Extracellular Water May Mask Actual Muscle Atrophy During Aging (Final draft (Post-print) version)

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Abstract

  Background. Skeletal muscle tissue holds a large volume of water partitioned into extracellular water (ECW) and intracellular water (ICW) fractions. As the ECW may not be related to muscle strength directly, we hypothesized that excluding ECW from muscle volume would strengthen the correlation with muscle strength.

  Methods. A total of 119 healthy men aged 20–88 years old participated in this study. Knee isometric extension strength, vertical jump, and standing from a chair were measured as indices of muscle strength and power in the lower extremities. The regional lean volume (LV), total water (TW), ICW, and ECW in the lower leg were estimated by anthropometry (skinfold and circumference measurements) and segmental multifrequency bioelectrical impedance spectroscopy (S-BIS). Then, we calculated the ECW/TW and ICW/TW ratios.

  Results. Although ICW and the LV index decreased significantly with age (p < .001), no significant changes in ECW were observed (p = .134). Consequently, the ECW/TW ratio increased significantly (p < .001) with age (young adult, 27.0 ± 2.9%; elderly, 34.3 ± 4.9%; advanced elderly, 37.2 ± 7.0%). Adjusting for this by including the ICW/TW ratio in our models significantly improved the correlation between the LV index and strength-related measurements and correlated with strength-related measurements independently of the LV index (p < .001).

  Conclusions. The ECW/TW ratio increases in the lower leg with age. The results suggest that the expansion of ECW relative to ICW and the LV masked actual muscle cell atrophy with aging.

  In the elderly, loss of muscle strength and power in the lower extremities is associated with functional limitations in the activities of daily life, such as walking, stair climbing, and chair standing. Muscle atrophy with aging (sarcopenia), the progressive loss of muscle mass, has been implicated as a primary factor in the loss of muscle

strength in older adults[1]. The maintenance of muscle volume (MV), therefore, seems to be critical in maintaining the activities of daily life in the elderly. Several previous studies indicated that MV is a strong independent predictor of physical disability or mortality[2] [3] [4]. Other studies, however, showed that MV measured by imaging methods, such as magnetic resonance imaging (MRI) and computed tomography (CT), or muscle mass measured by dual x-ray absorptiometry (DXA) had poor associations with physical function and mortality[5] [6] [7].

Skeletal muscle tissue holds a large amount of water, which is partitioned into intracellular water (ICW) and extracellular water (ECW; the sum of interstitial fluid and blood plasma) fractions. Therefore, skeletal muscle contains not only muscle cell mass but also ECW, which may not be related to muscle strength[8]. Therefore, we hypothesized that MV is correlated more strongly with muscle strength if the ECW volume is excluded from the MV. To our knowledge, however, due to the difficulty assessing regional ECW and ICW, previous studies have not examined ECW and muscle strength. Recently, estimating the regional muscle mass, ICW, and ECW became possible using segmental multifrequency bioelectrical impedance spectroscopy (S-BIS)[9] [10] [11] (see detail comments for S-BIS on Supplementary Material [S1]). The aims of this study were to examine the changes in ICW and ECW in the lower leg with age using S-BIS and to determine whether MV correlates more strongly with muscle strength when the

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ECW volume is excluded from the MV.

