MEASUREMENT OF BODY COMPOSTION FOR NUTRITIONAL ASSESSMENT IN MICROGRAVITY

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FINAL REPORT
NAG9-1039
PROJECT PERIOD: 7/15/98 – 6/30/01

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ABREVIATIONS

BIS, Bioelectrical impedance spectroscopy
BMI, body mass index
ECW, extracellular water
H, height
HDT, 6° head down tilt
ICW, intracellular water
L, length
MRI, magnetic resonance imaging
N, exchangeable water space
R, resistance
TBW, total body water
W, body mass
δ, relative isotopic abundance of deuterium = $1000[(D/H)_{sample}-(D/H)_{baseline}]/(D/H)_{baseline}$
ρ, specific resistivity

INTRODUCTION

Research Project Summary:

Long-term space flight increases the risk of reduced human performance due to loss of body cell mass resulting from the combined effects of microgravity and energy imbalance. Although countermeasures have been proposed, there are no valid means of monitoring these body composition changes in flight or monitoring the effectiveness of countermeasures prior to return to earth (1 g). As missions increase from weeks to years in length, the need for in-flight methods to monitor the nutritional status of astronauts becomes critical to health maintenance. To address this limitation, we undertook a systematic validation of hardware for measuring body composition during space flight. We completed ground-based studies to validate bioimpedance spectroscopy (BIS) to measure the compartmentalization of total body water between intra- and extracellular spaces under conditions of 1 g. We then completed ground-based studies using a head down tilt model to validate BIS insimulated microgravity. Next we undertook a study attempting to validate the ability of BIS to detect and quantitate changes body cell mass due to short-term negative nitrogen balance and negative energy balance. The criterion methods to be used during this validation are measurement of total body water by deuterium dilution and extracellular water by bromide dilution. In summary, we found that BIS validated for the measurement of water compartments at 1g and head down tilt simulated microgravity. Traditional BIS did not detect short-term changes in intracellular water volume secondary to negative protein and energy balance. Modification of electrode placement with segmental analysis, however, did detect the change, which was localized to the trunk.

In addition, we validated the use of BIS to measure peripheral skeletal muscle volume (calf and upper arm) using magnetic resonance imaging as the criterion method. In summary, we found that (mention results briefly). This ground-based, systematic approach to **clinical capacity** development demonstrated great potential for monitoring changes in human body composition during space flight. This technology should provide investigators with new capabilities to study the effectiveness of countermeasures and to assess nutritional status during space flight.

Hypotheses

Hypothesis I. Bioelectrical spectroscopy will provide an accurate measurement of water compartmentalization in humans under simulated microgravity environments.

Aims:
1) Correlate bioelectrical spectroscopy with deuterium oxide (TBW) and bromide dilution (ECW) during simulated microgravity (head down tilt).
2) Develop equations to calculate intra- and extracellular water volumes under conditions of simulated microgravity.

Hypothesis II. Bioelectrical spectroscopy will provide an accurate measurement of water compartmentalization in humans during simulated microgravity AND short-term under nutrition.

Aims: 1) Determine if simulated microgravity alters the calibration of bioimpedance for body water analysis
2) Optimize electrode placement to minimize interindividual variation in water redistribution to the trunk during undernutrition.

Hypothesis III. Bioelectrical spectroscopy will accurately and precisely measure changes in leg and arm muscle volume.

Aim: 1) Validate bioimpedance spectroscopy of peripheral segments against nuclear magnetic imaging of muscle volume.

Critical Path Questions Addressed by Project

The primary goal of this project is to increase **clinical capabilities** for in-flight assessment of an astronaut's nutritional status and health. We demonstrated that bioelectrical spectroscopy (BIS) can be used to measure the composition of weight loss in microgravity as well as the loss of peripheral muscle volume. This is vital information that can be used to prescribe appropriate countermeasures for loss of body mass or muscle strength that may occur during a long space flight. The bioelectrical spectrometer is small (volume less than 0.02 cubic meters and mass less than 2 kg) and???.

