Prediction of limb lean tissue mass from bioimpedance spectroscopy in persons with chronic spinal cord injury

Christopher M. Cirnigliaro¹, Michael F. La Fountaine¹⁻⁴, Racine Emmons⁵, Steven C. Kirshblum^{6,7}, Pierre Asselin¹, Ann M. Spungen^{1,2,4}, William A. Bauman^{1,2,4}

¹National Center of Excellence for the Medical Consequences of Spinal Cord Injury, James J. Peters VA Medical Center, Bronx, NY, USA, ²Medical & Spinal Cord Injury Services, The James J. Peters VA Medical Center, Bronx, NY, USA, ³Seton Hall University, South Orange, NJ, USA, ⁴Icahn School of Medicine at Mount Sinai, New York, NY, USA, ⁵William Paterson University, Wayne, NJ, USA, ⁶Kessler Institute for Rehabilitation, West Orange, NJ, USA, ⁷Department of Physical Medicine and Rehabilitation, New Jersey Medical School, Newark, NJ, USA

Background: Bioimpedance spectroscopy (BIS) is a non-invasive, simple, and inexpensive modality that uses 256 frequencies to determine the extracellular volume impedance (ECV_{Re}) and intracellular volume impedance (ICV_{Ri}) in the total body and regional compartments. As such, it may have utility as a surrogate measure to assess lean tissue mass (LTM).

Objective: To compare the relationship between LTM from dual-energy X-ray absorptiometry (DXA) and BIS impedance values in spinal cord injury (SCI) and able-bodied (AB) control subjects using a cross-sectional research design.

Methods: In 60 subjects (30 AB and 30 SCI), a total body DXA scan was used to obtain total body and leg LTM. BIS was performed to measure the impedance quotient of the ECV_{Re} and ICV_{Ri} in the total body and limbs.

Results: BIS-derived ECV_{Re} yielded a model for LTM in paraplegia, tetraplegia, and control for the right leg (RL) ($R^2 = 0.75$, standard errors of estimation (SEE) = 1.02 kg, $P < 0.0001$; $R^2 = 0.65$, SEE = 0.91 kg, $P = 0.0006$; and $R^2 = 0.54$, SEE = 1.31 kg, $P < 0.0001$, respectively) and left leg (LL) ($R^2 = 0.76$, SEE = 1.06 kg, $P < 0.0001$; $R^2 = 0.64$, SEE = 0.83 kg, $P = 0.0006$; and $R^2 = 0.54$, SEE = 1.34 kg, $P < 0.0001$, respectively). The ICV_{Ri} was similarly predictive of LTM in paraplegia, tetraplegia, and AB controls for the RL ($R^2 = 0.85$, SEE = 1.31 kg, $P < 0.0001$; $R^2 = 0.52$, SEE = 0.95 kg, $P = 0.003$; and $R^2 = 0.398$, SEE = 1.46 kg, $P = 0.0003$, respectively) and LL ($R^2 = 0.62$, SEE = 1.32 kg, $P = 0.0003$; $R^2 = 0.57$, SEE = 0.91 kg, $P = 0.002$; and $R^2 = 0.42$, SEE = 1.31 kg, $P = 0.0001$, respectively).

Conclusion: Findings demonstrate that the BIS-derived impedance quotients for ECV_{Re} and ICV_{Ri} may be used as surrogate markers to track changes in leg LTM in persons with SCI.

Keywords: Spinal cord injury, Bioimpedance spectroscopy, Lean tissue mass, Extracellular volume, Intracellular volume, Dual-energy X-ray absorptiometry

Introduction

Clinical observations following acute spinal cord injury (SCI) suggest that the adverse changes in body composition that occur can have a negative impact on rehabilitation, total body (TB) metabolism, and overall health.^{1,2} At the time of SCI, there is endogenous

release of cortisol, and as a possible elective component of routine care, the administration of methylprednisolone that further contributes to a catabolic state,³ contributing to the loss of lean tissue mass (LTM) below the level of lesion.^{4,5} Ongoing and dramatic muscle wasting results in a decreased resting energy expenditure and increased adiposity⁶ that persist into the chronic phase of SCI.⁷ As a result of these adverse body composition changes, persons with SCI may be at greater risk

Correspondence to: Christopher M. Cirnigliaro, Kessler Institute for Rehabilitation: Rm. L052m, 1199 Pleasant Valley Way, West Orange, NJ 07052, USA. Email: christopher.cirnigliaro@va.gov

for abnormal carbohydrate and lipid metabolism, which may predispose to cardiovascular disease.⁸ As evidence supporting these medical consequences of SCI continue to mount, routine clinical assessment of soft tissue mass has not been accepted as a standard of care in the SCI population.^{9,10} There are few practical and reliable methods to measure changes in LTM of the paralyzed limbs available for persons with SCI. In the research setting, magnetic resonance imaging (MRI), computerized tomography (CT), and dual-energy X-ray absorptiometry (DXA), as well as other methods have been utilized to assess fat and lean mass in the SCI population. Clinically, these methods are not feasible for routine assessment because they are expensive, expose patients to radiation (CT and DXA), and cannot be brought to the bedside or a doctor's office which makes access to this equipment difficult for acutely immobilized patients. Monitoring changes in LTM is essential in order for clinicians, therapists, and nutritionists to improve therapy and nutritional prescription in the acute and chronic SCI population.

The majority of clinical investigations utilize DXA to measure LTM in the SCI population. However, the validity of DXA in persons with chronic diseases and disability may be compromised due to non-uniform hydration.^{5,11,12} The relative hydration can vary considerably in persons with SCI, with observed decreases in intracellular volume (ICV) and increases in extracellular volume (ECV).^{13,14} The three-compartment (3-C) DXA model (FTM, LTM, and bone mineral) may not provide reliable and appropriate measures in populations with hydration disturbances.^{15,16}

