Bioimpedance Spectroscopy for the Estimation of Body Fluid Volumes in Mice

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Running head: Bioimpedance in mice
Conventional indicator dilution techniques for measuring body fluid volume (BFV) are laborious, expensive and highly invasive. Bioimpedance spectroscopy (BIS) may be a useful alternative due to being rapid, minimally invasive and allowing repeated measurements. BIS has not been reported in mice, hence we examined how well BIS estimates BFV in mice. Using C57/Bl6 mice, the BIS system demonstrated <5% inter-mouse variation in total body water (TBW), extracellular (ECFV) and intracellular fluid volume (ICFV) between animals of similar body weight. TBW, ECFV and ICFV differed between heavier male and lighter female mice, however, the ratio of TBW, ECFV and ICFV to BW did not differ between mice and corresponded closely to values in the literature. Furthermore, repeat measurements over one week demonstrated <5% intra-mouse variation. Default resistance coefficients used by the BIS system defined for rats produced body composition values for TBW that exceeded body weight in mice. Therefore, body composition was measured in mice using a range of resistance coefficients. Resistance values at 10% of those defined for rats provided TBW, ECFV and ICFV ratios to body weight that were similar to those obtained by conventional isotope dilution. Further evaluation of the sensitivity of the BIS system was determined by its ability to detect volume changes after saline infusion; saline provided the predicted changes in compartmental fluid volumes. In summary, BIS is a noninvasive and accurate method for the estimation of body composition in mice. The ability to perform serial measurements will be a useful tool for future studies.
INTRODUCTION

The assessment of body composition is extremely useful in studying the physiology of both man and animals. While indicator dilution is the reference method for determination of body fluid volumes (BFV) in animals, this technique is time-consuming, expensive, involves non-survival surgery, and requires bulky equipment. Bioimpedance spectroscopy (BIS) may be a useful alternative for BFV determination due to being rapid, minimally invasive and allowing repeated measurements.

BIS measures the resistance of the body to the flow of an electrical current, which is related to the amount of water in the body (total body water, TBW). A constant current at low frequencies cannot pass through cell membranes, however the current will travel through cell membranes at high frequencies. Therefore, over a range of frequencies (between 4 and 1000 kHz), the impedance at low frequency is related to the extracellular fluid volume (ECFV), while the impedance at high frequencies is related to TBW (2). Values for intracellular fluid volume (ICFV) are derived by subtracting ECFV from TBW.

BIS has been extensively validated in man (9, 14, 15) and in several animal species, including dogs, cats, sheep, and rats (8, 10, 11, 13). In particular, a recent study by Smith et al. (12) reported that BIS provided a precise and accurate means to determine TBW, fat mass and fat free mass in rats when compared to chemical carcass analysis. However, no previous studies have validated BIS in mice, a species in which body fluid volumes are especially difficult to assess. Consequently, the present study was designed to assess the utility of BIS in mice compared with the independent assessment of body composition by isotope dilution. We report that BIS accurately and reproducibly measures fluid compartment volumes in mice.
MATERIALS AND METHODS

*Mice.* All experiments were performed with approval from the Institutional Animal Care and Use Committee at the University of Utah. Wild-type C57BL6 mice were studied at 3-4 mo of age.

*Materials.* Tritiated water and $^{35}$SO$_4$ were obtained from PerkinElmer (Waltham, MA). All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO) unless stated otherwise.

*Isotope dilution analysis of body fluid volumes.* Total body water and ECFV were measured in mice by the distribution volumes of $^3$H$_2$O and $^{35}$SO$_4$, respectively. Values for ICFV were derived by subtracting ECFV from TBW. The mice were taken off feed and fluids in the morning prior to analysis. Two control mice were weighed, anesthetized, and blood samples (~0.5-0.7 ml) obtained by cardiac puncture. Twelve mice (6 male, 6 female) were weighed and orally gavaged with 0.25 ml of water containing $^3$H$_2$O at 200 μCi/ml, and 0.2 ml water containing $^{35}$SO$_4$ at 100 μCi/ml. Following administration of the isotopes, the animals were returned their cages. After 4 hrs to allow complete equilibration of the isotopes throughout the fluid compartments, a blood sample was collected by cardiac puncture under anesthesia. Plasma was frozen at -20°C until analysis in duplicate by liquid scintillation. The ratio of the initial concentration and volume of isotope administered to the final dispersed concentration allowed determination of the fluid compartment volume.