As indicated above, this development impacts two other critical paths. The clinical capacity provided by BIS can be used to detect **nutritional deficits** due to improper food intake. It can be used to differentiate between loss of body fat due to poor energy intake and loss of body water due to redistribution prior to the time when the energy deficit will compromise muscle mass. BIS can be used to determine the degree of deficit in extracellular water prior to return to a gravitational environment so that countermeasures can be prescribed to reduce the risk of poor **human performance** secondary to inadequate extracellular fluid volume. Finally, the ability to measure peripheral muscle volume and determine whether any change is due to intracellular fluid shift or loss of muscle cell volume will help investigators monitor strength and exercise countermeasures during flight as these apply to **human performance**.

Background material

Long-term space flight has the potential to produce losses of body tissue mass and function. These losses can limit strength and performance of duties and, more importantly, limit the ability to complete emergency procedures should they arise. Previous studies have shown that bone, water, muscle, and fat mass components of the body are altered in space flight (Rambaut et al, 1977; Leach et al, 1996). The first change in body mass is a rapid loss of 1-3 kg of body water that occurs 1-2 days after entering space (Leach et al, 1996). This change probably is secondary to a redistribution of extracellular water from the periphery to the trunk, including a 10% loss in plasma volume (Norsk, 1996; Convertino, 1996). As the duration of space flight increases, a state of negative nitrogen balance and loss of muscle mass occurs (Lutwak et al, 1969; Leonard et al, 1983). In addition, body fat stores may decline significantly with long-term space missions.

Despite the importance of monitoring body composition during flight, these measurements have not been made because of limitations imposed by space flight. Most

body composition methods take up too much room (e.g. DXA, hydrostatic weighing) or require earth-based lab analysis (e.g. isotope dilution). Bioelectric spectroscopy is a method that holds considerable promise because it does not suffer from these limitations. This technique was developed by Hoffer et al (1969) and is based on the principle that different body tissues have different electrical properties. The original method employed a single frequency, alternating current signal that was applied across the body at very low (safe) currents that are undetectable by subjects. The ionic media in fat-free mass conduct the current while the body fat's insulating properties provide resistance to current flow. The resistance of the body can be used to determine the percentage of fat-free mass. The validity of the technique is well documented (Kushner and Schoeller, 1986; Lukaski et al, 1985; Segal et al, 1985).

One important limitation of the original bioelectric spectroscopy model is that it only measures total body water and not changes in the compartmentalization of the water. As described above, the ability to differentiate between losses in extracellular and intracellular fluid is vital to the usefulness of body composition data in space flights. The development of multi-frequency bioelectrical impedance analyzers provides new hope that space flight body composition measurements can be made. These machines make use of the capacitive nature of intact human cells. At low frequencies, cells are largely non-conductive and thus, electrical currents run through extracellular water. As the frequency increases, cells begin to act as capacitors and become part of the current path. This method enables researchers to distinguish between intra- and extracellular water (Ackman and Seitz, 1984; Van Loan and Mayclin, 1992). This has two important implications. First, it improves the bioelectric spectroscopy model valid for space flight conditionsbecause the microgravity induced diuresis may alter the relationship between total body water fat-free mass. Second, it permits the measurement of changes in body water distribution and cell volume. This information is vital to determining whether changes in body composition are related to changes in plasma volume or cell volume, which has ramifications for the treatments prescribed to astronauts.

Bioelectric spectroscopy has been shown to be a valid method of measuring total body water (Kushner et al, 1992). In addition, new studies from our lab have validated the proximal electrode placement technique (at elbow and knee) as a way to reduce the effects of water redistribution on total body water measurements (Gudivaka et al, 1994). Lastly, multifrequency bioelectric impedance with analysis using the Cole-Cole model has been shown to be an accurate model for determination of extra- and intracellular fluid volume (Gudivaka et al, 1999).

The goal of this study is to validate bioelectric spectroscopy as a form of body composition analysis for space flight. If this method has acceptable precision and accuracy, it would allow for body composition analysis during space flights, rather than waiting for return to Earth. It would allow astronauts to design individualized treatment programs, including strength training or increased fluid intake, to resolve their specific health problems while still in space. This is particularly important during long space flights or during residency on a space station. Under these conditions, resolution of health problems should not, and can not, wait months or years.