There are studies that have validated fat mass (FM) and LM obtained from DXA with a 4-C model,¹⁷ and these models have demonstrated that DXA-derived soft tissue values appear to be unaffected by changes in hydration.¹⁸ In a study comparing DXA-derived LM values with MRI, Modlesky *et al.*⁵ found that DXA-derived LM measured 15% less leg muscle mass in men with long-term, complete SCI than that in able-bodied (AB) controls. The authors concluded that these findings may reflect poor prediction models used with DXA that assume skeletal muscle represents a constant proportion of the fat-free soft tissue and uniform hydration. However, TB DXA is one of the most widely available methods that can easily and accurately measure LTM and FTM in the limbs, is relatively inexpensive, associated with relatively low radiation exposure, and its use and findings have been established by numerous clinical investigations.¹⁹ As such, with these considerations in mind, body composition measurements that are developed and utilize ICV and ECV to predict body composition may

be useful in this population.²⁰ Multifrequency bioimpedance spectroscopy (BIS) is a non-invasive, simple, and inexpensive modality that has been well validated to determine the impedance (Ω) of the ECV (R_e) and the ICV (R_i) in the TB^{21–23} and limbs.^{24–26} The implementation of multiple currents with various frequencies, allows the signal to pass both through and around the cells. The commonly utilized bioelectrical impedance analysis (BIA), using a single frequency of 50 kHz, does not completely penetrate the cell membrane, resulting in an incomplete determination of ICV. By employing a variation of frequencies, the current can pass through and around the cell membrane, resulting in a more accurate measurement of ECV (R_e) and the ICV (R_i). Each measurement from BIS generates an impedance vector with both magnitude and direction; the accumulation of a number of impedance vectors from measurements obtained at different frequencies in a reactance–resistance graph forms a circular-shaped locus.^{21,27} This method has been well validated against tracer dilution techniques measuring total body water (TBW) and ECV in the healthy AB²² and hemodialysis²⁸ populations.

It should be appreciated that previous investigations have demonstrated disagreement between BIS and DXA-derived predicted values of FTM and fat-free mass (FFM), possibly due to the incorrect application of the standard BIS prediction equation in both diseased^{29,30} and athletic³¹ populations. Only through the fairly recent application of population-specific prediction equations have BIS predicted body composition values been validated against DXA and more advanced imaging techniques, such as MRI.³² In addition to evidence demonstrating raw impedance values correlation with the ECV and ICV, the ECV (R_e)/ICV (R_i) ratio has been demonstrated as an effective tool to monitor extracellular fluid changes in persons with lymphedema.^{24,25,33} The relative importance of this methodology may be greater in persons with SCI due to increased leg ECV and the absence of the leg skeletal muscle pump³⁴ and, on occasion, edema secondary due to regional thromboembolism.³⁵ Thus a method to monitor changes in LTM and fluid accumulation after SCI, independent of predictive algorithms, could be of clinical relevance and research interest.

To better understand the possible application of this technology, the relationship between BIS impedance quotients and DXA LTM in persons with SCI should be determined. Furthermore, the development and implementation of SCI-specific prediction equations with BIS, an easy to use and portable technology, could make bedside clinical body composition assessment a reality. As such, our investigation examined the

correlation between BIS ICV and ECV impedance quotients to DXA-derived LTM in the TB and limbs, and compared this relationship between SCI and healthy AB control subjects. In addition, we hypothesized that leg LTM could be predicted from BIS impedance quotients ECV_{Re} and ICV_{Ri} .

Materials and methods

Subjects

The demographics of the subjects are presented (Table 1). Thirty subjects with SCI (16 paraplegia and 14 tetraplegia) and 30 healthy AB controls between 20 and 64 years of age were recruited for participation in this study from an existing subject database and by physician referral at the James J. Peters Veterans Affairs Medical Center (JJPVAMC), Bronx, NY, USA and Kessler Institute for Rehabilitation (KIR), West Orange, NJ, USA. Individuals with SCI were included if they had a chronic injury (≥ 1 year), regardless of completeness or mechanism of injury, and were clinically stable. Subjects were excluded from participation if they had any type of metal implant, had been diagnosed by a physician with a pressure ulcer, diabetes, coronary heart disease, and/or any other chronic illness, or lacked mental capacity to provide informed consent. Using the American Spinal Injury Association Impairment Scale (AIS) to classify neurological impairment, 20 subjects were previously diagnosed with motor complete injury (AIS A or B) and 10 subjects with motor incomplete injury (AIS C and D). The study was approved by the Institutional Review Boards at the JJPVAMC and KIR, and informed consent was obtained for all subjects prior to beginning study participation.

Procedures

Studies were performed during a 1-day visit at the clinical centers (JJPVAMC and KIR). After obtaining informed consent, subjects performed TB and segmental BIS measurements followed by performing a TB DXA scan. All measures were conducted with subjects instructed to wear light clothing; shoes were removed prior to testing. Subjects were instructed to be free of all plastic and metal objects (i.e. rings, necklaces, etc.) and arrive in the morning between 8:00 and 10:00 am. Because it is appreciated that dietary factors may influence DXA and BIS results, subjects were instructed to present for testing procedures after a 12-hour fast, consume no more than 16 ounces of water 4 hours before the BIS measurement, and abstain from alcohol, caffeine, and strenuous exercise for 24 hours prior to testing.

Total body DXA

A TB scan was performed in accordance with the manufacturer guidelines using fan beam DXA (GE LUNAR iDXA, software version 13.3, Madison, WI, USA). DXA uses a congruent beam of stable dual-energy radiation that passes through the subject from the table below, while the differential absorption is measured above, permitting calculation of the attenuation coefficient for LTM. TB scans were performed and the energy level used for each TB scan was based on subject thickness (e.g. thin, standard, or thick) and lasted ~ 5 minutes. Due to the possibility of contractures and spasms in SCI subjects, particular effort was made to position each subject as precisely as possible. To maintain consistency of body composition measurements, body weight was recorded as the TB mass from

Table 1 Demographic characteristics of the study groups

	Para ($n = 16$)	Tetra ($n = 14$)	Control ($n = 30$)
Age	41 $\pm$ 13	45 $\pm$ 8	41 $\pm$ 12
DOI (years)	9 $\pm$ 11	20 $\pm$ 14*	NA
TB length (cm)	175.5 $\pm$ 7.6	179.8 $\pm$ 8.3**	171.8 $\pm$ 8.5
RA length (cm)	54.9 $\pm$ 2.7	57.4 $\pm$ 3.4*	56.5 $\pm$ 3.3
LA length (cm)	54.7 $\pm$ 2.6	56.9 $\pm$ 3.3*	56.4 $\pm$ 3.2
RL length (cm)	93.7 $\pm$ 4.7	96.7 $\pm$ 6.3	94.1 $\pm$ 5.0
LL length (cm)	94.0 $\pm$ 4.6	96.9 $\pm$ 6.2	94.0 $\pm$ 4.9
Weight (kg)	87.5 $\pm$ 15.9	81.1 $\pm$ 13.4	86.8 $\pm$ 21.6
BMI (kg/m ²)	28.1 $\pm$ 5.1	25.0 $\pm$ 3.3***	29.3 $\pm$ 6.4
Males/female	20/0	15/1	28/2
Motor complete (n)	13	7	NA
Motor incomplete (n)	3	7	NA

Values are expressed as group mean $\pm$ SD.

n , number of subjects; tetra, tetraplegia; para, paraplegia; Control, able-bodied control subjects; cm, centimeters; DOI, duration of injury; BMI, body mass index; kg, kilograms; m, meters; TB, total body; RA, right arm; LA, left arm; RL, right leg; LL, left leg.

