced by clinical conditions and treatments such as dialysis may alter as yet undefined parameters that influence the accuracy of measurements. Matthie et al. (1998) reported changes in C_m, f_c , and the Cole term α during fluid removal by ultrafiltration in hemodialysis patients, a finding later supported by Cox-Reijven et al. (2001). This suggests that treatment-induced changes in cell volume and/or cell membrane characteristics may have occurred. Furthermore, alterations in serum electrolytes, hematocrit, and blood flow are known to cause changes in Z and resistivity independent of fluid volume (Kushner et al., 1996). Failure of BIS to accurately measure volume changes in peritoneal dialysis (PD) was attributed to the change in conductivity due to the dialysate and to the relatively low contribution of the trunk to whole body impedance (Zhu et al., 2000). Using a segmental approach and modifying the resistivity constant, these researchers were able to detect 91–95% of the volume changes during PD with BIS. A better understanding the effect of different conditions/interventions on cell characteristics that influence resistance is needed before BIS can be adequately evaluated as a tool for use in the clinical setting.

In summary, BIA can provide accurate estimates of absolute TBW when correct procedures are followed and population-specific equations are employed. The ability to accurately assess water compartments by BIA is limited, especially for measures of acute and

long-term Δ ECW. This highlights the advantage of BIS in that BIS can discriminate among the water body compartments and appears to adequately measure change. The strength of BIS lies in the underlying theoretical basis of the methodology and the potential utility of the technique in a wide variety of settings.

BIA/BIS Measurement of Body Composition

For more than a decade, BIA has fulfilled the need for an accurate and non-invasive method to measure body composition in health and disease. Since the NIH Technology Conference proceedings were published (Anonymous, 1996), much research has focused on identifying the physical factors that influence the validity of the electrical measurements and improving models that yield meaningful estimates of body composition. In SF- and DF-BIA, answers have come primarily from attempts to validate and cross-validate equations in a range of populations, including clinical populations, children and the elderly. The only attempt to truly modify the method is the application of a parallel model to replace the series model implicit in the BIA measures of resistance and reactance data for impedance. BIS studies have become increasingly focused on identifying modifiable elements within the equation used to estimate body composition parameters. As such, research has produced some insight into alternate resistivity, conductivity, body density and geometry constants to better estimate body composition in a variety of populations.

Review of Body Composition Models and Assumptions

Estimation of body composition from body density is known as densitometry. The long-standing criterion method, hydrodensitometry, is based upon the classic two-component (2C) model in which body weight is divided into FFM and FM. The valid estimation of body composition from body density is dependent upon the following assumptions: 1) separate

densities of the body compartments are additive; 2) the densities of the body are constant from person to person; 3) the proportions of the constituents other than fat are constant from person to person and; 4) the person being measured differs from the standard reference body only in the amount of body fat or adipose tissue (Going, 1996). Given that the primary constituents of the FFM component (water, protein, and mineral) are known to vary considerably among different populations, the assumption of chemical constancy is a major limitation in the use of densitometry for body composition measurements.

Three- and four-component (3C, 4C) models of body composition evolved as technological advances allowed for measurement of the separate constituents of FFM (water, protein, and mineral). For example, equations for estimating percentage of body fat (%BF) based on 3C and 4C models necessitated the measurement of body density (hydrodensitometry), body water (isotopic dilution) and/or bone mineral (DXA). Despite the potential propagation of error introduced by multiple measurements, these models represented a significant improvement because they were able overcome some of the limitations imposed by the assumptions. These models became increasingly employed as criterion measures in validation studies for field methods in body composition research.

Since the 1994 NIH Technology Conference and subsequent publication of its findings, the “gold standard” of hydrodensitometry has been virtually abandoned in favor of DXA as the reference method to which BIA and BIS are compared. Essentially, DXA is a 3C model that measures bone mineral content (BMC), lean tissue and FM (Sutcliffe, 1996). Except for the assumed constancy of the hydration of non-fat soft tissue, it is not based on the assumptions regarding the density and composition of the FFM. However, variations in fat thickness and body thickness can affect the accuracy of the measurements (Laskey et al., 1992).

While DXA has been validated against *in vivo* neutron activation (Aloia et al., 1995), hydrodensitometry (Van Loan and Mayclin, 1992) and the 4C model (Prior et al., 1997), issues about hydration (Roubenoff et al., 1993), software version (Cordero-MacIntyre et al., 2002) and use of different DXA instruments (Modlesky et al., 1996; Van Loan et al., 1999) have been raised. Modlesky et al. (1996) found that the Lunar DXA gave lower values for body fat (2 kg) and higher values for FFM (2 kg) compared to the Hologic instrument in adult men. Within the Lunar models (DPX and DPX/L), Oldroyd et al. (1998) reported a significant difference in %BF and FM with the DPX giving higher values than the DPX/L. Nonetheless, DXA has a high degree of precision with reported coefficients of variation for %BF of 2.0–5.9% (Haarbo et al., 1991) and 1.0% for FFM (Slosman et al., 1992). Because DXA requires only moderate cooperation from the subject and delivers a low dose of radiation (Lohman, 1996; Pichard, Kyle and Slosman, 1999), DXA has become the reference method of choice for short- and long-term BIA validation studies of body composition in a variety of populations.

Precision and Accuracy of BIA/BIS

Accurate and precise estimates of body composition by BIA requires following well-defined standards for measurements and careful selection of the prediction equation. When applying standard BIA procedures and the appropriate population-specific equations, the SEE for FFM has been reported to be 1.7–3.5 kg (Baumgartner, 1996; Houtkooper et al., 1996). Ideally, prediction errors for FFM should not exceed 2.5 kg in men or 1.8 kg in women (Houtkooper et al., 1996). BIA prediction errors for %BF range from 3.5–5.0% (Houtkooper et al., 1996) which exceeds the acceptable level of 3% recommended by Lohman (1996).

The choice of the reference method by which a prediction equation is validated impacts the reported accuracy of the method. Comparison of BIA validity studies for commonly used

equations estimating FFM in general populations of men and women illustrates this point. When body density was used as the criterion measure, the coefficients of determination for FFM estimates ranged from 0.93–0.98 (SEE = 2.1–2.9 kg) (Lukaski et al., 1986; Deurenberg et al., 1991). When validated against 3C and 4C models, coefficients were similar ($R^2 = 0.91$ – 0.96) but the SEE were larger (2.47–3.06 kg) (Segal et al., 1985; Baumgartner et al., 1991). Lower correlations and SEE were reported when comparing FFM estimates from the Deurenberg ($r = 0.89$, SEE = 2.0 kg), Baumgartner ($r = 0.91$, SEE = 2.8 kg), and Geneva ($r = 0.89$, SEE = 1.6 kg) equations to Hologic DXA estimates (Pichard et al., 1999; Genton et al., 2001). As these studies indicate, consideration of the criterion measure employed is necessary when evaluating the accuracy of BIA.

The use of Bland-Altman analyses has become standard in body composition studies (Bland and Altman, 1986). This statistical procedure provides insight into the potential bias and limits of agreement between the criterion method and the new method being validated. Pichard et al. (1999) and Genton et al. (2001) reported limits of agreement between BIA and DXA estimates of FFM in a healthy population range from -3.25 to $+3.25$ kg for women, and -3.77 to $+4.15$ kg for men. No systematic bias between the methods was observed. In general, the mean differences between BIA and DXA measures of FFM were greater for women (-7.1 ± 2.3 to 4.3 ± 2.9 kg) than for men (-6.7 ± 2.7 to 1.4 ± 2.5 kg).

BIA prediction equations for %BF are less accurate than those for FFM, with reported coefficients of determination between 0.60 and 0.90 and SEE of 3.3–5.5% (Houtkooper et al., 1996). Ravaglia et al. (1999) demonstrated a significant overestimation of %BF by BIA in healthy men aged 20–95 years, which was most evident in those older than 80 years. Even with the use of age-specific equations, the limits of agreement were wide ($\pm 10.74\%$). Bolanowski and

Nilsson (2001) found gender-specific differences in %BF as measured by BIA with lower correlations to DXA measurement in men ($r = 0.80$) vs. women ($r = 0.92$). However, these investigators reported an underestimation of %BF compared to DXA. Similar findings were reported in patients with end-stage renal disease in whom BIA estimates of %BF were 6–7% less than DXA estimates (Flakoll et al., 2004). Both studies reporting underestimation of %BF used the Lunar DPX/L model, which may explain the conflicting results of the Ravaglia (1999) study which used the Hologic QDR 2000 DXA model. Nonetheless, the variability and relative inaccuracy of BIA estimates of %BF, perhaps compounded by the need for population-specific equations, brings into question its usefulness as a field method for static measures of body fat.

Less data on the accuracy and precision of BIS estimates for body composition in healthy populations are available. Van Loan et al. (1993) reported a correlation between FFM estimates from BIS and densitometry of 0.94 with an SEE of 2.6 kg in a small sample of men and women. BIS estimates of FFM were similar to densitometry and significantly higher than DXA estimates in a group of Caucasian and Chinese-American women (Van Loan, 1998). In children, a significant correlation between BIS and DXA estimates of FFM ($r = 0.83$), FM ($r = 0.92$), and %BF (0.73) were found (Fors et al., 2002). The mean differences in estimates were -2.31 kg for FFM, 2.95 kg for FM, and 7.1% for %BF with wide limits of agreement for all parameters. In a different study of children, Treuth et al. (2001) reported smaller limits of agreement between BIS and DXA (-4.4 to -0.10 kg for FM and -0.4 to 3.4 kg for FFM). At this time, there is insufficient evidence to adequately evaluate BIS measures of FFM, FM and %BF in the general population.

In summary, BIA can produce relatively accurate and reliable estimates of body composition when standardized techniques are employed and appropriate population-specific equations are selected. Although studied less than BIA, BIS has been used to accurately predict

body composition (Van Loan et al., 1993; Van Loan, 1996). Regardless of the technology, estimates of FFM and FM are second order and prone to error. Given the fundamental differences in these technologies, the potential theoretical advantage of BIS over BIA is further explored in the following sections.

BIA/BIS Measurement of Body Composition

The valid application of BIA is based upon the assumptions that the ratio of FFM to TBW is 73.2% and the ratio of ECW to ICW is constant (Deurenberg, 1996). Any changes in body water distribution, f_e , ionic concentration of the conductor, or specific resistivities of the body water compartments will affect the resistance measurements and subsequent estimates of body composition (Deurenberg, 1996). It is in addressing these sources of error that provide BIS with a theoretical advantage over BIA.

The use of Cole modeling for calculating resistance may be advantageous in conditions of altered body water distribution. As early as 1988, it was suggested that body fatness had an independent effect on resistance measurements (Segal et al., 1988). Baumgartner, Ross and Heymsfield (1998) argued that adipose tissue, when sufficiently large, may have a direct effect on measured resistance resulting in overestimation of FFM and underestimation of FM when equations developed on non-obese subjects are applied to obese subjects. In these and other studies (Deurenberg, 1996; Jakicic, Wing, and Lang, 1998), such findings can be explained by the relative expansion of the ECW space and the apparent overhydration of FFM in the obese (Waki et al., 1991; Deurenberg, 1996). Similar findings are reported for children in whom the relative amount of water in FFM is greater (77% for girls and 75% for boys) (Van Loan, 1996). When BIA prediction equations developed on adults are applied to children, FFM is overestimated and BF is underestimated (Okasora et al., 1999; Treuth et al., 2001). A decrease in the relative

amount of TBW, as is found in the elderly, results in an underestimation of FFM and overestimation of FM by BIA (Ravaglia et al., 1999; Kyle et al., 2001; Haapala et al., 2002). Due to the empirical relationship between TBW (FFM) estimates and impedance index, shifts in body water distribution will have a profound effect on the accuracy of BIA. In BIS, measured resistances that are translated into discrete volume estimates of ECW and ICW may moderate the impact of changing water distributions.

Volume estimates by BIA and BIS assume resistivity is constant. Resistivity is dependent on the tissue microstructure, level of hydration, and ionic concentration (De Lorenzo et al., 1997). Indeed, the apparent resistivity of ICW has been shown to be greater than ECW and is generally 4 to 5 times less conductive than ECW (Deurenberg, 1995). It has been found that a 5 mmol change in ion concentration can affect predicted ECW by 1–2% and ICW by 4–5% (Scharfetter et al., 1995). In standard man, 2% and 5% errors represent errors of 0.34 L and 1.2 L in ECW and ICW, respectively (Xitron Technologies Operating Manual 1.01, 1999).

Unlike BIA, the BIS model actually incorporates measures of resistivity in the form of constants that can be refined according to the population studied. Cox-Reijven and Soeters (2000) reported empirical relationships between the several BIS model constants and BMI. Accuracy of BIS was improved by adjusting the constants k_{ECW} to the degree of overweight or by calculating K_b with the use of anthropometric data. The influence of ρ_{ECW} was particularly evident in that it was the most strongly correlated with BMI of the 3 variables comprising the k_{ECW} constant.

