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New *specific* bioelectrical impedance vector reference values for assessing body composition in the Italian-Spanish young adult population

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Short title: Italo-Spanish bioelectrical reference values

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ABSTRACT

Objective: *Specific* bioelectrical impedance vector analysis (*sp*BIVA) is a recently proposed technique for the analysis of body composition. The aim of this study was to apply *sp*BIVA to a sample of Italian and Spanish young adults and to define the new bioelectrical references for this Mediterranean population.

Methods: A sample of 440 individuals (220 from Italy, 220 from Spain; 213 men, 227 women) aged 18 - 30 years was considered. Anthropometric (height, weight; relaxed upper arm, waist, and calf girths) and bioelectrical (resistance, reactance; 50 kHz, 800 μA) measurements were taken.- In order to verify the need of new references, *specific* bioelectrical values were compared to the reference values for U.S. adults and Italian elderly by tolerance ellipses and Student's t test.

Results: The mean *specific* bioelectrical values (resistivity, R*sp*, and reactivity, Xc*sp*, Ohm·cm) were: R*sp* (332.7±41.7 Ω·cm), Xc*sp* (44.4±6.8 Ω·cm), Z*sp* (335.6±41.9 Ω·cm) and phase (7.6±0.8°) in men; R*sp* (388.6±60 Ω·cm), Xc*sp* (43.7±7.5 Ω·cm), Z*sp* (391.0±60.3 Ω·cm) and phase (6.4±0.7°) in women. Italo-Spanish bioelectrical vectors were mainly distributed (>90%) in the lower part of the tolerance ellipses for U.S. young adults, due to a shorter impedance ($p<0.001$), indicative of lower percent fat mass. Compared to Italian elders, they were mainly located in the left side (>90%), due to a higher phase ($p<0.001$), indicative of higher body cell mass.

Conclusions: These population and age-related differences indicate the need of new *specific* tolerance ellipses that can be used as references for assessing body composition in young adults from Mediterranean populations.

Keywords: *specific* BIVA; body composition; human populations; reference values.

INTRODUCTION

The analysis of body composition has a theoretical and empirical interest, especially in the fields of human biology, nutrition and sports medicine, with possible applications in the clinical practice. Human populations differ for body composition (Lear et al., 2007b, Heo et al., 2012, Lear et al., 2007a) and fat distribution (Malina, 1996). However, large studies comparing different populations or different geographic regions are not very common (among them: (Lear et al., 2007b, Heo et al., 2012, Kyle et al., 2005, Böhm and Heitmann, 2013, Carpenter et al., 2013)). These studies apply different techniques for assessing body composition (imaging techniques, such as computed tomography, magnetic resonance imaging, and dual energy X-ray absorptiometry [DXA], bioelectrical impedance analysis [BIA], anthropometry), that differ for accuracy and reliability, invasiveness, procedural complexity, and costs (Baracos et al., 2012). Some of these methodological approaches are not adequate for use in the field or in routine applications. For this reason, most epidemiological studies on body composition have been conducted using the anthropometric or bioimpedance techniques, which are simple, quick and non-invasive.

Body mass index (BMI) has been largely used to evaluate obesity prevalence and worldwide trends (WHO, 2000, Finucane et al., 2011), due to its high usability and the availability of comparative data. In fact, BMI is correlated with fat mass. However, it does not actually disentangle body composition, as it is not able to distinguish fat from fat-free mass (Kyle et al., 2003, Zaccagni et al., 2014, Shah and Braverman, 2012, Barbieri et al., 2012). Further on the relation with fat mass percentage is different among populations (Norgan, 1994).

Bioelectrical impedance analysis is a practical and low cost technique (Ellis et al., 1999). The conventional BIA approach requires population-specific equations (Böhm and Heitmann, 2013). The main limitation of this approach is that the results of studies using different equations are not always comparable, and, on the other hand, it is not recommendable to use a single equation for comparative studies (Dehghan and Merchant, 2008).