* $P < 0.05$, tetra vs. para.

** $P < 0.01$ tetra vs. control.

*** $P < 0.05$ tetra vs. control.

Figure 1 Photograph of (A) BIS device and facsimile of (B) Cole-Cole plot illustrating the relationship between resistance and reactance. The impedance plot allows for the determination of a number of impedance measurements at key theoretical frequencies: R infinity (R_{∞}) and zero (R_0) (reprinted with permission from ImpediMed Limited, Imp™ SFB7).

the DXA scan. To analyze the results of LTM from each TB scan, proprietary software algorithms from the manufacturer were used to segment the body into the upper and lower extremities using the standard regions of interest. Body segments were then adjusted in the TB scan image the same way for each subject by one investigator at both JJPVAMC and KIR. Daily quality assurance testing was performed to determine the stability of the densitometer. The precision was determined at both study sites for 150 phantom scans in which the reference range was 30% fat; the coefficient of variation was <1%.

Total body and segmental multifrequency BIS

TB and segmental BIS were collected across a distribution of 256 frequencies ranging from 3–1000 kHz (ImpediMed Limited, Imp™ SFB7, software version 1.14 and 5.10, Brisbane, Australia) (Fig. 1A). Using the manufacturer's software, acquisition and analysis of the impedance R_e and R_i were determined by filtering and fitting the impedance of multiple impedance data points using the Cole-Cole plot (Fig. 1B).³⁶ From the apex of this locus, a theoretical extrapolation is made at the zero (0) and infinite (∞) frequencies, corresponding to the resistances (R) of ECV (R_e) and TBW (R_{∞}),

respectively. In addition to R_e and R_{∞} , BIS calculated the ICV impedance (R_i) by parallel subtraction using the values of R_e and R_{∞} .³⁷ To obtain TB and limb length for the numerator of the impedance quotient, the electronic caliper from the DXA TB scan was utilized to measure the length of the TB, right arm (RA), left arm (LA), right leg (RL), and left leg (LL), mirroring the TB and segment length of the standard region utilized for measures of LTM. The resistivity of the ECV (R_e) and ICV (R_i) fluid compartments were then calculated as the length (L) of the regions measured (surrogate measure of conducting length) squared over the impedance (cm^2/Ω) for all segments and presented throughout the text as the impedance quotient where applicable as follows^{22,20,38}:

ICV_{Ri}

- TB ICV_{Ri} = TB length squared (cm^2)/TB ICV impedance (Ω)
- RA ICV_{Ri} = RA length squared (cm^2)/RA ICV impedance (Ω)
- LA ICV_{Ri} = LA length squared (cm^2)/LA ICV impedance (Ω)
- RL ICV_{Ri} = RL length squared (cm^2)/RL ICV impedance (Ω)
- LL ICV_{Ri} = LL length squared (cm^2)/LL ICV impedance (Ω).

ECV_{Re}

- TB ECV_{Re} = TB length squared (cm^2)/TB ECV impedance (Ω)
- RA ECV_{Re} = RA length squared (cm^2)/RA ECV impedance (Ω)
- LA ECV_{Re} = LA length squared (cm^2)/LA ECV impedance (Ω)
- RL ECV_{Re} = RL length squared (cm^2)/RL ECV impedance (Ω)
- LL ECV_{Re} = LL length squared (cm^2)/LL ECV impedance (Ω).

Prior to each measurement, the instrument's calibration was verified using a test cell [resistor–resistor–111 capacitor (RRC) circuit] to check 256 frequencies (frequency range 3–1000 kHz) with a specific impedance tested for each frequency (impedance range 404–603 Ω). To allow for the equalization of body fluids, subjects were positioned supine with palms facing down for at least 15 minutes on a non-conducting surface before obtaining the BIS measurement. All measurement sites were cleaned with alcohol swabs before attachment of electrodes, and two electrodes were then placed on the right and left hand and foot. Using the standard wrist-to-ankle electrode placement to measure the impedance of the TB, current was applied to two driving leads placed on the midline of the dorsal surface of the right hand and foot proximal

Figure 2 Stick figure illustrations of electrode arrangement for segmental BIS for the (A) total body, (B) right arm, (C) left arm, (D) right leg, and (E) left leg.

to the metacarpophalangeal joints and the metatarsophalangeal joints, respectively. The sense leads were placed on the dorsal surface of the right hand and right foot directly on the metacarpal and metatarsal joint in line with the drive leads (Fig. 2A). Utilizing the law of equipotential explained elsewhere,^{21,39} keeping with electrodes placed in the midline of the limbs and opposing electrode, impedance of the RA was obtained by moving the metatarsal sense lead (blue) to the left metacarpal joint (Fig. 2B). For the LA, this lead configuration was reversed at the arms with the drive lead (black) remaining on the RA metatarsophalangeal joint (Fig. 2C). The impedance of the RL was obtained by placing the sense lead (yellow) on the metatarsal joint on the LL with the other sense lead (blue) on the RA metatarsal joint. The first drive lead (black) was then placed on the RL metatarsophalangeal joint with the second drive lead (red) placed on the RA metacarpophalangeal joint (Fig. 2D). For the LL, this lead configuration was reversed at the legs, with the drive lead (red) remaining on the RA metacarpophalangeal joint (Fig. 2E). The equipotential standard electrode arrangement was chosen as opposed to the direct method to improve precision of electrode placement between SCI and AB control subjects.²⁶

Statistical analysis

Results are expressed throughout as group mean $\pm$ standard deviation and 95% confidence intervals (95% CIs) where applicable. Duration of injury (DOI) was compared between the two SCI groups by use of an unpaired *t*-test. All other demographic characteristics (age, height,

weight, and body mass index (BMI)) and baseline DXA LTM values (TB, RA, LA, RL, and LL) and BIS extracellular and intracellular impedance (ECV_{Re} and ICV_{Ri}) were compared among the groups by one-factor analysis of covariance with TB length, arm length, and DOI (SCI only) used as covariates. To compare the extracellular to intracellular ratio between the SCI and control groups, paraplegia and tetraplegia were combined and unpaired *t*-tests were performed to identify group differences in the ECV_{Re} and ICV_{Ri} ratio. Pearson product moment correlation coefficients were used to test relationships between DXA LTM and impedance values between the groups. Simple linear regression analysis was used to determine linearity between RL and LL impedance values and LTM with the relative accuracy of each prediction determined by comparing the coefficients of determination (R^2) and the standard errors of estimation (SEEs). An *a priori* level of significance was set at $P \leq 0.05$. Statistical analyses were completed using Statview 5.0 (SAS Institute, Inc., Cary, NC, USA).

