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# Bioimpedance parameters in adolescent athletes in relation to bone maturity and biochemical zinc indices

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ABSTRACT

Phase angle (PA) is derived from resistance and reactance determined by bioimpedance analysis (BIA) and it appears to relate to cellular stability and integrity. Interpretation of PA values could be complemented by bioelectrical impedance vector analysis (BIVA), which relates to body hydration and structure. Body composition, age, sex, and nutrients are known to stabilize cell membranes, such as zinc, have been related to PA although information is scarce in adolescent athletes. The aim of the present study was to assess the association of body composition, skeletal maturity and zinc biochemical indices with phase angle and bioelectrical impedance parameters, in forty male adolescent soccer athletes (13.4 ± 0.6 years). BIA was performed with a single-frequency tetrapolar analyzer. PA and BIVA were determined using resistance and reactance BIA data. Plasma and erythrocyte zinc concentrations were measured using inductively coupled plasma-optical emission spectrometry. Body composition was determined by dual-energy X-ray absorptiometry, and bone age by hand X-ray measurements. PA was higher in adolescents classified by bone age as "Early" (6.8 ± 0.9°) compared to "Late" (5.7 ± 0.5°) ($p < 0.05$). PA correlated with bone age ($r = 0.562$), BMI ($r = 0.382$), fat-free mass ($r = 0.468$), and erythrocyte zinc ($r = 0.379$) ($p < 0.05$). BIVA confidence ellipses were sensitive to skeletal maturity status. Phase angle was higher in adolescents with erythrocyte zinc concentration above the median (> 0.66 μmol.g hemoglobin$^{-1}$) compared to those below the median. Multiple linear regression analysis showed that bone age ($B = 0.254$, $p = 0.001$) and erythrocyte zinc concentration ($B = 1.168$, $p = 0.047$) were significantly related to PA in this group, and accounted for 34% of its variability. Our results indicate that bone age and zinc erythrocyte contribute to PA values in the young male soccer athletes and that BIVA is influenced by skeletal maturity status in this group.

## 1. Introduction

PA has been related to cellular integrity and functionality, and it has been related to sex, age and body composition [1,2]. BIVA may be useful for clinical purposes because of its ability to detect changes in hydration or body composition, as has been shown in adults, adolescents, and adolescent athletes [3–5]. In a recent study, fat-free mass, height and extracellular to intracellular water ratio were the most significant PA predictors in healthy men and women adults [6]. However, in adolescents, information on PA predictors is scarce, particularly in adolescent athletes. In Brazilian non-athlete adolescents, PA values were higher in boys than in girls and were positively related to age, possibly due to increased biological maturity [7].

Skeletal age is widely recognized as the best-isolated indicator of biological maturity [8,9]. Bone age has been the most commonly used indicator in studies on growth and development [10]. Therefore, skeletal maturity assessed by bone age may contribute with relevant information in studies of PA determinants in adolescents.

Nutrients such as PUFA, alpha-tocopherol, magnesium, and zinc are recognized as cellular membrane stabilizers [11–14], although only few studies evaluated relationships with PA. An association between PA and erythrocyte PUFA was observed in swimmers [15], and between PA and serum and erythrocyte magnesium in judo athletes [16]. One randomized placebo-controlled study observed a significant increase in PA and in fat-free mass sensitive to BIVA, after zinc supplementation in pre-pubertal children (8–9 y) [17]. There are no studies evaluating PA and BIVA taking into account skeletal maturity, and relating PA to biochemical zinc indices in adolescent athletes.

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Considering zinc functions [18], and other variables related to PA values, the aim of the present study was to assess the associations of body composition, skeletal maturity and zinc biochemical indices with PA and BIVA in male adolescent soccer athletes.

## 2. Materials and methods

This was a cross-sectional study with 40 adolescent male soccer athletes (chronological age 13.4 ± 0.6 years) from Botafogo Soccer Club (Rio de Janeiro), who have joined the junior team in the last 5 years. Information regarding the period of time (hours per week) spent in soccer training sessions was collected from individual interviews. Participants were not taking any dietary supplements (vitamins or minerals) and were instructed not to change their diet during the study period.

This study was approved by the Ethics in Research Committee of the Pedro Ernesto Hospital at the State University of Rio de Janeiro (Brazil). After detailed explanation about the study, the athletes or a legal tutor for the athletes under legal age signed an informed consent form.

### 2.1. Dietary orientation

All athletes were receiving regular nutritional orientation at their Institution based on the Dietary Health Guide for the Brazilian Population [19] that encourages the intake of minimally processed foods. The recommendation consisted of all food groups in a normal Brazilian diet (grains, bread, vegetables, dairy, meat, fruits and fruit juice, and oil). The adolescents were instructed not to change their dietary habits during the study.