MATERIALS AND METHODS

Two human studies were performed. To simplify the report, these will be referred to as STUDY 1 and STUDY 2.

3 days

STUDY 1

DAY 1: Subjects reported to the GCRC by 8 am following an overnight fast. A pregnancy test was administered to all female subjects, but none required exclusion due to pregnancy. Weight and height were measured. A catheter with heparin lock was inserted and baseline blood was collected (10 mL). Subjects were orally dosed with a 100 mL mixture of deuterium oxide (4 g) and NaBr (10 mg/kg body weight) in tap water to drink. More blood (10 mL) was drawn twice during the morning (3 and 4 hours after consuming the tracers). Electrodes were placed and multifrequency bioelectrical impedance measured. Electrode placement was marked with waterproof ink. At approximately 1:00 p.m., subjects were given a liquid meal (Ensure™). They received a volume of supplement that provides approximately one third of daily energy needs (e.g. 1/3th of BEE x 1.2). After this ambulatory period, the subjects assumed the 6° head-down position (HDT) for the duration of the study. Blood was drawn, and weight and bioelectric impedance were measured four hours after assuming HDT. At approximately 5:00 p.m., subjects received a no added salt, low residue diet with an energy content at 1.2 x basal energy expenditure and a protein content of 15% of kcal. Subjects consumed 30 mL/kg fluids per each day of the study. Caffeine and alcohol consumption were not allowed.

DAY 2: Subjects remained in HDT and received the study diet. At 8:00 a.m., measurements of body water were repeated. This included collection of blood three times, assessment of bioelectric impedance two times, measurement of weight two times, and consumption of the 100 mL deuterium oxide/NaBr mixture for tracer dilution analysis.

DAY 3: Subjects remained in HDT and received the study diet. At 8:00 a.m., measurements of body water were repeated. This included collection of blood three times, assessment of bioelectric impedance two times, measurement of weight two times, and consumption of the 100 mL deuterium oxide/NaBr mixture. Subjects were given a full lunch and rehydration fluids before leaving. Staff ensured subjects could tolerate standing up before they are allowed to leave the GCRC.

U. of Wisconsin General Clinical Research Center

STUDY 2

DAY 1: Subjects reported to the GCRC by 6 p.m. A pregnancy test was administered to all female subjects, but none tested positive. Height and weight in hospital gown was measured. Subjects underwent an MRI of the calf and upper arm taken. Bioelectric impedance analysis of these same limbs was conducted. Electrode placement was marked with waterproof ink. At 10 p.m. subjects assumed the 6° HDT position in bed, which continued for the remainder of the study. A blood drawing catheter with heparin

lock was inserted. They did not receive food until after the next morning's study period is over (at approximately 12 noon). All urine was collected.

DAY 2: At 7:45 a.m., subjects had 10 mL blood drawn. An oral dose was administered to subjects with a 100 mL mixture of deuterium oxide (4 g) and NaBr (10 mg/kg body weight) in tap water. Additional blood was drawn twice during the morning. Weight, whole body, and segmental multifrequency bioelectrical impedance were measured. Electrode placement was marked with waterproof ink. At approximately 12 noon, subjects began receiving the study diet. This diet was a clear liquid, low calorie regimen designed to induce the negative nitrogen balance and glycogen depletion necessary for intracellular volume to decrease. Subjects received 800 kcal as carbohydrate with no additional kcal from either protein or fat. A multivitamin/mineral pill was given so that micronutrient needs were met. This diet continued for the duration of the study (approximately 32 hours). Blood was drawn daily to ensure electrolyte levels were maintained.

DAY 3: Subjects remained in HDT and received the study diet. Blood was drawn to ensure electrolyte levels were maintained.DAY 4: At 8 a.m., subjects repeated tracer dilution tests. This involved collection of blood three times, assessment of bioelectric impedance twice, measurement of weight twice, and consumption of the deuterium oxide/NaBr water solution. Body water was determined by the dilution of the tracer. Subjects had been completely deuterium depleted (approximately 2 kg) in the 3 days prior to the study and had been on a low calorie diet for 3 days. At 8 a.m., subjects were transported on a gurney to the clinic. Subjects were asked to have a second dinner at 5 p.m. the night before the study. Subjects were given a full dinner and rehydration fluids before leaving

introduced into a Finnigan Delta Plus Isotope Ratio Mass Spectrometer for deuterium analysis (Schoeller et al, 2000). The relative precision of the TBW determination was 1.2% (0.5kg).

