

**Serum osmolarity and haematocrit do not modify the association between the impedance index ( $Ht^2/Z$ ) and total body water in the very old: The Newcastle 85+ Study**

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## **HIGHLIGHTS**

- Bioimpedance analysis (BIA) is commonly used to measure total body water (TBW)
- Information on the accuracy of the leg-to-leg BIA method in the very old (80+ years) is limited
- Hydration status did not modify the association between impedance index and TBW in the very old
- BIA may provide a valid tool to assess nutritional status and improve disease-risk prediction in the very old

## ABSTRACT

Bioelectrical impedance is a non-invasive technique for the assessment of body composition; however, information on its accuracy in the very old (80+ years) is limited. We investigated whether the association between the impedance index and total body water (TBW) was modified by hydration status as assessed by haematocrit and serum osmolarity.

This was a cross-sectional analysis of baseline data from the Newcastle 85+ Cohort Study.

Anthropometric measurements [weight, height (Ht)] were taken and body mass index (BMI) calculated. Leg-to-leg bioimpedance was used to measure the impedance value (Z) and to estimate fat mass, fat free mass and TBW. The impedance index ( $Ht^2/Z$ ) was calculated.

Blood haematocrit, haemoglobin, glucose, sodium, potassium, urea and creatinine concentrations were measured. Serum osmolarity was calculated using a validated prediction equation.

677 men and women aged 85 years were included. The average BMI of the population was  $24.3 \pm 4.2 \text{ kg/m}^2$  and the prevalence of overweight and obesity was 32.6% and 9.5%, respectively. The impedance index was significantly associated with TBW in both men ( $n=274$ ,  $r=0.76$ ,  $p<0.001$ ) and women ( $n=403$ ,  $r=0.96$ ,  $p<0.001$ ); in regression models, the impedance index remained associated with TBW after adjustment for height, weight and gender, and further adjustment for serum osmolarity and haematocrit. The impedance index values increased with BMI and the relationship was not modified by hydration status in women ( $p=0.69$ ) and only marginally in men ( $p=0.02$ ).

The association between the impedance index and TBW was not modified by hydration status, which may support the utilisation of leg-to-leg bioimpedance for the assessment of body composition in the very old.

## 1. INTRODUCTION

The enhanced longevity of the human race is exemplified by the rising number of people aged 80 years or over (very old)(Oeppen and Vaupel, 2002). There are currently three million people aged more than 80 years in the UK and this is projected to almost double by 2030, reaching eight million by 2050(Cracknell, 2010). In 2009 102 million people worldwide were aged 80+ and the number is predicted to almost quadruple to 395 million by 2050(United Nations, 2009).

Accurate assessment of nutritional status in the very old is fundamental to address the lack of clear definition of nutritional requirements in this age group as well as the association between nutritional status with health, disability and survival(Loreck E et al., 2012).

However, nutritional assessment in older people is limited by the predictive inaccuracy of established anthropometric measurements such as body mass index (BMI), waist circumference (WC), and waist-hip ratio (WHR). In fact, these measurements become almost clinically meaningless in the very old(Reis et al., 2009; van Vliet et al., 2010) as many diagnostic features of anthropometry are masked by an increase in the central accumulation of fat and a decrease in appendicular fat mass with age(Enzi et al., 1986; Kyle et al., 2001). Therefore, the attention of nutritionists and geriatricians has shifted towards more detailed measurement of body composition to evaluate the association of adiposity and/or lean body mass with risk of disability and mortality(Woodrow, 2009).

The optimal mode of assessment of body composition in older subjects has to take into account several factors including portability of equipment, non-invasiveness and precision(Siervo and Jebb, 2010). The leg-to-leg bioelectrical impedance (BIA) fulfils all these criteria and therefore could represent a valuable method for assessment of body

composition the very old(Xie et al., 1999). The theoretical principle of BIA is based on the resistance offered by body tissues to the flow of a small electric current, which strongly correlates with the degree of tissue hydration (NIH Statement, 1996). Impedance ( $Z$ ) is a direct measure of tissue conductivity and the stature-adjusted impedance index ( $Ht^2/Z$ ) is utilised in predictive algorithms for the determination of body composition based on a robust association with total body water (TBW) (NIH Statement, 1996). Fat mass (FM) and fat free mass (FFM) can then be derived based on the assumption of a constant hydration of FFM ( $TBW/FFM \approx 0.73$ )(Wang et al., 1999a).