Methods

Participants

A total of 2,844 Japanese adults aged 18–97 years were recruited through the announcements in neighborhood associations, local community centers, and health promotion centers; local newspapers in Kyoto and neighboring cities; and to those who received a free routine physical fitness test from 2002 through 2008 at Kyoto Prefecture University of Medicine. From those participants, a total of 119 healthy male volunteers consisting of 50 young (20–31 years) and 69 independently living community-dwelling elderly (60–88 years) adults were randomly selected as participants for this study after providing written informed consent. The elderly participants were divided into two categories, elderly (60–74 years) and advanced elderly (≥ 75 years), based on the Japanese medical insurance system. The eligibility criteria were as follows: the ability to walk more than 400 m, climb stairs, and take a bath without assistance; the absence of dementia and the capability to understand the informed consent procedure; and no replacement arthroplasty or artificial pacemaker, pathological edema or lymphedema, definite kidney or digestive disease, hormone replacement therapy, any symptoms of dehydration or over hydration, or any acute or chronic diseases that influenced body composition or hydration status. The study protocol was approved by the ethics committee of Kyoto Prefectural University of Medicine. Body mass of each participant was measured to the nearest 0.1 kg, with the participants dressed in light clothing. Barefoot standing height was measured to the nearest 0.1 cm using a wall-mounted stadiometer.
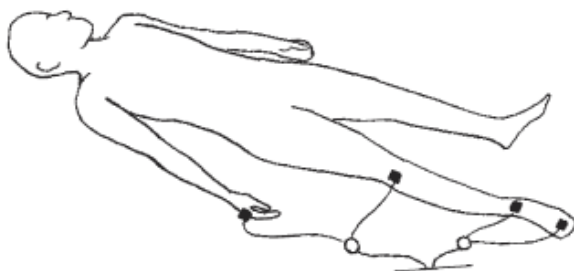

Figure 1. The setup for the measurement of segmental multifrequency bioelectrical impedance spectroscopy in the lower leg.

Impedance Spectroscopy

Bioelectrical impedance was measured using a logarithmic distribution of 140 frequencies ranging from 2.5 to 350 kHz (MLT-30; Sekisui Medical, Tokyo, Japan)

using disposable electrodes. MLT-30 is the successor to the MLT-100, which was validated against DXA to measure fat-free mass in previous studies that included elderly participants[12] [13]. The impedance of the right lower leg was measured between 5 and 10 minutes after the participant had laid down to avoid the immediate (1–2 minutes) shifts in body fluids from the extremities to the thorax when changing to a supine position but not the slow phase of the shifts that continues for over 3 hours. Two injecting electrodes were placed on the dorsal surfaces of the hand and foot on the right side of the body (Figure 1). Sensing electrodes were placed on the right articular cleft between the femoral and tibial condyles and the anterior surface of the ankle between the protruding portions of the tibial and fibular bones for lower leg measurements[14]. Participants were asked to abstain from strenuous exercise and alcohol intake for 24 hours and from eating a meal or drinking more than 0.5 L of water for 4 hours preceding the experiments. The room temperature was kept at about 22°C. Acquisition, storage, and analysis of data were performed with the software supplied with the bioimpedance analyzer. The instrument was calibrated against a reference resistance and checked before measurements. The resistance of the extracellular water compartment (RECW) and that of the total water compartment (RTW) for the lower leg was determined by extrapolation after fitting the spectrum of bioimpedance data to the Cole–Cole model using the supplied software. The resistance of the intracellular water compartment (RICW) was calculated as $1/[(1/RTW) - (1/RECW)]$. The segmental ECW and ICW in the lower leg were calculated using the following equations (10,11): ECW = rECW × L/RECW and ICW = rICW × L/RICW, where r represents factors for extracellular (rECW = 47 Wcm) and intracellular (rICW = 273.9 Wcm) resistivity, respectively, L is segmental length, RECW is segmental extracellular resistance, and RICW is segmental intracellular resistance. The volume of TW was calculated as the sum of ECW and ICW. The between-day coefficients of variation (CV) for repeated ECW, ICW, and TW measures in our laboratory were 2.0%, 3.4%, and 2.4%, respectively, and the day-to-day intra-class correlation coefficients (ICC[3,1]) were 0.969, 0.896, and 0.944.

Figure 1. The setup for the measurement of segmental multifrequency bioelectrical impedance spectroscopy in the lower leg.