The recently proposed *specific* bioelectrical impedance vector analysis (*sp*BIVA) (Buffa et al., 2013, Marini et al., 2013, Buffa et al., 2014) is an analytical variant of BIA that follows the approach of classic BIVA conceived by Piccoli et al. (Piccoli et al., 1994), in that it considers bioelectrical values directly, without the use of predictive equations. Thus, it avoids a potential source of error and enhances the possibility of inter-population comparisons. *Sp*BIVA differs from the classic BIVA approach, because bioelectrical values are standardized by cross-sections of the body, rather than by only height. *Sp*BIVA has showed to be effective in the evaluation of fat mass percentage (FM%) by comparison with DXA (Buffa et al., 2013, Marini et al., 2013, Buffa et al., 2014). It has also showed to be sensitive to variations of extracellular / intracellular water ratio

(ECW/ICW) and skeletal muscle mass (Marini et al., 2012, Buffa et al., 2013, Saragat et al., 2014). Further, *sp*BIVA showed to perform better than the classic approach in a sample of institutionalised elderly with dementia (Camina Martìn et al., 2014).

Specific bioelectrical references have been recently proposed for the U.S. young adult (Buffa et al., 2013) and the Italian healthy elderly populations (Saragat et al., 2014).

The aim of this study was to analyse the applicability of the existing *specific* bioelectrical reference values for U.S. adults and elderly Italians to a sample of Italo-Spanish young adults and, if not applicable, to propose new *specific* bioelectrical reference values for young populations of Mediterranean origin.

MATERIAL AND METHODS

The sample

A sample of 440 individuals of both sexes aged from 18 to 30 years was selected from two different countries of the Mediterranean area: Italy (220 subjects, 22.2±2.7 years for men; 22.8 ±2.9 for women) and Spain (220 subjects, 22.3 ± 2.5 years for men; 22.0 ± 2.5 years for women).

Data were collected in three settings: University of the Basque Country [UPV/EHU] (Spain), University of Cagliari (Italy) and University of Ferrara (Italy), on students born in different places of each country. The Italian sample was taken specifically for this research, whereas the Spanish one was selected from a large database of 967 individuals, in order to represent all the regions of Spain. Exclusion criteria were the presence of clinical conditions that may alter fluid status, i.e., renal, endocrine, or cardiovascular disease.

In accordance with the Helsinki Declaration, as revised in 2013, a written informed consent was obtained from all participants.

Measurements

The anthropometric measurements (height, weight, and three girths: relaxed upper arm, waist and calf) were taken in each setting by trained staff. Measurements were obtained according to the procedure described by Lohman et al. (Lohman et al., 1988), with the exception of relaxed upper arm and calf girths, which were taken on the left side of the body in Ferrara and the Basque Country settings (statistically adjusted as described below) according to Weiner and Lourie (Weiner and Lourie, 1981). Height was measured with a professional anthropometer (accuracy: 1 mm), weight with a portable scale (accuracy: 0.1 kg), and girths with an anthropometric tape accurate to 1 mm. The body mass index (BMI: weight/height^2, kg/m^2) was calculated and the WHO cut-offs (WHO, 2000) were applied for nutritional status assessment. Bioelectrical measurements (resistance, R, Ohm, and reactance, Xc, Ohm; at 50 kHz and 800 µA) were taken following the standard procedure (NIH, 1996). In all cases, a single-frequency BIA 101 analyser (Akern - RJL, Firenze; accuracy: 1 Ohm) was used.

The Italo-Spanish database is available at the Cagliari University institutional repository: http://veprints.unica.it/1057/.

Statistical analyses

Specific bioelectrical values (resistivity, R*sp*, Ohm·cm, and reactivity, Xc*sp*, Ohm·cm) were obtained by multiplying resistance and reactance by a correction factor (A/L), where area (A, m^2)

and length (L, m) were estimated as follows: A = (0.45 upper arm area + 0.10 waist area + 0.45 calf area) and L = 1.1 stature. The segment areas were calculated as $C^2/4Л$, where C (m) is the girth of the upper arm, waist, or calf (Buffa et al., 2013). *Specific* values were rescaled by a factor of 100. The phase angle (degrees) was calculated as arctan (Xc/R); and the impedivity vector (Z*sp*, Ohm·cm) as $(Rsp^2 + Xcsp^2)^{0.5}$.

The right upper arm girth values of the Ferrara and Basque Country samples were predicted from contralateral measurements, using regression equations derived from an independent large sample (650 individuals; 20 - 30 years) of the Italian population (men: right upper arm = 0.973 left upper arm + 1.183; R^2= 0.894; SEE= 0.84 cm; women: right upper arm = 0.950 left upper arm + 1.463; R^2= 0.882; SEE= 0.72 cm). Calf girths were not corrected as this anthropometric variable does not usually show lateral differences (Cuk et al., 2012).