The impedance index is the primary predictor of TBW in BIA-derived predictive algorithms and its accuracy is dependent largely on fluid volume and ionic solute concentrations(NIH Statement, 1996). Therefore, in theory, changes in whole-body hydration status may affect TBW measurements and consequently have an impact on the accuracy of FM and FFM measurements. Serum osmolarity is a biomarker of hydration status as it is directly related to the concentrations of osmotically active solutes impermeable to cell membranes (sodium (Na), glucose, potassium (K), and urea)(Armstrong, 2005). Changes in haematocrit may result from intravascular dehydration usually associated with diarrhoea, third space fluid loss (effusions or oedema) or diuretic use(Armstrong, 2005).

Several studies have indicated minimal changes in the hydration of FFM with ageing(Schoeller, 1989; Wang et al., 1999a). However, because of widespread use of diuretics and self-imposed restrictions in fluid intake, the very old may be at greater risk of dehydration(Hooper et al.) and it is not currently known whether changes in fluid volumes and shifts in extracellular/intracellular water distribution affect performance of the BIA in this age group. Here, we assessed whether objective measures of hydration (i.e. serum

osmolarity and haematocrit concentration) modified the association between the impedance index ( $Ht^2/Z$ ) and TBW using baseline data from the Newcastle 85+ Study.

## **2. MATERIAL AND METHODS**

### 2.1 Participants and Study Protocol

The protocol and baseline findings for the Newcastle 85+ Study have been described in detail elsewhere (Collerton et al., 2007). In short, the Newcastle 85+ Study is a population-based observational study which recruited people born in 1921, who were aged 85 in the year the study commenced (2006) and who were registered with a participating general practice in the Newcastle and North Tyneside area of the UK. Individuals in institutional care were also included. The study was approved by the Newcastle & North Tyneside Local Research Ethics Committee. Where individuals lacked the capacity to give informed consent, consultee approval was sought in accordance with the UK Mental Capacity Act 2005.

Recruitment and baseline assessment took place over a 17 month period in 2006-2007. A multidimensional health assessment (MDHA) - comprising questionnaires (including socio-economic status (income, level of education) and lifestyle), measurements and function tests (including anthropometry, BIA), and a fasting blood sample - was carried out by a trained research nurse at the participant's usual place of residence. General practice medical records were reviewed to obtain information about clinical diagnoses and prescribed medications.

Of the 854 individuals consenting to a baseline MDHA, 778 agreed to fasting blood sampling. For the present analyses, data from 677 participants with complete anthropometric, BIA and clinical biochemistry data were included.

## 2.2 Measurements

*2.2.1 Anthropometry:* Body weight was measured using a digital scale, with the participant wearing light clothes, and approximated to the nearest 0.1kg. In view of the well-recognized difficulties in measuring height in very old people, height was estimated from demi-span. Right arm demi-span was measured to the nearest 0.1cm; final data were the average of two measurements. Height was calculated as:  $[1.35 \times \text{demi-span} + 60.1]$  for women and  $[1.40 \times \text{demi-span} + 57.8]$  for men (Martin-Ruiz et al., 2011). BMI was calculated as body weight divided by squared height in meters and categorized as: underweight ( $<18.5\text{kg/m}^2$ ), normal weight ( $18.5\text{-}24.9\text{kg/m}^2$ ), overweight ( $25.0\text{-}29.9\text{kg/m}^2$ ) and obese ( $\geq 30.0\text{kg/m}^2$ ).

*2.2.2 Leg-to-leg Bioelectrical Impedance:* measurements were conducted using the Tanita-305 body-fat analyzer (Tanita Corp., Tokyo, Japan). Participants were measured standing erect with bare feet placed on the metal sole plates. The impedance value ( $Z$ ) was measured and the impedance index was calculated ( $Ht^2/Z$ ). Total body water, FM and FFM were estimated using the inbuilt prediction equations of the bioimpedance device.

*2.2.3 Clinical Biochemistry:* After an overnight fast, 40 ml blood was drawn from the antecubital vein between 7:00 and 10:30 am. Full blood count, electrolytes (sodium,  $\text{Na}^+$ ; potassium,  $\text{K}^+$ ), urea, creatinine and glucose were measured at the Department of Clinical Biochemistry at Newcastle Royal Victoria Infirmary. The modification of diet in renal disease formula (MDRD) was used to estimate glomerular filtration rate (eGFR) (Manjunath et al., 2001). Participants were classified as having severe renal impairment if eGFR was lower than  $30\text{ml/min}/1.73\text{m}^2$  (Levey et al., 2005).