Results

The characteristics of the subjects are presented (Table 1). Subjects with tetraplegia were injured significantly longer than those with paraplegia (20 ± 14 vs. 9 ± 11 years, respectively; $P < 0.05$). Subjects with tetraplegia were also significantly taller than the control group (179.8 ± 8.3 vs. 171.8 ± 8.5 cm, respectively; $P < 0.01$) and had a significantly longer RA and LA length compared with subjects with paraplegia (57.4 ± 3.4 vs. 54.9 ± 2.7 cm, respectively, $P < 0.05$, and 56.9 ± 3.3 vs. 54.7 ± 2.6 cm, respectively; $P < 0.05$). There were no significant differences in body weight; however, subjects with tetraplegia due to their longer body length had a significantly lower BMI than the control subjects (25.0 ± 3.3 vs. 29.3 ± 6.4 kg/m², respectively; $P < 0.05$).

LTM and impedance measures: Tetraplegia vs. paraplegia vs. control

For all regions, the average LTM and impedance quotients ECV_{Re} and ICV_{Ri} are presented (Table 2). Large LTM differences were found between the SCI and control groups. The tetraplegic and paraplegic groups had significantly lower TB LTM ($P < 0.01$), as well as RL and LL LTM ($P < 0.0001$ for each comparison), compared with that of AB controls. Furthermore, a significantly lower LTM in the tetraplegic group was observed for the RA and LA than in the paraplegic ($P < 0.05$) and control ($P < 0.001$) groups.

Similar to the observed LTM differences, the ICV_{Ri} impedance was significantly lower in tetraplegic group

Table 2 Total body and segmental lean tissue mass and impedance quotients

	Paraplegia (n = 16)	Tetraplegia (n = 14)	Control (n = 30)
LTM (kg)			
TB	53.0 ± 8.9 (48.3–57.7)*	48.8 ± 6.4 (45.1–52.5)*	58.4 ± 9.9 (54.7–62.1)
RA	4.3 ± 0.70 (3.9–4.7)	3.2 ± 0.97 (2.6–3.8) ^{†,§}	3.8 ± 0.88 (3.5–4.1)
LA	4.3 ± 0.75 (3.9–4.7)	3.0 ± 0.90 (2.5–3.5) ^{†,§}	3.7 ± 0.87 (3.4–4.0)
RL	7.2 ± 2.0 (6.1–8.3) [‡]	7.5 ± 1.3 (6.7–8.3) [‡]	10.1 ± 2.0 (9.4–10.8)
LL	7.4 ± 2.0 (6.3–8.5) [‡]	7.6 ± 1.3 (6.8–8.4) [‡]	10.0 ± 2.0 (9.3–10.7)
Impedance quotients			
ICV _{Ri} (cm ² /Ω)			
TB	22.7 ± 9.6 (17.6–27.8) [‡]	14.6 ± 5.3 (11.5–17.7) ^{¶,‡}	27.2 ± 5.6 (25.1–29.3)
RA	6.9 ± 1.7 (6.0–7.8)	3.7 ± 1.9 (2.6–4.8) ^{**,‡}	6.2 ± 1.8 (5.5–6.9)
LA	6.7 ± 1.3 (6.0–7.4)	6.3 ± 8.3 (1.5–11.1)	7.3 ± 3.0 (6.2–8.4)
RL	6.3 ± 3.3 (4.5–8.1) [‡]	7.1 ± 3.2 (5.3–8.9) [‡]	16.4 ± 4.2 (14.8–18.0)
LL	6.3 ± 3.2 (4.6–8.0) [‡]	7.3 ± 3.0 (5.6–9.0) [‡]	16.4 ± 3.1 (15.2–17.6)
ECV _{Re} (cm ² /Ω)			
TB	60.8 ± 11.9 (56.4–65.2) ^{††,‡‡}	53.9 ± 10.1 (48.1–59.7)	52.5 ± 8.3 (49.4–55.6)
RA	13.0 ± 1.6 (12.1–13.9) ^{§§}	10.9 ± 3.0 (9.2–12.6)	12.0 ± 2.6 (11.0–13.0)
LA	12.9 ± 1.6 (12.0–13.8) ^{§§}	11.1 ± 2.8 (9.5–12.7)	11.9 ± 1.9 (11.2–12.6)
RL	36.5 ± 11.0 (30.6–42.4)	34.2 ± 7.7 (29.8–38.6)	32.5 ± 5.8 (30.3–34.7)
LL	34.9 ± 11.2 (28.9–40.9)	34.4 ± 7.4 (30.1–38.7)	32.1 ± 5.2 (30.2–34.0)

Values are expressed as group mean ± SD (95% CIs).

SD, standard deviation; n, number of subjects; Control, able-bodied control subjects; LTM, lean tissue mass; cm, centimeters; kg, kilograms; Ω, ohms; TB, total body; RA, right arm; LA, left arm; RL, right leg; LL, left leg; ICV, intracellular volume; ECV, extracellular volume; R_i, resistance intracellular fluid; R_e, resistance extracellular fluid.

*P < 0.01 vs. control.

[†]P < 0.05 vs. control.

[‡]P < 0.0001 vs. control.

[§]P < 0.001 vs. paraplegia.

[¶]P < 0.01 vs. paraplegia.

^{**}P < 0.0001 vs. paraplegia.

^{††}P = 0.008 vs. control.

^{‡‡}P = 0.06 vs. tetraplegia.

^{§§}P < 0.05 vs. tetraplegia.

than in the paraplegic group ($P < 0.01$), and in both paraplegic and tetraplegic groups ($P < 0.0001$) than in the AB control group. In the RL and LL, the ICV_{Ri} impedance was also significantly lower in both paraplegic and tetraplegic groups ($P < 0.0001$ for each comparison) compared with the AB control group. Furthermore, the RA ICV_{Ri} impedance was lower in tetraplegic group ($P < 0.0001$) compared with paraplegic and AB control groups, with no difference in the LA. The TB ECV_{Re} impedance was significantly higher in the paraplegic group ($P = 0.008$) than in the AB control group, and approached significance compared with the tetraplegic group ($P = 0.06$). The ECV_{Re} impedance of the RA and LA was significantly higher in paraplegic group ($P < 0.05$) compared with tetraplegic group, with no differences found between these groups for the legs. A comparison of the ECV_{Re}/ICV_{Ri} impedance ratio revealed a significantly higher ratio in those with SCI compared with AB controls for the RL (6.6 ± 3.5 vs. 2.0 ± 0.14 , $P < 0.0001$) and LL (6.0 ± 3.2 vs. 2.0 ± 0.08 , $P < 0.0001$; Fig. 3) due to the lower ICV_{Ri} in the SCI group.

