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# Relationship between Lower-Limb Bioelectrical Impedance Indicators of Hydration Status and Muscle Function in Aged Population: A Cross-Sectional Study

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## Abstract

**Background:** Reduced Intracellular Water (ICW) has been related to poor muscle strength and functional capacity in aged populations. We aimed to evaluate hydration status and intracellular hydration of lower-limb lean mass in aged population and the relationship with thigh muscle function.

**Methods: Design and population:** Cross-sectional study of community-dwelling ≥70 year old subjects.

**Measurements:** Lower-limb hydration status was assessed by bioimpedance analysis and thigh muscle function was assessed by isokinetic evaluation. Frailty was established according to Fried criteria, sarcopenia according to EWGSOP-2 criteria, and physical performance according to the Timed Up-and-Go (TUG) test.

**Results:** Assessed were 117 individuals (mean age 75.0 years, 50.4% women). Women had lower Fat-Free Mass (FFM), Total Body Water (TBW), and Phase Angle (PhA) values than men. Age was negatively correlated with ICW ($r=-0.187$; $p=0.045$), the ICW/FFM ratio ($r=-0.192$; $p=0.039$), and PhA ($r=-0.311$; $p<0.001$). Extension and flexion strength and power of the knee were positively correlated with Total Water (TW) (L), FFM (kg), and the protein compartment (kg) of the lower-limb in both men and women, but not with TW as percentage of weight, ICW as percentage of TW, and the TW/FFM and ICW/FFM ratios, while the TUG test was negatively correlated with ICW as a percentage of TW.

**Conclusion:** Knee flexion and extension strength, power, and work increase as thigh FFM, TBW, and protein compartment values increase. No correlation was observed between intracellular hydration and muscle function, but correlation was observed with performance. As plausible hypotheses, further studies are required to test for these correlations.

**Keywords**: Dehydration; Intracellular water; Muscle function; Aged; Isokinetic test; Bioimpedance analysis

## Introduction

Water is the main component of the human body and an essential nutrient for life and health [1,2]. Water is distributed in the body into intracellular and extracellular compartments and flows from one compartment to the other according to osmotic pressure through transmembrane protein channels called aquaporins. Plasma or extracellular osmolarity must remain between 285-295 mOsm/L of water, as any increase in these values will induce intracellular dehydration, affecting cell structure and function [3]. It has been suggested that cell volume may be a metabolic signal, such that cell swelling favours anabolism and cell contraction, catabolism, and protein degradation [4,5]. Cells have different mechanisms to compensate for plasma hyperosmolarity (>295 mOsm/L) and restore osmotic balance, such as ion transporter mechanisms, osmolyte synthesis, aquaporin gene expression induction, and cytoskeleton rearrangement [3,6]. However, osmotic balance is regulated in the




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body mainly by arginine vasopressin acting at the renal level, favouring water reabsorption and urinary concentration. Hyperosmotic stress has been related to an inflammatory response [7], diabetes, insulin resistance, metabolic alterations, chronic renal failure, cardiovascular diseases [8], and with muscle function, especially in young adult athletes [9]. There is also growing evidence on the effect of hydration status on muscle function in aged populations.

Starting in adulthood, water content of the body progressively declines, mainly due to a relative reduction in Fat-Free Mass (FFM) and Muscle Mass (MM), but also attributed to chronic low-intensity dehydration in aged populations [10]. Muscle composition changes with ageing, so that a decrease in the ratio Muscle Cell Mass (MCM)/Skeletal Muscle Mass (SMM) and a relative expansion of ECW and extracellular space is observed in advanced ages [11]. Bio-Impedance Analysis (BIA) derived hydration parameters have shown to be useful markers of muscle performance [12]. Some authors have reported that an increased total body Extracellular Water/Intracellular Water (ECW/ICW) ratio is an independent predictor of poor muscle strength and decreased gait speed in the elderly [13-15]. Other authors have suggested that cellular dehydration, as measured by the total body ICW/FFM ratio, is associated with poorer muscle strength and functional capacity and a higher risk of frailty [16]. Moreover, when considering only specific group of muscles, other authors have observed that ICW/total water in the lower limbs is a predictor of dorsiflexion and plantar flexion strength [17]. These results, however, need to be corroborated and confirmed, especially with more accurate and precise measurements of muscle function and for the same specific muscle groups.

The objective of this study, based on the hypothesis that muscle function depends on hydration status, was to evaluate hydration status and intracellular hydration of lower-limb FFM in a community-dwelling aged population and to determine their relationship with thigh muscle function.

## Methodology

### Study Design and Population

An observational cross-sectional study was conducted of community-dwelling subjects aged 70 years and older. A random sample was pre-selected from the database of inhabitants ascribed to 3 primary care centres in the XX region (COUNTRY). Pre-selected subjects were invited by telephone to a visit with their primary care physician, who informed them about the study, checked eligibility criteria and, if they agreed to participate, obtained their signed consent. Exclusion criteria included active malignancy, neuromuscular disease, dementia, serious mental illness, life expectancy of less than 6 months, bilateral knee or hip prosthesis use, and in palliative care or institutionalized. Recruitment took place between January and March 2020, but the study was stopped due to the COVID-19 pandemic, so all assessments were postponed to between July and October 2021. The local ethics committee approved the study protocol (code CEIm CSdM 65/19).