BIA/BIS Measurement of Change in Body Composition

BIA has been shown to be insensitive to changes in body composition over 1 to 2 days of weight loss. Deurenberg, Westrate, and Hautvast, (1989) reported no change in impedance during

a 24-hour fast despite a water-weight loss of 1.2 to 1.3 kg. This can be attributed to the fact that impedance at 50 kHz does not fully penetrate the cell membrane and thus, it cannot measure the loss of ICW (water bound to glycogen) that occurs during the first days of weight loss (Deurenberg, 1996). Moreover, hydration states affect the conductive properties of ECW and ICW, resulting in changes in the specific resistance of these fluid compartments (Cox-Reijven et al., 2003). In a sample of 12 healthy volunteers who consumed a very-low calorie diet for 2 days, the mean change in FFM as determined by densitometry (1.2 kg) was not significantly different than the mean body weight change (1.3 kg). However, mean loss in FFM as calculated by three BIA equations consistently underpredicted measured weight changes (Deurenberg, Westrate, and van der Kooy, 1989). There are no definitive data for BIS on the effect of acute weight loss on FFM estimates in healthy populations. However, the incorporation of Cole modeling and mixture equation theory by BIS may reduce the prediction errors associated with changes in conductivity and resistivity (De Lorenzo et al., 1997).

The ability of to measure body composition changes over time using BIA is limited by the underlying assumptions that body fluid distribution will not change and changes will be distributed proportionately between the trunks and the limbs (Baumgartner, 1996). Since the trunk contributes 10 to 20% of total body impedance, fluid changes in the trunk will have less effect on measured resistance than comparable changes in the limbs (Cox-Reijven, van Kreel, and Soeters, 2002). This insensitivity of whole-body resistance measurements due to fluid loss in the trunk is particularly relevant for populations characterized by an expanded ECW space. Furthermore, disproportionate losses between the limbs and the trunk will alter body geometry and introduce error into the resistance measurement. Cole modeling of resistance in ECW and ICW, combined with the adjustments of the K_b constant which corrects for geometry effects on

resistivity, can potentially attenuate the error introduced by changes in volume and composition of body water compartments due to weight loss.

Any change in the ECW:ICW ratio will impact f_c . If f_c is greater than 50 kHz, reactance affects impedance because the capacitance of the cell membranes has not been fully overcome. Therefore, the measured impedance is not a measure of TBW but rather a measure of ECW and a partial measure of ICW. In a group of patients with gastrointestinal disease experiencing severe weight loss over 1 to 6 months, the f_c was 45 kHz greater, and the cell membrane capacitance was lower, in those without weight loss (Cox-Reijven et al., 2003). BIS prediction errors for TBW and BCM (as defined by ICW and closely related to FFM) were not affected by weight loss. The source of the prediction errors (which were significant for the non-depleted group but not for the depleted group) may have been in the failure of the investigators to scale the BIS measurements to the dilution method as suggested by Matthie et al. (1998). Since BIS can discern differences in characteristic frequencies and cell capacitance, it can provide insight into sources of error that may be correctable. In contrast, the empirical relationship between impedance index and TBW-dependent FFM estimates in BIA leaves no room for improvement in performance except by adding population-specific equations to an already overwhelming list.

Given the understanding of how obesity and weight loss can alter parameters that invalidate the underlying assumptions of BIA, it is no surprise that most (van der Kooy et al., 1992; Hendel et al., 1996; Carella et al., 1997; Fogelholm et al., 1997; Sartorio et al., 2000), but not all (Evans et al., 1999), studies reveal inaccuracies of BIA measures of FFM and FM loss over time. In general, FM loss was underestimated and FFM loss was overestimated independent of the equation used. In instances of measuring weight gain during treatment with growth hormone (Beshyah et al., 1995) and in AIDS patients (Paton et al., 1997), these inaccuracies are less pronounced and highly equation-dependent.

After a literature search, no studies on non-segmental BIS measurement of long-term weight loss in the obese were identified. In HIV-positive men, BIS was found to significantly underestimate absolute values of FFM compared to DXA at 0 and 6 weeks of nandrolone therapy (Van Loan et al., 1999). After 12 weeks of intervention, however, no statistically significant differences in FFM estimates were observed. DXA results showed an intermediate increase in FFM at 6 weeks (1.8 kg) compared to BIS (2.8 kg), with only a 1 kg increase in FFM at 12 weeks. BIS results demonstrated a continual increase in FFM throughout the 12-week period which most closely matched the projected lean tissue accretion from nitrogen retention data. These limited findings suggest that BIS may be useful in measuring change in body composition.

Despite the plethora of research on BIA estimates of body composition, little evidence has been presented in the last 10 years that would drastically change the conclusions of the NIH Technology Conference in 1994. BIA may be useful in characterizing long-term changes in groups of subjects, but it is not useful in measuring short-term changes in body composition in individuals. Its applicability is limited to populations in which major disturbances of water distribution are not prominent and severe obesity is not present. On the other hand, lack of research on BIS in non-clinical populations is a major limitation in evaluating its applicability. The promise and potential of BIS remains, but cannot be realized until more is known.

The Current Study

The current study is unique in several ways. No previous study has validated BIS against criterion dilution methods to measure short-term, acute changes in ECW in healthy adults. Given the complex nature of illness- or treatment-induced dehydration, findings from clinical studies are not applicable to the general population. Validating a field method for measuring short-term changes in hydration would be useful in situations where monitoring of water intake is essential

for maximum productivity and safety. Secondly, the criterion method of bromide dilution for ECW measurement has a 2–3% precision, 2–4.2% accuracy (Lukaski, 1987) which is 0.7 L for standard man with 17 L of ECW (Xitron Technologies Operating Manual 1.01, 1999) requiring approximately a 2 L change (10%) to be detectable (Ellis, 2000). A secondary aim of this study is to determine if the responsiveness of bromide dilution and measures of body weight to reflect Δ ECW during dehydration and rehydration are comparable. If so, the use of body weight as a simple, non-invasive method to estimate body water loss or gain may be considered appropriate in certain field and research situations.

Interest in body composition analysis has exploded in the current fitness/medical culture. Consequently, the use of BIA instruments by the general public has considerably increased. They can be found in every conceivable market to include health clubs, physicians' offices, and weight loss clinics. Accurate reporting of results is dependent on many factors, hydration status being critical. Most researchers are in agreement with the NIH Technology Conference consensus that BIA should not be used to measure acute changes in body composition. Its applicability is limited to studies of populations and is generally not recommended for measurement of short- or long-term change in body composition in individuals. However, no definitive study has been published that compares the effect of acute changes in hydration on BIS vs. consumer models of BIA for measurement of FFM and FM. Since BIS measures ECW and ICW separately, alterations in these compartments are, theoretically, less likely to impact the estimates of body composition. A validated field method that is minimally influenced by hydration status has the potential for use in general populations, for athletes and medical personnel.

CHAPTER 3

VALIDATION OF BIOIMPEDANCE SPECTROSCOPY TO ASSESS ACUTE CHANGES IN HYDRATION STATUS

Acute changes in hydration status can have deleterious effects on performance, health and functional capacity. Military personnel and athletes in training can lose 2 to 8% of their total body water during a single exercise bout (Sawka, 1992; Montain et al., 1995). This acute change in hydration status can result in loss of motor control, confusion, nausea and in some instances, death (Roth, 1967). To prevent this potential loss of health and physical performance, bioimpedance analysis (BIA) has been widely used as a noninvasive and inexpensive field method to monitor body water compartments.

Single frequency bioimpedance analysis (SF-BIA) was originally proposed as a method to measure total body water (TBW) (Hoffer et al, 1969), then evolved into a measure of extracellular water (ECW) (Lukaski and Bolunchuk, 1988), and body cell mass (BCM) (Kotler et al., 1996; Lukaski, 1996). In 1994, the National Institutes of Health (NIH) convened a technology assessment conference to evaluate the appropriate uses and applications of SF-BIA. It was concluded that, for groups and not for individuals, SF-BIA provided a reliable estimate of TBW under conditions in which body water distribution was not altered. However, it was not deemed useful for measuring TBW in states of body fluid flux, such as those that occur with acute changes in hydration.

In 1997, another panel was convened to evaluate the progress made on the concerns outlined by the 1994 NIH Technology Conference. After review of the most recent evidence, the panel concluded that SF-BIA body water estimates should be limited to studies in healthy

subjects or for disease states in which ECW/ICW ratio was normal (Ellis et al., 1999). The need to find more accurate methods for evaluating hydration status generated research efforts to investigate a promising new methodology known as bioimpedance spectroscopy (BIS). The ability to discriminate between the intracellular (ICW) and ECW compartments using BIS methodology, in addition to measuring TBW, was of particular interest. Preliminary research on BIS in both healthy subjects (van Marken Lichtenbelt et al., 1994) and hemodialysis patients (Ho et al., 1994) indicated that this technology could overcome many of the limitations of SF-BIA for monitoring body water compartments.

BIS (Xitron Technologies, Inc., San Diego) combines a modification of the Hanai mixture theory (Hanai, 1968) for volume estimation and a biophysical model in which a spectrum of frequencies is used to describe resistance and capacitance of the whole body (Cole, 1940). From this range of frequencies (5 kHz to 1,000 kHz), the resistance of ECW and ICW can be determined and volumes estimated. The resistance and reactance measurements are fit to an electrical circuit model (Cole-Cole) using iterative curve fitting software. The resistance/reactance curves identify the middle or characteristic frequency (f_c) of maximum reactance. In the single frequency BIA methods, the 50 kHz frequency used is considered to be the f_c . In fact, f_c can vary over a wide range of values for healthy individuals, changing with alterations in body water compartments or in cell membranes (Lofgren, 1951). BIS is the only impedance technology capable of identifying individual f_c .

BIS has been validated against criterion methods in healthy subjects (De Lorenzo et al., 1997; Ellis and Wong, 1998), and in certain clinical populations (van Marken Lichtenbelt et al., 1997; van den Ham et al., 1999). BIS has been shown to be responsive to the short-term, acute changes in hydration status in healthy individuals (Howell and Gaines, 1994; Koulman et al.,

2000; Berneis and Keller, 2000) and under certain clinical conditions (Ho et al., 1994; Matthie et al., 1998).

These studies speak to the potential for BIS to measure short-term changes in body water. However, validating instruments to monitor acute disturbances in body fluid distribution is limited by the accuracy of available reference methods, specifically deuterium isotope dilution for TBW and bromide dilution for ECW. Although this important issue was raised in the 1997 NIH Technology Conference follow-up, it has received little attention. There is a need for identifying accurate criterion and field methods to study acute changes in hydration. This study will produce preliminary evidence to gain insight into applicability of serial bromide dilution as a criterion measure for studies investigating acute changes in hydration. The use of body weight as a potential field method for use in studying acute body water will also be addressed.

The primary aim of this study was to validate BIS as a field method for measuring short-term, small changes (~3 % total body weight) in hydration status by assessing and comparing Δ ECW by BIS with a criterion method (bromide dilution), dual energy X-ray absorptiometry (DXA), and total body weight change during a fluid fast/rehydration manipulation. A secondary aim is to compare bromide dilution and measures of total body weight to predict acute changes in ECW.

Subjects and Methods

Subjects

Subjects were recruited from The University of Arizona community. Any healthy individual between the ages of 18 and 30 years, free of disease, weight stable and not taking medications known to affect hydration status was eligible. All women participants reported stable menstrual cycles and were not pregnant as confirmed by a pregnancy test performed in the

laboratory on the first day of the protocol. In addition, all women subjects were scheduled to start the protocol between 10 and 14 days after the beginning of their menstrual cycle to minimize the effects of hormonally-induced body water changes. Seventeen subjects (15 women, 2 men), between the ages of 19 and 28 years (mean age = 21.85 years) completed the protocol. Of the seventeen, two were Hispanic (12%); one was African-American (6%) and the remainder Caucasian (76%). The study protocol was approved by the Human Subjects Committee of The University of Arizona (Appendix A). Written informed consent was obtained from all participants.

Procedures

Subjects came to the Metabolic Monitoring Lab at The University of Arizona for an initial screening interview, to complete usual fluid intake and physical activity questionnaires and medical history forms (Appendix B). Participants were instructed to avoid exercise beyond that required for daily activities, and to abstain from alcohol and caffeine consumption for the entire 4-day protocol. Subjects were also instructed to continue other usual food and fluid intake prior to reporting for the first day of the 4-day sequence.

Fluid fast and rehydration protocol. After baseline measurements and specimen collections were taken on day 1 at the Metabolic Monitoring Laboratory, subjects were instructed to abstain from fluid intake until after measurements and collections were completed on day 2, approximately 29 hours. In addition to total abstinence from fluids, foods containing high amounts of water were eliminated or restricted. Specifically, only one serving of raw (small size) or drained ($\frac{1}{2}$ cup) fruit and vegetable or two servings of fruit and no vegetable or two servings of vegetable and no fruit were to be consumed during the fluid fast. Foods to be eliminated included soup, ice cream, fruit ices, sherbets, Jell-O, gelatin salads, yogurt of any kind, all sauces and

gravies, and puddings. The goal was to achieve a weight loss of approximately 0.5–2.1 kg. Verbal and written instructions were provided (Appendix C).

After the 29-hour fluid fast, participants resumed their usual fluid intake as defined in the baseline fluid intake questionnaire. Written instructions for the 48-hour rehydration phase of the study were individualized to include the specific amounts of fluid and high water containing foods each subject reported consuming on the baseline fluid intake questionnaire (Appendix C). The goal was to fully rehydrate back to baseline body weight values.

Anthropometric assessment. Subjects were scheduled to arrive at the Metabolic Monitoring Laboratory between 7 a.m. and 10 a.m. on days 1, 2 and 4. At each visit, body weight was measured to the nearest 0.05 kg with a digital platform scale (Kubota model K-10-300L-A, Chugai Boyeki (America) Corp., Commack, NY). Standing height was measured to the nearest 1 mm with a stadiometer (Narragansett Machine Co., Providence, RI) on day 1.