Relationship between BIS impedance and DXA LTM

The univariate relationship between TB and segmental LTM and impedance quotients from BIS is provided (Table 3). A highly significant positive correlation was

Figure 3 Comparison of the ECV_{Re}/ICV_{Ri} ratio in control and SCI subjects for the left leg (LL) (6.0 ± 3.2 vs. 2.0 ± 0.08 , $*P < 0.0001$) and right leg (RL) (6.6 ± 3.5 vs. 2.0 ± 0.14 , $*P < 0.0001$). Control, able-bodied control subjects; SCI, subjects with spinal cord injury.

Table 3 Univariate correlation coefficients for total body and segmental lean tissue mass and impedance values

TB LTM (kg)	RL LTM			LL LTM			RA LTM			LA LTM				
	r	P value	ICV _{RL}	r	P value	ICV _{RL}	r	P value	ICV _{RL}	r	P value	ICV _{RL}	r	P value
Para	0.72	<0.001	Para	0.92	<0.0001	Para	0.79	0.0001	Para	0.42	NS	Para	0.46	NS
Tetra	0.88	<0.0001	Tetra	0.78	0.0009	Tetra	0.76	0.001	Tetra	0.44	NS	Tetra	0.16	NS
Control	0.85	<0.0001	Control	0.65	<0.0001	Control	0.74	<0.0001	Control	0.87	<0.0001	Control	0.58	0.0006
ECV _{Re} (cm ² /Ω)	r	P value	ECV _{Re}	r	P value	ECV _{Re}	r	P value	ECV _{Re}	r	P value	ECV _{Re}	r	P value
Para	0.75	0.0004	Para	0.86	<0.0001	Para	0.87	<0.0001	Para	0.55	0.03	Para	0.54	0.03
Tetra	0.89	<0.0001	Tetra	0.88	<0.0001	Tetra	0.81	0.0004	Tetra	0.48	NS	Tetra	0.92	<0.0001
Control	0.86	<0.0001	Control	0.73	<0.0001	Control	0.72	<0.0001	Control	0.86	<0.0001	Control	0.86	<0.0001

Tetra, tetraplegia; Para, paraplegia; Control, able-bodied subjects; LTM, lean tissue mass; cm, centimeters; kg, kilograms; Ω, ohms; r , correlation coefficient; NS, not significant; TB, total body; RA, right arm; LA, left arm; RL, right leg; LL, left leg; ICV, intracellular volume; ECV, extracellular volume; R_e , resistance intracellular fluid; R_i , resistance extracellular fluid.

found for the TB impedance ICV_{RL} and TB LTM for paraplegia, tetraplegia, and AB controls ($r = 0.72$, $P < 0.001$; $r = 0.88$, $P < 0.0001$; and $r = 0.85$, respectively; $P < 0.0001$), as well as the RL ($r = 0.92$, $P < 0.0001$; $r = 0.78$, $P = 0.0009$; and $r = 0.65$, respectively; $P < 0.0001$), and LL ($r = 0.79$, $P = 0.0001$; $r = 0.76$, $P = 0.001$; and $r = 0.74$, respectively; $P < 0.0001$). In the paraplegic and tetraplegic groups, ICV_{RL} impedance had a weak non-significant correlation with RA and LA LTM, but in the AB control group a moderate-to-strong correlation was demonstrated for the RA LTM ($r = 0.87$, $P < 0.0001$) and LA LTM ($r = 0.58$, $P = 0.0006$). A positive correlation was also observed for impedance ECV_{Re} in those with paraplegia or tetraplegia and in AB controls for the TB LTM ($r = 0.75$, $P = 0.0004$; $r = 0.89$, $P < 0.0001$; and $r = 0.86$, respectively; $P < 0.0001$), RL LTM ($r = 0.86$, $P < 0.0001$; $r = 0.88$, $P < 0.0001$; and $r = 0.73$, respectively; $P < 0.0001$), and LL LTM ($r = 0.87$, $P = 0.0001$; $r = 0.81$, $P = 0.0004$; and $r = 0.72$, respectively; $P < 0.0001$). Compared with the ICV_{RL} impedance, a stronger relationship was found with the ECV_{Re} impedance in those with paraplegia and tetraplegia, and was similar to that found for AB controls for the RA LTM ($r = 0.55$, $P = 0.03$; $r = 0.48$, $P = NS$; and $r = 0.86$, respectively; $P < 0.0001$) and LA LTM ($r = 0.54$, $P = 0.03$; $r = 0.92$, $P < 0.0001$; and $r = 0.86$, respectively; $P < 0.0001$).

Prediction equations for ICV and ECV impedance regressed against LTM

Linear regression analysis findings for impedance ECV_{Re} and LTM in paraplegic, tetraplegic, and control groups significantly predicted LTM of the LL ($R^2 = 0.76$, SEE = 1.10 kg, $P < 0.0001$; $R^2 = 0.64$, SEE = 0.83 kg, $P = 0.0006$; and $R^2 = 0.54$, SEE = 1.34 kg, $P < 0.0001$, respectively) and RL ($R^2 = 0.75$, SEE = 1.02 kg, $P < 0.0001$; $R^2 = 0.78$, SEE = 0.65 kg, $P < 0.0001$; and $R^2 = 0.54$, SEE = 1.31 kg, $P < 0.0001$, respectively) (Figs. 4A, B, C and D). The impedance ICV_{RL} was equally predictive of LTM in those with paraplegia, tetraplegia, and in AB controls for the LL ($R^2 = 0.76$, SEE = 1.32 kg, $P = 0.0003$; $R^2 = 0.57$, SEE = 0.91 kg, $P = 0.002$; and $R^2 = 0.55$, SEE = 1.31 kg, $P < 0.0001$; respectively) and RL ($R^2 = 0.85$, SEE = 1.31 kg, $P < 0.0001$; $R^2 = 0.52$, SEE = 0.95 kg, $P = 0.003$; and $R^2 = 0.42$, SEE = 1.46 kg, $P = 0.0001$; respectively) (Figs. 3C and D).