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Anthropometric Measurements

Right calf skinfold thickness was measured in the standing position using a calibrated Eiken skinfold caliper (TK-11258; National Institute of Health and Nutrition, Meikosha, Japan) with a constant pressure of 10 g/mm2[15]. Right calf maximum circumference was measured by an investigator with appropriate training in the procedure using a standard measuring tape. Measurements were repeated twice, and the means were used in the data analysis. If the values disagreed by more than 5%, a new series of measurements was obtained. The fat and lean (muscle + bone) cross-sectional area (CSA) was estimated from calf skinfold (S) and circumference (C) using previously developed equations: fat = [S·C/2]− [p·S2/4] and lean = (C − p·S)2/4p [16]. The physiological CSA of muscle is linearly correlated with its volume in vivo[17], and maximal muscle torque is more closely related to MV than anatomical CSA [18]. Therefore, we also calculated

the lean volume (LV) index as the lean CSA multiplied by segmental length to match the volume dimension.

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Table 1. Physical Characteristics, Strength-Related Characteristics, and Regional Body Composition in Lower Leg of the Participants ($n = 119$)

	Young ($n = 50$), $M \pm SD$ (minimum, maximum)	Elderly ($n = 44$), $M \pm SD$ (minimum, maximum)	Advanced Elderly ($n = 25$), $M \pm SD$ (minimum, maximum)
Physical characteristics			
Age (y)	22 ± 2 (20, 31)	69 ± 3 (62, 73)***	79 ± 4 (75, 88)***,†††
Height (cm)	171.7 ± 5.3 (158.0, 183.0)	165.6 ± 6.3 (150.3, 182.6)***	159.8 ± 6.4 (146.7, 175.2)***,†††
Weight (kg)	65.7 ± 8.8 (50.0, 99.5)	64.1 ± 9.0 (42.9, 84.1)	56.3 ± 11.0 (38.1, 80.5)***,†
BMI (kg/m2)	22.3 ± 3.4 (17.3, 39.8)	23.3 ± 2.8 (17.4, 29.7)	22.0 ± 3.8 (16.1, 33.1)
Strength-related characteristics			
Vertical jump index (m·kg)	32.5 ± 5.4 (17.1, 44.2)	18.8 ± 4.9 (6.9, 28.2)***	14.5 ± 4.2 (6.5, 23.3)***,†
Muscle isometric strength (kg)	59.2 ± 8.4 (40.0, 79.0)	32.6 ± 8.6 (15.8, 48.8)***	27.5 ± 7.4 (9.2, 41.3)***,†
Chair stand (frequency/30 s)	38.6 ± 4.0 (30.0, 48.0)	23.7 ± 4.9 (16.0, 33.0)***	21.2 ± 6.5 (12.0, 39.0)***
Regional body composition in lower leg			
Fat CSA (cm2)	13.1 ± 9.1 (1.7, 44.2)	18.1 ± 8.9 (2.9, 41.3)*	17.1 ± 9.3 (3.4, 43.1)
Lean CSA (cm2)	98.4 ± 19.5 (63.7, 162.2)	86.3 ± 13.5 (62.6, 120.3)**	74.7 ± 14.1 (54.5, 118.2)***,†
LV index (m3)	3.78 ± 0.76 (2.33, 6.29)	3.17 ± 0.58 (2.22, 4.67)***	2.65 ± 0.52 (1.86, 4.08)***,††

Notes: Elderly: 60–74 years old; advanced elderly: 75–88 years old. Fat and Lean CSA was estimated by skinfold and circumference measurements. LV index was calculated as Lean CSA and segment length. BMI = body mass index; CSA = cross-sectional area; LV = lean volume.

Significantly lower than young adults (*p < .5, **p < .01, *** p < .001).

Significantly lower than younger elderly adults (†p < .05,††p < .01,†††p < 0.001).

