Electrical Properties Assessed by Bioelectrical Impedance Spectroscopy as Biomarkers of Age-related Loss of Skeletal Muscle Quantity and Quality

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Abstract

Skeletal muscle, in addition to being comprised of a heterogeneous muscle fiber population, also includes extracellular components that do not contribute to positive tensional force production. Here we test segmental bioelectrical impedance spectroscopy (S-BIS) to assess muscle intracellular mass and composition. S-BIS can evaluate electrical properties that may be related to muscle force production. Muscle fiber membranes separate the intracellular components from the extracellular environment and consist of lipid bilayers which act as an electrical capacitor. We found that S-BIS measures accounted for ~85% of the age-related decrease in appendicular muscle power compared with only ~49% for dual-energy x-ray absorptiometry (DXA) measures. Indices of extracellular (noncontractile) and cellular (contractile) compartments in skeletal muscle tissues were determined using the Cole–Cole plot from S-BIS measures. Characteristic frequency, membrane capacitance, and phase angle determined by Cole–Cole analysis together presented a S-BIS complex model that explained ~79% of interindividual variance of leg muscle power. This finding underscores the value of S-BIS to measure muscle composition rather than lean mass as measured by DXA and suggests that S-BIS should be highly informative in skeletal muscle physiology.

Keywords: Bioelectrical impedance spectroscopy—Contractile muscle tissue—Membrane capacitance

Skeletal muscle, the largest organ in the reference human body, is vital for any physical activity and also plays a role in maintaining whole-body metabolic homeostasis (1,2). Loss of skeletal muscle mass and function increases risk for impaired mobility, falls, fractures, metabolic dysfunction, and mortality and is a major concern in human health, acute and chronic diseases, immobility, and aging (3–6).

It has traditionally been hypothesized that the age-related reduction of muscle force and power results mainly from skeletal muscle mass loss. However, the age-related decrease in skeletal muscle mass estimated by computerized axial tomography (CT), magnetic resonance imaging (MRI), or dual-energy x-ray absorptiometry (DXA) is insufficient to explain the decline in muscle strength (7,8). Skeletal muscle tissue includes extracellular tissue and bundles of metabolically heterogeneous fiber types. Fibers atrophy with age and levels of fibrosis and intramuscular fat increase within and around the bundles (9). This age-related increase in noncontractile content of muscle is related to loss in muscle strength and power (10,11), however, age-related changes in muscle composition are not accessible using routine clinical measures (1). Current sarcopenia consensus definitions include DXA-measured appendicular lean mass (12–15). A major drawback of this technique is that fat-free mass (FFM) is largely the measurement of water and, moreover, it cannot inform about changes in skeletal muscle composition. In contrast, segmental bioelectrical impedance spectroscopy (S-BIS) has the ability to distinguish intracellular water (ICW) from extracellular water (ECW), the ratio of ICW/ECW (16–18), and additionally may evaluate other
changes in muscle composition. As such, we tested if the electrical properties of S-BIS could be reasonable metrics to estimate functional muscle mass and whether bioelectrical characteristics could provide insights into interindividual variance of maximum muscle power production that are not explained by DXA-measured lean mass.

Methods

Participants

Fifty-seven community-dwelling volunteers aged 26–76 years (13 men and 44 women) who participated in the national survey study “Midlife in the United States” (MIDUS), a study designed to evaluate health and well-being in midlife (19), were studied. MIDUS participants were identified through a nationally representative random-digit-dial sample of noninstitutionalized, English-speaking adults, aged 26–76 years, in the continental United States. This analysis uses a subsample of participants from the Refresher cohort who participated in biomarker data collection at the University of Wisconsin, Madison. Measurements were conducted between June 5, 2013 and October 19, 2013. This study was approved by the University of Wisconsin Health Sciences Institutional Review Board and conducted in compliance with global, national, and local regulations.