### Data Collection

Body composition and hydration status was assessed by BIA, a non-invasive, fast, reproducible, validated, and widely accepted method of analysis. BIA involves application of a low-intensity alternating electrical current at different frequencies and measurement of the opposition to the passage of this current by biological tissues.

**Impedance reflects 2 vectors:** Resistance (R), i.e., opposition to the flow of electrons, is inversely proportional to Total Body Water (TBW), and reactance (Xc), i.e., cell membrane opposition to the passage of electrical current, reflects the integrity of the cell membrane. BIA provides estimates, for the whole body and for each of its 5 segments (the limbs and trunk), of Fat Mass (FM), Fat-Free Mass (FFM), and Muscle Mass (MM) (in kg and as a percentage of body weight), TBW or Total Water of the limb (TW) (in litres and as a percentage of body or limb weight), and Extracellular Water (ECW) and Intracellular Water (ICW) (in litres and as a percentage of TBW). It also provides estimates of the protein compartment (in kg) and the Phase Angle (PhA), which is an indicator of cell membrane functionality and selective permeability.

zll no intense exercise in the previous 24 hours, no alcohol consumption in the previous 8 hours, strict fasting in the previous 2 hours, a toilet visit prior to the evaluation, and no evident clinical signs of dehydration. Used as indicators of right leg hydration and of cellular hydration were, respectively, the percentage of TW with respect to right leg weight and to FFM (TW/FFM), and the percentage of ICW with respect to TW and to FFM (ICW/FFM).

Muscle function was assessed by the isokinetic test (System 4 Pro device from PRIM), which allows objective measurements of muscle strength, work, and power. It allows the muscle to be exercised at constant speed throughout the range of joint movement. The isokinetic evaluation system has 3 elements: a goniometer, which measures the arc of movement; a tachymeter, which indicates the speed of movement; and a dynamometer, which measures strength at any given moment. The resistance produced by the system is always proportional to the exercised strength, with the corresponding muscles developing maximum tension and fibre activation. Strength (N), total work (J), and power (W) measurements were made for flexion and extension at the knee joint.

Sarcopenia was established according to the definition and criteria established by the European Working Group on Sarcopenia in Older People -2nd revision (EWGSOP-2) [18]. Sarcopenia is defined as low muscle strength measured according to grip strength (<27kg in men and <16kg in women using the handheld JAMAR dynamometer) and low muscle quantity measured as the Appendicular Skeletal Mass (ASM)/height$^2$ (<7.0Kg/m$^2$ in men and <5.5Kg/m$^2$ in women according to BIA). Sarcopenia is rated as severe if physical performance is also poor (gait speed ≤0.8m/s) [18]. Dynapenia was considered when low muscle strength was present (considering the mentioned grip strength cut-off points) accompanied with normal muscle mass.

**Frailty was established according to the Fried criteria [19], which consider that a person suffers from frailty if presents at least 3 of the following criteria:** weight loss, exhaustion, poor physical activity, weakness, and low gait speed. Physical performance was assessed according to the Timed Up-and-Go (TUG) test, which consists of getting up from a standard armchair, walking 3m, turning, walking back, and siting down again. The time to complete the task is measured and ten seconds or less is considered as normal mobility, and more than 20 seconds indicate the need for assistance. Other study variables included sociodemographic data (age, sex), comorbidities, and chronic medication use.

### Statistical Analysis

For sample size estimation, correlations between BIA and isokinetic parameters were main analyses considered. Accepting an alpha risk of 0.05 and a beta risk of 0.10 in a two-sided test, 113 subjects are necessary to detect as statistically significant a correlation coefficient of 0.3. Continuous variables were described using mean and Standard Deviation (SD) values, and categorical variables were described using percentages. Pearson (r) or Spearman ($r_s$) correlation coefficients were used to assess the relationship between hydration status indicators and isokinetic measurements, and also the relationship between other numerical variables and isokinetic measurements. The t-test or Mann-Whitney U-test was used to assess the relationship between dichotomous variables and muscle function parameters, depending on application conditions. Normality was assessed by the Kolmogrov-Smirnov test. Multivariate linear regression analysis was performed to adjust the effect of hydration indicators on muscle function for age. As body composition and muscle function differ for men and women, all analyses were performed separately by sex, and analyses were also performed for the 2 age groups of 70-79 years and ≥80 years. Statistical significance was set to p<.05.