Muscle Strength and Power in the Lower Extremities

The maximal muscle isometric strength (MS) was measured in the sitting position on a custom-made dynamometer chair at a knee angle of 90°, as described elsewhere[19]. The ankle was attached to a strain-gauge system via belts around the pelvis and shoulders. After familiarization with the test, participants were encouraged to produce maximal knee extension force. Three maximal efforts, separated by a 1-minute rest period, were conducted, and the highest recorded value was accepted as the result. Maximal leg power was assessed by a vertical jump test, as described elsewhere[20] [21],

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performed on a specially designed measuring scale (Jump Meter MD, TKK5106; Takei Scientific Instruments, Niigata, Japan)[22]. Each participant performed two maximal squat jumps. A trained investigator supported the participant after landing if they were likely to lose balance, although most did not lose balance on landing. The participants were given several familiarization trials before the test. The vertical jump index (VJI) was calculated using the following equation: VJI (m/kg) = jump height × body weight[23]. Chair stand (sit-to-stand) frequency was measured as participants stood up and sat down as quickly as possible on a firm, padded armless chair with a seat that was 0.43 m from the ground. The back of the chair was supported against a wall, and participants were instructed to fold their arms across their chest during the test. The number of repetitions over a period of 30 seconds was recorded as previously described[24]. The between-day CVs for repeated MS, VJI, and chair stand frequency measures were 11.8%, 11.5%, and 8.9%, respectively, and the intra-class correlation coefficients (ICC[3,1]) between two tests separated by approximately a month were 0.839, 0.856, and 0.838.

Data Analysis

Results are presented as the means ± SD. Group differences were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. Pearson's correlation coefficients were calculated between regional body composition and strength-related variables (VJI, MS, and chair stand). Partial correlation coefficients were also calculated with fat CSA as a covariate. The ICW/TW ratio was calculated and multiplied by lean CSA and the LV index to eliminate the relative expansion of ECW. Pearson's correlation coefficients were compared statistically using the methods described by Meng and colleagues[25]. Multiple linear regression analyses were applied to examine whether the ratio of ICW/TW significantly improved the relationships between the LV index and strength-related variables. All analyses were performed using SPSS 12.0 for Windows. For all analyses, an alpha of .05 was used to denote statistical significance. The data were judged to be distributed normally according to the

activity in 57 to 70-year-old women. Eur J Appl Physiol. 2006;96:10–16.

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Kolmogorov–Smirnov test (p > .05).

We compared our results with those of an epidemiological study (InCHIANTI)[26] that used quantitative CT to measure the calf muscle CSA of the participants aged 20–102 years. We obtained data for young men aged 20–29 years, elderly men aged 65–74 years, and advanced elderly men aged 75–85 years from Table 2 in the literature[27] and calculated the percent decrease from young adults to elderly and to advanced elderly.

Results

Table 1 shows the physical characteristics, strength-related characteristics, and regional body composition in the lower leg of the participants. Six young (12.0%), nine elderly (20.5%), and three advanced elderly adults (12.0%) had body mass indices (BMIs) >25 kg/m2, and one young and one advanced elderly had BMIs of >30 kg/m2. The height, weight, and BMI in the present study were not different from those described by the National Health and Nutrition Survey of Japan 2007. Further description of demographic status is shown in the Supplementary Material (S2) and Table S1. The physical function, demographic, and health status for each group were very similar to those of healthy participants in previous studies[28] [29].

Using one-way ANOVA, significant decreases in height and weight, but not in BMI, were observed with age. With the exception of fat CSA, all of the other strength-related variables and regional body composition variables decreased significantly as the age of group advances.

The water distribution in the lower leg in each age group is shown in Figure 2. Estimated ICW decreased significantly (p < .001) with aging (young 1,126 ± 222 mL; elderly, 784 ± 190 mL; and advanced elderly, 653 ± 233 mL), but no significant (p = .134) changes in estimated ECW were observed with aging (young 414 ± 72 mL; elderly, 402 ± 74 mL; and advanced elderly, 374 ± 101 mL; Figure 2A). Therefore, the ICW/TW ratio decreased significantly (p < .001) with aging (young 73.0 ± 2.9%; elderly, 65.7 ± 4.9%; and advanced elderly, 62.8 ± 7.0%), and the ECW/TW ratio increased significantly with age (young 27.0 ± 2.9%; elderly, 34.3 ± 4.9%; and advanced elderly, 37.2 ± 7.0%; Figure 2B).

