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 1800g 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 10,000g 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 $\mu mol\,L^{-1}$ for plasma zinc concentration, and $\mu mol\,g$ 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

transformation of the ratio Xc/R. Therefore, PA has been related to important cellular characteristics such as membrane integrity as well as permeability and water distribution between intra and extracellular spaces. Higher PA values are indicative of more intact cell membranes and higher body cell mass [2,28–30]. However, lower PA values usually are associated with disease severity and worse clinical prognosis [2].

A reliable hydration status assessment was obtained by instructing the volunteers not to exercise 24 h prior to the exam, to be in overnight fast, and to refrain from drinking 4 h before the exam. BIA measurements were taken after a 5-min rest, with the participants in the supine position, in a thermo-neutral environment of 25 °C. Before each testing session, the analyzer was checked with a calibration circuit of known impedance (resistance = 500.0 ohms; reactance = 0.1 ohms, 0.9% error). Total body water (kg) [31] (Eq. (1)) and PA [30] (Eq. (2)) were calculated according to the following the equations, respectively:

$$TBW = 0.286 + 0.195 \times H/R + 0.385 \times Wt + 5.086 \times Sex \qquad (1)$$

Where sex is 0 if female or 1 if male, Wt is weight in kg, H is height in cm, R is resistance.

$$PA\ (^0) = arctanXc(Ohms)/R(Ohms) \times 180°/\pi \qquad (2)$$

2.8. Body impedance vector analyses (BIVA)

The BIVA confidence, developed by Piccoli et al. [3], uses the plot of impedance parameters resistance (R) and reactance (Xc) determined as previously described, normalized per height as a bivariate vector in the RXc confidence graph. The normalization per height allows for the length of the conductor and thus provides a qualitative measure of soft tissue that does not depend on body size. The correlation between R and Xc determines the ellipsoidal form of the bivariate probability distributions. The position and length of the vector provides information on hydration status, body cell mass and cell integrity. The length of the vector indicates hydration status, from fluid overload (decreased resistance, short vector) to dehydration (increased resistance, longer vector). The vector distribution is described by its associated 95% confidence interval (confidence ellipse). In the present study, BIVA results were not compared with a reference population because there are no published data for male adolescent athletes.

2.9. Statistical analysis

Statistical analyses were performed using SPSS version 17.0 software package (IBM, USA). Continuous variables were expressed as means ± standard deviation (SD), and categorical variables as absolute numbers and percentages. One-way analysis of variance (ANOVA) and Duncan's post hoc test was used to determine whether there were any statistically significant differences between groups using "On time" as reference group. Independent *t*-test was used to compare "Early" and "Late" groups. The linear associations between PA and other continuous variables were measured with Pearson's correlation coefficient. Bone age, fat free mass and zinc-erythrocyte were initially included as independent variables in the linear regression model with backward elimination, adjusting for BMI *z*-score. Multiple linear regression analysis was performed to identify combined significant associations with PA, adjusting for BMI *z*-score. Results were presented as B unstandardized coefficients, 95% confidence intervals (95% CI) and p-values. Concerning BIVA confidence, all analyses were performed using BIVA software [4]. The differences between the mean impedance vectors in the different groups of male adolescent soccer athletes ("Late", "On time" and "Early") was determined using Hotteling T^2 test. The distances between ellipses were calculated using the Mahalanobis test; it is a descriptive statistics that provides a relative measurement of data point distances (residual) between vectors. The BIVA software was kindly provided by Dr. Antonio Piccoli (Institute of Internal Medicine,

Table 1
Anthropometric characteristics, body composition, zinc biochemical indices, and bioelectrical data according to bone maturity in male adolescent soccer athletes.