Isotope dilution volume. Bromide dilution was used as the criterion method for estimates of ECW. Immediately upon arrival at the Metabolic Monitoring Lab on days 1, 2 and 4 of the protocol, subjects voided completely and provided a urine sample. Body weight was then measured and venous blood samples obtained by a trained phlebotomist. Blood samples were collected in 10 ml tiger-top vacutainer tubes and centrifuged at 3,000 rpm for 20 minutes at 10°C to separate serum. Two 2.5 ml aliquots of each serum sample and three 5 ml aliquots of each urine sample were stored frozen at –80°C in airtight cryogenic vials until analysis.

After BIA and BIS measurements were completed, subjects drank a measured dose of sodium bromide (NaBr; Sigma, St. Louis, MO) solution, providing 1.0 ml of 3% (w/v) solution per kg of body weight. The solution was administered in paper cups and chased by a 15 ml wash of deionized, distilled water. Equilibration venous blood samples were obtained 5 hours after the initial dosing.

Frozen serum samples were shipped overnight to the Pennington Biomedical Research Center, Baton Rouge, LA for bromide analysis. Serum bromide enrichment was determined by the high-performance liquid chromatography (HPLC) method of Miller and Cappon (1984) using an HPLC (model 1090M; Hewlett-Packard, Palo Alto, CA) equipped with autosampler and diode array detector. The analytical precision of bromide measurements by this technique is approximately 2% (Miller and Cappon, 1984) with the accuracy estimated at 5% (van Kreel, 1994). The coefficient of variation for this analysis in the Pennington laboratory ranges from about 3% at high bromide concentrations to about 5% at low bromide concentrations. The ECW was calculated using a correction of 10% for nonextracellular distribution and 5% for Donnan equilibration (Miller, Cosgriff, and Forbes, 1989).

Bioimpedance measurements. After measurements of height and body weight were recorded, subjects assumed a supine position on a recliner, with limbs abducted to avoid current shunting. Disposable electrodes (IS4000; Xitron Technologies, Inc., San Diego, CA) were placed in the middle of the dorsal surface of the hands and feet just proximal to the metacarpophalangeal and metatarsophalangeal joints; a second set of electrodes was positioned between the distal prominence of the radius and the ulnar styloid and between the medial and lateral malleoli of the ankle (Kushner, 1992). All measurements were taken on the right side of the body and performed by the same trained investigator. The location of placement of electrodes was measured to the nearest millimeter and recorded to ensure electrodes were placed in the identical location for subsequent measurements. Calibration was confirmed according to manufacturer instructions. Triplicate BIS measurements were taken using the Hydra ECF/ICF Bio-Impedance Spectrum Analyzer (model 4200; Xitron Technologies, San Diego, CA) multi-frequency bioimpedance analyzer. Impedance spectral data were transmitted directly from the analyzer to a personal computer and controlled by the software programs (BIS 4200 Utilities Windows 95) supplied

with the instrument. Raw data were fit to the Cole model (Cole, 1972) and TBW, ECW and ICW volumes computed. The coefficients of variation for ECW and ICW volumes measured by the Xitron 4200 analyzer are reported to be 1.28% and 1.72%, respectively (Earthman et al., 2000).

Dual energy X-ray absorptiometry. Subjects reported to The University of Arizona Body Composition Laboratory between 12 noon and 5 p.m. on days 1, 2 and 4. Body weight (kg) with subjects dressed in lightweight clothing and without shoes, was measured on a calibrated digital scale (model 770, seca Corporation, Hanover, MD) accurate to 0.1 kg.

DXA measurements were made with a total body scanner (model DPX/L; Lunar Radiation Corp, Madison, WI) that uses constant potential X-ray source at 78 kVp and a rare-earth k-edge filter (cerium) to achieve a congruent stable dual-energy radiation with effective energies of 40 and 70 keV. Subject position and standard procedures were similar to those described by Peppler and Mazess (1981) and Mazess et al. (1990). The scanner was calibrated daily against the standard calibration block supplied by the manufacturer. All total body scans were completed by the same technician using medium scan mode to ensure appropriate image resolution.

Total body bone mineral content (BMC), fat mass (FM), and bone-free lean soft tissue mass (LTM) were derived from DXA according to computer algorithms (software version 1.36) provided by manufacturer (Lunar Radiation Corp). Fat-free mass (FFM) from DXA was calculated as the sum of BMC and LTM. Hence, FFM from DXA includes all lean tissue mass and bone mineral mass, but not FM. BW measured in the morning was not significantly different than TM measured by DXA in the afternoon, despite the 4–5 hour time difference.

Data analysis. Statistical analyses were carried out using SPSS for Windows (release 11.5.0) software (SPSS Inc., Chicago, IL). For descriptive statistics, means and SD were computed. One-way ANOVA was used to determine differences between the criterion measure

and all other measures of ΔECW . Bland-Altman plots (Bland and Altman, 1986) were used to visually assess agreement between the different methods to measure ΔECW . Limits of agreement derived from the Bland-Altman plots provided insight into the variability of the estimates. Regression analysis of the Bland-Altman plots was used to assess magnitude effects on accuracy. The difference between criterion and the method being tested was regressed against the independent variable of the mean of the Br- ΔECW values and the corresponding SF-BIA or BIS ΔECW values. To evaluate the congruence between the bromide-derived ΔECW and ΔECW measured by the other methods, Pearson's correlations and the root mean squared error (RMSE) were computed. The RMSE was computed by subtracting the average error of the measured ΔECW (E_{avchg} ; the difference between the predicted and the measured average ΔECW) from the error of each individual measurement, squaring each value, then computing the mean and its square root. The RMSE computed in this way serves as a measure of random error in each measure of ΔECW . For an infinitely large sample size, E_{avchg} is equivalent to systematic error of the method. For all other sample sizes, to estimate the systematic error in a method, the effects of random error on the measurement of systematic error must be determined. Systematic error was estimated by dividing the RMSE by the square root of the sample size, then adding and subtracting this term from the average error of the measured ΔECW . The root mean square error (RMSE) was used to compare variance among the methods.

To determine sample size, power estimates were based on reported data from our laboratory on the effects of a fluid fast/rehydration intervention on change in body weight (Howell and Gaines, 1994). In addition, data from our laboratory on average bromide-derived ΔECW were reviewed (Earthman et al., 2000). The data for body weight change indicated that, with a sample size of 10, the power to detect an effect of the fluid fast is 0.96 (population mean $\pm$

SD of $1.2 \text{ kg} \pm 0.9 \text{ kg}$). Because ΔECW has a high variability relative to the mean change ($0.4 \text{ L} \pm 1.2 \text{ L}$), our goal was to obtain a larger sample size of 20. The final sample size was 17.

Results

Subject characteristics are presented in Table 3-1. The majority of subjects had body weight changes within the expected magnitude ($0.5\text{--}2.0 \text{ kg}$; $< 3.0\%$). Although the average absolute values were in the expected direction for the phase (i.e. ECW loss during dehydration, ECW gain during rehydration), it is clear that some subjects had changes in the opposite direction (Figure 3-1), probably due to non-compliance. In addition, the magnitude of change was quite variable depending upon the individual. The average ΔECW measured by the criterion method of bromide dilution were $-0.11 \pm 1.00 \text{ kg}$ during dehydration and $1.05 \pm 1.29 \text{ kg}$ during rehydration. When bromide dilution ΔECW (Br- ΔECW) estimates were compared to those derived from bioimpedance spectroscopy (BIS- ΔECW), DXA (DXA- ΔECW), and body weight change (BW- ΔECW), phase dependent differences were found (Table 3-2). BIS-derived resistance values were significantly related to the Br- ΔECW values ($r = 0.93$, $P = 0.000$) and had directional shifts accordingly.

Dehydration Effects

During the dehydration phase, no significant differences were found between ΔECW measured by bromide dilution and BIS ($P = 0.605$), with the mean difference of 0.16 kg . However, Br- ΔECW was significantly different from BW- ΔECW ($P = 0.000$) and DXA- ΔECW ($P = 0.02$) with larger mean differences of 1.29 and 0.73 kg , respectively.

For the Bland-Altman analyses, the difference between Br- ΔECW and ΔECW measured by each method (BIS- ΔECW , DXA- ΔECW and BW- ΔECW) was regressed against the independent variable defined as the mean of Br- ΔECW and ΔECW measured by each method

(BIS- Δ ECW, DXA- Δ ECW and BW- Δ ECW). These analyses revealed narrower limits of agreement (± 2 SD) between Br- Δ ECW and BIS- Δ ECW (limits of agreement = -2.04 to 1.72 kg) vs. BW- Δ ECW (limits of agreement = -3.45 to 0.87 kg) and DXA- Δ ECW (limits of agreement = -3.03 to 1.58 kg) (Figure 3-2). Although the SEE for BIS- Δ ECW (0.81 kg) was smaller than that for either BW- Δ ECW (1.01 kg) or DXA- Δ ECW (1.19 kg), the slope of the regression line for BIS- Δ ECW (-0.771 , $r^2 = 0.299$) was significant ($P = 0.023$). Visual inspection of the Bland-Altman plot showed an increased variability in BIS- Δ ECW measurement with greater losses of ECW during dehydration. In contrast, the magnitude of the Δ ECW had no effect on the BW- Δ ECW or DXA- Δ ECW measurements.

The findings from the Bland-Altman analyses were further supported by the analysis of the RMSE (Table 3-3). The RMSE and the predicted average change comparing Br- Δ ECW to BIS- Δ ECW was better than the RMSE and predicted average change for DXA- Δ ECW and BW- Δ ECW. BIS- Δ ECW also had smaller systematic error (-0.22 kg) than either BW- Δ ECW or DXA- Δ ECW (-1.29 and -0.73 kg, respectively). For all methods, the random error (RMSE) was greater than Δ ECW predicted by these methods.

Rehydration Effects

During rehydration, there were no significant differences between Br- Δ ECW and the other methods, although BIS- Δ ECW did approach significance ($P = 0.056$). Smaller mean differences between the criterion measure and either BW- Δ ECW (-0.12 kg) or DXA- Δ ECW (0.11 kg), but not BIS- Δ ECW (0.58 kg), were found.

Bland-Altman analyses yielded comparable limits of agreement among the methods (BIS Δ ECW = -3.30 to 2.14 kg; BW- Δ ECW = -2.54 to 2.78 kg; DXA- Δ ECW = -2.79 to 2.57 kg) (Figure 3-3). The slope of the regression line was significant for BIS- Δ ECW (slope = -1.383 , P

= 0.001, $r^2 = 0.517$), and BW- Δ ECW (slope = -1.229, $P = 0.002$, $r^2 = 0.471$), but not DXA- Δ ECW (slope = -0.63, $P = 0.105$, $r^2 = 0.165$). The magnitude of Δ ECW impacted the accuracy of both BIS- Δ ECW and BW- Δ ECW estimates in that the methods tended to overestimate Δ ECW at values between 0.0 and 1.0 kg, and to underestimate change at values above 1.0 kg.

BW- Δ ECW and DXA- Δ ECW had smaller predicted average Δ ECW, systematic error and RMSE than BIS- Δ ECW (Table 3-3). The random error was similar among the three methods. Once again, all of the methods had a random error greater than what each predicted for Δ ECW.

Bromide vs. Body Weight Estimates

As previously described, significant differences among the methods (as compared to Br- Δ ECW change) prevailed during the dehydration ($P = 0.000$), but not during the rehydration phase ($P = 0.106$). However, using bromide dilution estimates as a criterion for excluding data from subjects whose Br- Δ ECW during dehydration was < -0.5 kg ($n = 10$), only BW- Δ ECW was significantly different from Br- Δ ECW ($P = 0.004$). In contrast, using bromide dilution estimates as the criterion for excluding data from subjects (non-compliers) whose Br- Δ ECW at rehydration was < 0.5 kg ($n = 12$), both BIS- Δ ECW and DXA- Δ ECW were significantly different from Br- Δ ECW ($P = 0.001$ and $P = 0.043$, respectively). Additionally, a comparison of the means of the bromide difference per individual for each day indicates an increased variability on day 4 (82.13 ± 10.96 μ g, range 62.9 to 101.3 μ g) compared to day 1 (87.71 ± 8.96 μ g, range = 74.2 to 107.8 μ g) and day 2 (86.10 ± 7.88 μ g, range = 72.2 to 100.1 μ g).

When using body weight as the reference method, more consistent results are seen (Table 3-4). Moderate, but significant, correlations using BW as the reference method (BIS: $r = 0.566$, $P = 0.018$; DXA: $r = 0.629$, $P = 0.003$) vs. bromide dilution (BIS: $r = 0.391$, $P = 0.121$; DXA: $r = 0.305$, $P = .233$) were found during dehydration. Additionally, BIS and DXA had more

variability when compared to bromide dilution ($SEE_{BIS} = 0.54$ kg and $SEE_{DXA} = 0.93$ kg) than when compared to BW ($SEE_{BIS} = 0.48$ kg and $SEE_{DXA} = 0.76$ kg).

During rehydration, significant correlations between BW and both BIS ($r = 0.645$, $P = 0.005$) and DXA ($r = 0.567$, $P = 0.018$) were found. For bromide dilution, there were no significant correlations for either BIS ($r = 0.056$, $P = 0.830$) or DXA ($r = 0.276$, $P = 0.284$). More variability was evident using bromide dilution ($SEE_{BIS} = 0.54$ kg; $SEE_{DXA} = 0.85$ kg) vs. BW ($SEE_{BIS} = 0.41$ kg; $SEE_{DXA} = 0.73$ kg) as the reference.