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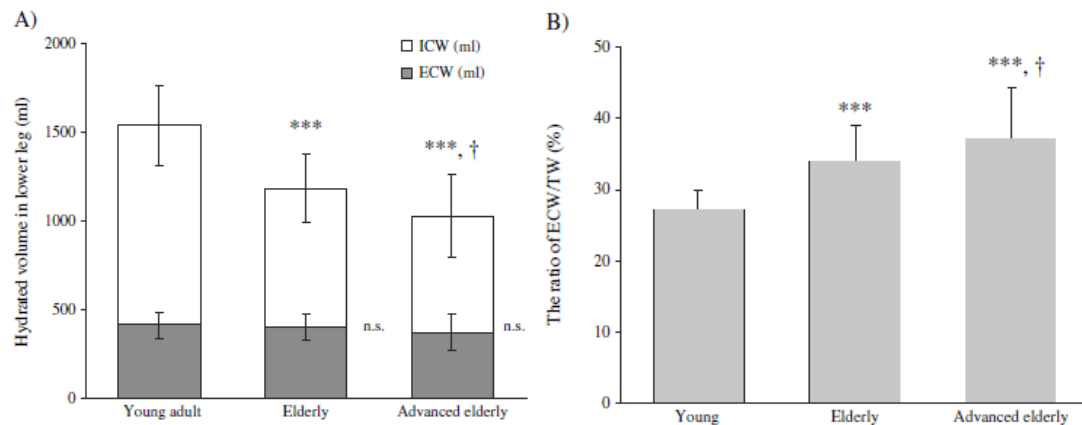
Figure 2. Water distribution in the lower leg estimated by S-BIS (mean ± SD). (A) ***significantly lower intracellular water (ICW) than young adult (p < .001); †significantly lower ICW than elderly adults (p < .05). No significant main effect was observed in extracellular water (ECW; p = .134). The total bar shows the sum of ICW and ECW (total water [TW]). (B) The ECW/TW ratio increased significantly (p < .001) with aging.

The ICW/TW ratio was significantly correlated with VJI (r = .640, p < .001), MS (r = .600, p < .001), and chair stand (r = .583, p < .001) in the 119 men enrolled in this study. Furthermore, partial correlations were calculated after adjusting for fat CSA. The ICW/TW ratio was still significantly correlated with VJI (r = .628, p < .001), MS (r = .582, p < .001), and chair stand (r = .567, p < .001).

Table 2 shows the correlation coefficients between strength-related variables and regional body composition for the entire study population. TW, lean CSA, and the LV index were significantly correlated with VJI, MS, and chair stand. All of these correlations improved significantly when the ICW/TW ratio was multiplied by lean CSA and the LV index (p < .01). In multiple regression analysis (Table 3), the ratio of ICW/TW was a significant predictive variable independently of the LV index for estimating strength-related variables (p < .001). The correlation coefficient between LV and ICW/TW was .484, and the variance inflation factor was 1.306 and did not reach the level for significant (>5.0) multicollinearity.

Table 4 shows the percent decreases in calf muscle area, lean CSA, and ECW-eliminated lean CSA (lean CSA × ICW/TW) from young adults to the elderly. The percent decrease of the lean CSA in our study was almost the same as the percent decrease in calf muscle area in the study by Lauretani and colleagues[30]. The percent decrease in the ECW-eliminated lean CSA in our study was higher than that in the lean

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CSA or calf muscle area.

