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Original Article

The Extracellular to Intracellular Water Ratio in Upper Legs is Negatively Associated With Skeletal Muscle Strength and Gait Speed in Older People

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Abstract

Skeletal muscles contain a large volume of water that is classified into intracellular (ICW) and extracellular (ECW) water fractions. Nuclear magnetic resonance-based biomarkers suggest that increased water T2 heterogeneities, as well as elevated water T2 relaxation in the quadriceps occurs in the elderly when compared with young adults. However, nuclear magnetic resonance is difficult to apply to a large-scale study or a clinical setting for sarcopenia and frailty screening. Segmental bioelectrical impedance spectroscopy is a unique tool used to assess the segmental ratio of ECW/ICW in the limbs. We evaluated 405 community-living people aged between 65 and 90 years. ECW and ICW in the upper legs were assessed by segmental bioelectrical impedance spectroscopy. Isometric knee extension strength, gait speed, and skeletal muscle mass were measured. Thigh ECW/ICW was negatively correlated with knee extension strength and gait speed (r = −.617 and −.431, respectively, $p < .001$) and increased with age ($p < .001$). Thigh ECW/ICW was a significant predictor of knee extension strength and gait speed independent of age, sex, body mass index, and skeletal muscle mass. Relative expansion of ECW against ICW in the thigh muscles is a factor in decreased muscle quality and a biomarker of muscle aging.

Keywords: Muscle strength—Muscle quality—Water fraction—Bioelectrical impedance spectroscopy

Skeletal muscle is the largest organ in the body and maintenance of its quality and mass is important for the prevention of age-related decline in metabolic function and physical frailty. It is well known that muscle strength or power decreases more rapidly with age when compared with skeletal muscle mass (SM), both cross-sectionally and longitudinally (1–4). This discrepancy is often referred as decreasing "muscle quality" and is calculated as the ratio of muscle strength to SM or as muscle strength adjusted by SM as a covariate (5–9). The qualitative change observed in skeletal muscle appears to be related to numerous morphological and/or neuromuscular aspects, such as fat infiltration of muscles (10–12), decreased lateral force transmission (13,14), change of pennate angle (15), fiber type change with increased co-expression of mixed myosin heavy chain characteristics (16,17), decreased sensitivity to calcium ions (18), and attenuated excitability of motoneurons (19,20).

Skeletal muscle holds a large volume of water, accounting for up to three-quarters of muscle mass (21). T2 relaxation measured by nuclear magnetic resonance is a biomarker of water in skeletal muscles, and Azzabou et al. (10) recently indicated that water T2 mean values and its heterogeneity indices, as well as fat fractions,

were significantly higher in the elderly when compared with young adults. That study, as well as previously conducted studies (22,23), have suggested that measurement of the water characteristics of skeletal muscle is important in order to clarify the effect of water distribution on muscle quality in skeletal muscle. However, nuclear magnetic resonance is difficult to apply in a large-scale study or indeed in a clinical setting for sarcopenia and frailty screening. Water in the skeletal muscle is distributed to both extra- and intracellular compartments (ECW and ICW, respectively) partitioned by muscle cell membranes (24). Segmental bioelectrical impedance spectroscopy (S-BIS) is a unique tool used to noninvasively assess the segmental ratio of ECW/ICW in the body (25). We previously reported that a relative expansion of ECW against ICW is observed in skeletal muscles with aging (24,26,27) The aim of the present study was to examine the relationship between ECW/ICW in the upper leg segments, as well as muscle strength or gait speed in the elderly.

Methods

Subjects

A total of 405 community-dwelling, healthy, elderly subjects (between 65 and 90 years of age) were enrolled in this study. These were the same subjects who were in our previous study (28), in which we examined the advantages of BIS over traditional single-frequency bioelectrical impedance analysis (BIA) for the assessment of appendicular skeletal muscles, as detailed in that publication. Inclusion criteria were as follows: (a) reported ability to walk more than 10 m with or without a cane, (b) ability to provide informed consent with no indication of dementia, (c) no history of any joint arthroplasty or current use of an artificial pacemaker, (d) no current medication for edema or lymphedema, and (e) absence of any definitive kidney, digestive, or other acute diseases. Height and weight were measured with the subjects dressed in light clothing and without shoes. This study protocol was approved by the ethics committee of Kyoto Prefectural University of Medicine.