Discussion

In this study BIS was validated as a field method for measuring short-term, small changes (~ 3 % total body weight) in hydration status by assessing and comparing ΔECW measured by BIS with a criterion method (bromide dilution), with DXA, and with total body weight change. Secondly, the responsiveness of bromide dilution was compared to measures of total body weight for the determination of acute changes in ECW.

The results indicate that BIS performs better when measuring ΔECW during dehydration compared to rehydration. It is unlikely that this discrepancy is due to the insensitivity of BIS to the change in resistance of the body water compartments. Cole modeling of resistance appears to be responsive to acute changes in body water compartments in that the resistance values were significantly related to the Br-ECW values ($r = 0.93$, $P = 0.000$) and had directional shifts accordingly. These findings are in agreement with studies on subjects undergoing ultrafiltration (Matthie et al., 1998; Cox-Reijven et al., 2001) where resistance of ECW increased after the procedure. Although the relationship between resistance and body water volumes is not simple, it is notable that in a previous study on healthy subjects (Howell and Gaines, 1994), the change in body water compartments during a controlled fluid fast/rehydration manipulation was detected by

BIS. Hence, Cole modeling of resistance does appear to be useful in measuring acute changes in hydration status.

In a study of healthy men undergoing exercise- or furosemide-induced water loss, O'Brien et al. (1999) concluded that BIS was sufficiently sensitive to detect moderate hypohydration (2.9–3.4 kg weight loss); however, the resolution of this technique diminished with isotonic fluid loss (as induced by furosemide whereby loss of solute and water results in hypohydration with no change in salt concentration of the blood). Although it is known that exercise influences resistance by as much as 3% (Kushner et al., 1996) due to the effects of increased blood flow on skin and temperature, the suggestion that tonicity affects the accuracy of BIS may provide some insight into the findings of the present study. Dehydration over a short period of time would result in a hypertonic state whereby salt concentration would be increased in the blood. It is likely that the subjects in the present study were in a hypertonic state upon completion of the 29-hour dehydration phase because only ECW, not ICW, changed significantly. During a return to the isotonic state over the 48-hour rehydration period, changing tonicity may have affected the accuracy of the volume estimates. O'Brien et al. (1999) speculated that differential resistivity changes occurred depending upon the degree of tonicity. Indeed, other authors have found improved volume estimates with resistivity adjustments to the model in populations characterized by expansion of the ECW space (Van Loan et al., 1995; Cox-Reijven and Soeters, 2000). The findings of the present study support this and implicate tonicity as a potential contributor to the increased error found during rehydration.

The suggestion that magnitude of ECW loss affects the accuracy of BIS estimates of Δ ECW in altered states of hydration results from studies reporting these effects related to long-term change in absolute body water volumes. As there are no studies that have reported magnitude effects on BIS accuracy during acute changes in hydration, long-term change studies

provide the only means for comparison. Van Loan et al. (1995) reported an increase in RMSE of the BIS- Δ ECW estimate with increasing gain of fluid during pregnancy compared to bromide dilution. Conversely, the postpartum RMSE decreased. Volume estimates were improved by applying a new resistivity constant calculated to accommodate for changes in plasma volume, hematocrit, and hemoglobin. Matthie et al. (1998) demonstrated that resistivity is a scalar term dependent upon the dilution method used. Cox-Reijven et al. (2001) lent further credence to this finding when the authors concluded that the discrepancy between BIS and dilution techniques in a population of patients undergoing hemodialysis was related to the relative magnitude of the body water compartments. One caveat in comparing such studies to the current study is that hemodialysis patients start in a state of relative over-hydration compared to the initial euhydration state of the subjects in the present study. Generally, the magnitude of water loss is much greater than was attained in this experiment.

Nonetheless, the data suggest that the accuracy of BIS estimates of Δ ECW decreases as the magnitude of loss or gain increases. Given that resistivity is an element of the mixture equations used by BIS to estimate volumes, change in the resistivity constant would be reflected in the body water estimates whereby the magnitude effects are attenuated. In the absence of other acceptable criterion measures, future studies must consider the use of different resistivity constants to accommodate the apparent discrepancies inherent in bromide dilution. In addition, research is needed to validate model adjustments that may improve accuracy of body water estimates under conditions of acute changes in hydration status.

It has frequently been noted that a major limitation in validating field techniques for measuring changes in hydration status is that isotope dilution reference methods are not without their own errors, and therefore, restrict the degree of precision and accuracy that can be achieved. The findings of this study suggest that bromide dilution may not be an appropriate criterion

method when measuring change during acute shifts in hydration. This is in contrast to the conclusion by Vaisman et al. (1987) that bromide dilution could be used for serial determination of ECW. This discrepancy is probably due to the fact that the serial measurements occurred over 3- and 4-day periods in static hydration states, unlike the current study in which the subjects received the bromide doses over a shorter timeframe (1 to 2 days) concurrent with dynamic shifts in hydration. Although there was no significant change in the corrected bromide space, Vaisman et al. (1987) did report a rise in the baseline level of bromide. Data from the current study indicate that there was an increased variability in the bromide measures on day 4 as compared to days 1 and 2. This may explain why BIS compared less favorably with bromide dilution during rehydration.

When using BW as a reference, more consistent results were found. However, BIS did not fare quite as well as did DXA. This is not surprising because DXA has been shown to have good agreement between change in BW and change in both total mass and soft tissue mass (Going et al., 1993). The volume effects on BIS estimates were only evident during dehydration suggesting that either BW is not sensitive enough to accurately detect magnitude effects, or given in context with the bromide results, it is a true effect present only during dehydration. The data are insufficient to draw definitive conclusions. Clearly, BW has limitations as a reference method for estimating body water changes that are beyond the scope of this discussion. Nonetheless, under certain field conditions BW may be an acceptable method for monitoring changes in hydration state.

Based on the results of the current study, it can be concluded that BIS provides an accurate measure, compared to the criterion of bromide dilution, of estimated Δ ECW during short-term dehydration. However, consideration must be given to the magnitude of Δ ECW when interpreting the data. Conflicting data seen on rehydration may be due to the use of repeated

bromide dilution studies over a period of 4 days. Such inconsistencies suggest that repeated bromide dilution studies over a short period of time, as was true in this study, may not be appropriate. The effect of administering three bromide doses over four days may obscure true dilution effects given the potential for bromide accumulation from one dose to another. This is a major limitation in this study. Further studies are necessary in which the dehydration and rehydration phases are separated with sufficient time to avoid potential cumulative effects of bromide dilution.

Table 3-1
Subject characteristics¹

	Baseline	Dehydration	Rehydration
Age (years)	21.8 ± 2.2		
Body mass index (kg/m ²)	22.2 ± 4.5		
Height (cm)	167.8 ± 7.8		
Weight (kg)	63.3 ± 17.3	61.9 ± 17.4	63.0 ± 17.5
Total mass measured by DXA ² (kg)	61.5 ± 16.1	60.6 ± 16.6	61.6 ± 16.4
Extracellular water (kg)			
Measured by bromide dilution	14.3 ± 2.9	14.2 ± 2.8	15.2 ± 3.0
Measured by bioimpedance spectroscopy	14.4 ± 3.8	14.1 ± 3.6	14.6 ± 3.8

¹Values are means ± SD; *n* = 17 (2 males, 15 females).

²DXA = Dual energy X-ray absorptiometry.

Table 3-2

Extracellular water change (Δ ECW) predicted by different methods compared to bromide dilution¹

Method	Dehydration		Rehydration	
	Average Δ ECW	Mean difference ²	Average Δ ECW	Mean difference ²
	----- kg -----			
Bromide dilution	-0.11 $\pm$ 1.00	---	1.05 $\pm$ 1.29	---
Bioimpedance spectroscopy	-0.27 $\pm$ 0.57	0.16	-0.05 $\pm$ 2.35	0.58
Body weight	-1.40 $\pm$ 1.02	1.43**	1.17 $\pm$ 0.56	-0.12
Dual energy X-ray absorptiometry	-0.84 $\pm$ 0.95	0.73*	0.94 $\pm$ 0.85	0.11

¹ Δ ECW values are means $\pm$ SD, n = 17 (2 males, 15 females).

²Mean difference = difference from bromide dilution estimate.

* $P \leq 0.05$.

** $P \leq 0.01$.

Table 3-3Extracellular water weight changes (Δ ECW) predicted by various methods¹

Method	Δ ECW (kg)	r	E_{avgchg}^2 (kg)	P	RMSE ³ (kg)	RMSE/ $\sqrt{n}$ (kg)	+ E_{sys}^4 (kg)	- E_{sys}^4 (kg)
<i>Dehydration</i>								
Br	-0.1 ± 1.0							
BIS	-0.3 ± 0.6	0.39	-0.16	0.121	0.91	0.22	0.06	-0.38
Body weight	-1.4 ± 1.0	0.43	-1.29	0.086	1.54	0.37	-0.92	-1.66
DXA	-0.8 ± 1.0	0.31	-0.73	0.233	1.25	0.30	-0.42	-1.03
<i>Rehydration</i>								
Br	1.1 ± 1.3							
BIS	0.5 ± 0.5	0.06	-0.58	0.830	1.33	0.32	-0.26	-0.90
Body weight	1.2 ± 0.6	0.15	0.12	0.563	1.47	0.36	0.48	-0.24
DXA	0.9 ± 0.9	0.28	-0.11	0.284	1.38	0.34	0.22	-0.45

¹ Δ ECW values are means $\pm$ SD.² E_{avgchg} = difference between predicted and measured average Δ ECW.³RMSE = root mean squared error computed by subtracting the average error of the measured Δ ECW from the error of each individual measurement, squaring each value, then computing the mean and its square root⁴ E_{sys} = systematic error computed as $E_{\text{avgchg}} \pm \text{RMSE}/\sqrt{n}$.

Table 3-4

Comparison of bromide dilution and body weight as reference methods for measurement of acute changes in extracellular water

Method	Bioimpedance spectroscopy			Dual energy X-ray absorptiometry		
	<i>R</i>	<i>P</i>	SEE (kg)	<i>r</i>	<i>P</i>	SEE (kg)
<i>Dehydration</i>						
Bromide	0.391	0.121	0.540	0.305	0.233	0.933
Body weight	0.566*	0.018	0.484	0.629*	0.003	0.761
<i>Rehydration</i>						
Bromide	0.056	0.830	0.544	0.276	0.284	0.848
Body weight	0.645*	0.005	0.413	0.567*	0.018	0.727

* $P \leq 0.05$

Figure 3-1. Body weight (kg) and extracellular water (ECW) measured by bromide dilution (kg) in each subject at three time points: baseline, dehydration, rehydration.

Figure 3-2. Bland-Altman comparison of measured change in extracellular water (Δ ECW) during dehydration by bromide dilution vs. the other methods: A) bioimpedance spectroscopy (BIS; mean difference of -0.16 kg, $P = 0.605$); B) body weight (BW; mean difference of -1.29 kg, $P = 0.000$); C) Dual energy X-ray absorptiometry total mass (DXA; mean difference of 0.73 kg, $P = 0.022$). Horizontal line indicates the mean differences and the dotted lines indicate limits of agreement (± 2 SD).

Figure 3-3. Bland-Altman comparison of measured change in extracellular water (Δ ECW) during rehydration by bromide dilution vs. the other methods: A) bioimpedance spectroscopy (BIS; mean difference of -0.58 kg, $P = 0.056$); B) body weight (BW; mean difference of -0.12 kg, $P = 0.809$); C) Dual energy X-ray absorptiometry (DXA; mean difference of 0.11 kg, $P = 0.822$). Horizontal line indicates the mean differences and the dotted lines indicate limits of agreement (± 2 SD).

CHAPTER 4

EFFECT OF ACUTE CHANGES IN HYDRATION ON BODY COMPOSITION ESTIMATES FROM BIOIMPEDANCE ANALYSIS AND BIOIMPEDANCE SPECTROSCOPY

The valid application of single frequency bioelectrical impedance analysis (SF-BIA) to body composition estimates is based upon the assumption that the water content of the fat-free mass (FFM) is constant and that body water compartment ratios remain static (Lukaski et al., 1985). SF-BIA measures resistance (R) that is transformed into estimates of total body water (TBW) through the use of the impedance index (H^2/R) with subsequent translation into estimates of FFM by the use of population-specific equations developed through regression analysis. Due to this empirical relationship between TBW (FFM) estimates and impedance index, shifts in body water distribution can have a profound effect on the accuracy of SF-BIA (Baumgartner, 1996).

Whole body BIA is based on the assumption that the human body is a conductor of uniform length and cross-sectional area. In reality, the geometrical shape of the human body more closely approximates a series of five cylinders: two arms; two legs; and the trunk. Because R is inversely proportional to cross-sectional area, the upper and lower extremities (which have the smallest cross-sectional area) have the most influence on whole body R measurements. The placement of electrodes determines the flow of current through the body and varies among different SF-BIA instruments. Given the disproportionate contribution of the limbs to the R, the placement of the electrodes is an important determinant of the accuracy of the R measurement.

In contrast to SF-BIA, bioimpedance spectroscopy (BIS) (Xitron Technologies, Inc., San Diego, CA) combines a biophysical model (Cole, 1972), to measure R, with a modification of the Hanai mixture theory (Hanai, 1968) for volume estimation. The use of Cole modeling for

calculating R may be advantageous in conditions of altered body water distribution. The R and reactance measurements are fit to an electrical circuit model (Cole-Cole) using iterative curve fitting software and used to identify the middle or characteristic frequency (f_c) of maximum reactance. In the SF-BIA methods, the 50 kHz frequency used is considered to be the f_c . In fact, f_c can vary over a wide range of values, changing with alterations in body water compartments or in cell membranes (Matthie et al., 1998). BIS is the only impedance technology capable of identifying individual f_c .