Table 2. Correlation Coefficients Between Strength-Related Variables and Regional Body Composition in the Lower Leg

	Water Measurements		Lean CSA		LV Index	
	TW	ICW	Lean CSA	Lean CSA × I/T	LV Index	LV Index × I/T
Vertical jump index	0.723	0.766***	0.661	0.741***	0.744	0.803***
Muscle isometric strength	0.663	0.703**	0.560	0.648***	0.633	0.701***
Chair stand	0.516	0.583***	0.414	0.528***	0.457	0.555***

Notes: ICW = intracellular water; I/T = ratio of intracellular water to total water; Lean CSA = lean cross-sectional area; LV index = lean volume index; TW = total water.

All correlation coefficients were significant (p < .001).

Significantly higher than the correlation coefficient in its left column (**p < .01, ***p < .001).

Table 3. Multiple Linear Regression Analysis for Predicting Muscle Function in the Lower Extremities

		Coefficients			
		Standardized	Standardized		
Dependent Variables	Independent Variables	b	B	SEE	p
Vertical jump index (m/kg)	LV index (m3)	.567	6.688	0.735	<.001
	ICW/TW (%)	.365	0.528	0.090	<.001
	Constant		−34.471	5.417	<.001
$R2 = .655$					
Muscle isometric strength (kg)	LV index (m3)	.447	9.419	1.560	<.001
	ICW/TW (%)	.383	0.990	0.191	<.001
	Constant		−55.976	11.506	<.001
$R2 = .513$					
Chair stand (frequency/30 s)	LV index (m3)	.229	2.729	0.996	<.01
	ICW/TW (%)	.472	0.689	0.122	<.001
	Constant		−26.571	7.344	<.001
$R2 = .380$					

Note: ICW/TW = ratio of intracellular water to total water in the lower leg; LV index = lean volume index; SEE = standard error of estimate

Discussion

At the whole-body level, although some studies have reported no age-related

differences[31], many others have indicated age-related increases in the ECW/ICW ratio[32] [33] [34]. The human body consists of various types of tissue, and we therefore hypothesized that the measurement of segmental water distribution would improve the relationship between muscle size and strength. Recent studies have indicated that S-BIS can be used to estimate the segmental ECW and ICW[35] [36] [37]. The traditional whole-body impedance method is based on the assumption that the hydrated portion of the body is cylindrical in shape. Although this assumption breaks down at the whole-body level[38], individual segments more closely resemble cylinders. Therefore, S-BIS can be used to estimate the segmental ICW and   ECW directly using only the resistance and segment length[39] [40] [41] [42]. Moreover, previous studies have indicated that S-BIS succeeds in monitoring the fluid changes in the segmental limbs during position changes, hemodialysis, exercise, and 72-hour head-down bed rest[43] [44] [45] [46].

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42 Bartolini ME, Wilson K, Raja M, et al. Dual X-ray absorptiometry model for characterizing water in the human forearm using multiple frequency bioimpedance analysis. Can J Physiol Pharmacol. 2006;84:181–193.

43 Bartok C, Schoeller DA. Estimation of segmental muscle volume by bioelectrical impedance spectroscopy. J Appl Physiol. 2004;96:161–166.

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For further discussion, regarding the scientific consensus related to bioelectrical impedance and the differences between whole-body methods and S-BIS, see the Supplementary Materials (S1).

We observed a relative increase in ECW in the lower leg in successively older cohorts when estimated using S-BIS (Figure 2). No previous reports have described age-related changes in segmental water distribution in vivo. Tanaka and colleagues[47] reported that people with cervical spinal cord injury (quadriplegia) had a higher ECW/ICW ratio in the limbs than noninjured individuals and that the relative expansion of ECW was related to the decrease in muscle mass. The retention of water in the extracellular space may occur concurrently with the loss of muscle contractile tissue.