According to *specific* BIVA, the bioelectrical values were analyzed by means of tolerance ellipses (representing the bivariate percentiles of the reference population), where the major axis refers to variations of FM% (higher values towards the upper pole) and the minor axis to variations of skeletal muscle mass and ECW/ICW (lower values on the left side). Italo-Spanish data were compared with the tolerance ellipses for the U.S. adults (21-49 years) (Buffa et al., 2013) (database freely available at: http://veprints.unica.it/809/) and the Italian elderly (>65 years) (Saragat et al., 2014) (database freely available at: http://veprints.unica.it/904/) by considering the distribution of individual vectors and comparing the mean bioelectrical values via the Student's t test.

The Italo-Spanish within sample variability was hence investigated by considering the distribution in the newly developed tolerance ellipses, and by means of the Student's t test.

The "r" coefficient between resistivity and reactivity was calculated by Pearson's correlation. Statistical analyses were performed using IBM SPSS Statistics 19 (IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp) and the newly assembled *specific* BIVA software (freely available at the website: http://specificbiva.unica.it/).

RESULTS

Italo-Spanish men and women were younger than U.S. adults and, especially, Italian elders (Table 1). Both sexes showed, on average, a normal nutritional status (mean BMI < 25 kg/m^2), while both the Italian elderly and the U.S. adults were overweight (Table 1). Men were lighter and shorter than U.S. adults but heavier and taller than Italian elders. Women had the lowest mean values for all the traits in comparison to U.S. adults, while they had higher height, calf girth and phase in comparison to Italian elders.

With respect to the tolerance ellipses for U.S. adults, the Italo-Spanish bioelectrical vectors were mainly located below the minor axis (men: 94.4%; women: 93.4%), and in the left side (men: 74.2%; women: 63.4%) (Fig. 1a). The impedance vectors were shorter in Italo-Spanish individuals of both sexes, while the phase was higher only in men (Table 1).

Compared to the healthy elderly Italians, the bioelectrical values of Italo-Spanish young individuals were mainly located on the left side of the ellipses (men: 98.1%, women: 91.6%), and showed a tendency, more accentuated in women, toward the lower half (men: 56.3%; women: 81.1%) (Fig. 1b). Accordingly, the phase angles were higher and the impedance vectors shorter in young than in elderly individuals of both sexes (Table 1).

Within-sample variability showed that Italian and Spanish individuals were similar in their anthropometric and bioelectrical characteristics, with the only exception of phase, which was higher in Spanish men and women, and waist circumference, which was higher in Italian women (Table 2). The distribution of bioelectrical values within the tolerance ellipses largely overlapped (Figure 2).

DISCUSSION

The shifted distribution of bioelectrical values of young Europeans in both reference ellipses for the U.S. adult (Buffa et al., 2013) and the Italian elderly populations (Saragat et al., 2014) is indicative of body composition peculiarities, according to *specific* BIVA (Buffa et al., 2013, Marini et al., 2013), and of the need of new reference values.

In particular, with respect to the U.S. adults, the shorter mean specific impedance vector indicates a lower FM% in the young European sample (Marini et al., 2013, Buffa et al., 2013). This result was confirmed by anthropometry. In fact, the U.S. sample was characterized by a higher BMI than the sample under study. Further, the higher phase in Italo-Spanish men indicates a tendency to a greater skeletal muscle mass (Marini et al., 2013, Buffa et al., 2013).

Our findings are consistent with earlier research on body composition showing that North Americans are characterized by higher values of FM and FM% than Europeans (Deurenberg et al., 2001, Kyle et al., 2005, Kelly et al., 2009, Böhm and Heitmann, 2013). Although bioelectrical differences between Europeans and North Americans have been already observed (Barbosa-Silva et al., 2005, Bosy-Westphal et al., 2006), no other studies using *specific* BIVA have been performed till now.

The tendency toward a greater skeletal muscle mass in Italo-Spanish men could be also due to body composition differences related to their lower age (22.3 vs. 34.2 years), that could appear even in young adults.

With respect to the elderly population (Saragat et al., 2014), the young Europeans showed more skeletal muscle mass, as indicated by the higher phase, and less FM%, as indicated by the shorter vector (Marini et al., 2013, Buffa et al., 2013). These results are consistent with the knowledge on bioelectrical and body composition changes in aging. In fact, an age-related trend toward lower phase angles has been observed in several studies (Barbosa-Silva et al., 2005, Bosy-Westphal et al., 2006, Buffa et al., 2003, Buffa et al., 2009, Saragat et al., 2014). This trend has been related to a tendency toward fat free mass reduction and to a possible incipient sarcopenia (Buffa et al., 2003, Buffa et al., 2009, Zaccagni et al., 2009, Saragat et al., 2014, Gueresi et al., 2014). In fact, the literature on body composition shows an age related loss of body mass, particularly lean mass, that is more accentuated in men (Buffa et al., 2011). Fat mass also decreases, but later and slower than fat free mass, so causing an increase of fat mass percentage in the first phases of aging (Buffa et al., 2011). However, the trend toward the increase of FM% can change in very advanced ages, slowing and even reversing in women (Saragat et al., 2014, Kelly et al., 2009).