SF-BIA has been shown to be insensitive to short-term changes in body composition (Deurenberg et al., 1989; Deurenberg, 1996). Given that FFM and fat mass (FM) are second order calculations, accurate estimation of body water compartments is a prerequisite for accurate estimates of body composition. Hydration states affect the conductive properties of ECW and ICW, resulting in changes in the specific resistance of these fluid compartments (Cox-Reijven et al., 2003). Preliminary studies have demonstrated that BIS appears to be responsive to small changes in hydration status (Howell and Gaines, 1994; O'Brien et al., 1999). It is possible that more accurate resistance measures by Cole modeling combined with volume estimation by advanced mixture theory may attenuate the prediction errors associated with changes in conductivity and resistivity.

No definitive study has been published that compares the effect of acute changes in hydration on BIS versus SF-BIA estimates of body composition estimation. The purpose of this study is to compare several SF-BIA instruments and BIS to dual energy X-ray absorptiometry (DXA) to assess the effects of short-term, small changes in hydration status on estimates of body composition, primarily FFM. A secondary purpose was to compare the ability of different SF-BIA models with different electrode arrangements to assess body composition in altered hydration states. We hypothesized that, given the potential advantage of BIS to accurately

measure body water volumes that are translated first into FFM, then into FM and percentage body fat (%BF), BIS would provide more accurate measures of body composition than SF-BIA during acute changes in hydration status.

Subjects and Methods

Subjects

Subjects were recruited from the University of Arizona community. Any healthy individual between the ages of 18 and 30 years, free of disease, weight stable and not taking medications known to affect hydration status was eligible. All women participants reported stable menstrual cycles and were not pregnant as confirmed by a pregnancy test performed in the laboratory on the first day of the protocol. In addition, all women subjects were scheduled to start the protocol between 10 and 14 days after the beginning of their menstrual cycle to minimize the effects of hormonally-induced body water changes. Seventeen subjects (15 women, 2 men), between the ages of 19 and 28 years (mean age = 21.85 years) completed the protocol. Of the seventeen, 2 were Hispanic (12%), one was African-American (6%) and the remainder Caucasian (76%). Of the 17 recruited subjects, one obese male subject was excluded from the data analysis due to the difficulty encountered while performing the DXA scan which may have compromised the accuracy of the scan. Thus, data analysis was performed and reported only on the remaining 16 subjects. The study protocol was approved by the Human Subjects Committee of The University of Arizona. Written informed consent was obtained from all participants.

Procedures

Subjects came to the Metabolic Monitoring Lab at The University of Arizona for initial screening interview, to complete usual fluid intake and medical history forms (Appendix B). Participants were instructed to avoid vigorous exercise, and to abstain from alcohol and caffeine

consumption for the entire 4-day protocol. Subjects were instructed to continue other usual food and fluid intake prior to the reporting for the first day of the 4-day sequence.

Fluid fast and rehydration. After baseline measurements and collections were taken on day 1 at the Metabolic Monitoring Laboratory, subjects were instructed to abstain from fluid intake until measurements and collections were completed on day 2, approximately 29 hours. In addition to total abstinence from fluids, foods containing high amounts of water were eliminated or restricted. Specifically, only one serving of raw (small size) or drained ($\frac{1}{2}$ cup) fruit and vegetable or two servings of fruit and no vegetable or two servings of vegetable and no fruit were to be consumed during the fluid fast. Foods to be eliminated include soup, ice cream, fruit ices, sherbets, Jell-O, gelatin salads, yogurt of any kind, all sauces and gravies, and puddings. The goal was to achieve a weight loss of 0.5–2.1 kg. Verbal and written instructions were provided (Appendix C).

After the 29-hour fluid fast, participants resumed their usual fluid intake as defined in the baseline fluid intake questionnaire. Written instructions for the 48-hour rehydration phase of the study were individualized to include the specific amounts of fluid and high water containing foods each subject reported consuming on the baseline fluid intake questionnaire (Appendix B). The goal was to fully rehydrate back to baseline values.

Anthropometric assessment. Subjects were scheduled to arrive at the Metabolic Monitoring Laboratory between 7 and 10 am on days 1, 2 and 4. At each visit, weight was measured to the nearest 0.05 kg with a digital platform scale (Kubota model K-10-300L-A, Chugai Boyeki (America) Corp., Commack, NY). Standing height was measured to the nearest 1 mm with a stadiometer (Narragansett Machine Co., Providence, RI) on day 1.

Single-frequency bioimpedance analysis

Bio-Resistance Body Composition Analyzer (Valhalla). After subjects voided, measurements of height and weight were recorded. Subjects assumed a supine position on a recliner, with limbs abducted to avoid current shunting. Disposable electrodes (IS4000; Xitron Technologies, Inc., San Diego, CA) were placed in the middle of the dorsal surface of the hands and feet just proximal to the metacarpophalangeal and metatarsophalangeal joints; a second set of electrodes was positioned between the distal prominence of the radius and the ulnar styloid and between the medial and lateral malleoli of the ankle (Kushner, 1992). Thus, the flow of current is hand to foot. Location of placement of electrodes was measured to the nearest millimeter and recorded to ensure electrodes were placed in the identical location for subsequent measurements. Calibration of the Bio-Resistance Body Composition Analyzer (model 1990B; Valhalla Scientific, San Diego, CA) was confirmed according to manufacturer instructions. Triplicate measurements were taken on the right side of the body and performed by the same trained investigator. The mean of the triplicate measures was used in the data analysis. Body water volumes, FFM, FM, and %BF were calculated by the instrument and recorded.

The Body Comp Scale (AW&M). After the DXA measurements were completed and the subjects voided, FFM, FM and %BF were measured by The Body Comp Scale (prototype model FS1535 & PAL101; American Weights & Measures, Rancho Santa Fe, CA). According to manufacturer's instructions, subjects stepped onto the scale, entered personal data as directed (age, height, sex) and grasped handrails for each measurement. Body composition estimates calculated from proprietary equations established by the manufacturer were recorded. Triplicate measurements were taken and the mean of the values was used in the data analysis. For this instrument, the current flows from hand-to-hand (vs. hand-to-foot).

Body Fat Analyzer (Omron). BMI and %BF were estimated using the hand-held Body Fat Analyzer (model HBF-306; Omron Healthcare, Inc., Vernon Hills, IL) and were automatically calculated by the instrument using proprietary equations. As detailed by the manufacturer, height and daily weight were entered. Subjects stood with feet shoulder width apart, grasped the instrument with both hands and held arms outstretched at shoulder height. Three consecutive measurements were made and recorded. Like The Body Comp Scale, the flow of current is from hand-to-hand.

Multi-frequency bioimpedance analysis

Bioimpedance Spectroscopy (BIS). Immediately subsequent to the Valhalla measurements, with the disposable electrodes still in place, triplicate measurements were taken by the same trained investigator on the right side of the body using the Hydra ECF/ICF Bio-Impedance Spectrum Analyzer (model 4200; Xitron Technologies, Inc., San Diego, CA). Calibration was confirmed according to manufacturer instructions. Impedance spectra data were transmitted directly from the analyzer to a personal computer and controlled by the software program (BIS 4200 Utilities Windows 95) supplied with the instrument. Estimates of body water volumes, FFM, FM, and %BF were calculated by the instrument and recorded on preprinted sheets. Raw data were fit to the Cole model and body water volumes, FM, FFM, and % BF were computed.

Dual energy X-ray absorptiometry (DXA). Subjects reported to the Body Composition Laboratory between 12 and 5 PM on days 1, 2, and 4. Weight (kg) with subjects dressed in lightweight clothing and without shoes, was measured on a calibrated digital scale (seca, model 770) accurate to 0.1 kg.

DXA measurements were made with a total body scanner (model DPX-L; Lunar Radiation Corp, Madison, WI) that uses constant potential X-ray source at 78 kVp and a rare-

earth k-edge filter (cerium) to achieve a congruent stable dual-energy radiation with effective energies of 40 and 70 keV. Subject position and standard procedures were similar to those described by Peppler and Mazess (1981) and Mazess et al. (1990). The scanner was calibrated daily against the standard calibration block supplied by the manufacturer. All total body scans were completed in medium scan mode to ensure appropriate image resolution.

Total body bone mineral content (BMC), fat, and bone-free lean soft tissue mass (LTM) were derived from DXA according to computer algorithms (software version 1.36) provided by manufacturer (Lunar Radiation Corp). FFM from DXA was calculated as the sum of BMC and LTM. Hence, FFM from DXA includes all lean tissue mass and bone mineral mass, but not FM.

Data analysis. Statistical analyses were carried out using SPSS for Windows (release 11.5.0) software (SPSS Inc., Chicago, IL). The mean of the triplicate measures was used for data analyses. For descriptive statistics, means and SD were computed. One-way ANOVA was used to determine differences between the criterion method (DXA) and the four impedance methods (BIS, Omron, Valhalla, and AW&M) for baseline, dehydration and rehydration body composition estimates. Bland-Altman (Bland and Altman, 1986) plots were used to look at the relative agreement of the impedance methods to DXA during dehydration and rehydration. Regression analysis and Pearson's correlations were used to describe relationships between criterion and impedance methods.

To determine sample size, power estimates were based on reported data from our laboratory on the effects of a fluid fast/rehydration intervention on change in body weight (Howell and Gaines, 1994). Our data for body weight change indicated that, with a sample size of 10, the power to detect an effect of dehydration was 0.96 (population mean $\pm$ SD of 1.2 kg $\pm$ 0.9 kg).

Results

The physical characteristics of the subjects are given in Table 4-1. Of the 17 recruited subjects, one obese male subject was excluded from the data analysis due to the difficulty encountered while performing the DXA scan which may have compromised the accuracy of the scan. Thus, data analysis was performed and reported only on the remaining 16 subjects. This group of subjects was relatively homogenous and within ideal body weight ($BMI = 21.2 \pm 3.0 \text{ kg/m}^2$; $\%BF = 24.6 \pm 8.9\%$). BIS-estimated TBW at baseline averaged 71% of the FFM from DXA which is similar to the assumed concentrations of 72–73% that underlie most body composition models. The expected directional changes in hydration status occurred as evidenced by the changes in body weight (BW) and extracellular water (ECW).

DXA detected changes in FFM during dehydration and rehydration but not from baseline to rehydration (Table 4-1). No significant differences in FM or $\%BF$ were found between any time points. In addition, changes between BMC measured during dehydration and rehydration were equivalent and nonsignificant.

At every time point, there was good agreement (within 1.3 kg) between measures of BW and total mass (TM) from DXA calculated as the sum of FFM (LTM + BMC) and FM. Highly significant correlations between TM and body weight (BW) were found regardless of hydration status ($r = 0.998\text{--}0.999$, $P < 0.000$). Descriptive statistics for FFM, FM, and $\%BF$ at baseline, dehydration and rehydration are given in Table 4-2.

Comparison of Fat-Free Mass (FFM) Estimates

At baseline, there were no significant differences between DXA-FFM and BIS-FFM estimates (mean difference = 2.41 kg, $P = 0.375$), Valhalla (mean difference = -2.28 kg, $P = 0.299$), or AW&M (mean difference = -2.27 kg, $P = 0.403$) (Table 4-4). Although BIS-FFM had

the highest correlation ($r = 0.975$), all methods were significantly related to DXA-FFM ($P = 0.000$). Bland-Altman analysis (Figure 4-1) revealed comparable, but wide limits of agreement (± 2 SD) among the methods (BIS = -8.01 to 3.19 kg; Valhalla = -2.74 to 8.38 kg; AW&M = 3.37 to 7.91 kg) compared to DXA-FFM. Of these, only the slope of the regression line for BIS-FFM (0.316 , $r^2 = 0.53$) was significant ($P = 0.001$). A visual inspection of the Bland-Altman plot for BIS-FFM indicates that, compared to DXA-FFM, BIS generally underestimates FFM when FFM is < 45 kg and the variability increases in subjects with larger amounts of FFM.

After the dehydration phase, there were no significant differences between DXA absolute estimates of FFM and the other methods. BIS-FFM had the highest correlation ($r = 0.98$), although both Valhalla and the AW&M did reasonably well ($r = 0.95$ and $r = 0.91$ kg for Valhalla and AW&M, respectively). There was a decline in the correlation of both BIS-FFM and Valhalla to DXA-FFM ($r = 0.69$ and $r = 0.59$, respectively) suggesting that rehydration had some effect on the ability of each instrument to estimate FFM.

Fat Mass (FM) and Percentage Body Fat (%BF)

The results comparing DXA measures for FM and %BF to the other methods are summarized in Table 4-3. For FM, there were no significant differences at any time point and all the methods were highly correlated ($r = 0.94$ – 0.99) with DXA-FM throughout the protocol.

Significant differences between DXA-%BF and BIS-%BF (mean difference = -6.55 to -5.92% , $P = 0.022$ to 0.04), but not Valhalla-%BF (mean difference = 2.52 to 3.53% , $P = 0.34$ to 0.23) were found throughout the protocol. Differences at baseline and dehydration were also found for AW&M (mean difference = 5.57 to 6.04% , $P = 0.03$ to 0.04). Omron-%BF estimates approached significance during rehydration (mean difference = 5.15% , $P = 0.06$) and reached significance during dehydration (mean difference = 5.98% ; $P = 0.03$).