## Results

### Main Sample Characteristics

Of the initial 237 candidate participants who attended the recruitment visit, 117 who completed baseline BIA and isokinetic test assessments constituted the study sample. Mean (SD) age was 75.0(4.1) years, 50.4% were women, 25.6% lived alone, 62.1% had an educational level of primary education or lower, 55.2% never smoked, 15.4% had no outdoor life, 7.0% were frail, 3.4% were sarcopenic (all rated as severely so), 17.9% presented dynapenia, and 6.0% had fallen in the previous 3 months. The main comorbidities were arterial hypertension (62.9%), arthritis (57.8%), dyslipidaemia (49.6%), gastro-oesophageal reflux (19.8%), diabetes (15.5%), peripheral artery disease (15.5%), cancer (14.8%), and depression (14.8%), and mean medication consumption was 4.37(3.02) drugs.

### Body Composition and Muscle Function by Sex

Table 1 describes the main body composition parameters (including hydration indicators) for the full sample, by sex, and by age group. Differences in body composition between men and women were significant, with women having higher FM and lower FFM, TBW and PhA values than men. Men lost more TBW and gained more FM than women as they aged. Age was negatively correlated with ICW (r=-0.187; p=0.045), the ICW/FFM ratio (r=-0.192; p=0.039), and PhA (r=-0.311; p<0.001).

Table 2 shows thigh-muscle isokinetic test results for the full sample, by sex, and by age group. For both knee flexion and extension, muscle strength, work, and power values for men were higher than for women, and decreased with age in both sexes.

### Correlation between Hydration and Muscle Function

Table 3 shows correlations, by sex, between hydration status parameters and muscle function indicators for the right leg. In both sexes, muscle strength and power were positively correlated with TW(L), ICW(L), FFM(kg) and Protein(kg), while no correlations were observed between muscle function parameters and ICW as a percentage of TW, the TW/FFM and ICW/FFM ratios, or PhA. These hydration indicators also did not show any correlation with muscle strength and power in men or in wom-