The within-sample variability was quite low. In fact, most of the anthropometric characteristics corresponding to Italian and Spanish populations did not show significant differences (indeed, the mean BMI values were quite identical). Furthermore, in terms of mean BMI, they are also very similar to other groups of European young adults (Kyle et al., 2005). The distribution of *specific* bioelectrical values was also similar, without differences of resistivity and reactivity, indicating a similar amount of FM% (Marini et al., 2013, Buffa et al., 2013). However, the different phase angle could indicate a lower ECW/ICW and a greater skeletal muscle mass in the Spanish sample (Marini et al., 2012, Buffa et al., 2013).

A possible limitation of this study, that may have affected the comparison between the U.S. and European samples, is related to the different instrumental devices used for bioelectrical measurements (U.S. adults: Model 4200; Xitron Technologies, Inc, San Diego, California, USA; Europeans young and elderly: BIA 101; Akern - RJL, Firenze). In fact, using different devices can reduce comparability across studies (Smye et al., 1993). However, Bosy-Westphal et al., (Bosy-Westphal et al., 2006) did not found significant differences between Xitron and RJL 101 devices in resistance, i.e. the main determinant of the different impedivity vector observed in this study between U.S. and Europeans samples.

CONCLUSIONS

This study proposes new *specific* bioelectrical reference values for the Mediterranean population, as the already existing ones (for U.S. adults and Italian elders) don't fit this sample of young population. The new references can be used to evaluate variations of body composition and nutritional status in young individuals or groups belonging to Mediterranean countries.

Further, this study showed that *specific* BIVA can detect age and population related differences in body composition: a lower content of skeletal muscle mass in elderly Italians and a greater content of FM and FM% in U.S. adults, with respect to Italo-Spanish young adults.

Hence, *specific* BIVA can represent a new useful tool for research in the field of human biology. More studies are needed to ascertain if these reference values are useful for other populations.

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Legends

Figure 1. Distribution of bioelectrical vectors of the sample under study within the tolerance ellipses for the U.S. adults (a) and the healthy Italian elderly (b)

Legend: the major axis refers to variations of FM% (higher values towards the upper pole) and the minor axis to variations of skeletal muscle mass and ECW/ICW (lower values on the left side).

Figure 2. Distribution of bioelectrical vectors within the tolerance ellipses for the Italo-Spanish population (a: men; b: women; white dots: Italy; black dots: Spain).

Legend: the major axis refers to variations of FM% (higher values towards the upper pole) and the minor axis to variations of skeletal muscle mass and ECW/ICW (lower values on the left side).

Table 1: Descriptive and comparative statistics for anthropometric and bioelectrical variables in European and North American samples