*2.2.4 Serum Osmolarity:* The equation for serum osmolarity (mOsm/L) developed by Khajuria and Krahn (Khajuria and Krahn, 2005) was used:

$1.86 \times (\text{Na} + \text{K}) + 1.15 \times \text{glucose} + \text{urea} + 14$ , where all components were measured in mmol/L. The equation has been validated in 172 frail older people with and without diabetes (age:  $85.8 \pm 7.9$

years) and was best able to predict serum osmolality compared with 35 other predictive equations (Siervo et al., 2014). Participants were categorised as being normally hydrated (serum osmolality <295 mOsm/L; euhydration), having impending dehydration (serum osmolality 295 to 300 mOsm/L; pre-dehydration), or dehydrated (>300 mOsm/L) (Thomas et al., 2008).

*2.2.5 Data Analysis:* Data are described as mean±s.d. (continuous variables) and percentage (categorical variables). Q-Q plots and the Shapiro-Wilk test were used to test for normality. Variables were normalised before analysis using appropriate transformations. Male and female participants were compared using the T-test for independent samples. Univariate analysis of variance was used to assess the interactive effect of BMI, as a measure of body size, and serum osmolality on impedance index values. Linear regression was used to determine whether hydration status modified the association between the impedance index and TBW. Multiple regression models adjusted for height, weight and gender investigated the influence of biomarkers of hydration (serum osmolality, haematocrit) on the association between the impedance index (independent variable) and TBW (dependent variable, Models 1 and 2, respectively). Next, participants with impaired renal function, diuretic use and previous diagnosis of cancer, heart failure and myocardial infarction were excluded to determine whether diseases or medications affecting water balance modified the association (Model 3). All statistical analyses were carried out using PASW 19 for Windows (Polar Engineering and Consulting, formerly known as SPSS). Statistical significance was set at  $p < 0.05$ .

### **3. RESULTS**

The average BMI of participants was  $24.3 \pm 4.2 \text{ kg/m}^2$  with no significant difference between men and women ( $p=0.18$ ). FFM and TBW were higher in men ( $p < 0.001$  for both), but FM was



higher in women despite lower body weight ( $p < 0.001$ ). These differences in body composition resulted in significant gender-differences in impedance ( $p < 0.001$ ) and impedance index ( $p < 0.001$ ) values. Average serum osmolality was  $294.0 \pm 6.8$  mOsm/L, and was higher in men than women ( $p = 0.01$ ) amongst whom there was a greater prevalence of pre-hydration and dehydration ( $p = 0.03$ ) (**Table 1**).

The impedance index was significantly associated with TBW in both men ( $n = 274$ ,  $r = 0.76$ ,  $p < 0.001$ ) and women ( $n = 403$ ,  $r = 0.96$ ,  $p < 0.001$ ). A regression model including height, weight, gender and the impedance index explained 97% of the TBW variance in the population and all independent variables were significantly associated with TBW ( $p < 0.001$ ). The regression coefficient for the impedance index was  $1889 \pm 38$  m<sup>2</sup>/ohm (Model 1). The addition of serum osmolality and haematocrit to the model did not modify the results. Explained variance and impedance index were essentially unaltered (97% and  $1894 \pm 38$  m<sup>2</sup>/ohm, respectively), whereas serum osmolality and haematocrit were both not significant ( $p > 0.05$ ) (Model 2). The exclusion of participants with conditions affecting whole-body hydration did not modify the results and the impedance index ( $1896 \pm 70.6$  m<sup>2</sup>/ohm) was unchanged (Model 3). Results are detailed in **Table 2**.

The lack of influence of hydration status on the association between the impedance index and TBW was confirmed by the overlap of the regression lines fitted to the data stratified by hydration status (euhydration, pre-dehydration, dehydration) in both men (**Figure 1A**) and women (**Figure 1B**). Finally, the impedance index was significantly associated with BMI in both men and women ( $p < 0.001$ ) but a marginal, significant interaction with hydration status was observed only in men (**Figure 1C and 1D**).