Although we did not measure the MV anatomically, if this MV of young adults is 1,300 cm3 [48] and the ECW is 400 cm3, the ECW-eliminated MV is 900 cm3. If this MV of the elderly adults were 1,100 cm3 and the ECW is still 400 cm3, the ECW-eliminated MV were 700 cm3 and age-related muscle atrophy were expressed using the percent decrease from the value of young adults, then the percent decrease in MV is (200/1,300) = ~15%, whereas the percent decrease in ECW-eliminated MV is (200/900) = ~22%. Therefore, if we did not eliminate ECW from the total MV, the rate of actual MV decline would be underestimated. As shown in Table 4, the percent decrease in the lean CSA in our study was similar to the percent decrease in calf muscle area in the study by Lauretani and colleagues[49]. However, the percent decrease in the ECW-eliminated lean CSA in our study was higher than that in the lean CSA or calf muscle area. These results support the idea that relative expansion of the ECW would mask the actual muscle cell atrophy in the previous studies.

stage 5 chronic-kidney-disease patients after haemodialysis. Nephrology. 2008;13:109–115.

47 Tanaka T, Yamada Y, Ohata K, Yabe K. Intra- and extra-cellular water distribution in the limbs after cervical spinal cord injury [in Japanese with English abstract]. J Exercise Sports Physiol. 2008;15:11–17.

48 Miyatani M, Kanehisa H, Masuo Y, Ito M, Fukunaga T. Validity of estimating limb muscle volume by bioelectrical impedance. J Appl Physiol. 2001;91:386–394.

49 Lauretani F, Russo CR, Bandinelli S, et al. Age-associated changes in skeletal muscles and their effect on mobility: an operational diagnosis of sarcopenia. J Appl Physiol. 2003;95:1851–1860.

Table 4. Change in the Segmental Composition of the Lower Leg During Adulthood

	Calf Muscle Area* (cm2)	Lean CSA (cm2)	Lean CSA × I/T (cm2)
Young adults	83.3	98.4	71.8
Elderly adults	72.2	86.3	56.7
Advanced elderly adults	64.8	74.7	46.9
Present decrease from young to elderly adults	13.3	12.3	21.1
Present decrease from young to advanced elderly adults	22.2	24.1	

Notes: I/T = ratio of intracellular water to total water; Lean CSA = lean cross-sectional area measured at the largest outer calf diameter.

* The values from the literature of Lauretani colleagues (6) InCHIANTI study, derived from quantitative computerized tomography scans obtained at 66% of the tibia length, proximal to the anatomic marker that is the region with the largest outer calf diameter.

Several recent studies have indicated changes in muscle composition (ie, intramuscular adipose tissue and muscle density) with aging in vivo. Kent-Braun and colleagues[50] reported that the intramuscular fat in the tibialis anterior muscle as estimated by MRI increased with aging, and Galban and colleagues[51] reported that the diffusion tensor–magnetic resonance imaging (DT-MRI) signal intensity decreased significantly in the muscles of the lower leg with aging. Other studies have found significant decreases in the muscle density estimated by CT with aging related to lower extremity function[52] [53]. Although the relationships among ECW, intramuscular fat, muscle density of CT, and DT-MRI remain unknown, the previous and present results suggest that quantification of the intramuscular noncontractile tissue is critical for accurate assessment of muscle quality. Furthermore, S-BIS is the least costly of these methods.

The present results suggest that the relative expansion of ECW with aging may be

50 Kent-Braun JA, Ng AV, Young K. Skeletal muscle contractile and noncontractile components in young and older women and men. J Appl Physiol. 2000;88:662–668.

51 Galban CJ, Maderwald S, Stock F, Ladd ME. Age-related changes in skeletal muscle as detected by diffusion tensor magnetic resonance imaging. J Gerontol ABiol Sci Med Sci. 2007;62:453–458.

52 Sipila S, Koskinen SOA, Taaffe DR, et al. Determinants of lower-body muscle power in early postmenopausal women. J Am Geriatr Soc. 2004;52:939–944.