	Men								Women							
	Italo-Spanish N=213		U.S. Adults N=836		Italian Elderly N=265				Italo-Spanish N=227		U.S. Adults N=754		Italian Elderly N=295			
	mean	s.d.	mean	s.d.	mean	s.d.	t†	t‡	mean	s.d.	mean	s.d.	mean	s.d.	t†	t‡
Age (y)	22.3	2.6	34.24	8.6	77.0	7.2	-20.19**	-105.58**	22.5	2.8	35.5	8.4	76.0	7.1	-23.0**	-107.48**
Height (cm)	175.0	6.5	175.7	7.7	162.0	8.5	-1.24	18.47**	161.4	6.2	162.6	6.7	150.2	8.0	-2.38*	18.12**
Weight (kg)	71.8	10.1	84.3	16.2	69.5	11.1	-10.74**	2.37*	58.2	9.7	74.8	18.9	60.1	11.0	-12.82**	-2.10*
BMI (kg/m^2)	23.4	2.9	27.3	4.8	26.4	3.3	-11.20**	-10.36**	22.3	3.3	28.3	7.0	26.6	4.1	-12.62**	-12.85**
Upper arm crf (cm)	28.9	2.9	33.8	4.0	28.0	3.3	-16.74**	3.14**	26.0	3.0	32.0	5.3	28.2	3.8	-16.21**	-7.03**
Waist crf (cm)	79.1	7.5	95.7	12.9	95.7	9.2	-18.00**	-21.24**	71.1	9.1	92.9	15.4	92.2	10.9	-20.27**	-23.49**
Calf crf (cm)	37.0	2.6	39.1	3.6	34.6	3.4	-8.07**	8.32**	35.4	2.9	38.3	4.8	33.8	3.7	-10.02**	7.8**
R*sp* (Ohm · cm)	332.7	41.6	402.4	62.9	391.8	57.9	-15.4**	-12.54**	388.6	60.0	492.0	95.9	462.0	80.1	-15.37**	-11.55**
Xc*sp* (Ohm · cm)	44.4	6.8	52.5	9.5	42.6	9.9	-11.68**	2.33*	43.7	7.5	55.4	12.3	47.9	11.2	-13.53**	-4.78**
Z*sp* (Ohm · cm)	335.6	41.9	405.9	63.4	394.2	58.2	-15.34**	-12.33**	391.0	60.3	495.2	96.5	464.6	80.5	-15.37**	-11.50**
Phase (degrees)	7.6	0.8	7.5	0.7	6.2	1.2	3.15**	15.03**	6.4	0.7	6.5	0.7	5.9	1.0	0.27	6.77**
r R*sp*-Xc*sp*	0.77**		0.84**		0.59**				0.79**		0.88**		0.75**			

Legend: s.d.: standard deviation; †: Italo-Spanish vs U.S. Adults; ‡: Italo-Spanish vs Italian Elderly; y: years; BMI: body mass index; crf: circumference; R*sp*: resistivity; Xc*sp*: reactivity; Z*sp*: impedivity; *= $p< 0.05$; **= $p< 0.001$;

Table 2: Descriptive and comparative statistics for anthropometric and bioelectrical variables in the Italo-Spanish sample

	Men					Women				
	Italy N=103		Spain N=110			Italy N=117		Spain N=110		
	Mean	s.d.	Mean	s.d.	t	Mean	s.d.	Mean	s.d.	t
Height (cm)	174.9	6.5	175.1	6.4	-0.287	161.6	6.6	161.2	5.8	0.492
Weight (kg)	70.6	10.0	72.8	10.0	-1.593	58.3	10.8	58.0	8.5	0.273
BMI (kg/m^2)	23.1	2.8	23.7	3.0	-1.703	22.3	3.8	22.3	2.7	0.110
Upper arm crf (cm)	29.3	3.2	28.6	2.5	1.737	26.3	3.4	25.8	2.3	1.154
Waist crf (cm)	78.8	6.6	79.5	8.3	-0.625	72.7	10.5	69.5	7.0	2.729*
Calf crf (cm)	37.0	2.7	37.0	2.4	0.134	35.6	3.2	35.1	2.4	1.427
R*sp* (Ohm · cm)	334.3	36.7	331.1	45.8	0.550	394.6	65.6	382.1	50.3	1.572
Xc*sp* (Ohm · cm)	43.6	6.6	45.2	7.0	-1.748	42.8	8.2	44.7	6.5	-1.905
Z*sp* (Ohm · cm)	337.1	37.0	334.2	46.2	0.506	397.0	67.9	384.7	50.5	1.529
Phase	7.4	0.9	7.8	0.6	-3.366*	6.2	0.7	6.7	0.6	-5.689**

Legend: s.d.: standard deviation; y: years; BMI: body mass index; crf: circumference; R*sp*: resistivity; Xc*sp*: reactivity; Z*sp*: impedivity.
*= $p< 0.05$; **= $p< 0.001$