Bioelectrical Impedance Spectroscopy

The basic theory of BIS is described elsewhere (16,18) and the detailed method is provided in the Supplementary Material. Briefly, bioelectrical impedance was measured using a logarithmic distribution of 256 frequencies, ranging from 4 to 1,000 kHz (SF7, ImpediMed, Pinkenba, QLD, Australia), using disposable tab-type monitoring electrodes (2 cm x 2 cm, Medtronic, Minneapolis, MN). The $R_0$ and $R_\infty$ for the whole-body and leg were determined by extrapolation after fitting the spectrum of bioimpedance data to the Cole–Cole model (Bioimp software, ImpediMed; Figure 1B). For BIS, the $R_I$ was calculated using $1/(1/R_\infty - 1/R_0)$. Segment length ($L_\text{cm}$) was measured from the most lateral aspect of the lateral greater trochanter of the femur to lateral tibial malleolus. Intracellular impedance index was calculated as $L^2/R_I$, and extracellular resistance index was calculated as $L^2/R_\infty$. Segmental ICW, and $L^2/R_I$ reflects segmental ICW, and $L^2/R_\infty$ reflects segmental ECW in the leg. The resistance ratio, calculated as $R_0/R_I$, is the index of the ratio of ICW/ECW (16,17) (Figure 1C). The membrane capacitance ($C_m$), characteristics frequency ($f_c$), and phase angle ($\phi$) were also obtained from the Cole–Cole model (20) (Figure 1B,D).

Dual-Energy X-Ray Absorptiometry

A Lunar iDXA (GE Healthcare, Madison, WI) densitometer was used for whole-body and leg composition assessment. Routine densitometry quality assurance procedures were conducted and no instrument drift or shift was detected during the study period. All scans were acquired and analyzed with enCORE software; version 13.31 in accordance with International Society for Clinical Densitometry (ISCD) recommendations (21). All measurements were performed by ISCD-certified technologists. Participant tissue composition, including fat, bone, and lean mass, was obtained as described previously for the whole-body and leg regions (22,23).

Jumping Mechanography

Jumping mechanography has been described elsewhere (24,25), and the detailed method is in the Supplementary Material. Briefly, countermovement jumps were performed on a ground reaction force...
platform (Leonardo Mechanograph; Novotec Medical, Pforzheim, Germany). Leonardo software version 4.2 (Novotec Medical) was used to record voltage and carry out performance calculations. Volunteers were instructed to jump as high as possible, making effort to touch the ceiling with their head. Two trained staff members were present during jumping to explain the procedure and help the participant with regaining balance if necessary. Jump power ($P_{\text{max}}$) was calculated by the software using a model previously described (24,25).

**Statistical Analysis**

The IBM SPSS Statistics, Version 22, was used for all statistical analyses. Participants’ characteristics are given as mean (SD) in Table 1. Normal distribution was tested using a Kolmogorov–Smirnov test and variables showed statistically normal distribution. Regression analysis was applied to examine the relationship between age and $P_{\text{max}}$, DXA and S-BIS variables for men and women separately, and the relative decrease rate per decade was calculated from the regression equation using a 30-year-old normative value as reference. Relationships between $P_{\text{max}}$ and DXA and S-BIS variables were examined using correlation analysis. Correlation coefficients were compared using Meng’s statistical method (26). The partial correlation analysis was also conducted to examine the relationship between $P_{\text{max}}$ and the intracellular impedance index, impedance differential, $C_m$, $f_c$, and $\phi$ with age, sex, height, and weight as control variables. Stepwise multivariate linear regression analysis was performed using intracellular resistance index ($L^2/R_I$), resistance ratio ($R_0/R_I$), membrane capacitance ($C_m$), characteristics frequency ($f_c$), phase angle ($\phi$) of S-BIS as independent variables and $P_{\text{max}}$ was dependent variable with 0.05 entry and 0.10 removal probability of $F$.

**Results**

**Impact of Age on Muscle Power, DXA, and S-BIS**

We examined the impact of age on leg maximum muscle power output, DXA leg lean mass, whole-body impedance, and S-BIS in the leg. Regression analysis revealed that the correlation between leg maximum power outputs and age was strong and negative (Men: $r = -0.817, p < .001$; Women: $r = -0.700, p < .001$) (Figure 2A).