Extracellular Water

The bromide dilution space was determined at 3 and 4h after the dose and averaged.Blood was collected in dry heparin, stored briefly on ice, then centrifuged to separate the plasma. A loading dose of 5 mg sodium bromide per kg body mass was administered by mouth. Blood plasma was obtained again at 3h and 4h. The ECWs at 3 and 4h were calculated from the bromide dilution space.

$$ECW = 0.99 \times 0.95 \times 0.90 N_{Br}$$

where 0.99 is the fraction of water in ultrafiltered plasma, 0.95 is the Donan equilibrium correction, and 0.90 is the estimated correction for penetration of bromide into the intracellular space (Cheek, 1954; Manery, 1954). The fraction of water in ultrafiltered plasma was gravimetrically determined in four freeze-dried plasma samples after passage through a 50,000 Dalton exclusion filter. The bromide dilution space was calculated from the change in bromide concentration in plasma.

$$N = \frac{d}{[Br]_f - [Br]_b} - N_{out}\frac{[Br]_{out} - [Br]_b}{[Br]_f - [Br]_b} + N_{in}\frac{[Br]_{in} - [Br]_b}{[Br]_f - [Br]_b}$$

where the symbols are defined as above except that bromide concentration ([Br], M/L) is used in place of the deuterium abundance. During the initial 3-h equilibration, the only adjustment was that for urinary loss.

Bromide concentration was measured by HPLC using the technique of Miller et al (1989). Briefly, plasma was filtered using a 50,000 Dalton Centricon exclusion filter. A 10μL aliquot was introduced via a fixed volume injection loop after a 30μL flush. The bromide was isolated by liquid chromatography on a 250x4.6mm Partisil SAX-10 ion exchange column (Whatman Inc., Clinton, NJ). A 30mmol/L aqueous KH_2PO_4 mobile phase at 1 mL/min was used. Bromide was detected at 180nm using a variable wavelength UV detector calibrated against gravimetric sodium bromide standards prepared fresh each week. The relative precision of the ECW determination was 4% (0.7kg). The bromide dose was corrected for urinary bromide losses. Urine was decolored using carbon black and then filtered using an anion exchange Sep-Pak filtration unit (company). Bromide concentration was determined using the HPLC method described above.

(2) Bioelectrical Impedance Analysis

Electrode placement was proximal as described by Scheltinga et al (1991). This was used in preference to typical wrist/ankle electrode placement because the proximal

placement is less sensitive to temperature and orthostatic effects (Gudivaka et al, 1994). After cleaning the electrode site with isopropopol alcohol, electrode patches (7.6 x 1.9cm) with self-adhesive conducting gel (IS 4000, Xitron Tech, San Diego, CA) were attached to the dorsal surface of the right foot and right hand for current injection. Detector electrodes were attached to the dorsal surface of the right leg with the proximal edge 1cm distal to the center of the knee cap and on the dorsal surface of the right forearm with the proximal edge 1cm distal to the mid-crease of the antecubital fossa.

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Muscle Volume Modeling

Selection of arm and leg segments to model: A 13.3 cm section of the upper right arm and a 16.1 cm section of the right leg were modeled using both magnetic resonance imaging and bioelectrical impedance spectroscopy. For the arm segment, the elbow was bent at a 90° angle and a straight edge was applied to the underside of the forearm. A ruler edge was applied to the backside of the upper arm so that it bisected the straight edge at a 90° angle. A line was drawn 10 cm up from the tip of the ventral side of the elbow. The arm was straightened then abducted so that it was parallel to the floor, and the mark was extended to circumscribe the arm in a circle that was perpendicular to the floor. Another circumscribing line was drawn 13.3 cm proximal to the first line. For the leg segment, a mark was drawn 2 cm distal to the tibial tuberosity. While subjects were standing, the mark was extended to circumscribe the leg in a circle that was parallel to the floor. Another circumscribing line was drawn 16.1 cm distal to the first line.