*Bioimpedance measurement of body fluid volumes.* The ImpediVet™ Vet BIS1 system (ImpediMed, San Diego, CA) analyzes whole body bioimpedance data providing determination of TBW, ECFV and ICFV body composition estimates. Prior to analysis, animals were
anesthetized and measured for length and weight. The animal details (length, weight, age, gender and species) were then entered into the BIS software system. The animal was placed on its abdomen and shaved to allow good skin contact at the sites of needle placement. Four needles (25g x 25mm) bent at 90º about 3 mm from the tip were inserted under the skin of the animal to be analyzed at each of the standard placement sites. The needle placement sites include the intercept between the front of the ears and the longitudinal midline, at the base of the tail, and 0.5 cm from these sites toward the nose and tip of the tail respectively. After the leads were attached to the needles, measurements were begun.

_Jugular cannulation and infusion._ Following induction of general anesthesia, the jugular vein was cannulated with PE-10 polyethylene tubing filled with 0.9% sodium chloride containing 50 mg of EDTA disodium salt/ml. Saline was infused intravenously in increments of 0.1, 0.4, 0.5, 1, and a further 1ml for accumulative totals of 0.1, 0.4, 0.5, 1, 2 and 3ml for each mouse. Impedance measurements were taken immediately after each infusion.

_Calculations and statistics._ While $^3$H$_2$O provides an accurate indication of TBW, no tracer provides an exact measure of ECFV. Results for ECFV obtained by $^{35}$SO$_4$ dilution were multiplied by 0.90, 0.95, and 0.94, where 0.90 is the correction for non-extracellular distribution, 0.95 is the Donnan equilibration factor, and 0.94 is the correction factor for the water content of serum (1).

Saline infusion data were analyzed within a group between the different treatments using one-way analysis of variance (ANOVA) with the Bonferroni correction. Differences between groups were compared using one-way ANOVA or unpaired Student’s $t$-test. $p<0.05$ was taken as significant. Data are expressed as mean ± SEM.
RESULTS

BIS values for TBW and ECFV are derived using a resistance coefficient that is specific for the particular species being analyzed. Since mice had not previously been studied using BIS, no such resistance coefficient existed. The resistance coefficient for rats, as supplied by the manufacturer, yielded body composition values for TBW that exceeded body weight in mice. Therefore, we initially empirically estimated the resistance coefficient for mice. To do this, we evaluated mice as a simple cylinder shape; in this case, impedance is proportional to length and inversely proportional to cross-sectional area. Therefore, since adult mice are approximately one tenth the weight of adult rats, and presuming a similar body proportionality between the species, resistance coefficients ranging from 5-15% of those defined for rats were assessed. Resistance values at 10% of those defined for rats provided TBW, ECFV and ICFV ratios to body weight with no difference from either textbook published body composition data (7) or to fluid volumes measured by isotope dilution using $^3$H and $^{35}$S (Figure 1). Consequently, a resistant coefficient that was 10% of that for rats was used in all subsequent studies.

Bioimpedance demonstrated small inter-mouse variation in TBW, ECFV and ICFV between mice of similar weight (Table 1). Body weights were different (p < 0.05) between heavier male (N = 8, 28.6 ± 1.2 g) and lighter female (N = 8, 22.9 ± 0.3 g) mice. Similarly, TBW, ECFV and ICFV differed between heavy and light mice. However, the ratios of TBW, ECFV and ICFV to body weight were not different between genders and corresponded closely to textbook published body composition values (7) (Table 1 and Figure 1).
In the next study (Figure 2), repeat measurements of TBW, ECFV and ICFV to body weight ratios were determined in the same mice one week apart. Body fluid composition values did not differ in the same mice over the course of one week (N=8).