Discussion

The relationships presented herein demonstrate the differences in the ICV_{RL} impedance among those with paraplegia, tetraplegia, and in AB controls, which corresponded well with lower LTM values for TB and extremity measurements by DXA. Furthermore, TB and leg

Figure 4 Scatter plots of SBIS extracellular volume (ECV Re) and intracellular volume (ICV Ri) impedance values regressed on lean tissue mass (LTM) for (A) left leg (LL) ECV_{Re} (para: $LTM (kg) = 0.16 \times R_e (cm^2/\Omega) + 1.76$, slope 95% CI: 0.11–0.21, $R^2 = 0.76$, $SEE = 1.10$ kg, $P < 0.0001$; tetra: $LTM (kg) = 0.14 \times R_e (cm^2/\Omega) + 2.63$, slope 95% CI: 0.08–0.21, $R^2 = 0.64$, $SEE = 0.83$ kg, $P = 0.0006$; and control: $LTM (kg) = 0.28 \times R_e (cm^2/\Omega) + 1.17$, slope 95% CI: 0.18–0.37, $R^2 = 0.54$, $SEE = 1.34$ kg, $P < 0.0001$), (B) right leg (RL) ECV_{Re} (para: $LTM (kg) = 0.15 \times R_e (cm^2/\Omega) + 1.61$, slope 95% CI: 0.10–0.21, $R^2 = 0.75$, $SEE = 1.02$ kg, $P < 0.0001$; tetra: $LTM (kg) = 0.15 \times R_e (cm^2/\Omega) + 2.32$, slope 95% CI: 0.10–0.20, $R^2 = 0.78$, $SEE = 0.65$ kg, $P < 0.0001$; and control: $LTM (kg) = 0.24 \times R_e (cm^2/\Omega) + 2.24$, slope 95% CI: 0.16–0.33, $R^2 = 0.54$, $SEE = 1.31$ kg, $P < 0.0001$), (C) left leg (LL) ICV_{Ri} (para: $LTM (kg) = 0.51 \times R_i (cm^2/\Omega) + 4.19$, slope 95% CI: 0.28–0.73, $R^2 = 0.76$, $SEE = 1.32$ kg, $P = 0.0003$; tetra: $LTM (kg) = 0.335 \times R_i (cm^2/\Omega) + 5.11$, slope 95% CI: 0.15–0.52, $R^2 = 0.57$, $SEE = 0.91$ kg, $P = 0.002$; and control: $LTM (kg) = 0.47 \times R_i (cm^2/\Omega) + 2.31$, slope 95% CI: 0.31–0.64, $R^2 = 0.55$, $SEE = 1.31$ kg, $P < 0.0001$), and (D) right leg (RL) ICV_{Ri} (para: $LTM (kg) = 0.55 \times R_i (cm^2/\Omega) + 3.79$, slope 95% CI: 0.42–0.68, $R^2 = 0.85$, $SEE = 1.31$ kg, $P < 0.0001$; tetra: $LTM (kg) = 0.299 \times R_i (cm^2/\Omega) + 5.37$, slope 95% CI: 0.12–0.48, $R^2 = 0.52$, $SEE = 0.95$ kg, $P = 0.003$; and control: $LTM (kg) = 0.30 \times R_i (cm^2/\Omega) + 5.27$, slope 95% CI: 0.16–0.43, $R^2 = 0.42$, $SEE = 1.46$ kg, $P = 0.0001$). Circle indicates Para, subjects with paraplegia, Letter X indicates Tetra, subjects with tetraplegia, and Filled triangle indicates Control, AB control subjects.

LTM values were lower in subjects with tetraplegia and paraplegia compared with AB controls, and LTM could be predicted quite well from both ICV_{Ri} and ECV_{Re} impedance values. Studies in a variety of populations with non-uniform hydration have successfully validated the use of BIS-predicted body composition values.^{32,22} Using MRI and TB potassium counting, BIS prediction equations have been developed and validated for skeletal muscle in the TB and limbs in hemodialysis patients,⁴⁰ but not in the paralyzed extremities to date. The calculation of volumes from BIS is based on tissue impedance, which is affected by the tissue composition, hydration, and geometry of the conducting cylinder. In TB BIS, the body is the conducting cylinder; in segmental BIS, each limb is the conducting cylinder, with both limbs assumed to have a homogeneous geometry.^{41,26} In a study by Zhu *et al.*,⁴² the prediction of

TBW, ECV, and ICV was significantly improved in 29 hemodialysis patients by applying segment-specific impedance values for the trunk, arms, and legs compared with predicted values from TB BIS. In persons with SCI, the integration of segmental impedance values may improve the accuracy of SCI-specific prediction equations that take into account and apply a correction factor for the abnormal composition and geometry of the limbs.

Buchholz *et al.*³⁸ tested the validity of single and multifrequency BIA compared with tracer dilution techniques for TBW and ECV in 31 subjects with paraplegia and 60 healthy AB controls; these investigators found that TBW and TB ECV can be reasonably well predicted in persons with SCI using single-frequency BIA. Furthermore, arm ECV_{Re} impedance correlated well with tracer dilution measures in the control

group, but not the paraplegic group, concluding that the use of the arm impedance to predict TB composition assumes the arm is representative of the remainder of the body, which may be true in the healthy AB population but not be the case in the SCI population. Our results also demonstrated a weak relationship in the SCI group between arm ICV_{Ri} and ECV_{Re} impedance and LTM. A possible explanation for this finding may be that absolute skeletal muscle mass in the arms for the SCI group was below a threshold for optimum conduction employing the existing technology. However, this explanation is highly suspect because the group with paraplegia was found to have higher arm LTM than that of the AB control group, and there was a strong correlation demonstrated between arm impedance values and LTM in the control group. Because ICV_{Ri} impedance had a strong correlation with LTM in the AB control group, it may be speculated that the poor conduction is attributable to abnormal geometry and/or composition in the arms of subjects in the SCI group (see Table 2).

In our study, the ECV_{Re} impedance was significantly higher in the paraplegic group compared with the tetraplegic and AB control groups in the TB and arms, but not in the legs. Furthermore, the ECV_{Re}/ICV_{Ri} ratio was significantly higher in the combined SCI group due to the lower ICV_{Ri} impedance value, with no significant difference in the ECV_{Re} . These findings were unexpected because only the leg region was expected to demonstrate higher ECV_{Re} impedance, possibly due to peripheral edema. Peripheral edema is a common clinical finding in persons with SCI due to increased orthostatic pressure on the venous vasculature that can result in interstitial fluid accumulation. Previous reports have demonstrated BIS-derived ECV_{Re} impedance as more effective than circumference measurements in diagnosing peripheral lymphedema in post-surgical cancer patients when compared with pre-operative values and healthy AB controls.^{43,33} However, there are no studies to date that have validated the use of impedance methodology in diagnosing peripheral edema in the SCI population. These findings cannot be explained from the measures obtained in this study and future studies are needed to validate the hydration distribution observed in this SCI cohort.