Magnetic resonance imaging procedure: Gel beads (containing oil) were applied to the dorsal side of the leg and the ventral side of the upper arm at the sites of the circumscribing lines to allow for detection of desired limb regions. T1 weighted scans (TE = 25, TR = 600, NEX = 1.0) were conducted to differentiate between muscle, fat, bone, and air. Using the gel beads as a guide, 23 seven mm thick leg slices and 19 seven mm thick arm slices were selected for analysis. The slices were processed using the custom 3-D modeling package of an Advantage Windows workstation (software version 2.0.20). In brief, thresholds were manually set to eliminate air, bone, and subcutaneous fat from the scans. Then, the software integrated the slices to produce a muscle volume estimate for the entire set of slices. The muscle volume modeling procedure was conducted in a blind fashion on three separate occasions. For outlying points the procedure was repeated a fourth time. The average of volumes (excluding outliers) was used as the criterion measure of muscle volume for further statistical analysis.

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BIS procedure: The areas of the arm and leg containing the circumscribing lines were cleaned with isopropyl alcohol. Then 0.5 cm wide electrode tape (Electro-Diagnostic Instruments, Valencia, CA) for current detection was wrapped around the arm and leg directly on the circumscribing lines. Current injection electrodes were place 8 cm away from the electrode tape. BIS scans of the arm and leg segments were completed in triplicate.

RESULTS

Subject characteristics for both studies are shown in Table 1. The subjects were healthy young adults of both genders. Of these, 39 were Caucasian, 6 Hispanics and 1 African American.

Table 1. Subject Characteristics (mean and range)

	STUDY 1		STUDY 2	
	Males	Females	Males	Females
Number	14	14	10	8
Age, y	27.9 (21-44)	25.7 (19-41)	31.5 (20-45)	25.8 (20-35)
Height, cm	179 (163-193)	166 (159-175)	178 (158-191)	162 (155-167)
Weight, kg	83.4 (61-110)	59.6 (46-81)	81.4 (61-98)	57.9 (49-66)
BMI, kg/m^2	26.6 (19-36)	21.9 (23-31)	25.7 (22-32)	21.9 (20-25)

BMI=Body mass index

Generation of BIS Water Space Prediction Equations

The accuracy and precision of bioelectrical impedance spectroscopy (BIS) was tested against bromide and deuterium oxide dilution during the ambulatory (1g) phase in STUDY 1. This was tested against a previously published BIS prediction equation to determine if we could reproduce the accuracy and precision that we observed in our preliminary studies performed at the University of Chicago (Gudivaka et al, 1999). We found that BIS accurately measured extracellular water (ECW) and total body water (TBW), but that there was systematic bias for intracellular water (ICW) (Table 2).

Table 2. Accuracy and Precision of BIS at 1g Using BIS Prediction Equations of Gudivaka et al (1999).

	ECW (kg)	ICW (kg)	TBW (kg)
BIS	15.1±2.8	24.6±6.7	39.6±9.4
Dilution	15.5±3.1	23.46±6.7	39.0±9.0
p-value	NS	0.02	NS

Mean±between subject SD
p-values are entered when <0.05

The bias in ICW may result from a slight difference in either the human protocol or the bromide/deuterium analyses from when we performed the preliminary and thus we generated a new set of predictive equations for BIS. A new set of prediction equations was generated by linear regression of Ht^2/R against either the dilution measured ECW or ICW where R is the resistance of the extracellular or intracellular pathway, respectively.

The water space prediction equations generated in this manner are:

$$ECW\ (kg) = 0.104H^2/Re + 4.1,\ r = 0.91$$
$$ICW\ (kg) = 0.248H^2/Ri + 6.1,\ r = 0.95$$
$$TBW\ (kg) = ECW + ICW$$

These equations are used for the BIS measurements throughout the remainder of this report.