Change in Fat-Free Mass (FFM), Fat Mass (FM), and Percentage Body Fat (%BF)

The comparison of changes in DXA derived body composition estimates and the other methods are summarized in Table 4-4. There were no significant differences between DXA-FFM change estimates and any of the other methods. Although variability was comparable during dehydration for all methods, much larger variability was seen in BIS and Valhalla estimates of FFM during rehydration. AW&M-FFM change estimates had the highest correlation to DXA-FFM change estimates for both the dehydration ($r = 0.505$, $P = 0.046$) and rehydration phases ($r = 0.713$, $P = 0.002$).

Phase dependent differences were evident for change estimates of FM and %BF. Valhalla-FM change estimates were significantly correlated with DXA-FM during dehydration ($r = 0.725$, $P = 0.002$) but not rehydration ($r = -0.241$, $P = 0.368$) and no significant differences were noted at any time point. BIS-FM change estimates were significantly different during dehydration ($P = 0.043$) but not rehydration ($P = 0.60$). AW&M had significant differences from DXA estimates for both FM and %BF during rehydration (FM: $P = 0.03$; %BF: $P = 0.008$), but not dehydration, (FM: $P = 0.384$; %BF: $P = 0.26$). Omron-%BF change estimates were not correlated with ($r = -0.045$ to 0.346), and significantly ($P \leq 0.05$) different from the criterion for both phases.

Discussion

Because of ability of BIS to discriminate between the intracellular (ICW) and extracellular water (ECW) compartments, and provide accurate measures of total body water (TBW) that are translated into body composition estimates (De Lorenzo et al., 1997), we hypothesized that BIS would provide more accurate measures body composition during acute changes in hydration status than single frequency bioimpedance analysis (SF-BIA). We tested

this hypothesis by inducing small changes in body weight by a fluid fast/rehydration manipulation. We used DXA as the criterion method because it has been shown to be useful for monitoring small changes in individual components of body composition (Going et al., 1993). The results of this study were comparable to those of Going et al. (1993) in that DXA detected changes only in FFM as would be expected during short-term changes in body water compartments. No significant differences in FM or %BF were found during dehydration, rehydration or from baseline to rehydration, and BMC was unchanged as would be expected.

BIS appears to provide accurate estimates of absolute FFM at baseline that are within acceptable error limits (Houtkooper et al., 1996). Our results are slightly better than those presented by Van Loan et al. (1993) who reported a correlation of 0.92 (SEE = 2.3 kg) in a small sample of healthy men and women. BIS performed equally well during dehydration, but not during rehydration. In a previous study performed in this sample by this lab (unpublished results), resistance values were significantly related to the Br-ECW values ($r = 0.93$, $P = 0.000$) and had directional shifts accordingly. Therefore, it is unlikely that the differences in accuracy due to hydration status can be attributed to the resistance measures since the Cole modeling of resistance used by BIS appears to be responsive to acute changes in body water compartments that are ultimately reflected in FFM estimates.

The magnitude effects observed here implicate the errors in the volume estimates derived from mixture theory for the inaccuracies in BIS-FFM estimates. This speculation is supported by previous studies (Cox-Reijven et al., 2001; Cox-Reijven et al., 2002) demonstrating significant bias in estimates of ECW by BIS in populations characterized by fluctuating body water compartments.

The equation used by BIS to calculate FFM is: $FFM = d_{ECW} (V_{ECW}) + d_{ICW} (V_{ICW})$ where d_{ECW} (1.106) is the mean density of ECW and its associated materials; V_{ECW} = volume of ECW,

d_{ICW} (1.521) is the mean density of ECW and its associated materials and V_{ICW} = volume of ECW. Resistivity is an element of the volume equations. It is possible that changes in resistivity occurred with changes in hydration. O'Brien et al. (1999) concluded that BIS was sufficiently sensitive to detect moderate hypohydration in healthy men undergoing exercise- or furosemide-induced water loss. However, the resolution of this technique diminished with isotonic fluid loss. The authors suggested that the tonicity (the concentration of salt in the blood) affects the accuracy of BIS estimates of body water because tonicity impacts resistivity. This is supported by research showing improved volume estimates when resistivity adjustments to the mixture model in populations characterized by expansion of the extracellular water space are made (Cox-Reijven and Soeters, 2000; Van Loan et al., 1995). Any error in ECW and ICW volume estimates introduced by the resistivity constant will be reflected in the FFM estimate.

SF-BIA did not perform as well as BIS in estimating FFM at baseline, although the results for SF-BIA were comparable to previous observations (Pichard, 1999; Bolanski, 2001). Our findings are consistent with those of Deurenberg et al. (1989) who induced a mean change in BW (1.3 kg) and FFM (1.2 kg) in individuals consuming a very-low caloric diet for 2 days. The reported mean loss in FFM as calculated by three BIA equations consistently underpredicted the change in measured weight. It appears that the whole-body resistance measurements by SF-BIA are not sensitive enough to detect small changes in body water volumes as evidenced by the non-significant changes in resistance, reactance and impedance between baseline and dehydration ($P = 0.22$) and the significant difference between rehydration and baseline ($P = 0.01$). Similar findings were reported in a previous study (Deurenberg et al., 1989), whereby impedance by SF-BIA had not changed during a 24-hour fast despite a water-weight loss of 1.2 to 1.3 kg. This can be attributed to the fact that impedance at 50 kHz does not fully penetrate the cell membrane and that any change in the ECW:ICW ratio will impact f_c . If f_c is greater than 50 kHz, reactance is

affecting impedance because the capacitance of the cell membranes has not been fully overcome. Therefore, the measured impedance is not a measure of TBW but rather a measure of ECW and a partial measure of ICW. BIS overcomes this limitation because it is the only impedance technology capable of identifying individual f_c . Due to the empirical relationship between TBW (FFM) estimates and impedance index (H^2/R), shifts in body water distribution will have a profound effect on the accuracy of SF-BIA. Moreover, hydration states affect the conductive properties of ECW and ICW, resulting in changes in the specific resistance of these fluid compartments (Cox-Reijven et al., 2003). Concomitant changes in resistance should occur with changes in FFM. Thus, the inability of SF-BIA to distinguish small changes in TBW as reflected in ECW shifts contributes to the inaccuracies. Nonetheless, SF-BIA does appear to provide accurate absolute FFM estimates when body water is stable.

Although BIS performed better than AW&M for estimating FM, Valhalla SF-BIA provided the most accurate results at all three time points. In BIS, FM is the difference between weight and FFM. If all methods use the same equation, one would expect that BIS would also more accurately measure FM. Without access to the proprietary equation used by Valhalla, it is difficult to explain this finding. Nonetheless, there were no significant differences between FM estimates from any of the methods and the criterion. However, BIS consistently underestimated FM compared to DXA-FM through all phases. Despite this, BIS-FM estimates were highly correlated with the criterion regardless of hydration status.

BIS was less accurate for measuring %BF. Although BIS was moderately correlated with DXA measures of %BF, the significant mean differences and large error estimates render it less useful as a field method for measuring %BF. This is also true for AW&M and Omron because both models displayed inconsistencies in measurements across the three time points.

At baseline, Valhalla-%BF measures had lower correlations compared to previous studies (Bolanowski and Nilsson, 2001). It performed equally well during dehydration and rehydration phases. This is in contrast to findings in athletes (Saunders, Blevins, and Broeder, 1998) in which significant differences in Valhalla-%BF estimates compared to hydrostatic weighing were found in dehydrated states induced by exercise and in rehydrated states produced by consumption of water and/or electrolyte solution. These investigators failed to consider the known effects of exercise on resistance due to the increased blood flow effects on skin and temperature (Kushner et al., 1996). In our protocol, rehydration was more gradual, allowing for a more equilibrated state than one produced by consumption of water over a short period of time. It is possible, therefore, that the significant differences attributed to change in hydration status may be due instead to the protocol used. In general, however, BIA prediction equations for %BF have been shown to be of questionable accuracy with reported coefficients of determination between 0.60 and 0.90 and SEE's of 3.3–5.5% (Houtkooper et al., 1996). In contrast, our findings suggest that Valhalla SF-BIA provides reasonably accurate %BF results in young, healthy populations regardless of hydration status.

In summary, the findings of this study suggest that BIS performs better than SF-BIA in measuring absolute FFM at baseline and in a dehydrated state in healthy, young, non-obese subjects. Rehydration appears to have an effect on the ability of BIS and SF-BIA to accurately measure FFM. In contrast, all methods performed reasonably well in measures of FM regardless of hydration status. Valhalla SF-BIA provided the most accurate estimates of %BF at all time points. Further research is needed to identify the modifiable elements of the Hanai mixture equation, specifically the resistivity constant that would allow more accurate estimation of body composition under conditions of acute changes in hydration status.

Table 4-1
Subject characteristics¹

	Baseline	Dehydration	Rehydration
Age (years)	22.0 ± 2.1		
Body mass index (kg/m ²)	21.2 ± 3.0		
Height (cm)	167.2 ± 7.5		
Weight (kg)	60.3 ± 12.8	58.9 ± 12.6 ²	60.1 ± 12.9 ³
Dual energy X-ray absorptiometry (kg)			
Total mass	59.0 ± 12.7	58.0 ± 12.8 ²	59.0 ± 13.0 ³
Fat-free mass	43.7 ± 6.5	42.9 ± 6.3 ²	43.9 ± 6.7 ³
Fat mass	15.3 ± 9.2	15.2 ± 9.3	15.2 ± 9.8
Percentage body fat (%)	24.6 ± 8.9	24.6 ± 8.9	24.3 ± 9.5
Extracellular water (kg)			
Measured by bromide dilution	13.8 ± 2.3	13.7 ± 2.1	14.8 ± 2.4 ^{2,3}
Measured by bioimpedance spectroscopy	13.7 ± 2.4	13.4 ± 2.4	13.9 ± 2.5 ³

¹Values are means ± SD; *n* = 16 (1 male, 15 females).

²Significantly different than baseline (*P* ≤ 0.05)

³Significantly different than dehydration (*P* ≤ 0.05)

Table 4-2

Comparison of estimates of fat-free mass (FFM), fat mass (FM), and percentage body fat (%BF) with the corresponding DXA estimates at baseline, after dehydration, and after rehydration¹

Body compartment and method	Baseline	Dehydration	Rehydration
Total body mass (kg)			
Body weight	60.3 ± 12.8	58.9 ± 12.6	60.1 ± 1.9
Dual energy X-ray absorptiometry	59.0 ± 12.7	58.0 ± 12.8	59.0 ± 13.0
Fat-free mass (kg)			
Dual energy X-ray absorptiometry	43.68 ± 6.46	42.92 ± 6.29	43.89 ± 6.63
Bioimpedance spectroscopy	41.27 ± 8.71	40.72 ± 8.82	39.94 ± 10.84
Valhalla	46.51 ± 7.96	45.74 ± 7.76	45.04 ± 11.18
American Weights & Measures	45.95 ± 6.91	45.41 ± 6.77	45.92 ± 6.90
Fat mass (kg)			
Dual energy X-ray absorptiometry	15.29 ± 9.15	15.05 ± 9.32	15.15 ± 9.78
Bioimpedance spectroscopy	19.06 ± 8.16	18.15 ± 8.17	18.40 ± 8.27
Valhalla	13.78 ± 7.25	13.21 ± 7.40	13.01 ± 6.55
American Weights & Measures	11.70 ± 7.78	11.23 ± 7.64	11.80 ± 7.87
Percentage body fat (%)			
Dual energy X-ray absorptiometry	24.64 ± 8.92	24.60 ± 8.94	24.27 ± 9.45
Bioimpedance spectroscopy	31.19 ± 8.14 ²	30.42 ± 8.48 ²	30.18 ± 8.59 ²
Valhalla	22.12 ± 6.65	21.40 ± 6.38	20.91 ± 6.10
American Weights & Measures	19.07 ± 7.38	18.56 ± 7.30 ²	19.20 ± 7.37
Omron	20.55 ± 5.94	18.61 ± 6.83 ²	19.11 ± 6.84

¹Values are means ± SD, *n* = 16

²Significantly different from corresponding DXA estimates, *P* ≤ 0.05 (One-way ANOVA)

Table 4-3

Correlation of bioimpedance estimates of fat-free mass (FFM), fat mass (FM), and percentage body fat (%BF) with the corresponding DXA estimates at baseline, after dehydration, and after rehydration

Body compartment and method	Baseline			Dehydration			Rehydration		
	Mean difference	P^1	r^2	Mean difference	P^1	r^*	Mean difference	P^1	r^2
Fat-free mass (kg)									
Bioimpedance spectroscopy	2.41	0.38	0.98	2.20	0.41	0.98	2.23	0.42	0.69 ³
Valhalla	-2.28	0.30	0.95	-2.83	0.29	0.94	-3.28	0.24	0.59 ⁴
American Weights & Measures	-2.27	0.40	0.91	-2.50	0.35	0.91	-2.10	0.46	0.90
Fat mass (kg)									
Bioimpedance spectroscopy	-3.77	0.17	0.96	-3.10	0.27	0.94	-3.25	0.25	0.95
Valhalla	1.50	0.58	0.98	1.84	0.51	0.99	2.14	0.45	0.98
American Weights & Measures	3.59	0.19	0.98	3.83	0.18	0.98	3.35	0.24	0.98
Percentage body fat (%)									
Bioimpedance spectroscopy	-6.55	0.02	0.81	-5.82	0.04	0.77	-5.92	0.03	0.82
Valhalla	2.52	0.34	0.93	3.19	0.24	0.95	3.35	0.23	0.94
American Weights & Measures	5.57	0.04	0.92	6.04	0.03	0.92	5.06	0.07	0.93
Omron	4.09	0.13	0.89	5.98	0.03	0.93	5.15	0.06	0.95