Bioelectrical Impedance Spectroscopy

Bioelectrical impedance was measured using a logarithmic distribution of 256 frequencies ranging from 4 to 1,000 kHz (SFB7, ImpediMed, Pinkenba, QLD, Australia) using disposable tab-type monitoring electrodes (2 cm × 2 cm, Red Dot, 3M, St. Paul, MN). Before the test, the system was checked against a series of precision resistors provided by the manufacturer. Impedance of the upper leg segments was measured by placing an injecting electrode on each side of the body on the dorsal surface of the feet, proximal to the second and third metatarsal-phalangeal joints, while a sensing electrode was placed on each side of the body on the articular cleft between the femoral and tibial condyles. Segment length (L) was calculated as twice the length of the right upper leg when measured from the articular cleft between the femoral and tibial condyles to the greater trochanter of the femur. Resistance of zero (R_0) and infinity (R_∞) frequencies was determined by extrapolation after fitting the spectrum of bioimpedance data to the Cole–Cole model using specialized software (ImpediMed, Pinkenba, QLD, Australia). The analysis parameters included minimum frequency, 5 kHz; maximum frequency, 500 kHz; and rejection limit, 0%. For S-BIS, the R_{ICW} was calculated using $1/[(1/R_\infty) - (1/R_0)]$ (28). Thigh ECW was calculated as ECW = $\rho_{ECW} \times L^2/R_0$, where ρ_{ECW} represents factors for extracellular resistivity (47 Ωcm). Thigh ICW was calculated as ICW = $\rho_{ICW} \times L^2/R_{ICW}$, where ρ_{ICW} represents factors for intracellular resistivity (237.9 Ωcm) (29,30). Therefore, the ratio of ECW/ICW was calculated as ECW/ICW = $[\rho_{ECW} \times L^2/R_0]/[\rho_{ICW} \times L^2/R_{ICW}] = 0.197 \times R_{ICW}/R_0$. The details of S-BIS have previously been described (25,29,31).

Knee Extension Strength and Gait Speed

Maximal knee extension strength (KES) at a knee angle of 90° was measured with the subject in a sitting position on a custom-made dynamometer chair, as previously described (28,32). The ankle was attached to a strain-gauge system (TKK5710e; Takei Scientific Instruments, Niigata, Japan). After familiarization with the test, subjects were encouraged to exert maximal knee extension force. The test consisted of two maximal efforts, each separated by a 1-minute rest period, with the highest value recorded. The length from the articular cleft between the femoral and tibial condyles to the ankle attachment was measured. Knee extension torque (Nm) was calculated as the strength multiplied by the length, and the mean KES of right and left knee joints was additionally calculated.

Subjects were instructed to walk a distance of 10 m as quickly as possible in order to determine their maximal gait velocity. This walking time was measured using a digital stopwatch for a distance of 6 m, following an initial 2 m of acceleration and prior to a final 2 m of deceleration. The speed was calculated as the distance divided by the walking time. Gait speed was measured twice, and the mean of the two trials was used (32).

Skeletal Muscle Mass

SM was calculated using the equation developed by Janssen et al. (33) where SM (kg) = $[(\text{height}^2/R_{50} \times 0.401) + (\text{sex} \times 3.825) + (\text{age} \times -0.071)] + 5.102$. Height was measured in centimeters, R_{50} was measured in ohms between the right wrist and ankle in a supine position (men = 1 and women = 0), and age was measured in years. This BIA equation was developed against magnetic resonance imaging (MRI) measures of whole-body muscle volume in a sample of 269 men and women who varied widely in age (between 18 and 86 years) and adiposity (body mass index [BMI], 16–48 kg/m^2). In that study, the correlation between BIA-predicted and MRI-measured muscle mass was 0.93 with a standard error estimated at 9%.

Statistical Analysis

Results are presented as the mean ± *SD*. Variables between men and women were compared using analysis of variance. Pearson's correlation coefficients were calculated for the comparison of ECW/ICW in the upper legs and KES, gait speed, and age. Multiple linear regression analysis was conducted using KES or gait speed as a dependent variable. In the linear model, age, sex, BMI, and SM were entered in model 1, and ECW/ICW in the upper legs was entered in model 2, in order to investigate the contribution of ECW/ICW in the upper legs to muscle strength and gait speed. All analyses were performed using SPSS software (Version 22.0 for Windows, IBM Corp. Armonk, NY).