Accuracy and Precision for BIS During Simulated Microgravity

The accuracy and precision of BIS under conditions of simulated microgravity were investigated in STUDY 1. After the subjects completed the above ambulatory phase, they lay in bed with a 6° head down tilt (HDT) for 48h. Water spaces were measured by BIS and dilution at 4, 24, and 48 of HDT. BIS was accurate under conditions of simulated microgavity (Table 3). It should be pointed out that accuracy was obtained using the proximal electrode placement on knee and elbow, and that accuracy would not be expected using the traditional wrist-ankle electrode place because of the shift of extracellular fluid out of the calf (Gudivaka et al, 1999).

Table 3. Accuracy and precision of BIS during simulated microgravity

	ECW (kg)		ICW (kg)		TBW (kg)	
	absolute[a]	residual[b]	absolute	residual	absolute	residual
4h HDT						
Dilution	15.2 (3.1)		23.3 (6.9)		38.5 (9.2)	
BIS	15.3 (2.6)	0.1 (1.8)	23.6 (6.3)	0.4 (2.8)	39.0 (8.8)	-0.5 (2.7)
p-value	NS	NS	NS	NS	NS	NS
24h HDT						
Dilution	14.9 (3.1)		23.1 (6.6)		38.0 (9.1)	
BIS	15.0 (2.6)	0.1 (1.5)	23.3 (6.4)	0.3 (2.8)	38.4 (8.9)	-0.4 (2.4)
p-value	NS	NS	NS	NS	NS	NS
48h HDT						
Dilution	14.7 (3.2)		22.9 (7.0)		37.6 (9.4)	
BIS	14.7 (2.7)	0.0 (1.5)	23.4 (6.4)	0.5 (3.1)	38.1 (9.0)	-0.6 (2.8)
p-value	NS	NS	NS	NS	NS	NS

[a] mean and between subject SD

[b] mean and SD for the within-subject difference between BIS and dilution (BIS – DIL)

Although the accuracy of BIS as shown in Table 3 is excellent, the precision is only modest. This imprecision can theoretically be due to instrument errors, within subject changes in bioelectrical properties, imprecision in the dilution methods, or between subject variations in geometry or bioelectrical properties. The latter two are important because if they explain a significant amount of the variance in Table 3, then BIS can be used to track within-subject changes in water spaces with great precision. To test this, we compared the within-subject changes in water spaces between the ambulatory phase and the HDT to determine whether BIS can follow the changes in water spaces associated with simulated microgravity. In Table 4, we demonstrate the variance is reduced when following within individual changes. This shows that BIS can be used to track within-subject changes in water space with a high degree of precision. Weight is included as measure of change in TBW because we were feeding the subjects and maintaining them in energy balance. Thus all the weight change can be assumed to be a change in TBW for this two-day period. Weight indicates that TBW begins dropping immediately upon entering HDT and reaches a 0.8kg loss by 48h. This loss of water is detected by deuterium dilution at all time point, but not by BIS until the 24h time point when the loss is 0.7 kg. BIS measurements of loss do not differ from dilution or weight

change. The within-subject variance of BIS is smaller than that of dilution indicating that validation of BIS may be limited by the inherent precision of the dilution techniques.

Based on these results, we can estimate the detection limit of BIS for within-subject change in fluid spaces. When this is done for the individual, the detection limit (2 SD) for within-subject changes are 3% for within-day changes and 5% for between-day changes.

Table 4. Within Subject Changes in Water Spaces During 48h of HDT Simulated Microgravity.

	4h HDT	p-value	24h HDT	p-value	48h HDT	p-value
ECW						
BIS	-0.2 (0.2)	NS	-0.5 (0.4)	<0.01	-0.8 (0.8)	<0.01
DIL	-0.3 (0.8)	0.05	-0.6 (1.2)	0.02	-0.8 (1.2)	<0.01
ICW						
BIS	0.0 (0.6)	NS	-0.4 (0.5)	<0.01	-0.3 (0.6)	0.02
DIL	-0.2 (0.8)	NS	-0.4 (0.5)	<0.01	-0.6 (2.0)	NS
TBW						
BIS	-0.2 (0.7)	NS	-0.8 (0.6)	<0.01	-1.0 (1.0)	<0.01
DIL	-0.5 (0.6)	<0.01	-1.0 (0.8)	<0.01	-1.4 (1.5)	<0.01
Weight	-0.2 (0.3)	<0.01	-0.7 (0.5)	<0.01	-1.0 (0.5)	<0.01