	Skeletal maturity		
	On time (n = 18)	Early (n = 10)	Late (n = 12)
Anthropometric characteristics			
Total body mass, kg	50.2 ± 4.4	59.9 ± 9.6[#]	36.9 ± 4.8[#, *]
Height, m	1.63 ± 0.06	1.61 ± 0.05	1.52 ± 0.06[#,*]
BMI, kg/m^2	18.7 ± 1.4	21.2 ± 2.2[#]	16.6 ± 1.2[#,*]
BMI, z-score	0.01 ± 0.44	0.69 ± 0.54[#]	−0.73 ± 0.46[#,*]
Body composition			
Fat mass$_{DXA}$, kg	7.0 ± 3.7	7.2 ± 4.6	4.9 ± 1.9
Fat mass$_{DXA}$, %	13.9 ± 7.5	12.1 ± 5.6	13.2 ± 4.5
Fat free mass$_{DXA}$, kg	42.3 ± 4.1	48.4 ± 6.4[#]	30.5 ± 4.4[#,*]
Total body water$_{BIA}$, kg	24.7 ± 0.7	28.4 ± 3.7[#]	19.6 ± 1.9[#,*]
Zn biochemical indices			
Plasma zinc, μmol L^{-1}	12.1 ± 1.8	11.5 ± 1.4	11.7 ± 1.6
Erythrocyte zinc, μmol g hemoglobin^{-1}	0.69 ± 0.16	0.71 ± 0.19	0.66 ± 0.17
Bioelectrical data			
R, Ohms	528.3 ± 61.2	497.0 ± 60.7	645.2 ± 70.7[#,*]
R/H, Ohms m^{-1}	321.6 ± 37.0	300.5 ± 38.5	438.0 ± 55.0[#,*]
Xc, Ohms	59.9 ± 9.4	59.6 ± 9.9	62.4 ± 9.6
Xc/H, Ohms m^{-1}	37.2 ± 3.8	36.1 ± 6.8	43.7 ± 5.3[#,*]
Phase angle, °	6.5 ± 0.6	6.8 ± 0.9	5.7 ± 0.5*

Values are expressed as mean ± SD. BMI = body mass index; R = resistance; Xc = reactance. Comparisons between groups were done by one-way ANOVA followed by Duncan's post hoc test using the "On time" group as reference. Comparisons between "Late" and "Early" were done by Student *t* test. Significant difference compared to "On time" was indicated with #, and difference of "Late" compared to "Early" was indicated with * (p ≤ 0.05).

Division of Nephrology, and Clinical Nutrition Unit, University of Padova, Italy). Statistical tests were considered significant at the usual significance level of 5% (p ≤ 0.05).

3. Results

The adolescent soccer athletes were classified according to skeletal maturity: n = 10 (25%) adolescents were classified as "Early maturity" (bone age 15.47 ± 0.90 years); n = 18 (45%) as "On time" maturity (bone age 13.46 ± 0.80 years); and n = 12 (30%) were considered "Late" maturity (bone age 10.69 ± 1.22 years), (p = 0.001). All participants were apparently healthy and practiced soccer during at least 20 h per week (training and/or competition). The athletes were not anemic (erythrocyte count 4.9 ± 0.3 × 10^6 cell μL^{-1}; hemoglobin 13.2 ± 0.9 mg dL^{-1}) (normal value: 4.7–6.1 × 10^6 cell μL^{-1} and > 12 mg dL^{-1}, respectively) [32].

Body composition compartments (fat mass and fat-free mass) were adequate for adolescent athletes (Table 1). BMI *z*-scores were normal for age and gender for all participants (> −1 < +1) [20]. As expected, BMI was higher in "Early" (21.2 ± 2.4 kg/m^2) stage of skeletal maturity adolescents compared to the "Late" (16.6 ± 1.2 kg/m^2) and "On time" (18.7 ± 1.4 kg/m^2) groups (p = 0.005). Total body water was significantly different among the groups (p = 0.001).

The biochemical measurements of zinc indices showed that seven (17%) participants presented zinc deficiency (plasma zinc < 10 μmol L^{-1}) [33], four of whom were classified as "Late", two as "On time" and only one as "Early". Plasma zinc was positively related to zinc-erythrocyte (r = 0.55, p = 0.0001). Plasma zinc and erythrocyte-zinc concentrations were not significantly different according the stage of skeletal maturity.