Results

The physical characteristics of study subjects are presented in Table 1. Age and BMI did not differ significantly between sexes. Height, weight, SM, KES, and gait speed were significantly higher in men when compared with women. ECW/ICW in the upper legs was significantly lower in men when compared with women. The relationships between KES or gait speed and the ECW/ICW in the upper legs are presented in Figure 1. ECW/ICW in the upper legs was significantly and negatively correlated with KES and gait

Table 1. Physical Characteristics of Subjects (240 Women and 165 Men)

	Women	Men
Age (y)	73.8 ± 4.9	74.7 ± 5.2
Height (cm)	151.1 ± 5.4	163.9 ± 6.2*
Weight (kg)	51.7 ± 8.1	61.5 ± 8.9*
BMI (kg/m²)	22.7 ± 3.4	22.8 ± 2.8
SM (kg)	17.1 ± 2.3	27.0 ± 3.2*
Knee extension strength (N m)	88.3 ± 24.1	145.2 ± 37.6*
Gait Speed (m/s)	1.8 ± 0.3	2.0 ± 0.4*
ECW/ICW in the upper legs	0.514 ± 0.091	0.442 ± 0.088*

Notes: BMI = body mass index; ECW = extracellular water; ICW, intracellular water; SM = skeletal muscle mass.

Data are expressed as mean ± *SD*.

*Significantly different between women and men ($p < .001$).

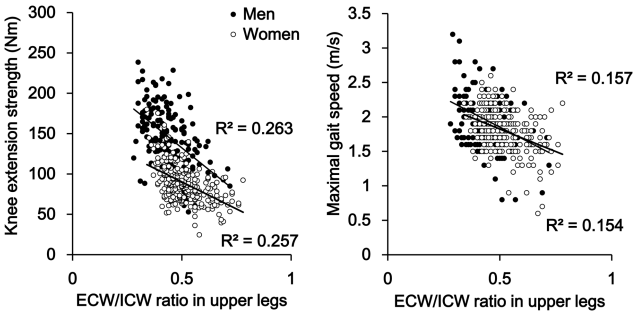

Figure 1. The relationships between the ratio of extra- and intracellular water (ECW/ICW) in the upper legs as assessed by segmental bioelectrical impedance spectroscopy (S-BIS) and isometric knee extension strength (A) and maximal gait speed (B). ○ women and • men.

speed ($r = -.617$ and -0.431, respectively; $p < .001$) and ECW/ICW increased with age ($r = .395$, $p < .001$).

When ECW/ICW in the upper legs was not taken into account, age and sex contributed towards explaining the variance of KES independent of SM (Table 2). The ECW/ICW in the upper legs significantly explained the variance of KES independent of age, sex, BMI, and SM. In model 2, age, sex, and BMI did not significantly contribute towards KES variance; SM and the ECW/ICW in the upper legs only were selected as significant variables.

The results of multivariate analysis for gait speed are presented in Table 3. Age, BMI, and SM were selected as significant variables to explain the variance in gait speed observed in model 1. The ECW/ICW in the upper legs was selected as a significant variable in addition to those variables, and the standardized coefficient of ECW/ICW in the upper legs was higher than that of other variables for gait speed.

Discussion

In the present study, the ratio of ECW/ICW in the upper legs was assessed by S-BIS and was found to be significantly associated with both KES and gait speed, independent of age, sex, BMI, and SM. Our most novel finding is that the ECW against ICW in skeletal muscle is correlated with gait speed independently from age, sex, BMI, and SM.

Traditionally, BIA (including single- and multi-frequency) and BIS have been used to estimate body composition including total body water, fat and fat-free mass, as well as SM (34,35). These studies have established or validated estimated SM by BIA using computed tomography, MRI, or dual-energy X-ray absorptiometry as a criterion method for SM. Normal imaging methods such as T1-weighted spin-echo MRI, computed tomography, or dual-energy X-ray absorptiometry, are regarded as a criteria for the assessment of SM in terms of the present consensus for sarcopenia (36–39). However, SM estimated by these simple imaging methods cannot differentiate ECW from other skeletal muscle components within the muscle tissue. ECW within skeletal muscle tissue is not a component of muscle cell mass (MCM) and is therefore not related to muscle strength, that is, the relative expansion of ECW against SM may have an adverse effect on muscle quality.

S-BIS is a unique tool for differentiating ECW from intracellular components. Skeletal muscle cell components are isolated by cell membranes that are organized by phospholipid bilayers. The cell membrane works as a capacitor in alternating the circuit current (40,41). Low-frequency currents cannot penetrate the cell membrane; however, these membranes are permeable to high-frequency currents. S-BIS operates using a series of frequency currents on the principle of the Cole–Cole model, which characterizes the measurement segment with parallel circuits for ECW and ICW and further accounts for the capacitive effect introduced by the nonconducting membrane separating the ICW from the ECW. A plot of reactance versus resistance at different frequencies results in a semicircular arc. To fit the measured impedance data to this model, resistance at infinity (R_∞) and zero (R_0) frequencies are obtained by extrapolation. In S-BIS, the reciprocal of R_0 reflects the ECW component and the reciprocal of R_{ICW} (the reciprocal of R_∞ subtracting by the reciprocal of R_0) reflects the ICW component in a given segment. Intriguingly, the index of ECW/ICW can be obtained simply as a proportion of R_{ICW}/R_0 (see the Methods section in detail).