Mean (SD)

Body Composition During Short-term Malnutrition

Accuracy and Precision of BIS for Measuring Change During Malnutrition

During STUDY 2, subjects were placed on a low energy, very low protein diet for 48h while undergoing HDT. This treatment produced a 1.2 kg weight change (Table 5). This was smaller than the weight change observed during STUDY 1, but unlike STUDY 1, the initial measurement period for STUDY 2 was after 12 h of HDT. Thus, this was after the initial diuresis of HDT. When adjusted for the initial 4 h HDT weight loss, the weight changes observed in STUDY 2 were greater than STUDY 1 (1.2 vs 0.8kg, p=0.02). Unlike the STUDY 1, the treatment induced a larger change in ICW than ECW as measured by dilution, which was the expected result. This change in ICW, however, was not detected by BIS using the proximal (knee-elbow) electrode placement (Table 5). We conclude that this was not due to any errors in dilution because the dilution TBW agrees with the weight change.

Table 5. Accuracy and precision of BIS for detecting change during short-term malnutrition

	48 h Change, kg	p-value
ECW		
BIS	-0.2 (0.3)	0.05
Dilution	-0.2 (0.3)	0.05
ICW		
BIS	-0.1 (0.6)	NS
Dilution	-1.4 (0.9)	<0.01
TBW		
BIS	-0.4 (0.7)	0.03
Dilution	-1.6 (0.6)	<0.01
Weight	-1.2 (0.4)	<0.01

Mean (SD)

Segmental Analysis

We hypothesized that BIS was insensitive to the changes in ICW during short-term undernutrition because the major effect was localized to the trunk. Even the elbow-knee electrode placement underestimates fluid changes in trunk, although the error is smaller than the wrist-ankle placement. To test this hypothesis, we analyzed our segmental BIS data to obtain the independent resistance of the upper arm, trunk and thigh. These were then multiplied by the average water spaces of each segment assuming that 14% of TBW is in the upper arm, 39% in the thigh and 42% in the truck (Table 6). When this was done it was found that the largest fractional changes were did indeed occur in the trunk. When calculated segment–by-segment, the changes in water spaces are in reasonable agreement with those measured by dilution (Table 6). This supports the hypothesis that the short-term changes in water spaces were not detected by BIS because they were localized to the large, low resistance trunk region.

Table 6. Segmental changes in resistances water spaces during 48 h of HDT and undernutrition.

	%Change Re	ECW Change (kg)	%Change Ri	ICW Change (kg)	TBW Change (kg)
Elbow-shoulder	2.7	-0.05	0.5	-0.02	-0.7
Knee-hip	1.6	-0.09	3.7	-0.34	-0.43
Shoulder-hip	7.2	-0.42	6.2	-0.60	-1.01
Total Body		-0.56		-0.95	-1.51

Mean (SD)

Validation of BIS for Measurement of Peripheral Muscle Volume

When measuring a cylindrical body, bioelectrical impedance analysis can be used to directly calculate volume (V) when the specific resistivity of the medium is known. The equation is :

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

where ρ is the specific resistivity (ohm •cm), L is the length (cm) and R is the resistance (ohm). The specific resistivities of ECW provided by Xitron are 40.5 and 39 ohm·cm for males and females, respectively. Those for ICW are 264.9 and 273.9 ohm·cm for males and females, respectively. Total volume is then obtained by adding ECW and ICW.

This absolute measurement of muscle volume by BIS was validated against Magnetic Resonance Imaging (MRI) (Table 7). The comparison was excellent. BIS volume was about 18% greater than MRI ($P<0.01$), but the effect was systematic and thus mathematically correctable. The two measures were highly correlated for both the calf ($r=0.96$) and the upper arm ($r=0.93$).

Table 7. Comparison of BIS Measured Muscle Volume with MRI During HDT and short-term undernutrition.