#### 4. DISCUSSION

To our knowledge this is the first study to investigate the performance of a leg-to-leg BIA device in a large sample of octogenarians. Specifically, we tested whether BIA measurements were influenced by hydration status, as assessed by haematocrit and serum osmolality concentration. The association between impedance index and TBW was unmodified by hydration status in both men and women. A small but significant interaction between impedance index and both BMI and hydration status was observed in men, which appears to be related to differences in impedance index values between BMI groups. The very old population is characterised by a high prevalence of multi-morbidity and multi-drug therapy(Collerton et al., 2009) and these factors need to be considered in the analysis of body composition data. Our analyses specifically evaluated the influence of these factors, showing that the association between impedance index and TBW was not influenced by differences in hydration status, diuretic use or multi-morbidity. Therefore, our results may support the use of the leg-to-leg BIA device for the measurement of body composition in the very old and show no evidence of systematic bias related to hydration status.

There is an urgent need for more reliable methods to assess body composition in the very old due to the limited predictive accuracy of anthropometric measurements such as BMI and waist circumference (Sanchez-Garcia et al., 2007; van Vliet et al., 2010). Non-invasive and user-friendly techniques such as the leg-to-leg BIA method may represent valuable solutions for the assessment of body composition in cohorts of very old participants(Siervo and Jebb, 2010; Xie et al., 1999). Furthermore, leg-to-leg BIA devices are portable and can therefore be employed in large epidemiological studies including home-based body composition assessments. Newer leg-to-leg BIA devices provide measurements of segmental body composition (i.e. central fat mass and appendicular lean body mass), which may improve risk prediction models linked to obese and/or sarcopenic phenotypes(Pietrobelli et al., 2004).

The paucity of body composition data from very old populations complicates the comparison of our results with previous studies. Powers et al reported no correlation between urine osmolality and TBW measured by tetrapolar BIA in 63 older people aged 78.5 years (age range: 66-95years)(Powers et al., 2012). The use of a tetrapolar BIA device precludes a direct comparison of the results of Powers et al. with our findings. In addition, they did not investigate the relationship between urine osmolality and the impedance index. Our study found a weak correlation ( $r=0.14$ ,  $p<0.001$ ,  $n=677$ , data not shown) between serum osmolality and TBW which may be explained by a greater sensitivity of blood-derived biomarkers in assessing differences in hydration status(Armstrong, 2005).

Our study did not test the accuracy of the leg-to-leg BIA device for the measurement of FM, FFM and TBW, which would require the utilisation of reference body composition methods such as deuterium dilution, dual-energy X-ray absorptiometry (DXA) or 4-compartment models(Siervo and Jebb, 2010). The limitations of the BIA methodology are recognised and several reviews have discussed these issues(Kyle et al., 2004; NIH Statement, 1996). To our knowledge three studies have attempted to validate the leg-to-leg BIA in older subjects(Mally et al., 2011; Ritchie et al., 2005; Xie et al., 1999). The first from Ritchie et al. recruited 50 subjects aged 55 years and older (age range: 56-94 years) but the interpretation of the results is biased by the lack of a body composition reference method since the leg-to-leg BIA was compared with a tetrapolar BIA device(Ritchie et al., 2005). Second, Xie et al. recruited post-menopausal women aged 51 - 63 years and employed DXA as a reference method; the considerably younger age group studied and methodological differences with our study limit the comparability of the results(Xie et al., 1999). In addition, the study from Xie et al(Xie et al., 1999) reported highly reproducible measurements of the leg-to-leg BIA at a population

level but the accuracy at individual level was modest. The third study from Mally et al. assessed the reliability and accuracy of segmental leg-to-leg BIA against DXA in 72 healthy older people (mean age: 69 years). Again they found a modest accuracy of the leg-to-leg BIA for the assessment of segmental muscular mass and FM at the individual level(Mally et al., 2011).

The measurement of TBW is a cornerstone for the assessment of body composition using BIA. The calculation of FFM from TBW depends on an assumption of constant hydration of the FFM compartment, which may be modified in the very old(Schoeller, 1989; Wang et al., 1999b). However, the impact of hydration status in the very old on body composition by BIA is largely unknown. Regardless of the attempt to minimize errors by the use of age-specific equations, the majority of studies included participants who were < 85 years.

Our study has some limitations which need to be taken into account for the interpretation of the results. First, the limitations of the BIA method for the assessment of TBW need to be acknowledged. BIA provides an indirect assessment of TBW and other body composition outcomes (FM, FFM), which are then calculated using specific predictive algorithms validated against body composition reference methods such as deuterium dilution or DXA. Reflecting the composition of the 85+ population in Newcastle upon Tyne (which is similar to that of the UK as a whole), the results are largely derived from Caucasian participants (~99% of the study population) and therefore the application of our results to other ethnic groups may not be appropriate. All participants were of same birth cohort 1921 and assessed around 85<sup>th</sup> birthday and therefore the extrapolation of these results to people in their 90's and beyond may be inaccurate, though the mean impedance index value for women in our study was similar to values measured in younger women reported in the study of Xie et al<sup>14</sup>. Serum osmolarity was determined using a validated prediction equation(Khajuria and Krahn, 2005), chosen from 36 others, and the equation was validated in a sample of 172 older subjects (mean age: 85 years).