| Table 1. Physical and Electrical Characteristics of Study Participants |
|-------------------------|-------------------------|-------------------------|-------------------------|
|                        | Men (Mean (SD))         | Women (Mean (SD))       | $p$ Value               |
| Age (y)                | 48.8 (12.4)             | 52.0 (14.8)             | .482                    |
| Height (cm)            | 178.3 (6.5)             | 164.5 (7.4)             | <.001                   |
| Weight (kg)            | 98.4 (31.9)             | 88.3 (29.5)             | .289                    |
| BMI (kg m$^{-2}$)      | 30.6 (8.4)              | 32.6 (11.1)             | .559                    |
| $P_{\text{max}}$ (kW)  | 3.42 (0.94)             | 2.03 (0.64)             | <.001                   |
| DXA measurements       |                         |                         |                         |
| Percent body fat (%)   | 29.2 (10.2)             | 40.4 (9.3)              | <.001                   |
| FFM (kg)               | 68.8 (13.3)             | 49.1 (8.8)              | <.001                   |
| ALST (kg)              | 30.6 (6.3)              | 21.3 (4.9)              | <.001                   |
| Leg lean mass (kg)     | 21.9 (4.3)              | 16.2 (3.7)              | <.001                   |
| S-BIS measurements in the leg |                   |                         |                         |
| Intracellular resistance index (cm$^2$ Ω$^{-1}$) | 11.5 (3.5)             | 7.9 (2.5)               | <.001                   |
| Extracellular resistance index (cm$^2$ Ω$^{-1}$) | 28.6 (6.5)             | 22.9 (7.1)              | .011                    |
| Resistance ratio       | 0.40 (0.08)             | 0.35 (0.08)             | .069                    |
| $C_m$ (nF)             | 6.0 (2.4)               | 4.6 (1.7)               | .067                    |
| $f_c$ (kHz)            | 32.6 (8.6)              | 35.0 (6.0)              | .258                    |
| Phase angle (deg)      | 4.2 (0.9)               | 3.7 (0.8)               | .052                    |

Note: Cohort $N = 57$; men $n = 13$, women $n = 44$.

ALST = appendicular lean soft tissue; BMI = body mass index; $C_m$ = membrane capacitance; DXA = dual-energy x-ray absorptiometry; Extracellular resistance index was calculated as $L^2/R_J$; $f_c$ = characteristic frequency; FFM = fat-free mass; Intracellular resistance index was calculated as $L^2/R_J$; $P_{\text{max}}$ = maximum leg muscle power; Resistance ratio was calculated as $(R_0/R_I)$; S-BIS = segmental bioelectrical impedance spectroscopy. Bold values are statistically different between men and women ($p < .05$).
Men had a steeper negative slope for maximum jump power output as a function of age than women (0.6 and 0.3 kW less per decade, respectively), although the relative decrement was similar for both men and women (13.1% and 11.1% per decade, respectively). A weaker correlation was detected between age and leg lean mass obtained by DXA (LLM Dx A) (Men: \( r = -0.301, p = .08 \); Women: \( r = -0.337, p = .03 \)) (Figure 2B). The relative decrement (percent slope) of leg lean mass obtained using DXA (LLM Dx A) (7.2% and 4.8% per decade, in men and women, respectively) was only \(-49\%\) of the relative decrements of muscle functional leg muscle power against age.

Intracellular resistance index (\( L/RI \)) in the leg was calculated for each individual in the cohort using the Cole–Cole plot of resistance against reactance (Figure 2C). \( L/RI \) correlated strongly or moderately with age for both men and women (Men: \( r = -0.601, p = .03 \); Women: \( r = -0.531, p < .001 \)) and the correlation coefficients were significantly greater than with LLM Dx A (\( p < .01 \)) (Figure 2B). The relative decrement of intracellular resistance index was 11.5% and 9.2% per decade and reached 87.8% and 82.9% of the relative decrements of leg muscle power against age in men and women, respectively. In contrast, the extracellular resistance index (\( L/RE \)) in the leg was not related to age. The resistance ratio, calculated as the \( R_c \) divided by \( R_e (R_c/R_e) \), in the leg was strongly and negatively correlated with age (Men: \( r = -0.819, p < .001 \); Women: \( r = -0.713, p < .001 \); Figure 2D).

Traditional approaches to estimating skeletal muscle mass by bioelectrical impedance analysis (BIA) typically use a 50-kHz signal and this partially penetrates the intracellular space (27–30). This traditional BIA does not differentiate between intracellular and extracellular compartments. Moreover, the gold standard for using the BIA approach requires regression against total lean tissue or FFM as measured by DXA or MRI (28–30). Not surprisingly, the ability of standard BIA to detect the age-related decline in muscle power was similar to that of appendicular lean soft tissue of DXA (Supplementary Figure 1). These data demonstrate that S-BIS is a more sensitive method for evaluation of age-related loss in muscle power, outperforming traditional BIA and DXA.