**Table 1:** Description of bioelectrical impedance analysis parameters by sex and age.

| Bioelectrical Impedance Analysis | | | | |
|---|---|---|---|---|
| | **Full sample** | **Women** | **Men** | **p** |
| | **N=116** | **N=59** | **N=57** | |
| Whole body | | | | |
| TBW (% weight) | 46.9(5.5) | 44.2(5.2) | 49.8(4.3) | <0.001 |
| TBW/FFM (L/kg) | 0.736(0.003) | 0.735(0.003) | 0,737(0.003) | <0.001 |
| ICW (% TBW) | 61.1(0.76) | 61.0(0.73) | 61.3(0.77) | 0.077 |
| FM (% weight) | 36.2(7.5) | 39.9(7.1) | 32.4(5.8) | <0.001 |
| FFM (kg) | 47.2(8.7) | 40.5(4.7) | 54.0(6.1) | <0.001 |
| ECW/ICW | 0.64(0.02) | 0.64(0.02) | 0.63(0.02) | 0.077 |
| ICW/FFM (L/kg) | 0.45(0.01) | 0.45(0.01) | 0.45(0.01) | <0.001 |
| Phase angle | 5.0(0.63) | 4.7(0.50) | 5.3(0.59) | <0.001 |
| Protein (kg) | 9.2(1.73) | 7.9(0.94) | 10.6(1.21) | <0.001 |
| Right leg | | | | |
| TW (% weight) | 7.2(1.0) | 6.5(0.78) | 7.9(0.71) | <0.001 |
| TW/FFM (L/kg) | 0.782(0.002) | 0.781(0.002) | 0.781(0.002) | 0.054 |
| ICW (% TBW) | 60.9(0.85) | 60.8(0.83) | 61.0(0.86) | 0.242 |
| FFM (kg) | 6.8(1.6) | 5.6(0.86) | 8.1(1.1) | <0.001 |
| ECW/ICW | 0.642(0.02) | 0.645(0.02) | 0.638(0.02) | 0.242 |
| ICW/FFM (L/kg) | 0.48(0.01) | 0.475(0.005) | 0.477(0.005) | 0.261 |
| Phase angle | 5.0(0.73) | 4.8(0.66) | 5.2(0.75) | <0.001 |
| **70-79 year old group** | | | | |
| Whole body | | | | |
| | **N=103** | **N=52** | **N=51** | |
| TBW (% weight) | 47.3(5.7) | 44.2(5.4) | 50.4(4.0) | <0.001 |
| TBW/FFM (L/Kg) | 0.736(0.003) | 0.735(0.003) | 0.737(0.003) | 0.123 |
| ICW (%TBW) | 61.2(0.71) | 61.0(0.60) | 61.3(0.78) | 0.168 |
| FM (% weight) | 35.8(7.7) | 39.9(7.4) | 31.6(5.5) | <0.001 |
| FFM (kg) | 47.8(8.6) | 41.0(4.7) | 54.6(5.9) | <0.001 |
| ECW/ICW | 0.64(0.02) | 0.64(0.02) | 0.63(0.02) | 0.178 |
| ICW/FFM (L/kg) | 0.45(0.01) | 0.448(0.005) | 0.452(0.005) | 0.084 |
| Phase angle | 5.1(0.61) | 4.8(0.45) | 5.4(0.59) | <0.001 |
| Protein (kg) | 9.3(1.71) | 8.0(0.91) | 10.7(1.16) | <0.001 |
| Right leg | | | | |
| TW (% weight) | 7.2(1.0) | 6.5(0.82) | 7.9(0.64) | <0.001 |
| TW/FFM (L/kg) | 0.781(0.002) | 0.782(0.002) | 0.781(0.002) | 0.490 |
| ICW (%TBW) | 61.0(0.79) | 60.9(0.7) | 61.1(0.88) | 0.390 |
| FFM (kg) | 6.9(1.6) | 5.7(0.85) | 8.1(1.1) | <0.001 |
| ECW/ICW | 0.641(0.02) | 0.643(0.02) | 0.638(0.02) | 0.406 |
| ICW/FFM (L/Kg) | 0.48(0.005) | 0.476(0.004) | 0.477(0.005) | 0.371 |
| Phase angle | 5.0(0.71) | 4.8(0.60) | 5.2(0.77) | 0.025 |
| **≥80 year old group** | | | | |
| Whole body | | | | |
| | **N=13** | **N=7** | **N=6** | |
| TBW (% weight) | 44.4(3.5) | 44.2(3.8) | 44.6(3.5) | 0.013 |
| TBW/FFM (L/Kg) | 0.736(0.004) | 0.734(0.003) | 0.738(0.003) | <0.001 |
| ICW (%TBW) | 60.9(1.1) | 60.7(1.4) | 61.2(0.71) | 0.056 |
| FM (% weight) | 39.3(4.7) | 39.7(5.3) | 38.8(4.4) | 0.020 |
| FFM (kg) | 42.2(7.9) | 36.5(3.3) | 48.9(6.1) | <0.001 |
| ECW/ICW | 0.64(0.03) | 0.65(0.04) | 0.64(0.02) | 0.056 |
| ICW/FFM (L/Kg) | 0.45(0.01) | 0.445(0.011) | 0.451(0.006) | <0.001 |
| Phase angle | 4.6(0.67) | 4.3(0.71) | 5.0(0.46) | 0.001 |
| Protein (kg) | 8.2(1.61) | 7.0(0.73) | 9.5(1.20) | <0.001 |
| Right leg | | | | |
| TW (% weight) | 6.7 (0.74) | 6.4 (0.43) | 7.1 (0.86) | <0.001 |
| TW/FFM (L/Kg) | 0.783 (0.003) | 0.783 (0.004) | 0.782 (0.002) | 0.065 |
| ICW (% TBW) | 60.7 (1.2) | 60.4 (1.5) | 60.9 (0.82) | 0.264 |
| FFM (kg) | 6.1 (1.6) | 4.9 (0.67) | 7.4 (1.4) | <0.001 |
| ECW/ICW | 0.650 (0.03) | 0.655 (0.04) | 0.643 (0.02) | 0.264 |
| ICW/FFM (L/Kg) | 0.47 (0.01) | 0.473 (0.009) | 0.476 (0.005) | 0.408 |
| Phase angle | 4.7 (0.83) | 4.4 (0.99) | 4.9 (0.53) | 0.054 |

**Note:** % weight refers to percentage of body weight.

**Abbreviations:** TBW: Total Body Water; ICW: Intracellular Water; ECW: extracellular Water; FM: Fat Mass; FFM: Fat Free Mass.