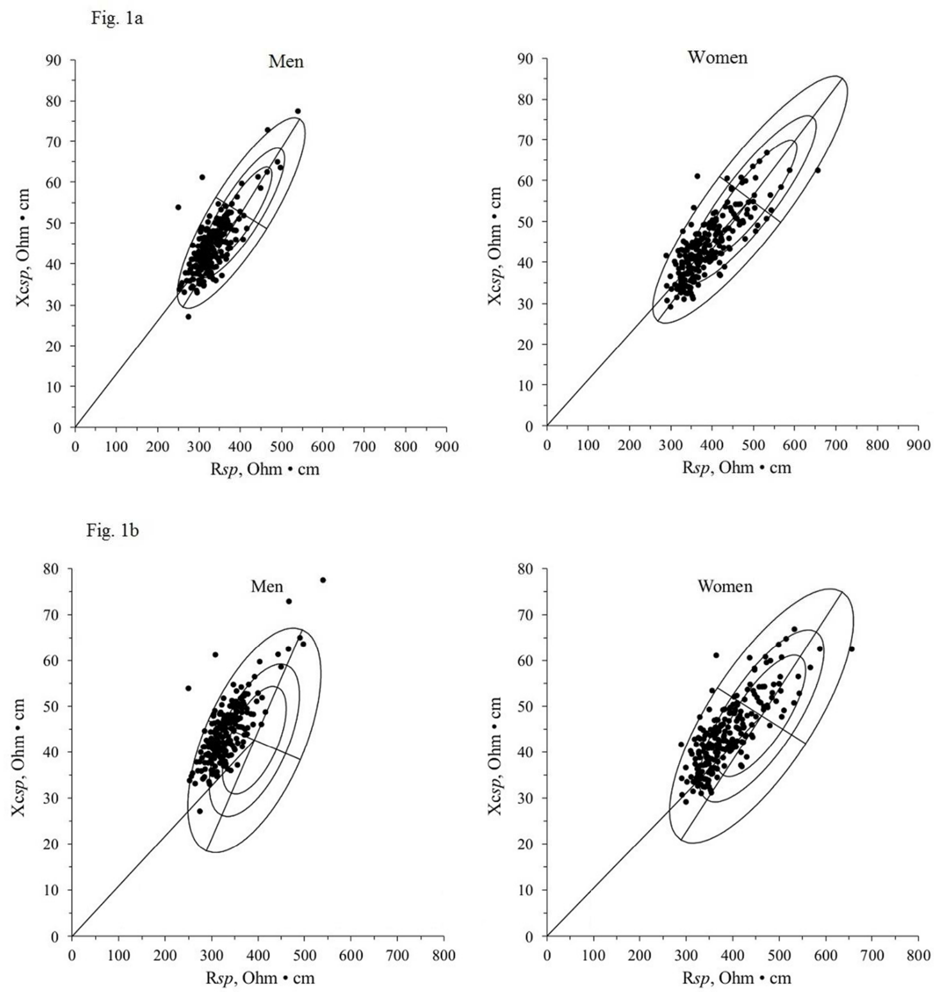

Figure 1. Distribution of bioelectrical vectors of the sample under study within the tolerance ellipses for the U.S. adults (a) and the healthy Italian elderly (b)
Legend: the major axis refers to variations of FM% (higher values towards the upper pole) and the minor axis to variations of skeletal muscle mass and ECW/ICW (lower values on the left side).
332x364mm (96 x 96 DPI)

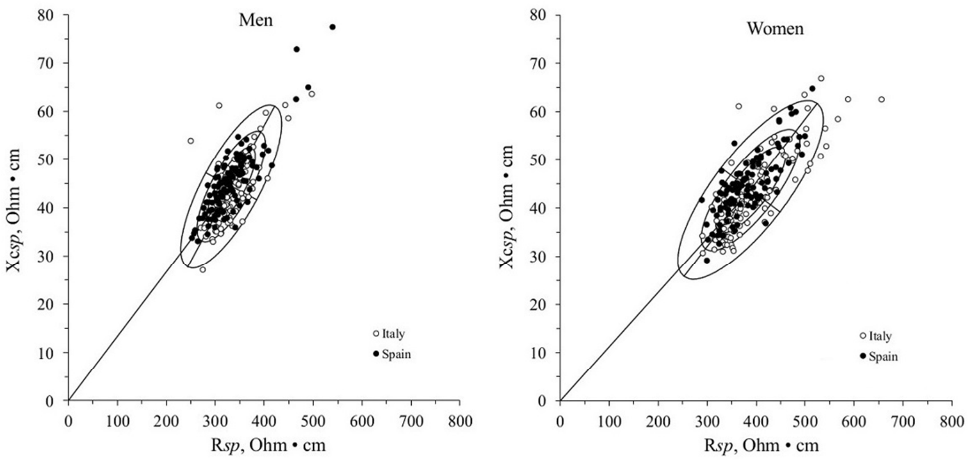

Figure 2. Distribution of bioelectrical vectors within the tolerance ellipses for the Italo-Spanish population (a: men; b: women; white dots: Italy; black dots: Spain).
Legend: the major axis refers to variations of FM% (higher values towards the upper pole) and the minor axis to variations of skeletal muscle mass and ECW/ICW (lower values on the left side).

315x166mm (96 x 96 DPI)