Strengths of this study include the large sample size of our study provides further support to these results by minimising error variability.

## **5. CONCLUSIONS**

The association between the impedance index and TBW was not modified by hydration status, assessed by serum osmolarity and haematocrit, in a large population-based sample of octogenarians. The leg-to-leg BIA system can be recommended in cohorts of very old participants to obtain reproducible measurements of body composition and improve the assessment of nutritional status and disease-risk prediction in the very old. Targeted nutritional and lifestyle interventions could be developed to correct and/or maintain normal body composition in the very old as well as monitor the efficacy of the interventions by integrating body composition measurements into geriatric multi-dimensional protocols for the assessment of health status, mental performance and physical capability. The results of these studies could assist with the early prediction of health deterioration to maintain physical independence and increase life expectancy in good health. However, we also recognise that further studies are warranted to evaluate the accuracy of the leg-to-leg BIA with reference body composition methods (i.e., deuterium dilution, DXA or multi-compartment models) in the very old and study the biological and lifestyle factors associated with age-related changes in the composition of FFM.

## **AUTHOR CONTRIBUTIONS**

The analysis was conceived by MS and CJ. MS and CP analysed the data and co-wrote the manuscript. All authors contributed to subsequent analyses and interpretation. All authors contributed to the final revision of the manuscript.

The Corresponding Author (MS) is the guarantor for the manuscript and had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final version of the paper.

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## **CONFLICT OF INTEREST STATEMENT**

MS has received conference travel expenses from Tanita UK Ltd. All other authors have no conflicts of interest to declare.

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## FIGURE LEGENDS

**Figure 1:** Linear regression to evaluate whether osmolarity modified the association between impedance index and total body water (TBW) in male (Fig 1A) and female (Fig 1B) subjects. Difference in impedance index between males (Fig 1C) and females (Fig 1D) stratified by osmolarity and body mass index (BMI) category. Univariate analysis of variance was used to detect difference between groups (BMI, Osmolarity) and evaluate their interaction (BMI\*Osmolarity). Regression equations ( $y = \text{intercept} \pm \text{regression coefficient} * x$ ) and coefficient of determinations ( $R^2$ ) are showed.