**Table 2:** Description of isokinetic test parameters by sex and age.

| Isokinetic Test (Right Leg) | | | | |
|---|---|---|---|---|
| | **Full sample** | **Women** | **Men** | **p** |
| | **N=112** | **N=54** | **N=58** | |
| Extension strength (N) | 86.4(28.4) | 68.1(17.6) | 103.3(26.1) | <0.001 |
| Extension work (J) | 342.5(138.9) | 275.5(92.5) | 404.9(146.2) | <0.001 |
| Extension power (W) | 49.7(17.8) | 40.2(12.4) | 58.5(17.6) | <0.001 |
| Extension fatigue (%) | 9.0(29.7) | 6.2(27.1) | 11.6(31.9) | 0.170 |
| Flexion strength (N) | 44.3(18.3) | 33.4(10.0) | 54.3(18.5) | <0.001 |
| Flexion work (J) | 187.0(96.2) | 142.0(60.9) | 228.9(104.2) | <0.001 |
| Flexion power (W) | 27.0(15.4) | 19.9(8.0) | 33.7(17.6) | <0.001 |
| Flexion fatigue (%) | 1.8(36.3) | 5.4(31.1) | -1.6(40.5) | 0.435 |
| **70-79 year old group** | | | | |
| | **N=99** | **N=48** | **N=51** | |
| Extension strength (N) | 88.3(27.7) | 70.4(16.0) | 105.2(25.7) | <0.001 |
| Extension work (J) | 352.1(138.6) | 283.9(88.3) | 416.4(147.0) | <0.001 |
| Extension power (W) | 50.9(17.5) | 41.6(11.3) | 59.6(17.9) | <0.001 |
| Extension fatigue (%) | 10.4(26.3) | 9.2(14.8) | 11.5(33.8) | 0.936 |
| Flexion strength (N) | 46.2(17.8) | 34.8(9.3) | 57.0(17.1) | <0.001 |
| Flexion work (J) | 196.2(94.1) | 148.3(58.2) | 241.3(99.4) | <0.001 |
| Flexion power (W) | 28.4(15.2) | 20.9(7.4) | 35.8(17.3) | <0.001 |
| Flexion fatigue (%) | 2.4(35.7) | 5.9(30.3) | -0.90(40.2) | 0.441 |
| **≥80 year old group** | | | | |
| | **N=13** | **N=6** | **N=7** | |
| Extension strength (N) | 71.3(31.0) | 49.9(21.0) | 89.7(26.5)* | 0.022 |
| Extension work (J) | 269.0(122.2) | 208.7(106.8) | 320.7(116.7) | 0.063 |
| Extension power (W) | 40.3(18.0) | 28.9(15.5) | 50.1(14.3)* | 0.032 |
| Extension fatigue (%) | -1.9(48.8) | -18.0(70.3) | 12.0(12.8) | 0.775 |
| Flexion strength (N) | 29.3(15.3)* | 22.7(10.1)* | 35.0(17.4)* | 0.224 |
| Flexion work (J) | 116.6(85.2)* | 91.0(63.3)* | 138.5(99.8)* | 0.475 |
| Flexion power (W) | 16.7(13.0)* | 11.9(8.7)* | 20.8(15.2)* | 0.317 |
| Flexion fatigue (%) | -3.2(41.9) | 1.1(40.0) | -6.8(46.3) | 0.775 |

*Significant differences between age groups.

en after adjusting for age. Muscle work was correlated with TW as a percentage of body weight in both men and women, and was also positively correlated in women with TW(L), ICW(L), FFM(kg), and Protein(kg), but not with ICW (as a percentage of TW), or PhA. Figure 1 shows right-leg extension strength association with MM, protein compartment, and cell hydration indicators stratified by sex.

### Muscle Mass Association with Muscle Strength

Right leg MM was related to right leg knee extension strength in both men ($r=0.260$; $p=0.051$) and women ($r=0.363$; $p=0.007$). MM was closely correlated with TW(L) ($r=0.868$; $p<0.001$ for men and $r=0.847$; $p<0.001$ for women), as well as with ECW(L) ($r=0.867$; $p<0.001$ for men and $r=0.834$; $p<0.001$ for women) and ICW(L) ($r=0.856$; $p<0.001$ for men and $r=0.849$; $p<0.001$ for women). However, ICW(and ECW) as percentage of TW was independent of both MM ($r=-0.064$; $p=0.637$ for men and $r=-0.062$; $p=0.638$ for women) and of strength ($r=0.039$; $p=0.774$ for men and $r=0.219$; $p=0.112$ for women). Moreover, as muscle strength increased, the protein compartment also increased ($r=0.268$; $p=0.044$ for men and $r=0.475$; $p<0.001$ for women).

### Hydration Indicator Associations with Sarcopenia, Frailty, and Physical Performance

Table 4 shows BIA parameter associations with sarcopenia, dynapenia and frailty. It shows that sarcopenia is related with TBW/FFM, ICW/FFM and ICW(%) in women, and that dynapenia is related with ICW/FFM and ICW(%) in both men and women. TUG values were correlated with ICW as a percentage of TBW ($r=-0.255$; $p=0.056$) in the whole sample, and the ICW/FFM ratio ($r=-0.245$; $p=0.066$) in men, and with ICW as a percentage of TBW ($r=-0.332$; $p=0.012$) and PhA ($r=-0.276$; $p=0.040$) in women.

## Discussion

This study confirms well-known sex differences in body composition and muscle function [20,21]. Regarding body composition, women had lower TBW, ICW, FFM, and PhA and higher FM values than men, while no significant differences were observed in ICW as a percentage of TBW and the ECW/ICW ratio, and only minor differences were observed in the TBW/FFM and ICW/FFM ratios between the sexes. These results suggest that, while men had more TBW due to a greater amount of FFM (and MM), water flowed through the cell membrane in the same way in men and women. Body composition also changed with age in both sexes, through an important reduction in FFM. Although the TBW/FFM ratio remained quite stable in individuals at more advanced ages, the ECW/ICW ratio was slightly higher in the ≥80 year group, possibly explained by a higher prevalence of oedema (ECW) or a relatively reduced ICW due to cell dehydration. As for muscle function, strength, work, and power were greater in men than in women, mainly due to a greater MM, with which those values are strongly correlated. Muscle function also decreased significantly in the older group.