Finally, intravenous infusion of isotonic saline caused a proportional increase in TBW and TBW/BW in mice (Figure 3). Infusion of 0.5 ml of saline produced an increase in ECFV, ECFV/BW and ECFV/TBW that was detectable by BIS. BIS could detect an increase in TBW and TBW/BW after 1 ml of saline infusion. Changes in ICFV were more difficult to detect with BIS wherein 3 ml of saline infusion was required to see changes in ICFV; however changes in ICFV/TBW could be detected after 1 ml of saline infusion.
The current study demonstrates that BIS is an accurate method to assess body fluid volume compartments in mice. A resistance coefficient that was 10% of that used for rats gave BIS values for TBW and ECFV in mice that compared closely to results obtained by conventional $^3$H$_2$O and $^{35}$SO$_4$ dilution methods. Furthermore, both BIS and isotope dilution techniques gave body fluid composition values in mice that compared closely to textbook values. BIS has several advantages over indicator dilution techniques. First, while conventional indicator dilution methodologies are the current state of the art for determining body fluid volume compartments, they are, in of themselves, subject to errors (3). In addition, indicator dilution studies can only be done one time in a given mouse as they require removal of large blood volumes. Furthermore, isotope dilution has an inherent variability that make detection of small differences in body fluid volume compartments problematic. In contrast, BIS is non-destructive and requires only about five minutes per mouse. This permits repeated measurements in the same animal. Importantly, our study demonstrated that BIS yielded highly reproducible values, i.e., very small intra-mouse variability. Furthermore, BIS is sufficiently sensitive to detect fluid compartment differences in mice of different weights and changes in fluid compartment volumes following acute intravenous fluid administration, albeit changes in body fluid volume compartments $\leq 0.5$-1 ml can not be reliably detected. Taken together, these findings indicated that BIS could be useful in studying longitudinal changes in fluid volume compartments. This utility would be particularly relevant to studies involving difficult to obtain mice, such as transgenic or knockout mouse lines. For example, the use of endothelin A receptor antagonists for the control of hypertension, or thiazolidinediones to treat type II diabetes mellitus,
have lead to edema in humans(16, 17). Transgenic and knockout mice have been used to study these systems (4-6, 18); BIS could be useful in elucidating the underlying cause of fluid retention in these animals.

BIS appears to have relatively few disadvantages. One is that it requires purchasing the bioimpedance device and the necessary software. However, this expense would be offset by the ongoing costs of conventional techniques for measuring BFV. Furthermore, the ease and rapidity with which BIS can be performed will aid data collection and reduce operator training, while avoiding the use of radioisotopes. Another issue is that the BIS device measures body composition from impedance data using parameters calculated in rats; this required our having to empirically derive the resistance coefficients for mice. Hence, while it seems likely that our resistance coefficient values would likely be valid in all strains of mice with reasonably comparable body sizes, this has not been definitively determined.

In summary, the results of the current study suggest that BIS is a valid laboratory tool when compared to conventional methods for estimating body fluid composition in mice. Its ability to non-invasively and longitudinally assess body fluid volume compartments will likely make it useful in studying genetically engineered mice.
ACKNOWLEDGEMENTS

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REFERENCES


Table 1. Body weight (BW), total body water (TBW), extracellular fluid volume (ECFV), intracellular fluid volume (ICFV), TBW/BW, ECFV/BW and ICFV/BW in male and female mice measured by bioimpedance spectroscopy (BIS).

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<th>ECFV (ml)</th>
<th>ICFV (ml)</th>
<th>TBW/BW (%)</th>
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*p<0.05 between male and female groups.
**FIGURE LEGENDS**

**Figure 1.** Total body water/body weight ratio (TBW/BW) (Panel A), extracellular fluid volume (ECFV)/body weight ratio (Panel B), and intracellular fluid volume (ICFV)/body weight ratio (Panel C) in mice (n = 8) using isotope dilution ($^3$H and $^{35}$SO$_4$) and 5-15% default BIS system resistance constants (rats). Comparisons to textbook body fluid volume compartments in humans are shown. *p<0.05 vs. indication dilution and textbook values.

**Figure 2.** Comparisons of repeat measurement of total body water (TBW), extracellular fluid volume (ECFV) and intracellular fluid volume (ICFV) to body weight (BW) ratios in mice (n = 8 each data point) at days 0 and 7.

**Figure 3.** Effect of intravenous infusion of saline on body fluid compartment volumes measured by bioimpedance spectroscopy (BIS) in mice. Total body water (TBW), extracellular fluid volume (ECFV) and intracellular fluid volumes (ICFV) are shown in Panel A. TBW, ECFV and ICFV to body weight (BW) ratio are shown in Panel B. ECFV/TBW and ICFV/TBW are shown in Panel C. Saline was infused intravenously with 0.1, 0.4, 0.5, 1, and 1 ml of normal saline to an accumulative total of 3ml. N = 12 each data point). *p<0.05 compared to pre-infusion volumes.
Day 0

Day 7

% BW

TBW/BW

ECFV/BW

ICFV/BW