¹One-way ANOVA

²Pearson's correlation coefficient; $P = 0.000$ with the exceptions noted

³ $P = 0.003$

⁴ $P = 0.03$

Table 4-4
Comparison of changes in body weight and composition by all methods¹

Body compartment and method	Change period		
	Dehydration – Baseline	Rehydration – Dehydration	Rehydration – Baseline ³
Total body mass (kg)			
Body weight	-1.46 ± 1.02^2	1.18 ± 0.58	-0.28 ± 0.82
Dual energy X-ray absorptiometry	-1.00 ± 0.70	1.07 ± 0.68	0.07 ± 0.52
Fat-free mass (kg)			
Dual energy X-ray absorptiometry	-0.77 ± 1.28	0.98 ± 1.18	0.21 ± 0.95
Bioimpedance spectroscopy	-0.56 ± 1.21	-0.78 ± 7.07	-1.34 ± 7.16
Valhalla	-0.76 ± 1.07	-0.70 ± 8.41	-1.47 ± 8.73
American Weights & Measures	-0.54 ± 0.49	0.51 ± 0.49	-0.03 ± 0.33
Fat mass (kg)			
Dual energy X-ray absorptiometry	-0.24 ± 1.03	0.10 ± 0.84	-0.14 ± 1.15
Bioimpedance spectroscopy	-0.90 ± 0.96^2	0.24 ± 0.85	-0.66 ± 1.05
Valhalla	-0.57 ± 0.88	-0.20 ± 1.31	-0.78 ± 1.03
American Weights & Measures	-0.47 ± 0.30	0.58 ± 0.39^2	0.11 ± 0.30
Percentage body fat (%)			
Dual energy X-ray absorptiometry	-0.04 ± 1.53	-0.33 ± 1.41	-0.38 ± 1.61
Bioimpedance spectroscopy	-0.77 ± 1.57	-0.24 ± 1.47	-1.01 ± 1.66
Valhalla	-0.72 ± 1.03	-0.49 ± 1.08	-1.21 ± 1.38
American Weights & Measures	-0.51 ± 0.55	0.64 ± 0.44^2	0.13 ± 0.45
Omron	-1.93 ± 1.96^2	0.50 ± 1.33^2	-1.44 ± 2.01

¹Values are means $\pm$ SD, $n = 16$

²Significantly different from corresponding DXA estimate $P \leq 0.05$

³No analysis of significance provided

Figure 4-1. Bland-Altman comparison of fat-free mass (FFM) at measured baseline by dual energy X-ray absorptiometry (DXA) vs. the other methods: A) bioimpedance spectroscopy (BIS; mean difference of 2.41 kg, $P = 0.375$); B) Bio-Resistance Body Composition Analyzer from Valhalla Scientific (Valhalla; mean difference of -2.28 kg, $P = 0.299$); C) The Body Comp Scale from American Weights & Measures (AW&M; mean difference of -2.24 kg, $P = 0.403$). Horizontal line indicates the mean differences and the dotted lines indicate limits of agreement (± 2 SD).

CHAPTER 5

SUMMARY

In this study bioimpedance spectroscopy (BIS) was validated as a field method for measuring short-term, small changes in hydration status by assessing and comparing change in extracellular water (Δ ECW) measured by BIS with a criterion method (bromide dilution). A secondary aim was to compare body weight (BW) to bromide dilution as a method for estimating acute Δ ECW. Finally, BIS was compared to dual energy X-ray absorptiometry (DXA) and single frequency bioimpedance analysis (SF-BIA) methods to determine differential hydration effects on body composition estimates after acute dehydration and rehydration.

The major limitation of this study was the use of bromide dilution as the criterion measure for estimating Δ ECW during acute shifts in hydration. The findings suggest that bromide dilution may not be an appropriate criterion method when inducing short-term changes in hydration status. There appeared to be a cumulative effect from administering the bromide three times over the 4-day protocol. This is not surprising given that the biological half-life for bromide is 12 days. Because it is actually the difference between the “baseline” and “final” bromide measures that are used to determine the bromide space, it is unclear as to why the accumulation of bromide would affect the ECW estimates. In addition, when inducing comparable changes in ECW during each phase, the difference in bromide concentration for each phase should also be comparable. However, this study indicated significant differences between bromide concentrations from baseline to dehydration compared to bromide concentrations from dehydration to rehydration. One possible explanation is that the correction factors used to determine the “corrected bromide space” are not valid in different hydration states if, for

example, the non-extracellular sites become saturated with bromide. In addition, the accuracy of the bromide analysis may be decreased. As the concentration of bromide in the baseline sample rises, the relative difference between the magnitude of the change being measured and the baseline bromide concentration decreases. Ideally baseline concentrations should be as low as possible. Also, the kinetics of bromide elimination from the body is likely altered and may contribute to the inaccuracy of the measured concentration change.

In the absence of other validated and acceptable criterion methods, bromide dilution still remains the best method for measuring ECW in the research setting. To overcome the limitations of the criterion measure, future studies of acute changes in hydration status should be designed such that the dehydration and rehydration phases are separated with sufficient time to allow true baseline measures by bromide dilution for each phase.

Given the potential variation in bromide dilution measures that may be dependent upon hydration status, it will be difficult to completely standardize the BIS prediction of ECW. Because the sensitivity of bromide dilution diminishes with weight changes less than or equal to 0.5 kg, the use of bromide dilution for measuring acute body water changes should be limited to studies wherein expected ECW change is greater than 0.5 kg. In this study, BW did appear to be an acceptable method for estimating acute ECW change. Since BW has not been validated as a criterion measure for estimates of ECW change, it could not be used to validate BIS in this study. Still, BIS was significantly related to BW estimates of ECW change during both the dehydration and rehydration phases.

The resistivity constant used in the BIS equation has been identified as a potential source of error for estimating body water volumes and, as such, requires further examination. To establish a different resistivity constant, resistivity should be computed from the deuterium

dilution and sodium bromide dilution measures collected from a large, well-standardized, multiple laboratory study. Subsequent studies could then use the same methods to modify the constant according to the population studied. To determine if tonicity affects resistivity or other elements related to conductance, future studies could employ measures of serum osmolality as a measure of tonicity.

Although the power estimates performed prior to initiating this project indicated that the final sample size was adequate to distinguish significant effects, a larger sample size would provide greater insight into the research questions. The finding that random error in estimating ECW change was greater than the predicted ECW change by each instrument needs further scrutiny. Collecting data on a larger sample size would provide a more definitive answer as to the contribution of random and systematic error to the inaccuracies of the instruments.

In summary, BIS appears to provide accurate measures of ECW change when compared to bromide dilution or body weight, especially in the direction of dehydration. The criterion measure of bromide dilution needs further study to identify its validity for use in measuring ECW change during acute shifts in hydration. BIS also provides accurate measures of FFM and FM regardless of hydration status that are comparable to, or better than, estimates by SF-BIA. However, more research is needed to identify the modifiable elements within the BIS equation that may allow more accurate measures of ECW change and body composition during acute shifts in hydration.

APPENDIX A

**HUMAN SUBJECTS COMMITTEE APPROVAL
AND SUBJECT'S CONSENT FORM**

Human Subjects Protection Program

11 December 2001

Wanda Howell, Ph.D.
Department of Nutritional Sciences
Shantz 326
PO BOX 210038

THE UNIVERSITY OF
ARIZONA.
TUCSON ARIZONA

1350 N. Vine Avenue
P.O. Box 245137
Tucson, AZ 85724-5137
(520) 626-6721

RE: **HSC #01-242 VALIDATION OF BIOIMPEDANCE SPECTROSCOPY TO ASSESS
ACUTE CHANGES IN HYDRATION STATUS (USDA)**

Dear Dr. Howell:

We received your 7 December 2001 letter and accompanying revised consent form and advertisements for the above referenced project. All of the conditions as set out in our 13 November 2001 letter to you have been met. Therefore **full Committee approval** for this subjects-at-risk project is granted effective 11 December 2001 for a period of one year.

The Human Subjects Committee (Institutional Review Board) of the University of Arizona has a current assurance of compliance, number M-1233, which is on file with the Department of Health and Human Services and covers this activity.

Approval is granted with the understanding that no further changes or additions will be made either to the procedures followed or to the consent form(s) used (copies of which we have on file) without the knowledge and approval of the Human Subjects Committee and your College or Departmental Review Committee. Any research related physical or psychological harm to any subject must also be reported to each committee.

A university policy requires that all signed subject consent forms be kept in a permanent file in an area designated for that purpose by the Department Head or comparable authority. This will assure their accessibility in the event that university officials require the information and the principal investigator is unavailable for some reason.

Sincerely yours,

David G. Johnson, M.D.
Chairman
Human Subjects Committee

DGJ:rs

cc: Departmental/College Review Committee

SUBJECT'S CONSENT FORM

**Metabolic Monitoring Laboratory
The University of Arizona
Department of Nutritional Sciences**

Validation of Bioimpedance Spectroscopy to Assess Acute Changes in Hydration Status

I AM BEING ASKED TO READ THE FOLLOWING MATERIAL TO ENSURE THAT I AM INFORMED OF THE NATURE OF THIS RESEARCH STUDY AND OF HOW I WILL PARTICIPATE IN IT, IF I CONSENT TO DO SO. SIGNING THIS FORM WILL INDICATE THAT I HAVE BEEN SO INFORMED AND THAT I GIVE MY CONSENT. FEDERAL REGULATIONS REQUIRE WRITTEN INFORMED CONSENT PRIOR TO PARTICIPATION IN THIS RESEARCH STUDY SO THAT I CAN KNOW THE NATURE AND RISKS OF MY PARTICIPATION AND CAN DECIDE TO PARTICIPATE OR NOT PARTICIPATE IN A FREE AND INFORMED MANNER.

PURPOSE

I am being invited to participate voluntarily in the above-titled research project. The purpose of this project is threefold: 1) to determine the usefulness of a machine called a multiple frequency bioimpedance analyzer (Xitron, Hydra ECF/ICF Bio-Impedance Spectrum Analyzer) for measuring short-term, small changes (about 3% total body weight) in body water content (hydration); 2) to evaluate the usefulness of several single frequency bioimpedance analyzers (Valhalla Bio-Resistance Body Composition Analyzer, Omron hand-held Body Fat Analyzer, and American Weights & Measures The Body Comp Scale) in comparison with the Xitron analyzer for measuring short-term, small changes in body water content; and 3) to compare how well a technique called bromide dilution and measures of total body weight and lean tissue mass reflect changes in body water outside of the cells (extracellular water) during the loss of body water (dehydration) and during the restoration of normal body water content (rehydration).

SELECTION CRITERIA

I am being invited to participate because I am a healthy adult between the ages of 18 and 30. My body weight is stable. I am not taking any medications that might affect the amount of water in my body and I do not consume more than 4 liters (136 ounces) of fluid daily. I am not an athlete in training and do not engage in strenuous daily exercise. If I am a woman, I am not pregnant or trying to conceive and I have regular menstrual periods. Approximately 40 subjects will be enrolled in this study.

PROCEDURE(S)

If I agree to participate, I will be asked to consent to the following:

1. Three visits to The University of Arizona campus over a four calendar day period. Each visit will take about 3 hours.
2. Have my medical history reviewed.
3. Consume a caffeine-free diet and avoid taking "water pills" or diuretics during the 4-day testing period.

4. Refrain from exercise beyond that of normal daily activity during the 24 hours before the first test measurement and throughout the entire 4-day study period.
5. Refrain from drinking any fluids from about 12:00 PM on Day 1 of the study until about 1:00 PM on Day 2.
6. Resume normal fluid intake during the 48-hour rehydration period.
7. Keep a 4-day activity diary over the testing period.
8. Keep a 4-day food diary over the testing period.
9. Provide urine samples for pregnancy testing during the pre-study screening interview and on Days 1, 2 and 4 for a check of fluid balance and to be analyzed for deuterium oxide concentration for the measurement of total body water.
10. Allow 2 teaspoons of blood to be taken from a vein in my arm two times (5 hours apart) on Days 1, 2 and 4 to be analyzed for bromide concentration for the measurement of extracellular water. A total of 12 teaspoons of blood will be collected.
11. Have my height and weight measured.
12. Have my total body water measured by drinking a small dose of "heavy water" or deuterium oxide. This type of water is present in all drinking water in small amounts, and is present naturally in my body. Even though the amount of deuterium in the dose will be much higher than the amount usually present in Tucson tap water, it is well within safety limits.
13. Have my extracellular water measured by drinking a small dose of 3% sodium bromide solution. Sodium bromide is a naturally occurring salt which is colorless, odorless, tastes like table salt, and is nontoxic in the amounts that I will be taking. Even though the amount of sodium bromide in the dose will be much higher than the amount usually present in Tucson tap water, it is well within safety limits.
14. Have my body water and fat-free mass assessed by a bioelectrical impedance analyzer (Omron) that will require me to stand and hold the analyzer with both hands while a low electrical current will be passed through my body (arms) for less than 1 minute per measurement.
15. Have my body water and fat-free mass assessed by a bioelectrical impedance analyzer (American Weights & Measures) that will require me to stand on a scale while a low electrical current will be passed through my body (legs) for less than 1 minute per measurement.
16. Have my body water and fat-free mass assessed by two bioelectrical impedance analyzers (Xitron and Valhalla) that will require me to lie in a reclining chair and have 2 electrodes attached to my right hand and 2 electrodes attached to my right foot. A low electrical current will be passed through my body for less than 1 minute per measurement.
17. Have the number of calories burned at rest (resting energy expenditure) measured by a metabolic cart that measures the amount of oxygen consumed and carbon dioxide produced. This will involve lying in a reclining chair and having a ventilated hood placed over my head for 20 minutes while the measurement is taken.
18. Have my bone density and fat mass measured by an x-ray scanner. This will involve lying on a padded table for 20 minutes while a scanner moves slowly over my body.