Knee flexion and extension strength, power, and work increased as thigh FFM (mainly represented by MM) also increased, corroborating most of the scientific literature that states that MM is strongly and positively correlated with muscle strength and function [22]. Since TBW and ICW increased as FFM increased, TBW(L) and ICW(L) were – similarly to FFM – associated with muscle function. These results agree with those reported in a similar study of the same age group [12]. However, TBW as a percentage of body weight, which reflects not only FFM but also hydration status, was correlated with muscle work but not with strength or power. Work occurs when strength produces movement and reflects the energy needed to ensure functional capacity. An inability to generate movement means no maintenance or improvement in functional capacity, so the concept of muscle work is of great clinical relevance. In our study, an increased total water percentage in the right leg increased movement capacity (both flexion and extension), and therefore, functional capacity. As just mentioned, the TBW percentage with respect to body weight is mainly an indicator of FFM and is less influenced by hydration status. However, note that a one-litre loss due to dehydration leads to a 1.25-1.50% reduction in TBW as a percentage of body weight, and that a 1% weight loss due to dehydration is enough to affect muscle endurance, power, and strength [23].

Regarding ICW as a percentage of TBW (reflecting cell hydration, or the presence/absence of oedema), this was not correlated with any of the muscle function indicators. The TBW/FFM and ICW/FFM ratios, as indicators of lower-limb hydration status and intracellular hydration status, respectively, showed very small variations, and were not significantly correlated

**Table 3:** Correlations between right-leg bioelectrical impedance analysis parameters and isokinetic parameters (r [p]).

| | TW (L) | TW (%) | ICW (L) | ICW (%) | FFM (kg) | Protein (Kg) | TW/FFM (L/kg) | ICW/FFM (L/Kg) | ICW/ Protein | PhA |
|---|---|---|---|---|---|---|---|---|---|---|
| Women (n=54) | | | | | | | | | | |
| Extension strength | 0.421 (0.002) | 0.177 (0.200) | 0.431 (0.001) | 0.166 (0.230) | 0.423 (0.001) | 0.475 (<0.001) | -0.173 (0.212) | 0.158 (0.253) | -0.419 (0.002) | 0.220 (0.110) |
| Extension work | 0.349 (0.010) | 0.318 (0.019) | 0.341 (0.012) | -0.111 (0.425) | 0.347 (0.010) | 0.378 (0.005) | 0.116 (0.403) | -0.099 (0.475) | -0.353 (0.009) | 0.082 (0.558) |
| Extension power | 0.366 (0.007) | 0.202 (0.143) | 0.370 (0.006) | 0.070 (0.613) | 0.367 (0.006) | 0.406 (0.002) | -0.088 (0.528) | 0.067 (0.628) | -0.431 (0.001) | 0.149 (0.282) |
| Flexion strength | 0.427 (0.001) | 0.125 (0.369) | 0.423 (0.001) | -0.061 (0.660) | 0.427 (0.001) | 0.459 (<0.001) | -0.014 (0.918) | -0.087 (0.533) | -0.370 (0.006) | 0.031 (0.821) |
| Flexion work | 0.335 (0.013) | 0.188 (0.174) | 0.321 (0.018) | -0.166 (0.229) | 0.333 (0.014) | 0.366 (0.006) | 0.140 (0.314) | -0.186 (0.178) | -0.345 (0.011) | 0.013 (0.923) |
| Flexion power | 0.337 (0.013) | 0.090 (0.520) | 0.333 (0.014) | -0.019 (0.892) | 0.338 (0.013) | 0.382 (0.004) | -0.031 (0.825) | -0.049 (0.725) | -0.407 (0.002) | 0.044 (0.750) |
| Men (n=57) | | | | | | | | | | |
| Extension strength | 0.304 (0.021) | 0.207 (0.122) | 0.304 (0.022) | 0.043 (0.748) | 0.305 (0.021) | 0.268 (0.044) | -0.040 (0.770) | 0.044 (0.747) | -0.062 (0.648) | 0.200 (0.135) |
| Extension work | 0.235 (0.078) | 0.304 (0.021) | 0.207 (0.122) | -0.241 (0.070) | 0.230 (0.085) | 0.200 (0.136) | 0.221 (0.098) | -0.245 (0.066) | 0 (1.000) | 0.025 (0.851) |
| Extension power | 0.294 (0.026) | 0.201 (0.133) | 0.278 (0.036) | -0.123 (0.362) | 0.291 (0.028) | 0.248 (0.063) | 0.127 (0.345) | -0.122 (0.367) | -0.030 (0.827) | 0.105 (0.438) |
| Flexion strength | 0.303 (0.022) | 0.236 (0.078) | 0.309 (0.019) | 0.110 (0.416) | 0.305 (0.021) | 0.304 (0.021) | -0.136 (0.312) | 0.102 (0.450) | -0.054 (0.692) | 0.213 (0.111) |
| Flexion work | 0.172 (0.200) | 0.334 (0.011) | 0.163 (0.227) | -0.049 (0.716) | 0.171 (0.202) | 0.164 (0.224) | 0.011 (0.937) | -0.059 (0.665) | -0.053 (0.698) | 0.101 (0.453) |
| Flexion power | 0.258 (0.053) | 0.246 (0.065) | 0.255 (0.056) | 0.025 (0.852) | 0.257 (0.053) | 0.239 (0.073) | -0.029 (0.831) | 0.026 (0.846) | -0.140 (0.298) | 0.169 (0.208) |

**Abbreviations:** ECW: Extracellular Water; FFM: Fat Free Mass; ICW: Intracellular Water; PhA: Phase Angle; TW: Total Right Leg Water.