Considering skeletal maturation, PA values were higher in those classified as "Early" compared to "Late" stage (p = 0.010) (Table 1). Resistance (R) value was lower (p = 0.001) in "Late" adolescents in
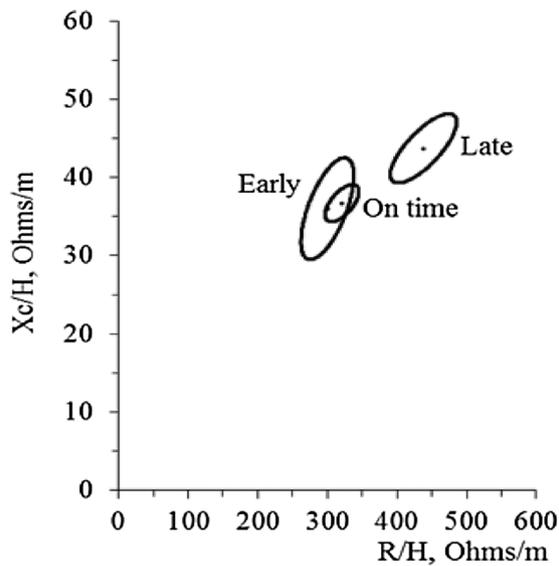
Fig. 1. Mean impedance vectors by bone maturity classification with 95% confidence ellipses.

	T^2	F	p	D
"Early" *vs* "On time"	2.4	1.2	0.327	0.61
"Late" *vs* "Early"	48.9	23.2	0.001	2.99
"Late" *vs* "On time"	49.3	23.8	0.001	2.62

Bone maturity classification: Early (n=10), On time (n=18) and Later (n=12).

T^2 = Hotteling´s test; F = variance analysis; p = significance level; D = Mahalanobis Distance

comparison to "On time" and "Early" adolescents. However, reactance value was similar between the groups. The adolescents classified as "Late" skeletal maturity, presented higher values of R/H, whereas the PA was lower in this group (Table 1). Pearsoń́s correlation coefficient between R/H and Xc/H for the each group was $r = 0.647$ for "Early", $r = 0.620$ for "On time", and $r = 0.762$ for "Late" stage.

BIVA confidence ellipses of male adolescent soccer athletes were calculated according to skeletal maturity stage. The Mahalanobis distance is a descriptive statistics that provides a relative measurement of data point distances (residual) between vectors. The Mahalanobis distances of male adolescent soccer athletes classified as "Late" was different from those in the "On time" and "Early" groups, according to skeletal maturity ($p = 0.001$). However, no difference was observed between "On time" and "Early" skeletal maturity groups ($p = 0.327$) (Fig. 1).

There are no reference values of erythrocyte zinc concentration for nutritional status classification [18]. Therefore, in order to evaluate the influence of the level of this indicator on PA, two erythrocyte zinc concentration categories were considered, lower and higher than the median (0.66 μmol/g hemoglobin) for the whole group ($n = 40$), as no differences were found in erythrocyte zinc according to skeletal maturity classification.

Table 2 presents bioelectrical impedance data according to erythrocyte zinc concentration categories considering all adolescents ($n = 40$). Resistance was lower whereas PA was higher in the adolescents with higher median erythrocyte zinc concentration ($p = 0.014$ and $p = 0.001$, respectively).

Table 3 presents all significant Pearsoń́s correlation coefficients ($p < 0.05$). There were positive correlations between PA and bone age ($r = 0.562$), BMI ($r = 0.382$) and BMI *z*-score ($r = 0.320$), fat free mass ($r = 0.468$) and erythrocyte zinc concentration ($r = 0.379$).

The multiple linear regression analysis showed that erythrocyte zinc concentration ($p = 0.047$) and bone age ($p = 0.001$) were positively

Table 2
Bioelectrical data according to erythrocyte zinc concentration levels in male adolescent soccer athletes.

	Erythrocyte Zinc concentration	
	< Median[a]	> Median[a]
Resistance, Ohms	588.7 ± 86.0	522.4 ± 76.0*
R/H, Ohms m^{-1}	376.1 ± 71.9	331.9 ± 68.7
Reactance, Ohms	60.2 ± 5.3	61.5 ± 8.5
Xc/H, Ohms m^{-1}	38.7 ± 5.1	38.9 ± 6.8
Phase angle, °	5.9 ± 0.61	6.7 ± 0.8**

t-Test independent, *p = 0.014; **p = 0.001.