	Calf, cc		Upper Arm, cc	
	12h HDT	60hHDT	12h HDT	60h HDT
BIS	937 (210)	808 (200)	474 (220)	463 (221)
MRI	801 (178)	758 (145)	397 (172)	380 (178)
Within-subject difference	-135 (98)	-141 (110)	-76 (71)	-82 (73)
p-value	<0.01	<0.01	<0.01	<0.01

Mean (SD)

Of greater interest, BIS was equally as sensitive as the very expensive MRI for tracking changes in the muscle volume during the 48h of HDT with undernutrition. The MRI recorded a 49 (69) cc decrease in the calf volume and BIS recorded a 36cc decrease (N.S.). For the arm, MRI recorded a 17 (23) cc decrease compared to the 11 (24) cc decrease by BIS. The standard deviations (indicated in parentheses) were virtually identical for the two techniques, but only the BIS instrument is small enough for space flight.

DISCUSSION

Clinical Capacity Development

The primary focus of this research project has been to develop a method to measure body composition that could be used during space flight. This technique is Bioelectrical Impedance Spectroscopy (BIS). The instrument has a volume of less than 0.02 cubic meters, weighs less than 2 kg, and consumes little power. Our studies demonstrate that the instrument could measure ECW, ICW and TBW with an accuracy of 1.2kg for ECW, 2.2kg for ICW and 2.4kg for TBW. We demonstrated that accuracy was not altered by the effects of simulated microgravity. More importantly, we demonstrated that BIS was able to follow the changes in ECW and ICW that occur when a persons moves from standing to HDT and that it can measure these changes with a relative precision (SD) of 2 to 3% in a single individual. Thus, BIS can provide in flight technology for monitoring body fluids shifts. The ability to independently measure ECW and ICW means that the BIS can be used to monitor loss of extracellular fluids that often occurs in microgravity. The ability of measure ICW provides clinical capacity to monitor

the changes in nutritional status. Finally we demonstrated that BIS provides clinical capacity to measure changes in peripheral muscle volume. This is limited to peripheral muscles, however, because BIS requires that the object be presented as a cylinder in order to make an absolute measure of volume. It is also notable that BIS should be able to distinguish between muscle volume changes resulting from changes in ECW or ICW and thus distinguish between movement of extracellular water and loss of muscle fiber volume.

We did find that BIS was not as effective at detecting a sudden decrease in intracellular volume that is concentrated in the trunk. This could be circumvented through the use of segmental analysis, but this increases the complexity of the measurement and decreases the precision. It is likely that some of the problem lay in the choice of a somewhat unphysiologic model in which we severely restricted the subjects with regard to protein and energy for 48h. This is likely to produce a relatively specific loss of liver volume due to protein breakdown and glycogen utilization. We are collaborating with a French investigator to access the accuracy and precision of BIS to monitor microgravity induced changes in ICW using a chronic 90-day, HDT, bed rest model that is likely to provide a more physiologic alteration of body composition.

Nutritional Deficiencies

The BIS instrument now offers the clinical capacity to study and diagnose clinical deficiencies during space flight. Until now, measurement of body composition could only be performed before launch and after reentry. The BIS technology provides a means to distinguish between the various causes of weight loss and to test countermeasures in space flight. Fluid loss secondary to exposure to microgravity is primarily due to extracellular fluid loss. BIS is very accurate at measuring ECW and thus determining what percentage of weight loss is due to simple diuresis. BIS can also be employed in real time to monitor the effectiveness of countermeasures such as lower body negative pressure and saline ingestion.

In contrast, chronic, mild undernutrition will induce a loss of body protein and or body fat. The former will be accompanied by a loss of ICW while the latter will not, because fat is anhydrous. BIS can be used for differential diagnosis of fat loss or body cell mass loss and this information can be used to make prescriptions for dietary change.

Human Performance

The BIS instrument also provides clinical capacity for the study and diagnosis of loss of muscle mass during space flight. Loss of muscle mass usually results in loss of strength, which can be detrimental upon the return to gravity. The ability to monitor whole body ICW and peripheral muscle mass changes during flight will allow investigators and medical personnel to monitor the effectiveness of exercise countermeasures on muscle mass.

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