**Table 4:** Relationship between hydration status indicators and sarcopenia, dynapenia and frailty.

| Mean (SD) | Sarcopenia | | p | Dynapenia | | p | Frailty | | p |
|---|---|---|---|---|---|---|---|---|---|
| | Yes | No | | Yes | No | | Yes | No | |
| Women | | | | | | | | | |
| TBW (%) | 47.7 (1.37) | 44.0 (5.21) | 0.180 | 45.5 (5.35) | 43.8 (5.10) | 0.242 | 43.6 (3.01) | 44.6 (5.26) | 0.596 |
| TBW/FFM (L/kg) | 0.729 (0.005) | 0.735 (0.002) | 0.040 | 73.5 (0.36) | 73.5 (0.24) | 0.789 | 73.4 (0.23) | 73.5 (0.26) | 0.266 |
| ICW (%) | 59.8 (0.06) | 61.0 (0.70) | 0.033 | 60.4 (1.04) | 61.1 (0.52) | 0.026 | 60.7 (1.28) | 61.0 (0.65) | 0.534 |
| FFM (kg) | 32.6 (0.42) | 40.8 (4.56) | 0.017 | 40.2 (5.93) | 40.6 (4.40) | 0.694 | 38.2 (5.05) | 40.4 (4.26) | 0.223 |
| ICW/FFM (L/Kg) | 0.44 (0.00) | 0.45 (0.01) | 0.026 | 0.44 (0.01) | 0.45 (0.004) | 0.010 | 0.45 (0.01) | 0.45 (0.01) | 0.354 |
| PhA | 3.50 (0.14) | 4.76 (0.45) | 0.019 | 4.46 (0.67) | 4.79 (0.43) | 0.161 | 4.43 (0.58) | 4.73 (0.50) | 0.193 |
| Protein (kg) | 6.15 (0.07) | 7.91 (0.90) | 0.020 | 7.74 (1.19) | 7.88 (0.87) | 0.694 | 7.37 (1.06) | 7.82 (0.84) | 0.227 |
| Men | | | | | | | | | |
| TBW (%) | 49.1 (2.50) | 49.8 (4.36) | 0.729 | 48.9 (3.84) | 50.0 (4.41) | 0.448 | 42.5 (1.02) | 55.1 (4.13) | 0.030 |
| TBW/FFM (L/kg) | 0.74 (0.001) | 0.74 (0.003) | 0.265 | 73.8 (0.29) | 73.7 (0.25) | 0.182 | 73.6 (0.14) | 73.7 (0.26) | 0.419 |
| ICW (%) | 60.7 (0.34) | 61.3 (0.77) | 0.225 | 60.8 (0.86) | 61.4 (0.70) | 0.021 | 62.0 (0.04) | 61.3 (0.77) | 0.129 |
| FFM (kg) | 41.8 (0.92) | 54.5 (5.76) | 0.022 | 48.6 (6.57) | 55.3 (5.31) | 0.003 | 54.3 (7.00) | 54.02 (6.16) | 0.983 |
| ICW/FFM (L/Kg) | 0.45 (0.00) | 0.45 (0.01) | 0.488 | 0.45 (0.01) | 0.45 (0.005) | 0.082 | 0.46 (0.0002) | 0.45 (0.01) | 0.193 |
| PhA | 4.55 (0.35) | 5.36 (0.58) | 0.056 | 4.90 (0.58) | 5.44 (0.55) | 0.013 | 5.40 (0.14) | 5.33 (0.60) | 0.845 |
| Protein (kg) | 8.15 (0.07) | 10.6 (1.13) | 0.021 | 9.41 (1.20) | 10.8 (1.05) | 0.002 | 10.8 (1.34) | 10.5 (1.21) | 0.879 |

with strength, work, or power, which all showed much greater between-subject variability. These results may indicate that, as thigh muscle function increases, FFM increases in very similar proportions to TBW and ICW. Hence, muscle function seems to be more related with MM than with hydration status (when measured by BIA).

Protein content is positively correlated with strength in men and with strength, power, and work in women, while the ICW/protein ratio showed no correlation in men and negative correlations in women. These results suggest that the increased thigh MM that accompanied increased muscle strength also increased TBW and ICW, but even more so the protein compartment, especially in women. Our results also indicate that muscle function is much more significantly related to protein content in women compared to men. It is possible that muscle function depends on a protein content threshold that is frequently exceeded by men but not by women.