Mingtone et al. (21) established a model of skeletal muscle composition at the cellular level. In that model, muscle mass contains not only MCM but also ECW and intramuscular adipose tissue. The theoretical ratio of ICW/MCM is likely to be approximately 0.72 and is relatively constant between subjects, although hydration of total skeletal muscle tissues varies as a result of variance in lipid contents. Thus, ECW/ICW in a given segment is proportional to ECW/MCM in the skeletal muscle tissue and could be a biomarker of muscle quality. Indeed, ECW/ICW in the upper legs was a significant variable for clarifying the inter-individual variance of muscle strength and gait speed, independent of age, sex, BMI, and SM.

Most recently, Azzabou et al. (10) examined the differences in nuclear magnetic resonance T2 imaging between younger and older adults and found that, aside from fat fraction, elevated water T2 and increased T2 heterogeneities in quadriceps were observed in the elderly. An age-related increase in T2 in the calf muscles was further reported by Hatakenaka et al. (22) and Schwenzer et al. (23) and in the tibialis anterior muscles in humans and mice by Esposito et al. (42). Hatakenaka et al. (22) stated that the T2 relaxation time of fast-twitch muscle increases with aging and that this is mainly attributable to increased extracellular space, reflecting age-related type II fiber atrophy. Recent studies reported that the metabolic profiles assessed by liquid chromatography-tandem mass spectrometry and muscle bioenergetics assessed by 31P-MRS are related to muscle quality or gait speed (43,44), it is interesting to examine the relationship between the heterogeneities of skeletal muscle composition and those factors using S-BIS.

Lexell et al. (17) examined the cross-sections of autopsied whole vastus lateralis muscle from 43 previously physically healthy men

Table 2. Multivariate Analysis: Linear Model With Knee Extension Strength as a Dependent Variable

	Model 1			Model 2		
	Unstandardized	Standardized		Unstandardized	Standardized	
Factors Included	*B* (95% CI)	β	*p*-value	*B* (95% CI)	β	*p*-value
Constant	132.1 (83.6, 180.6)		<.001	130.4 (86.0, 174.8)		<.001
Age (y)	−1.5 (−2.0, −0.9)	−0.182	<.001	−0.4 (−0.9, 0.2)	−0.046	.191
Sex (male =1, female = 0)	17.2 (4.3, 30.2)	0.206	.009	6.4 (−5.7, 18.5)	0.076	.297
BMI (kg/m^2)	−0.2 (−1.1, 0.8)	−0.014	.698	−0.7 (−1.6, 0.2)	−0.052	.131
SM (kg)	4.1 (2.9, 5.3)	0.559	<.001	4.1 (3.1, 5.2)	0.561	<.001
ECW/ICW in the upper legs				−135.5 (−165.6, −105.4)	−0.316	<.001

Note: BMI = body mass index; CI = confidence interval; ECW = extracellular water; ICW = intracellular water; SM = skeletal muscle mass.

Table 3. Multivariate Analysis: Linear Model With Gait Speed as a Dependent Variable

	Model 1			Model 2		
	Unstandardized	Standardized		Unstandardized	Standardized	
Factors Included	*B* (95% CI)	β	*p*-value	*B* (95% CI)	β	*p*-value
Constant	3.8 (3.24, 4.35)		<.001	3.785 (3.258, 4.312)		<.001
Age (y)	−0.023 (−0.03, −0.017)	−0.348	<.001	−0.014 (−0.021, −0.008)	−0.213	<.001
Sex (male =1, female = 0)	0.018 (−0.128, 0.165)	0.027	.807	−0.07 (−0.214, 0.073)	−0.102	.335
BMI (kg/m^2)	−0.023 (−0.034, −0.012)	−0.216	<.001	−0.027 (−0.038, −0.017)	−0.253	<.001
SM (kg)	0.015 (0.002, 0.029)	0.251	.025	0.015 (0.003, 0.028)	0.254	.019
ECW/ICW in the upper legs				−1.108 (−1.466, −0.751)	−0.315	<.001

Note: BMI = body mass index; CI = confidence interval; ECW = extracellular water; ICW = intracellular water; SM = skeletal muscle mass.