[a] Median erytrhocyte zinc concentration: 0.66 μmol Zn g hemoglobin^{-1}.

Table 3
Significant Pearson correlation coefficients between phase angle and bone age, body composition, and zinc indices, in male adolescent soccer athletes ($n = 40$).

	r	p
Phase angle *vs*		
Bone age	0.562	0.001
Body mass index *z*-score	0.32	0.044
Body mass index	0.382	0.015
Fat free mass	0.468	0.002
Erythrocyte zinc	0.379	0.016

associated to PA. In the conditions tested, these combined independent variables accounted for 34% of the PA variability in the adolescent soccer athletes studied (Table 4).

4. Discussion

To our knowledge, this is the first study to demonstrate that bone

Table 4
Phase angle determinants according to a linear regression model in male adolescent football athletes (n = 40).

	B	CI (95%)	P
Bone age	0.254	0.166–0.343	0.001
Erythrocyte zinc	1.168	0.107–2.228	0.047

Bone age, fat free mass and erythrocyte zinc were initially included as independent variables in the linear regression model with backward elimination, adjusting for BMI z-score. Variables kept in the model were those with p < 0.05. Final model: R^2 adjusted = 0.34; p = 0.019.

age and erythrocyte zinc are significantly associated to PA in adolescent soccer athletes, accounting for 34% of the PA variability in the study group in the conditions tested. This study also showed a significant influence of a delayed skeletal maturity on BIVA confidence. These results contribute to a better understanding of PA within an integrated nutritional assessment system in adolescent athletes. The PA theory and applications in the clinical practice have been discussed in several reviews [2,28–30].

The mean PA values found in the present study were similar to those found in previous studies conducted in Brazilian adolescent athletes [5] and non-athletes [7]. Higher PA (6.9 ± 0.9°) values have been previously reported in adolescents participating in various sports modalities (athletics, swimming, water polo, triathlon and basketball) when compared to soccer athletes (6.3 ± 0.8°) [5]. Additionally, in Brazilian male non-athlete adolescents of different age groups, PA values ranged from 5.99 ± 0.57° (12 years; n = 35) to 6.19 ± 0.69° (16 years; n = 32) [7]. Nonetheless, stage of skeletal maturity was not evaluated in these studies. In the present study, we found that "Early" adolescents have higher PA than those categorized as "Late" or "On time", considering the stage of skeletal maturity. This result is possibly related to the higher BMI and fat free mass in the "Early" adolescents. In agreement with our findings, PA values in male adolescent athletes had positive correlations with BMI and age [5,15]. These correlations are consistent with the increase in metabolic tissues and BMI that occur during biological maturation [15,34].

Zinc is essential for adequate growth due to critical roles in the body, including endocrine function and the bone matrix structure [35]; maturation of spermatozoa, testicular growth and testicular steroidogenesis [18]; and cell contractility being key to sustain appropriate muscular contraction and performance in athletes [4,17]. In the present study, it was observed that 17% of male adolescent soccer athletes were zinc-deficient according to plasma zinc concentrations (< 10 μmol L^{-1}, [33]). In spite of limitations, plasma zinc is considered the most useful indicator of zinc nutritional status [36]. The majority of these zinc-deficient adolescents were classified as "Late"; it is understandable, since the zinc deficiency seems to be related to deleterious effects in biology maturation. However, this result should be viewed with caution, because exercise has a pronounced effect on zinc metabolism [37]. Aerobic exercise may increase zinc uptake by muscle tissue, in order to meet the increased zinc demand for muscle repair mechanisms following exercise [37]. The increased uptake of zinc by muscle cell may be associated with a decrease in plasma zinc, and this result is not related to nutritional zinc status. In our adolescent athletes, plasma zinc and zinc erythrocyte concentration were similar among the three groups classified by skeletal maturity.

The positive correlation between PA and erythrocyte zinc, and the higher PA at higher erythrocyte zinc concentration observed in the present study are consistent with the significant increase in PA after zinc supplementation in pre-pubertal children [17], and with the role of zinc on cell membrane stability and antioxidant capacity [14,21]. Erythrocytes are cells strongly exposed to oxidative stress [38] and our results indicate that they appear to be a good cell model to study the relationship between cell zinc and PA. Moreover, PA was also positively related to fat free mass, possibly due to the greater intracellular water

content in this compartment. This result is in accordance with a recent study in adult population [6].

