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Original article

# Estimation of total body water and extracellular water with bioimpedance in athletes: A need for athlete-specific prediction models

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SUMMARY

*Purpose:* Bioelectrical impedance analysis (BIA) equations can predict total body water (TBW) and extracellular water (ECW) in non-athletic healthy populations. This study aimed: a) to develop BIA-based models for TBW and ECW prediction based on dilution methods in a sample of national level athletes; and b) to validate the new models with a cross-validation approach in a separate cohort using dilution methods as criterion.

*Methods:* Two hundred and eight highly trained athletes (21.3 ± 5.0 years) were evaluated during their respective competitive seasons. Athletes were randomly split into development (n = 139) and validation groups (n = 69). The criterion method for TBW was deuterium dilution and for ECW was bromide dilution, where ICW was the respective difference between both. Resistance (R) and reactance (Xc) were obtained with a phase-sensitive 50 kHz BIA device and used for the estimation of TBW and ECW.

*Results:* Athletic BIA-based models were developed for TBW and ECW [$TBW = 0.286 + 0.195*S^2/R + 0.385*Wt + 5.086*Sex$; $ECW = 1.579 + 0.055*S^2/R + 0.127*Wt + 0.006*S^2/Xc + 0.932*Sex$, where sex is 0 if female or 1 if male, Wt is weight (kg), S is stature (cm), and R and Xc are in ohm (Ω)]. Cross validation revealed $R^2$ of 0.91 for TBW and $R^2$ 0.70 for ECW and no mean bias.

*Conclusions:* The new equations can be considered valid, with no observed bias, thus affording practical means to quantify TBW and ECW in national level athletes.


## 1. Introduction

The benefits and importance of an appropriate hydration for health and performance are well known with total body water (TBW) being comprised of both intracellular (ICW) and extracellular (ECW) with a flux existing between the two [1]. Criterion methods for measuring TBW (deuterium oxide) and ECW (sodium bromide) are expensive, impractical for most settings, and impossible for determining fluid shifts in short, discreet time intervals [2].

The aforementioned limitations revealed the need for easier but valid field-based instruments, hence bioelectrical impedance serves this purpose (BIA) [3]. BIA is generally cheaper than more laboratory based methods and the maintenance is fairly straightforward. These advantages prompted the development of several predictive models based on 50 kHz single frequency phase-sensitive BIA using tracer dilution techniques as criterion. As reviewed by Kyle [3,4], for TBW estimation, 50 kHz single frequency BIA-based models were developed in children, in adults, in obese participants, and in chronic disease patients, while for the ECW estimation, 50 kHz single frequency BIA-based models were developed in healthy subjects, in paraplegia and in surgical

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patients. In addition ECW has been predicted using other frequencies, namely 5 kHz, 224 kHz, and the resistance of the extracellular fluid (R0 or Re) obtained by modelling procedures in bioelectrical impedance spectroscopy.

The ICW compartment is determined as the difference between the TBW and ECW compartments. It has been recently shown that reductions in the ICW compartment decrease strength and power in elite judo athletes and leg strength and jumping height over a season in basketball, handball and volleyball players [5–7]. These findings further support the important role of an effective monitoring of the water distribution volumes (TBW, ECW, and ICW) in physical performance.

Recently it has been suggested that assessment of water distribution volumes among athletes should use a phase-sensitive BIA instrument that provides measurements of R and Xc instead of volume estimations automatically calculated by the BIA device with generalized regression equations without justification or equations that were not validated for this specific population [8]. Athletes differ from the general population specifically with sport-specific differences in body geometry, that almost always are ignored by researchers despite awareness that geometry is a factor affecting bioimpedance measurements, and that BIA prediction equations are sample-specific [3]. Thus, to date there are no phase-sensitive BIA-based predictive models for TBW and ECW that have been specifically developed for athletes using reference dilution methods, and cross-validated in an athletic sample [8].

The purpose of this study was two-fold: develop a 50 kHz BIA-based model for estimating TBW and ECW in a convenient sample of national level athletes (male and female) using dilution techniques as the criterion method, and cross-validate the new prediction models, in a separate group of national level male and female athletes. A secondary objective was to determine the performance of previous non-specific published models in our sample of athletes.