Our results demonstrate a strong correlation between impedance of the ECV_{Re} and ICV_{Ri} in the TB and legs in both SCI groups and AB controls. These findings confirm those of Baumgartner *et al.*,⁴⁴ which demonstrated that the impedance within a limb is determined by the amount of skeletal muscle and the resultant increased conductivity. Related to this concept, our

prediction equations for predicting leg LTM from ECV_{Re} and ICV_{Ri} impedance was strong due to the higher absolute quantity of skeletal muscle in the legs. Unexpectedly, both ECV_{Re} and ICV_{Ri} impedance values were equally effective in predicting leg LTM. This is most likely due to the fact that the R_i is derived from the R_e and has overlap with the tissue impedance. Other investigators have developed prediction equations of leg LTM using similar feasible methods, such as anthropometrics. Gorgey *et al.*⁴⁵ developed prediction models for TB and segmental FFM using body weight in 63 subjects with motor complete SCI. The author concluded that body weight could significantly predict up to 53, 56, and 25% of TB FFM, trunk FFM, and leg FFM, respectively. Our prediction equations fared well in comparison to other reports, with our findings revealing the ECV_{Re} impedance can determine up to 76, 64, and 54% of the LL LTM and 75, 78, and 54% of the RL LTM in those with paraplegia, tetraplegia, and in AB controls, respectively. Equally predictive was the ICV_{Ri} impedance, which was able to determine up to 76, 57, and 55% of the LL LTM and 85, 52, and 42% of the RL LTM in those with paraplegia or tetraplegia, and in AB controls, respectively. Previous investigations that regressed BIS impedance quotients against FFM or skeletal muscle mass had prediction errors of 3.65 kg using hydrostatic weighing⁴⁴ and 1.16 kg using MRI.⁴⁰ The results from our study are similar to those regressed against MRI with prediction errors for LTM ranging from 0.65–1.34 kg for the ECV_{Re} impedance and from 0.91–1.46 kg for the ICV_{Ri} impedance for all groups and RL and LLs combined (see Fig. 4).

Our findings yielded equations to predict leg LTM in persons with SCI and AB controls from BIS-derived impedance measurements. This is of clinical relevance because physicians and therapists can use BIS methodology to easily monitor LTM in the lower extremities, the region where muscle atrophy is the most prominent and ongoing in those with SCI. However, the implications of this investigation were limited by a relatively small sample size, and this work needs to be expanded to a larger cohort of SCI subjects with participants stratified by completeness of lesion, gender, and ethnicity. Additional limitations include the absence of gold standard measures (i.e. MRI and/or tracer dilution technique) and anthropometric measurements to cross-validate these prediction equations and support their potential use in the clinical setting. Finally, precision studies are needed to assess the test–retest of BIS impedance measures compared with criterion measures and determine the reproducibility of this method in the SCI population.

Conclusion

In summary, the impedance values obtained for TB and limb ICV_{Ri} correspond with the lower LTM values for TB and limb observed in persons with SCI compared with these values in AB controls. However, ECV_{Re} impedance values did not appear to capture peripheral edema in the SCI group, and both ICV_{Ri} and ECV_{Re} impedance values were strong predictors of LTM in the leg. Thus, the findings herein support the utility of BIS as a simple, non-invasive, and portable method that can track these adverse body composition changes that occur soon after acute SCI and accurately assess regional extremity muscle endpoints over the course of rehabilitation. Additional studies are needed to validate the accuracy and precision of the BIS-derived prediction equations against gold standard measures of LTM.

Acknowledgements

The authors thank the James J. Peters VA Medical Center, Bronx, NY, the Department of Veterans Affairs Rehabilitation Research & Development (RR&D) Service, and the Kessler Institute for Rehabilitation, West Orange, NJ for their support. This work was funded by a RR&D National Center of Excellence for the Medical Consequences of Spinal Cord Injury (#B2648C & B4162C).

References

- Spungen AM, Adkins RH, Stewart CA, Wang J, Pierson RN Jr, Waters RL, *et al.* Factors influencing body composition in person with spinal cord injury: a cross-sectional study. *J Appl Physiol* 2003;95(6):2398–407.
- Spungen AM, Wang J, Richard N, Pierson J, Bauman WA. Soft tissue body composition differences in monozygotic twins discordant for spinal cord injury. *J Appl Physiol* 2000;88(4):1310–125.
- Wilmet E, Ismail AA, Heilporn A, Welraeds D, Bergmann P. Longitudinal study of the bone mineral content and of soft tissue composition after spinal cord section. *Paraplegia* 1995;33(11):674–7.
- Castro MJ, Apple DF Jr., Hillegass EA, Dudley GA. Influence of complete spinal cord injury on skeletal muscle cross-sectional area within the first 6 months of injury. *Eur J Appl Physiol* 1999;80(4):373–8.
- Modlesky CM, Bickel CS, Slade JM, Meyer RA, Cureton KJ, Dudley GA. Assessment of skeletal muscle mass in men with spinal cord injury using dual-energy x-ray absorptiometry and magnetic resonance imaging. *J Appl Physiol* 2004;96(2):561–5.
- Sedlock DA, Laventure SJ. Body composition and resting energy expenditure in long term spinal cord injury. *Paraplegia* 1990;28(7):448–54.
- Cardus D, McTaggart WG. Body sodium and potassium in men with spinal cord injury. *Arch Phys Med Rehabil* 1985;66(3):156–9.
- Spungen A, Bauman WA. Carbohydrate and lipid metabolism in chronic spinal cord injury. *J Spinal Cord Med* 2001 Winter;24(4):266–77. Review.
- Gater D, Clasey JL. Body composition assessment in spinal cord injury clinical trials. *Top Spinal Cord Inj Rehabil* 2006;11(3):36–49.
- Kocina P. Body composition of spinal cord injured adults. *Sports Med* 1997;23(1):48–60.
- Hansen RD, Raja C, Aslani A, Smith RC, Allen BJ. Determination of skeletal muscle and fat-free mass by nuclear and dual-energy x-ray absorptiometry methods in men and women aged 51–84 y (1–3). *Am J Clin Nutr* 1999;70(2):228–33.
- Brunton J, Bertolo R, Winthrop A, Atkinson S. Body composition by dual energy x-ray absorptiometry (DEXA) compared to chemical analysis of fat, lean, and bone mass in small piglets. Paper presented at International In Vivo Body Composition Conference 1992; Houston, TX, USA.
- Cardus D, McTaggart W. Total body water and its distribution in men with spinal cord injury. *Arch Phys Med Rehabil* 1984;65(9):509–12.
- Spungen AM, Bauman WA, Wang J, Richard N, Pierson J. The relationship between total body potassium and resting energy expenditure in individuals with paraplegia. *Arch Phys Med Rehabil* 1993;74(9):965–8.
- Kim J, Heshka S, Gallagher D, Kotler DB, Mayer L, Albu J, *et al.* Intermuscular adipose tissue-free skeletal muscle mass: estimation by dual-energy x-ray absorptiometry in adults. *J Appl Physiol* 2004;97(2):655–60.
- Shen W, St-Onge M-P, Pietrobelli A, Wang J, Wang Z, Heshka S, *et al.* Four compartment cellular level body composition model: comparison of two approaches. *Obesity Res* 2005;13(1):58–65.
- Sopher AB, Thornton JC, Wang J, Richard N, Pierson J, Heymsfield SB, *et al.* Measurement of percentage of body fat in 411 children and adolescents: a comparison of dual energy x-ray absorptiometry with a four compartment model. *Pediatrics* 2004;113(5):1285–90.
- Going SB, Massett MP, Hall MC, Bare LA, Root PA, Williams DP, *et al.* Detection of small changes in body composition by dual-energy x-ray absorptiometry. *Am J Clin Nutr* 1993;57(6):845–50.
- Krueger D, Binkley N. Total body DXA: on the cusp of clinical care. *J Clin Densitom* 2012;15(4):387–88.
- Yamada Y, Schoeller DA, Nakamura E, Morimoto T, Kimura M, Oda S. Extracellular water may mask actual muscle atrophy during aging. *J Gerontol A Biol Sci Med Sci* 2010;65(5):510–6.
- Cornish B. Bioimpedance analysis: scientific background. *Lymphat Res Biol* 2006 Spring;4(1):47–50.
- van Marken Lichtenbelt WD, Westerterp KR, Wouters L, Luijckendijk SC. Validation of bioelectrical-impedance measurements as a method to estimate body-water compartments. *Am J Clin Nutr* 1994;60(2):159–66.
- Cornish BH, Ward LC, Thomas BJ, Jebb SA, Elia M. Evaluation of multiple frequency bioelectrical impedance and Cole-Cole analysis for the assessment of body water volumes in healthy humans. *Eur J Clin Nutr* 1996;50(3):159–64.
- Cornish BH, Thomas BJ, Ward LC, Hirst C, Bunce IH. A new technique for the quantification of peripheral edema with application in both unilateral and bilateral cases. *Angiology* 2002;53(1):41–7.
- Cornish BH, Chapman M, Hirst C, Mirolo B, Bunce IH, Ward LC, *et al.* Early diagnosis of lymphedema using multiple frequency bioimpedance. *Lymphology* 2001;34(1):2–11.
- Organ LW, Bradham GB, Gore DT, Lozier SL. Segmental bioelectrical impedance analysis: theory and application of a new technique. *J Appl Physiol* 1994;77(1):98–112.
- KS C, RH C. Dispersion and absorption in dielectrics. *J Chem Phys* 1941;9:341–51.
- Chertow GM, Lazarus JM, Lew NL, Ma L, Lowrie EG. Bioimpedance norms for the hemodialysis population. *Kidney Int* 1997;52(6):1617–21.
- Ellegard LH, Ahlen M, Korner U, Lundholm KG, Plank LD, Bosaeus IG. Bioelectric impedance spectroscopy underestimates fat-free mass compared to dual energy X-ray absorptiometry in incurable cancer patients. *Eur J Clin Nutr* 2009;63(6):794–801.
- Berstad P, Randby A, Seim Ekland G, Ulveland H, Omland T, Almendingen K. Body fat and fat-free mass measured by bioelectric impedance spectroscopy and dual-energy X-ray absorptiometry in obese and non-obese adults. *Br J Nutr* 2011;107(8):1192–200.
- Svantesson U, Zander M, Klingberg S, Slinde F. Body composition in male elite athletes, comparison of bioelectrical impedance