RISKS

My participation in this study may involve risks which are currently unforeseeable. Several risks already known are the following:

1. Dehydration may cause headache, dizziness or sleepiness. These effects may become extreme if I participate in excessive physical activity while I am dehydrated.
2. Each dual-energy x-ray absorptiometry scan involves exposure to x-rays equal to less than 1/1000 of a chest x-ray and is similar to the amount of radiation one would obtain from living in Tucson for about 1 month. Such exposure is not thought to pose any risk.
3. During blood collections, there may be a slight discomfort from the needle puncture of the skin. There is also a chance that a bruise or swelling may occur where the needle is placed in my arm.
4. Deuterium oxide (heavy water) is colorless, odorless, tasteless, present in all drinking water, and is not toxic in the amount given.
5. Sodium bromide is colorless, odorless, tastes like table salt, is found normally in nature, and is not toxic in the amount given.
6. There are no known risks from measurements of resting energy expenditure or bioelectrical impedance. Bioelectrical impedance delivers an amount of electricity less than the average standard D cell battery.

BENEFITS

There are no guaranteed benefits to participating in this study. I will be told about aspects of my nutritional status which might be useful to me. I will be referred to my personal physician if any clinically significant medical/psychological findings are observed during the course of this study.

CONFIDENTIALITY

All information obtained from this research (including questionnaires, medical history, laboratory findings, or physical examination) will be kept confidential within the limits imposed by law. However, according to FDA regulations, records will be open to FDA representatives to review, if necessary. This may include questionnaires, medical history, laboratory findings/reports, statistical data, and/or notes taken throughout this study. Records of the research may also be subpoenaed by court order or may be inspected by federal regulatory authorities. Data derived from this study may be used in reports, presentations, and publications. However, subjects will not be individually identified, unless written consent is obtained. All information concerning my participation and specific test results will be kept strictly confidential. All information will be filed according to a number identification coding system.

PARTICIPATION COSTS AND SUBJECT COMPENSATION

I will not be charged for any of the procedures or materials used in this study. I will receive \$50 for participation in this study for 4 days. If I am an enrolled student at The University of Arizona I will not receive any academic credit for participation in this study.

CONTACTS

I can obtain further information from the principal investigator, Wanda H. Howell, PhD, RD at (520) 621-1619. If I have questions concerning my rights as a research subject, I may call the Human Subjects Committee office at (520) 626-6721.

LIABILITY

Side effects or harm are possible in any research program despite the use of high standards of care and could occur through no fault of mine or the investigator involved. Known side effects

have been described in this consent form. However, unforeseeable harm also may occur and require care. I do not give up any of my legal rights by signing this form. In the event that I require or am billed for medical care that I feel has been caused by the research, I should contact the principal investigator Wanda H. Howell, PhD, RD at (520) 621-1619. If I have questions concerning my rights as a research subject, I may call the Human Subjects Committee office at (520)626-6721.

AUTHORIZATION

BEFORE GIVING MY CONSENT BY SIGNING THIS FORM, THE METHODS, INCONVENIENCES, RISKS, AND BENEFITS HAVE BEEN EXPLAINED TO ME AND MY QUESTIONS HAVE BEEN ANSWERED. I MAY ASK QUESTIONS AT ANY TIME AND I AM FREE TO WITHDRAW FROM THE PROJECT AT ANY TIME WITHOUT CAUSING BAD FEELINGS. MY PARTICIPATION IN THIS PROJECT MAY BE ENDED BY THE INVESTIGATOR OR BY THE SPONSOR FOR REASONS THAT WOULD BE EXPLAINED. NEW INFORMATION DEVELOPED DURING THE COURSE OF THIS STUDY WHICH MAY AFFECT MY WILLINGNESS TO CONTINUE IN THIS RESEARCH PROJECT WILL BE GIVEN TO ME AS IT BECOMES AVAILABLE. THIS CONSENT FORM WILL BE FILED IN AN AREA DESIGNATED BY THE HUMAN SUBJECTS COMMITTEE WITH ACCESS RESTRICTED TO THE PRINCIPAL INVESTIGATOR, WANDA H. HOWELL, PhD, RD OR AUTHORIZED REPRESENTATIVE OF THE NUTRITIONAL SCIENCES DEPARTMENT. I DO NOT GIVE UP ANY OF MY LEGAL RIGHTS BY SIGNING THIS FORM. A COPY OF THIS SIGNED CONSENT FORM WILL BE GIVEN TO ME.

Subject's Signature

Date

Parent/Legal Guardian (if necessary)

Date

Witness (if necessary)

Date

INVESTIGATOR'S AFFIDAVIT

I have carefully explained to the subject the nature of the above project. I hereby certify that to the best of my knowledge the person who is signing this consent form understands clearly the nature, demands, benefits, and risks involved in his/her participation and his/her signature is legally valid. A medical problem or language or educational barrier has not precluded this understanding.

Signature of Investigator
1/2000

Date

APPENDIX B

**MEDICAL HISTORY, FLUID INTAKE AND
PHYSICAL ACTIVITY QUESTIONNAIRES**

**Metabolic Monitoring Lab
Bioimpedance Validation Study**

MEDICAL HISTORY QUESTIONNAIRE

NAME _____ DATE ____/____/____

Date of Birth ____/____/____ ID _____

Address _____

City _____ State _____ Zip _____

TELEPHONE: Home _____ Work _____

Height ____ cm Weight ____ kg BMI ____ kg/m²

1. Check any of the following medical conditions you have ever had:

____ Diabetes	____ Stroke
____ Heart disease	____ Thyroid disease
____ Rheumatic heart disease	____ Stomach ulcers
____ Heart murmur	____ Arthritis
____ Heart surgery	____ Depression
____ Congestive heart failure	____ Nervous or emotional problems
____ Dizziness or fainting spells	____ Asthma
____ Hypertension (high blood pressure)	____ Allergies
____ Epilepsy	____ Gastrointestinal disease
____ Kidney disease	____ Autoimmune disease
____ Liver disease	____ Lung disease
____ Injuries to back, neck, etc.	____ Female problems
____ High blood cholesterol	____ Hospitalization

____ Eating disorders (anorexia, bulimia)

If you answered yes to any of the above, please explain (use back of sheet if necessary).

2. Check any of the following symptoms if you have experienced them during the past 12 months:

____ Irregular heartbeat

____ Swollen, stiff or painful joints

____ Chest pressure or pain

____ Extreme fatigue

____ Shortness of breath

____ Nervousness

____ Heart fluttering

____ Fainting

____ Cough on exertion

____ Back pain

____ Difficulty sleeping

____ Calf pain with exercise

____ Swollen ankles or legs

If you answered yes to any of the above, please explain (use back of sheet if necessary).

3. Do you have any other health problems? Y N

If yes, explain (use back of sheet if necessary):

4. At what age did you have your first menstrual period? _____ years

5. How many menstrual periods have you had in the past year? _____

6. How long is your menstrual cycle? _____ - _____ days

7. How long do your periods last? _____ - _____ days
8. When was the first day of your last period? _____
9. Are you currently taking any prescription medications? Y N

Drug Name	Dosage (per unit)	Amount/day

10. Are you currently taking any over-the-counter medications? Y N

Drug Name	Dosage (per unit)	Amount/day

11. Check any supplements which affect body composition that you are currently taking:

☐ Creatine ☐ DHEA ☐ Androstene
☐ Ephedrine (eg. MetaboLife) ☐ Other

If "Other" is checked, explain:

**Metabolic Monitoring Lab
Bioimpedance Validation Study**

FLUID INTAKE QUESTIONNAIRE

NAME _____ **ID** _____

DATE ____/____/____

1. How much do you usually drink with each meal?

Breakfast _____ Specify kind(s) of drink _____

Lunch _____ Specify kind(s) of drink _____

Dinner _____ Specify kind(s) of drink _____

2. Do you usually drink between breakfast and lunch? YES NO

Specify kinds of drinks and usual amount _____

Do you usually drink between lunch and dinner? YES NO

Specify kinds of drinks and usual amount _____

Do you usually drink after dinner and before bed? YES NO

Specify kinds of drinks and usual amount _____

3. How often and in what amounts do you use the following caffeinated beverages?

	Times/Day					Usual Serving Size (in cups)				
a) Colas (regular or diet)	1	2	3	4	5+	1	2	3	4	5
b) Regular coffee	1	2	3	4	5+	1	2	3	4	5
c) Decaffeinated coffee	1	2	3	4	5+	1	2	3	4	5
d) Hot tea (regular)	1	2	3	4	5+	1	2	3	4	5
e) Iced tea	1	2	3	4	5+	1	2	3	4	5
f) Hot cocoa	1	2	3	4	5+	1	2	3	4	5
g) _____	1	2	3	4	5+	1	2	3	4	5

4. Indicate how often and specify the usual amounts you eat of the following foods:

Food	Daily	4-6x/week	2-3x/week	1x/week	1-3x/month	Rarely
Yogurt (any kind)						
Citrus fruits						
Jello						
Melons						
Ice cream/Sherbet						
Soup						
Sauces/Gravies						
Pudding						

**Metabolic Monitoring Lab
Bioimpedance Validation Study**

PHYSICAL ACTIVITY QUESTIONNAIRE

NAME _____

ID _____

DATE ____/____/____

1. How many city blocks or their equivalent do you walk each day?
(12 city blocks = 1 mile) _____ blocks/day
2. What is your usual walking pace? (Check only one)
 - a. _____ Casual or strolling (less than 2 mph)
 - b. _____ Average or normal (2-3 mph)
 - c. _____ Fairly brisk (3-4 mph)
 - d. _____ Brisk or striding (4 mph or faster)
3. How many flights of stairs do you climb **UP** each day?
(1 flight = 10 steps) _____ flights/day
4. List any sports or recreation that you have actively participated in during the past **YEAR**.
Please remember seasonal sports or events.

Sport, Recreation or Other Physical Activity	Number of Times/Year	Average Time/Episode		Number of Years of Participation
		Hours	Minutes	

5. Do you engage in regular activity such as brisk walking, jogging, bicycling, swimming, etc.
at least **ONCE A WEEK** long enough to get out of breath or to get your heart thumping?

YES

NO

6. How would you rate your level of exertion (degree of effort) when you are exercising in your usual fashion?

- ___ 0 = Nothing at all
 ___ 0.5 = Very, very weak
 ___ 1 = Very weak
 ___ 2 = Weak
 ___ 3 = Moderate
 ___ 4 = Somewhat strong
 ___ 5 = Strong
 ___ 6 =
 ___ 7 = Very strong
 ___ 8 =
 ___ 9 =
 ___ 10 = Very, very strong (almost maximal)

7. On a usual weekday and weekend day, how much time do you spend involved in the following activities? (Total for each day should add up to 24 hours)

Activity	Weekday Hours/Day	Weekend Hours/Day
Vigorous activity: digging, chopping wood, heavy carpentry, strenuous sports, jogging, sustained swimming, brisk walking, vigorous bicycling, etc.		
Moderate activity: housework, yard work, painting, repairing, light carpentry, light sports, regular walking, golf, dancing, easy bicycling, etc.		
Light activity: office work, driving a car, strolling, standing with little motion, personal care, etc.		
Sitting activity: desk work, eating, reading, watching TV, listening to radio, etc.		
Sleeping or reclining		
TOTAL HOURS		

APPENDIX C
DEHYDRATION AND REHYDRATION INSTRUCTIONS

**Metabolic Monitoring Lab
Bioimpedance Validation Study**

FLUID FAST INSTRUCTIONS

After baseline measurements are taken on Day 1, you will be placed on a fluid fast until measurements on Day 2 are complete. This is approximately 24 hours.

In addition to abstinence from fluids, foods containing high amounts of water cannot be consumed. The foods/fluids which cannot be consumed during this 24-hour fluid fast are as follows:

**Fluids of any kind
Yogurt
Ice Cream or Sherbet
Soup
Jello
Sauces or Gravies
Pudding**

During the 24-hour fast, you may consume 1 serving of raw (small size) or drained ($\frac{1}{2}$ cup) fruit and 1 serving of raw or drained vegetable, or 2 fruits, or 2 vegetables.

Some suggestions to help you with this fluid fast are to suck on hard candy, jelly beans, and lemon wedges (a few per day), chew gum, eat cold food instead of hot food, perform activities that will not make you perspire, and keep busy.

Strict adherence to this fluid fast is crucial to the results of this study. If you have any questions about what you may drink or eat, please do not hesitate to ask. You may contact Karen Higgins at 621-9904 or (623) 203-2894.

**Metabolic Monitoring Lab
Bioimpedance Validation Study**

REHYDRATION INSTRUCTIONS

After the second set of measurements are taken on Day 2, you will be placed on a rehydration regimen for 48 hours at which time final measurements will be taken.

During this 48-hour rehydration period you should resume consuming your normal fluid intake. Based on analysis of your food/fluid questionnaire, your normal fluid intake is as follows:

FOOD	SERVING SIZE	SERVINGS/DAY
Fluid	8 oz	
Fruit	½ cup	
Vegetables	½ cup	
Yogurt	8 oz	
Jello	½ cup	
Soup	1 cup	
Ice Cream or Sherbet	½ cup	
Sauces or Gravies	¼ cup	
Pudding	½ cup	

You should try as much as possible during this 48 hours not to consume more than this amount.

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