53 Visser M, Kritchevsky SB, Goodpaster BH, et al. Leg muscle mass and composition in relation to lower extremity performance in men and women aged 70 to 79: the Health, Aging and Body Composition Study. J Am Geriatr Soc. 2002;50:897–904.

one reason for the age-related decrease in specific force. Metter and colleagues[54] noted that the relationship between specific force and age is dependent on how muscle mass is estimated. Even the most reliable methods, such as CT or MRI, have several assumptions and limitations. Bartok and Schoeller[55] reported that the decreasing MV estimated by MRI in the lower leg after short-term head-down bed rest mainly reflected the decreasing ECW. Berg and colleagues[56] also demonstrated that calf muscle area estimated by CT significantly decreased by 5.5% and the radiological density of muscle showed a simultaneous increase of 4.8% during the initial 120 minutes of bed rest. MV estimated by CT decreased significantly after changing position from standing to lying down. Therefore, the MV estimated using these imaging methods includes not only the muscle cell volume but also ECW, which is not related to muscle strength[57]. Simultaneous measurement using S-BIS and imaging methods should be effective for assessing actual muscle tissue changes.

Although the proportion is small, fat tissue also holds water. We examined partial correlations with the fat CSA as a covariate. The partial correlation coefficients were not different from the single correlation coefficients. Therefore, the differences in subcutaneous fat CSA did not affect the results obtained in the present study. We did not measure muscle mass CSA but instead measured lean (muscle + bone) CSA; thus, quantifying the effects of bone mass was not possible in this study. However, the correlation coefficients between strength-related variables and the LV index were almost the same as those between strength-related variables and TW, which is mainly associated with the MV. Therefore, although further investigations are required, this suggests that the bone tissue mass did not influence our results.

A limitation of our study is that the vertical jump test and chair stand test rely not only on the muscles of the lower legs but also on the muscles in the thighs and hips, which were not measured using bioelectrical impedance spectroscopy. If we could measure the thigh segments as well as the lower legs by S-BIS or measure the muscle power of each muscle groups separately, our results might be reinforced. We also measured S-BIS for the thigh segment, but the reactance showed poor reproducibility at

54 Metter EJ, Lynch N, Conwit R, Lindle R, Tobin J, Hurley B. Muscle quality and age: cross-sectional and longitudinal comparisons. J Gerontol ABiol Sci Med Sci. 1999;54:B207–B218.

55 Bartok C, Schoeller DA. Estimation of segmental muscle volume by bioelectrical impedance spectroscopy. J Appl Physiol. 2004;96:161–166.

56 Berg HE, Tedner B, Tesch PA. Changes in lower limb muscle cross-sectional area and tissue fluid volume after transition from standing to supine. Acta Physiol Scand. 1993;148:379–385.

57 Chamney PW, Wabel P, Moissl UM, et al. A whole-body model to distinguish excess fluid from the hydration of major body tissues. Am J Clin Nutr. 2007;85:80–89.

both low and very high frequencies, especially in the advanced elderly (data not shown). This poor reproducibility may be attributable to the difficulty in distinguishing between trunk and thigh segments due to the noncylindrical shape of the pelvis or instrumental limitations for these values of reactance. This poor reproducibility markedly affected Cole–Cole modeling in the thigh segment because this segment presented a smaller resistance than the lower leg due to its large CSA. Improvements in the instrument are needed. The measurement of the size and strength of specific muscle groups is important for discussing the effect of the relative expansion of ECW on the age-related change in a specific force. Other potential limitations of this study were that the recruitment method used was not entirely random and that the study sample was small. We enrolled only generally healthy individuals. Further studies are required to provide standardized values for each generation.

In conclusion, our study found that the ICW/TW ratio decreased and the ECW/TW ratio increased significantly in the lower leg with aging. The ICW/TW ratio was significantly correlated with muscle strength and power in the lower extremities. This indicated that the expansion of ECW relative to ICW and the LV masks actual muscle cell atrophy during aging. S-BIS is an affordable, noninvasive, easy-to-operate, and fast method and would complement anthropometric or other measurements when assessing sarcopenia in the limbs. The findings reported here suggest that measurement of ECW and ICW using S-BIS may fill a gap in the measurement of muscle atrophy with aging.