- spectroscopy with dual energy X-ray absorptiometry. *J Negat Results Biomed* 2008;7:1.
- 32 Tengvall M, Ellegard L, Malmros V, Bosaeus N, Lissner L, Bosaeus I. Body composition in the elderly: reference values and bioelectrical impedance spectroscopy to predict total body skeletal muscle mass. *Clin Nutr* 2009;28(1):52–8.
 - 33 Halaska MJ, Mala I, Chod J, Strnad P, Novotny J, Stankusova H, *et al.* A method for the detection of post-operative lymphedema after operation for breast cancer: multifrequency bioelectrical impedance analysis. *J Appl Biomed* 2006;4:179–85.
 - 34 Seem E, Stranden E, Stiris MG. Computed tomography in deep venous thrombosis with limb oedema. *Acta Radiol Diagn (Stockh)* 1985;26(6):727–30.
 - 35 Monreal M, Martorell A, Callejas JM, Valla R, Llamazares JF, Lafoz E, *et al.* Venographic assessment of deep vein thrombosis and risk of developing post-thrombotic syndrome: a prospective study. *J Intern Med* 1993;233(3):233–8.
 - 36 Cole KS, Cole RH. Dispersion and absorption in dielectrics. *J Chem Phys* 1941;9:341–51.
 - 37 Jaffrin MY, Maasrani M, Le Gourrier A, Boudailliez B. Extra- and intracellular volume monitoring by impedance during haemodialysis using Cole-Cole extrapolation. *Med Biol Eng Comput* 1997; 35(3):266–70.
 - 38 Buchholz AC, McGillivray CF, Pencharz PB. The use of bioelectric impedance analysis to measure fluid compartments in subjects with chronic paraplegia. *Arch Phys Med Rehabil* 2003;84(6):854–61.
 - 39 Cornish BH, Jacobs A, Thomas BJ, Ward LC. Optimizing electrode sites for segmental bioimpedance measurements. *Physiol Meas* 1999;20(3):241–50.
 - 40 Kaysen GA, Zhu F, Sarkar S, Heymsfield SB, Wong J, Kaitwatcharachai C, *et al.* Estimation of total-body and limb muscle mass in hemodialysis patients by using multifrequency bioimpedance spectroscopy. *Am J Clin Nutr* 2005; 82(5):988–95.
 - 41 Jaffrin MY, Morel H. Body fluid volumes measurements by impedance: a review of bioimpedance spectroscopy (BIS) and bioimpedance analysis (BIA) methods. *Med Eng Phys* 2008;30(10): 1257–69.
 - 42 Zhu F, Kuhlmann MK, Kaysen GA, Sarkar S, Kaitwatcharachai C, Khilnani R, *et al.* Segment-specific resistivity improves body fluid volume estimates from bioimpedance spectroscopy in hemodialysis patients. *J Appl Physiol* 2006;100(2):717–24.
 - 43 Halaska MJ, Novackova M, Mala I, Pluta M, Chmel R, Stankusova H, *et al.* A prospective study of postoperative lymphedema after surgery for cervical cancer. *Int J Gynecol Cancer* 2010; 20(5):900–4.
 - 44 Baumgartner RN, Chumlea WC, Roche AF. Estimation of body composition from bioelectric impedance of body segments. *Am J Clin Nutr* 1989;50(2):221–6.
 - 45 Gorgey AS, Dolbow DR, Gater DR, Jr. A model of prediction and cross-validation of fat-free mass in men with motor complete spinal cord injury. *Arch Phys Med Rehabil* 2012;93(7):1240–5.