Regarding PhA, we found no correlation with muscle function, contradicting the findings of other authors, e.g., Yamada et al. [24], who suggested that PhA could be a good indicator of muscle function, and who reported that individuals with dynapenia and sarcopenia compared to those without had significantly lower PhA values, while no difference was observed for pre-sarcopenic individuals. In our study, PhA was likewise correlated with sarcopenia, but no correlation was observed between PhA and muscle strength, work, or power. This lack of correlation may be due to the fact that most of our study participants had quite a robust phenotype, with PhA values above the threshold proposed by Yamada et al. [24] to determine sarcopenia and dynapenia. Thus, the lack of agreement between results of our study versus those of Yamada et al. [24] may be due to our lower mean age (75.0 years vs 81.1 years) and higher mean PhA and lower PhA variability (5.3±0.59 vs 3.96±0.93 in men and 4.7±0.50 vs 3.8±80.76 in women, respectively). Hetherington-Rauth et al. [12], analysing men and women together, reported a significant positive correlation of PhA with lower-limb strength and power. We did not observe a significant correlation between PhA and strength and power, probably because we analysed men and women separately; sex may act as a confounder as it is related with both PhA and muscle function. Kilic et al. [25] reported an independent effect of PhA on sarcopenia; however, their study included both community-dwelling and hospitalized aged subjects, and there was no adjustment for disease severity.

Overall, our study failed to confirm the hypothesis that low-grade dehydration, especially intracellular dehydration, is related to muscle function in older people. The lack of correlation in our study between right-leg hydration indicators and right-thigh muscle function parameters has several possible explanations. Firstly, BIA may not be capable of detecting small differences in hydration status, and especially in intracellular hydration. BIA only directly measures resistance and reactance, so all other BIA parameters are indirectly estimated using formulas and algorithms based on resistance and reactance measurements, on other parameters such as age and sex, and on assumptions regarding specific reference populations. In other words, BIA parameter estimations are based on the same source data and on some assumptions and so are closely related and prone to error. Plasma and urine osmolarity and sodium concentrations may be better indicators of slight variations in hydration status [26,27]. Secondly, our study participants showed similar hydration status, with very small between-subjects variability, which made it difficult to find associations between dehydration parameters and muscle function. Studies are therefore needed that include individuals with a wider range of hydration indicators, as well as prospective longitudinal studies with sufficient follow-up to allow effects on muscle function to emerge. Thirdly, muscle function is determined by a variety of factors, including training, exercise type, nutritional status, inflammation, and comorbidities. The relatively small sample size in our study reflects poor statistical power to identify small differences and to identify independent effects in the multivariate analyses. Finally, the possibility also remains that our hypothesis is faulty, and that muscle function depends, not on hydration status, but on MM and protein compartment size. Nonetheless, we are the opinion that, although not confirmed, this hypothesis cannot be ruled out. As mentioned, the lack of association between hydration indicators and muscle function may be due to low variability in hydration indicators in the study participants, who overall showed good hydration status.

**It is also worth noting the relationship between physical performance (measured with the TUG test) and two indicators of intracellular hydration:** physical performance was negatively and significantly correlated with ICW as a percentage of TBW in both men and women, and almost correlated with the ICW/FFM ratio in men (p=0.066). This result agrees with other published works reporting that intracellular dehydration indicators were related with strength, performance, and frailty risk in aged populations [12-14,16]. The fact that intracellular hydration indicators are related to physical performance and not to muscle strength and power may seem contradictory. This contradiction may be explained by the fact that dehydration not only affects muscles but also concomitantly affects other organs and systems – such as the cardiovascular and central nervous systems, metabolism, thermoregulation, vision and hearing – that also more broadly play a role in coordination, balance, and physical performance. Moreover, apart from published evidence showing that decreased ICW is related with impaired muscle function in young athletes [28], other studies have reported that hypohydration is related to both poor handgrip strength [29] and exhaustion [30] in aged individuals, and especially women. Thus, further well-powered and prospective studies are needed to deepen our understanding of the role played by chronic low-grade dehydration in muscle function in aged populations.

In conclusion, suggesting a process of intracellular dehydration with age is the fact that age is negatively correlated with TBW and ICW (mainly due to FFM loss) and also with the ICW/FFM ratio and PhA values. While, in our study, muscle function was correlated with FFM, MM, and protein compartment size, the correlation with intracellular hydration indicators in aged population was not conclusively demonstrated. Further studies are needed to explore the role played by hydration status in muscle function and physical performance in aged populations.

## Author Statements

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**Conflict of Interest**

None to declare.

**Author Contribution**

MSP contributed to the conception and design of the research; IL, JM, PF and EPa contributed to the acquisition and analysis of the data; MC, EB, EPl contributed to the interpretation of the data; and NSP drafted the article. All authors critically revised the article, agree to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final article.

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**Data Availability**

The data that support the findings of this study are not openly available due to confidentiality norms, but are available from the corresponding author upon reasonable request and Ethical Committee approval.

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