### 2.2. Anthropometric measurements

Trained research staff measured adolescents' height and weight using standardized procedures and equipment. Height was measured to the nearest millimeter using a portable stadiometer in millimeter (Sanny®). Body weight was determined to the nearest 0.1 kg, using an electronic scale (Filizola®). BMI was calculated as total body mass (kilograms) divided by height (meters) squared. The nutritional status of the participants was evaluated by BMI *z*-score according to WHO [20].

### 2.3. Body composition

Dual-energy X-ray absorptiometry (DXA) measurements were taken using a total-body scanner (Lunar Prodigy Advance – General Electrics TM, Chalfont St. Giles, United Kingdom). All scanning was performed by the same trained operator and followed standard quality control procedures according to the manufacturer's technical data. Measurements on the calibration block (daily) and on the calibration spine phantom (weekly) supplied by the manufacturer had coefficients of variation < 0.7%. Body composition, i.e., fat mass (kg), body fat mass (percentage), and fat free mass (kg) were derived from the total body scan in each participant.

### 2.4. Sample collection

Blood (5 mL) samples were collected after an overnight fast and after 24 h without physical exercise. The blood was collected using antecubital vein puncture into heparinized (30U per tube) mineral-free tubes. Blood samples were centrifuged at 1800*g* for 10 min for separation of plasma, and the erythrocyte cells were washed three times with ice-cold 0.9% NaCl. The washed cells were lysed with an equal volume of ice-cold de-ionized water. Aliquots of erythrocyte lysate samples were stored at −20 °C until analyzed.

### 2.5. Laboratory assays

According to Oliveira et al. [21] the plasma was separated from the whole blood by centrifugation at 4 °C. The buffy coat was removed from the erythrocyte pellet, and an equal volume of ice-cold 0.9% NaCl was added. After being inverted several times, the tubes were centrifuged again; this process was repeated twice. The washed cells were lysed by addition of ice-cold doubly deionized water (1:1.4).

To separate the erythrocytes and then measure zinc levels the method described by Argaval & Henkin [22] was used. The erythrocyte mass obtained was washed three times with 5 mL of 0.9% saline, slowly homogenized by inversion and centrifuged again at 10,000*g* for 10 min (Sorvall® RC-SB) at 4 °C, after which the supernatant was discarded. Following the final centrifugation, the saline solution was aspirated and the erythrocyte mass was carefully extracted using a micropipette, transferred into demineralized Eppendorf tubes and stored at −20 °C, until measurement of zinc levels. Zinc in lysed erythrocytes was determined after overnight nitric acid (Suprapur, Merck) digestion of samples at 105 °C and appropriate dilution with deionized water. Plasma and erythrocyte zinc concentrations were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES, Optima 4300 DV, Perkin Elmer, USA PerkinElmer). Accuracy was validated by concordant results obtained from a reference material (whole blood, Seronorm™, lot 4040109, NY AS, Pharma Diagnostics). Results were expressed as μmol $L^{-1}$ for plasma zinc concentration, and μmol g hemoglobin $^{-1}$ for zinc erythrocyte concentration.

Hemoglobin, hematocrit percentage and erythrocyte count in blood were measured by using an electronic hematology analyzer (Cell-Dyn/Cobas Vega, USA).

All measurements were taken in triplicates and intra-assay coefficients of variations (CV%) were lower than 5%.

### 2.6. Skeletal maturity

Skeletal maturity was evaluated using bone age, according to Tanner-Whitehouse 3 method (TW3) [23] based on X-ray measurements in 13 bones of the left hand. The X-ray radiation dose was within the range of 3–5 mrem (0.003–0.007 rads), corresponding approximately to 5% of the allowed annual dosage [24]. The test-retest reproducibility of bone maturity assessments was very high in correlation within observers ($r = 0.9610$, $p < 0.0001$; $t = 0.4118$; $p = 0.68$) and between observers ($r = 0.7003$, $p < 0.0001$; $t = 0.1033$; $p = 0.91$). Chronological age (decimal age) was calculated as the difference between dates of birth and of the radiograph.

The participants were classified into three maturity categories according to the skeletal stage calculated as the difference between bone age and chronological age, both in years: "On time", when difference was between −1 to +1 years; "Late" (delayed), when difference was < −1 year; and "Early" (advanced), when difference was > +1 year [25].

### 2.7. Bioelectrical impedance analysis (BIA)

The bioelectrical parameters R (Ohms) and Xc (Ohms) were determined with a single-frequency tetrapolar impedance analyzer (RJL, model 101 Quantum; RJL Systems, Clinton Township, MI), which applies a current of 800 μA at an operating single frequency of 50 kHz. Whole body impedance measurements were taken using the standard positions with outer and inner electrodes on the right hand and foot [26].

PA is derived of the relation between R and Xc, where R is the opposition to alternating electric current flow exerted by intracellular and extracellular ionic solutions, and Xc is defined as the delay in the conduction of the applied current by cell membranes and tissue interfaces [2,27]. Since part of the electric current is temporarily stored in cell membranes, a phase shift or PA can be quantified as the angular