| <b>Table 1: Characteristics of the study population stratified by gender</b> |                        |                         |                           |          |
|--|------------------------|-------------------------|---------------------------|----------|
|  | <b>All<br/>(N=677)</b> | <b>Male<br/>(N=274)</b> | <b>Female<br/>(N=403)</b> | <b>p</b> |
| <b>Weight (kg)</b>   | 64.0±12.8              | 70.6±11.7               | 59.5±11.5                 | <0.001   |
| <b>Height (m)</b>  | 1.61±0.08              | 1.69±0.05               | 1.57±0.04                 | <0.001   |
| <b>BMI (kg/m<sup>2</sup>)</b>  | 24.3±4.2               | 24.6±3.7                | 24.2±4.6                  | 0.18     |
| <b>BMI Groups (%)</b>  |                        |                         |                           |          |
| <i>Underweight</i>   | 6.5                    | 3.3                     | 8.7                       | 0.01     |
| <i>Normal Weight</i>   | 51.4                   | 50.7                    | 51.9                      |          |
| <i>Overweight</i>  | 32.6                   | 37.6                    | 29.3                      |          |
| <i>Obese</i>   | 9.5                    | 8.4                     | 10.2                      |          |
| <b>Diuretic Use (%)</b>  | 41.9                   | 34.7                    | 46.9                      | 0.001    |
| <b>Heart Failure (%)</b>   | 11.1                   | 11.7                    | 10.7                      | 0.38     |
| <b>Myocardial Infarction (%)</b>   | 14.8                   | 21.9                    | 9.9                       | <0.001   |
| <b>Cancer (%)</b>  | 25.0                   | 29.6                    | 21.8                      | 0.01     |
| <b>FM (kg)</b>   | 18.7±7.5               | 17.2±7.0                | 19.7±7.7                  | <0.001   |
| <b>FFM (kg)</b>  | 45.3±9.1               | 53.4±6.4                | 39.8±6.0                  | <0.001   |
| <b>TBW (L)</b>   | 33.1±6.5               | 39.1±4.7                | 29.0±4.1                  | <0.001   |
| <b>Impedance (ohm)</b>   | 502.2±106.0            | 467.0±84.3              | 526.0±112.5               | <0.001   |
| <b>Impedance Index (m<sup>2</sup>/ohm)</b>                                   | 0.005±0.001            | 0.006±0.001             | 0.004±0.001               | <0.001   |
| <b>Haemoglobin (g/dL)</b>  | 13.1±1.5               | 13.6±1.6                | 12.8±1.4                  | <0.001   |
| <b>Haematocrit (%)</b>   | 40.2±4.9               | 42.5±5.1                | 39.4±4.5                  | <0.001   |
| <b>Glucose (mM/L)*</b>   | 5.3±1.4                | 5.2±1.0                 | 5.3±1.6                   | 0.56     |
| <b>Sodium (mM/L)</b>   | 139.0±2.9              | 139.1±2.8               | 138.9±3.0                 | 0.44     |
| <b>Potassium (mM/L)</b>  | 4.2±0.3                | 4.3±0.3                 | 4.2±0.3                   | 0.01     |
| <b>Creatinine (µM/L)*</b>  | 107.6±41.8             | 122.2±54.9              | 97.7±25.6                 | <0.001   |
| <b>Urea (mM/L)*</b>  | 7.4±3.5                | 7.9±4.1                 | 7.0±2.9                   | 0.001    |
| <b>eGFR</b>  | 54.8±13.7              | 57.3±14.9               | 53.0±12.5                 | <0.001   |
| <b>Serum Osmolarity (mOsm/L)</b>   | 294.0±6.8              | 294.8±6.6               | 293.5±6.9                 | 0.01     |
| <b>Hydration Group (%)</b>   |                        |                         |                           |          |
| <i>Euhydration</i>   | 53.5                   | 47.4                    | 57.6                      | 0.03     |
| <i>Pre-Dehydration</i>   | 28.7                   | 31.8                    | 26.6                      |          |
| <i>Dehydration</i>   | 17.9                   | 20.8                    | 15.9                      |          |

Data are described as mean±s.d. (continuous variables) and percentage (categorical variables). BMI= body mass index; FM= fat mass; FFM= fat free mass; TBW= total body water; eGFR= estimated glomerular filtration rate. Difference between male and female groups was assessed using the unpaired t test (continuous variables) and Chi-Square test (categorical variables). \*Variables were transformed before the analysis.

| <b>Table 2: Multiple regression analysis to evaluate whether measures of hydration (serum osmolarity, haematocrit concentration) modify the association between the impedance index and total body water (dependent variable). All models were adjusted for height, weight and gender.</b> |                 |        |                 |        |                 |        |
|--|-----------------|--------|-----------------|--------|-----------------|--------|
|  | Model 1 (N=677) |        | Model 2 (N=677) |        | Model 3 (N=252) |        |
|  | B±SE            | p      | B±SE            | p      | B±SE            | p      |
| Height (cm)  | 0.07±0.01       | <0.001 | 0.07±0.01       | <0.001 | 0.08±0.01       | <0.001 |
| Weight (kg)  | 0.1±0.005       | <0.001 | 0.1±0.005       | <0.001 | 0.1±0.007       | <0.001 |
| Gender   | -4.3±0.1        | <0.001 | -4.3±0.1        | <0.001 | -4.5±0.2        | <0.001 |
| Impedance Index (m <sup>2</sup> /ohm)  | 1889±38         | <0.001 | 1894±38         | <0.001 | 1896±71         | <0.001 |
| Serum Osmolarity (mOsm/L)  | -               | -      | -0.007±0.006    | 0.24   | 0.01±0.01       | 0.40   |
| Haematocrit (%)  | -               | -      | 0.97±0.92       | 0.29   | -3.5±1.4        | 0.02   |
| R <sup>2</sup>   | 0.97            | <0.001 | 0.97            | <0.001 | 0.97            | <0.001 |

B= unstandardized regression coefficient; SE= standard error; R<sup>2</sup>= coefficient of determination. Model 1 (independent variables): height, weight, gender, impedance index; Model 2 (independent variables): Model 1+ serum osmolarity, haematocrit; Model 3 (independent variables): same as Model 2 but participants with impaired renal function, diuretic use and previous diagnosis of cancer, heart failure and myocardial infarction were excluded from the analysis.