between 15 and 83 years of age. According to our calculation using Table 1 in that article (17), whole muscle cross-sectional area (CSA) of vastus lateralis was ~26% lower in subjects in their 70's when compared with those in their 20's. This value is consistent with a previous study by Janssen et al. (45) that measured SM in 468 men and women aged between 18 and 88 years using MRI and found a ~26% difference in lower body SM when comparing men in the same age brackets as Lexell et al. However, Lexell et al. (17) also examined the total number of fibers, proportions of type I fibers, and the mean fiber size of types I and II in vastus lateralis. The total number of fibers was ~41% lower in subjects in their 70's compared with those in their 20's. The mean fiber size of type I did not differ between the two age groups (0% difference), however, that of type II was ~25% lower in subjects in their 70's compared with those in their 20's. We are furthermore able to calculate the so-called "total muscle cell CSA" using the total number of fibers, proportion of type I fibers, and mean fiber size of types I and II. Total muscle cell CSA was ~48% lower in subjects in their 70's, despite the fact that a difference of just ~26% was observed in whole muscle CSA between the two age groups. These results support the notion that extracellular space in skeletal muscle tissue increases with age.

An important finding of the present study was that age, sex, and BMI were not significant factors while considering inter-individual variance in muscle strength after inclusion of ECW/ICW in the upper legs. Previous studies show that muscle quality, calculated as the ratio of muscle strength to SM or muscle strength adjusted by SM, as a covariate, decreases with age, even in cohorts comprised solely of an older adult population (5–9,46). Multivariate analysis without taking into account ECW/ICW in the upper legs (Table 2, model 1) supports this previous finding. In contrast, after including ECW/ICW in the upper legs as an independent variable, age was no longer significantly associated with muscle strength. This result suggests that the decreased muscle quality observed with aging can be explained at least partly by the relative expansion of ECW against MCM in skeletal muscle tissue.

In addition, ECW/ICW in the upper legs was significantly associated with gait speed independent of age, sex, BMI, and SM. No studies have previously examined the relationship between gait speed and the segmental ratio of ECW/ICW. ECW and ICW can be measured using bromide and stable isotope dilution at a whole body level, and Ritz et al. (47) found that elderly patients had a lower ICW and a higher ECW at this level when compared with both young and elderly healthy adults. Wang et al. (48) measured ECW/ICW, body cell mass by total body potassium count, and fat-free mass by dual-energy X-ray absorptiometry and reported a significant increase in ECW/ICW, as well as decreased body cell mass /fat-free mass during aging. The results of the present study are in line with these findings. Bromide and stable isotope dilution techniques are a reliable method for assessing water distribution at a whole body level; however, it cannot be used for the assessment of segmental measurements. While Pietrobelli et al. (49) have demonstrated the usefulness of forearm potassium counting by ^{40}K, this is a large, expensive, and inconvenient instrument that is furthermore not widely available. S-BIS is a portable, noninvasive, and rapid tool for the assessment of segmental water distribution and may be used on a daily basis within the clinical setting.

The equations of segmental ECW = $\rho_{ECW} \times L^2/R_0$ (ρ_{ECW} = 47 Ωcm) and ICW = $\rho_{ICW} \times L^2/R_{ICW}$ (ρ_{ICW} = 237.9 Ωcm) were used in the present study. The specific resistivity are determined empirically. The ρ_{ECW} and ρ_{ICW} is determined by Kaysen et al. (29) with a reference of

Zhu et al. (30). Zhu et al. (50) reported that segment specific resistivity improves the body fluid measurement for whole leg (ρ_{ECW} = 99 Ωcm and ρ_{ICW} = 281 Ωcm) in hemodialysis patients. We, however, assessed only thigh segments in the current study, and the specific resistivity may be differ from it. The correlation coefficients and standardized regression coefficients between ECW/ICW and muscle strength or gait speed is independent of the specific resistivity. The conclusion will not be influenced, although the further studies are needed to determine the specific resistivity of the segments.

Conclusion

The ratio of ECW/ICW in the upper legs assessed by S-BIS is significantly associated with muscle strength and gait speed in the elderly. The index of ECW/ICW can be easily obtained as a proportion of R_{ICW}/R_0 without the need for any undisclosed equation dependent on a manufacturer. As S-BIS is a portable, noninvasive, and rapid methodology that can be used daily in the clinical setting, assessment of water distribution in limb segments is an attractive tool for the assessment of both muscle quality and quantity. Increased extracellular water in skeletal muscle tissue is an important contributor towards decreased muscle quality during aging.

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