Muscle Power Output and S-BIS Versus DXA

To determine if bioelectrical impedance measures could explain differences in leg maximum muscle power output independent of age, we conducted a regression analysis. Maximum power output of the leg significantly correlated with LLM Dx A (Men: \( r = 0.691, p = 0.09 \); Women: \( r = 0.727, p < .001 \); Supplementary Figure 2A). A very strong positive correlation was detected between maximum power output and the intracellular resistance index (Men: \( r = 0.808, p < .001 \); women: \( r = 0.881, p < .001 \); Supplementary Figure 2B). In agreement with the previous analysis, a strong or moderate positive correlation between maximum power output and the resistance ratio was also detected (Men: \( r = 0.873, p < .001 \); Females: \( r = 0.382, p < .001 \).

S-BIS Parameters and Muscle Power

We next investigated if other parameters derived from S-BIS measures would be informative in defining the impact of age on maximum power output. The Cole–Cole model fits the measured resistance with parallel circuits and calculates the electrical capacitance introduced by the nonconducting membrane. The membrane capacitance (\( C_m \)) is indicative of the retention capacity of membrane potential gradient and the depolarization reactivity of the muscle cell membranes. The characteristics frequency (\( f_c \)) is determined by the energy required to supply a constant current though the tissue and reflects heterogeneity in the density of the tissue. The phase angle \( (\theta) \) has been linked to adioposity in whole-body measures and is thought to positively reflect cell membrane content (31). In this study, \( C_m \) and \( \theta \) both decreased with aging, whereas \( f_c \) increased with aging (Figure 3). Perhaps not surprisingly, \( C_m \) and \( \theta \) correlated positively with maximum power output, and \( f_c \) correlated negatively with maximum power output (Figure 4A–C). These data are indicative of broad differences in muscle composition. Correlations between Cole–Cole parameters and maximum power output remained significant even after controlling for age, sex, height, and weight, suggesting that the physiological properties reflected in these measures are fundamentally linked to muscle power production. Stepwise regression analysis of aggregate S-BIS measures (Supplementary Table 1) identified a statistical model where the intracellular impedance index, resistance ratio, \( C_m \), and \( f_c \) in combination were significant predictors for maximum power output and explained 77.6% and 80.7% of individual variance of leg muscle power in men and women respectively (Figure 4D).

Discussion

The stronger correlations between S-BIS measures and power and functional capacity reported herein suggest that S-BIS may be sensitive to differences in muscle composition, including fiber shrinkage, aging, fibrosis, and adipose infiltration. Previous studies have reported changes in muscle composition with aging in vivo, including changes in intramuscular adipose tissue and muscle density. Intramuscular fat estimated by MRI in the tibialis anterior muscle increases with aging (10), and diffusion tensor MRI (DT-MRI) signal intensity decreases significantly in the muscles of the lower leg with aging (32). More recently, water T2 mean values assessed by MRI and its heterogeneity were significantly higher in the elderly participants compared with young adults (33). These methods require complex and/or expensive measurement techniques and cannot be conducted in routine clinical care. In contrast, S-BIS is an affordable alternative to CT or MRI, noninvasive, easy-to-operate, portable, and fast. We suggest that S-BIS offers a highly effective method for detection of age-related declines in muscle function and as such may be a superior method for clinical assessment of sarcopenia as well as basic medical research for skeletal muscle assessment.

Skeletal muscle undergoes profound changes in composition with age including fiber atrophy and fibrosis that may not be detected by simple measures of muscle girth. S-BIS is an alternative strategy to DXA that takes advantage of the partitioning of water in skeletal muscle between intracellular and extracellular spaces (16,17,34) (Figure 2A). Muscle fiber cell membranes are phospholipid bilayers and act as capacitors that insulate the intracellular compartment
from electrical current applied at low frequencies. At higher frequencies, in contrast, the membranes are permeable to the current so that ICW and ECW both contribute to electrical conductance. A series of frequencies applied across appendicular muscle groups allows for separate detection of resistance along the intracellular and extracellular conductivity pathways (35). In addition, S-BIS can also evaluate other electrical properties of muscle tissues and membrane which may be related to tensional force production. Recent studies confirm that the upper leg ECW to ICW ratio obtained by BIS is related to muscle function, but a direct comparison of BIS to other techniques (43) or biopsy analysis (44) to delineate age-related changes in muscle composition and how those parameters relate to change in muscle function.

In conclusion, the results of the present study indicate the value of S-BIS to measure muscle composition rather than appendicular lean mass as measured by DXA and suggest that S-BIS should be highly informative in biological and medical researches of skeletal muscle physiology.

Supplementary Material
Supplementary data is available at The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences online.

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References