In contrast with healthy adult populations, PA determinants in adolescent athletes have not been completely examined. In adult men and women, age is the most important biological determinant of PA, and aging is related to a decrease in PA values [6]. However, in adolescents, the correlation between PA and age is positive, due to growth and biological maturation [5,7]. In spite of this, age was not considered a significant contributor to PA variability in the present study, which could possibly be explained by the narrow chronological age range (12.2–14.2 years) among the adolescents enrolled in the study. On the other hand, bone age was a significant factor affecting PA in the soccer adolescents, suggesting that the degree of skeletal maturity, rather than chronological age, affects PA in male adolescent athletes. In the present study, linear regression analysis adjusted by BMI z-score, indicated that zinc-erythrocyte and bone age are important independent variables contributing to PA variability (34%) in the adolescent soccer athletes studied.

The present study also found that the BIA parameter R had a higher value in adolescents classified as "Late" maturity than in "Early" and "On time". This is in agreement with the expectations since male mature adolescents have lower fat free mass than non-mature adolescents and fat free mass is less resistant to electrical current flow, due to muscle tissue hydration [7]. The Xc did not differ among the adolescents classified by skeletal maturity stages, possibly because all participants were eutrophic healthy athletes with similar training load. R/H and Xc/H were higher in "Late" maturity adolescents, considering the lower height in this group.

Impedance analysis of body hydration and body composition constitutes a very promising methodology [29]. However, it is necessary to choose population-specific equations for the group being studied [28], which is considered the major weakness of conventional BIA [27]. Piccoli et al. [3] proposed impedance (Z) to be plotted in a Cartesian plane as a bivariate vector originating from R and Xc standardized by height (bioelectrical impedance vector analysis − BIVA). A shortening or lengthening of the vector over the confidence ellipses means a fluid overload or dehydration, respectively. Therefore, PA and BIVA can be used together to indicate cell integrity and hydration. The vector length in BIVA confidence graph is influenced by the amount of total body water and fat free mass. PA is influenced by soft tissue (which changes depending on age and clinical conditions), hyperhydration and fat mass (which lead to a progressive shortening of the vector) [3,4]. In the present study, different ellipse forms were observed due to the correlation coefficient between R/H and Xc/H. Moreover, a significant shortening of the impedance vector according to skeletal maturity to "Early" and "On time" groups (overlapped ellipses) was observed. This result emphasizes the difference in body composition between non-mature and mature (more fat free mass) male adolescent soccer athletes. The "Late" maturity group presented higher lengthening vector than "Early" and "On Time", possibly because of dehydration, as shown by the lower total body water in the "Late" group. This result is consistent with Silva et al. [39] in Brazilian adolescents and with Phillips et al. [40] in European youth soccer players during consecutive training sessions. Besides that, this result can be related to body composition.

A limitation of the study is the lack of information on dietary zinc intake and the overall nutrient composition of the diets of the adolescent athletes. However, national data [41] indicates that the average dietary zinc intake of male adolescents in Brazil is 10% above zinc recommendation for this age group [33]. Moreover, the adolescent athletes studied were instructed to have a healthy diet according to the Dietary Health Guide for the Brazilian Population [19].

Taken together, these results strongly suggest that bone age and zinc erythrocyte are important variables to consider in the interpretation of BIVA and PA values in male adolescent soccer athletes.

5. Conclusions

This study indicates that bone age and erythrocyte zinc are associated with PA values in young male soccer athletes, taking into account BMI z-score. The data obtained contribute to a better understanding of the interaction between zinc status and PA in adolescent athletes. The associations found in our study relate to soccer athlete adolescents used to intense chronic training, and may not be extrapolated to other sports modalities or to untrained male adolescents.

Ethical approval

All procedures involving human participants were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study protocol was approved by the Research Ethics Committee (Ethics Committee of the Pedro Ernesto University Hospital; CEP/HUPE 649.202).

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

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