## 2. Methods

### 2.1. Participants

Using a cross-sectional design, a total of 208 athletes (138 males, 70 females) were evaluated during their respective competitive seasons. The sample consisted of athletes spanning 15 sports: basketball (21 females, 26 males), handball (2 females, 16 males), judo and wrestling (2 females, 3 males), karate and taekwondo (4 females, 6 males), pentathlon (1 female, 2 males), rugby (9 males), soccer (1 male), swimming (14 females, 19 males), track and field athletics (sprinters, hurdlers, and jumpers) (6 females, 11 males), triathlon (5 females, 24 males), and volleyball (15 females, 17 males); tennis (2 males); sailing (2 males). The inclusion criteria were as follows: 1) Tanner stage V or greater (determined by self-evaluation) [9]; 2) > 10 h of sport specific training per week; 3) free from performance-enhancing drugs specifically and any medication in general and 4) free from the consumption of alcohol and caffeinated beverages for at least 15 h prior to testing. Informed consent was obtained from each participant and/or guardian if under the age of legal consent prior to testing. All procedures were approved by the Ethics Committee of the University of Lisbon and the investigation was conducted according to the guidelines reported in the Declaration of Helsinki [10].

### 2.2. Body composition measurements

Testing began promptly at 08:00 after an overnight fast lasting at least 12 h with at least 15 h from the last exercise session.

#### 2.2.1. Anthropometric measurements

All participants were weighed (nearest 100th of a gram) in minimal clothing (i.e. swimsuit) using the scale interfaced with the plethysmograph (BOD POD© Cosmed, Rome, Italy), while stature was measured to the nearest 10th of a cm using a wall stadiometer (Seca, Hamburg, Germany) using standardized procedures as reported elsewhere [11].

#### 2.2.2. Hydration status

The specific gravity (USG) was determined using a refractometer (Urisys 1100, Roche Diagnostics, Portugal) from a fasting baseline urine sample to ensure that all athletes were euhydrated (well hydrated USG <1.010) [12]. The coefficient of variation (CV) of the urine specific gravity procedure in our laboratory based on 10 active adults is 0.1% [13].

#### 2.2.3. Fat mass (FM) and fat free mass (FFM)

Body Composition, specifically % fat mass (%FM), total fat mass (FM), and fat free mass (FFM), was determined by dual-energy X-ray absorptiometry (Hologic Explorer W, QDR for windows version 12.4, Waltham, MA, USA) as described elsewhere [14]. In our laboratory, in ten healthy adults, the test–retest CV for both FM and FFM is 0.8% and 1.7%, respectively [14].

#### 2.2.4. Reference total body water

Following the collection of a baseline urine sample, each participant was given an oral dose of 0.1 g of 99.9% $^2H_2O$ per kg of body weight (Sigma–Aldrich; St. Louis, MO) for the determination of TBW by deuterium dilution using a Hydra stable isotope ratio mass spectrometer (PDZ, Europa Scientific, UK). Subjects were encouraged to void their bladder prior to the 4-h equilibration period and subsequent sample collection, due to inadequate mixing of pre-existing urine in the bladder [2]. Urine samples were prepared for $^1H/^2H$ analyses using the equilibration technique by Prosser and Scrimgeour [15] as described by our group previously [16]. Our laboratory has reported a CV in ten subjects for TBW of 0.3% [16].

#### 2.2.5. Reference extracellular water

ECW was assessed from a baseline saliva sample using the sodium bromide (NaBr) dilution method after the subject consumed 0.030 g of 99.0% NaBr (Sigma–Aldrich; St. Louis, MO) per kg of body weight, diluted in 50 mL of distilled-deionized water as described by our group previously [17].

The test–retest CV in 7 participants for the ECW using high performance liquid chromatography in our laboratory is 0.4% [17].

#### 2.2.6. Reference intracellular water

ICW was calculated as the difference between TBW and ECW.

#### 2.2.7. Single frequency bioelectrical impedance analysis

Whole body resistance (R) and reactance (Xc) were obtained by BIA using a single frequency, phase-sensitive 50 kHz (BIA-101, RJL/Akern Systems, Firenze, Italy), as described by others [18]. All subjects were instructed to lie in a supine position for 10 min (serving as an equilibration period) with electrodes placed on the right ankle and wrist. Using a 50 kHz frequency, a 0.8-mA alternating current was placed into the distal electrode (source electrode) of each pair, whereas using the proximal electrode (detector electrode) the voltage drop through the body is determined. Prior to each test the analyzer was calibrated with the calibration deemed successful if R value is 383 Ω and Xc equal to 46 Ω. The test–retest CV in 10 participants in our laboratory for R and Xc is 0.3% and 0.9%, respectively.