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PHASE ANGLE AND VECTOR ANALYSIS FROM MULTIFREQUENCY SEGMENTAL BIOELECTRICAL IMPEDANCE ANALYSIS: NEW REFERENCE DATA FOR OLDER ADULTS

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Phase angle (PA) and bioelectrical impedance vector analysis (BIVA) have been recommended as useful prognostic markers in various clinical settings. However, reference data for older adults measured by the novel segmental multifrequency bioelectrical impedance analysis (SMF-BIA) technique are currently lacking. This study examined 567 (286 men, 281 women) healthy older adults (65 – 97 years) and new SMF-BIA-based PA and BIVA reference values were generated stratified according to gender and 3 age groups (65 – 75 years, 76 – 85 years, > 85 years). Mean PA-values (women: 4.30 ± 0.6°, men: 4.77 ± 0.7°) were significantly lower than those previously reported for a younger reference population. Age and gender were significant determinants of PA and BIVA. PA showed a significant decrease with increasing age in both genders. The greatest changes occurred in the age group > 85 years. Men had higher PAs compared to women (except for the oldest age group), but showed a substantially steeper decline in PA, possibly due to a more pronounced reduction of muscle mass. Compared to published reference data for younger adults, there was a clear downward migration of the BIVA vector points in older adults, indicating an age-related reduction of body cell mass. Accordingly, the equation for the BIVA chart generation was modified by adding the factor age. In conclusion, this is the first study to present SMF-BIA-determined PA and BIVA reference data for healthy subjects aged ≥ 65 years. These data can be used for clinical purposes to identify individuals at increased risk for adverse health events or to monitor treatment responses.

Key words: *bioimpedance analysis, body composition, nutrition status monitoring, health risk diagnostic and prediction, geriatric medicine, age and gender stratification*

INTRODUCTION

It is well documented that aging affects many physiological functions including significant changes in body composition. In general, total body water, bone mineral density and muscle mass decrease across the life course while body fat stores typically increase and become redistributed more to the abdominal region (1, 2). These changes in body composition are linked to endocrine and metabolic alterations as part of the normal aging process (2-4) and can be substantially influenced and accelerated through chronic inflammatory diseases (5) and lifestyle changes that commonly occur with advancing age, such as a decline in physical activity (6) and altered dietary habits (4). In contrast, targeted physical activity interventions have shown beneficial effects on body composition in different age groups (7, 8) and can counteract muscle mass loss during aging. However, studies have shown, for example, that many older adults are not meeting the recommended physical activity levels (9, 10) and that the prevalence of malnutrition is increasing with age (11, 12). Given that an age-related gradual reduction of muscle mass, bone substance and deficiencies or excesses of body fat are believed to be major risk factors for the loss of functional independence, the development of disability, several diseases and premature mortality (13, 14), it is important to routinely monitor body composition in older adults. Particularly in light of the demographic change in many industrialized countries, appropriate screening methods for monitoring body composition and nutrition status for older adults are becoming increasingly important.

Bioelectrical impedance analysis (BIA) is a non-invasive, relatively inexpensive and easy applicable method to assess body composition, which renders it an attractive alternative diagnostic tool for routine examinations in clinical and research settings (15). The methodology is based on the concept that body impedance (Z, the opposition of the human body to an alternating current) is made up of two components: resistance (R, the body's opposition to the flow of an applied electrical current, inversely related to the water and electrolyte content of tissue) and reactance (Xc, caused by the capacitance of cell membranes and tissue interfaces) (16, 17). In traditional BIA

systems, the human body is considered as a single cylinder with constant resistivity and the impedance is only measured of the half (usually the right side) of the body. Thus, the accuracy of measurements may be limited by the assumption that the various body regions do not significantly differ in composition. Moreover, it has been reported that conventional whole-body BIA methods strongly depend on the muscle mass in the distal part of limbs and that fluid changes in the trunk cannot be detected accurately (18). Improvements in technology and the development of new segmental multifrequency BIA (SMF-BIA) devices, however, have enhanced the precision of this method (16-21). SMF-BIA takes into account that the body consists of five heterogeneous cylinders (trunk and four limbs). In contrast to conventional whole-body BIA-methods, SMF-BIA measures the impedance of the left and the right side of the body and can thus correct for differences in body shape by using the mean values of both body sides. This technique enables to estimate muscle mass for each limb separately (18, 21). Given that BIA-outcomes are generally dependent on which predictive equations have been used for the calculation of body compartments and that measurement accuracy can be influenced by hydration status (19, 21), the use of raw impedance data has recently gained popularity, particularly when monitoring individuals with abnormalities of body water. Raw impedance data are not biased by the choice of a specific regression equation and not affected by the assumption of constant tissue hydration (22). The phase angle (PA) and the bioelectrical impedance vector analysis (BIVA) are the most commonly used parameters based on the sole use of raw impedance data. PA is directly calculated from R and Xc using the formula $arctan(Xc/R) \times (180/\pi)$ and provides information about soft tissue hydration and cell membrane permeability through the electrical tissue properties. Higher PA-values reflect a larger amount of healthy, intact cell membranes, whereas lower values suggest a loss of membrane integrity and diminished cell function (22). In BIVA, R and Xc are plotted as a vector point in a coordinate system standardized to body height (R/h and Xc/h, respectively). The position of the vector point within three tolerance ellipses corresponding to the 50th, 75th and 95th percentiles of a healthy reference population in the graph provides information about the hydration status and the body cell mass (23). Several studies suggest that PA and BIVA are useful prognostic markers in various clinical settings (24-35).

However, the proper application of PA and BIVA in research and health care is convenient only as far as population reference values are available. To date, such reference data for newly developed SMF-BIA devices have only been established in a population aged 18 – 65 years (36). Published data on PA and/or BIVA in older adults (65+ years) are all based on more conventional BIA-technologies (37-44) and thus, the use of SMF-BIA for monitoring older adults is currently limited by the fact that age-specific PA and BIVA normal ranges are still lacking. Comparing older adults to normal ranges derived from a younger population could consequently lead to misinterpretations of individual data. The aim of the present study was, therefore, to establish new reference values for PA and BIVA in a population of healthy older adults (≥ 65 years) using SMF-BIA.

MATERIAL AND METHODS

Participants

Participants were recruited through newspaper advertisements and from medical practices and retirement homes in the area of Erlangen, Germany. All adults from the age of 65 years onwards were generally eligible for the study. Exclusion criteria for study participation were: any acute or serious chronic diseases (especially severe heart or kidney failure, cancer, severe neurological diseases), paralysis (*e.g.* after stroke), amputation of limbs, electrical implants (*e.g.* cardiac pacemakers, defibrillators), insulin pumps and metallic implants (except tooth implants).

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the ethical committee of the Friedrich-Alexander University Erlangen-Nuernberg (Reg.Nr.127_17B). All participants were fully informed about the objectives and methods of the study and provided a written consent before participation.

Anthropometrics

Body height was obtained to the nearest centimeter using a stadiometer (seca 274, Seca GmbH & Co., KG, Hamburg). Waist circumference (WC) was measured at the approximate midpoint between the lower margin of the last palpable rib and the upper iliac crest along the midaxillary line with a measuring tape while participants were in a standing position.

Bioelectrical impedance analysis

The measurements were performed using a SMF-BIA device (seca mBCA 515; Seca GmbH & Co., KG, Hamburg, Germany) that operates with a current of 100 µA at frequencies ranging between 1 and 1000 kHz. The device consists of a platform with an integrated scale and a handrail system. The handrail system contains 6 electrodes, of which 2 pairs were chosen depending on the participant's height. Two further pairs of electrodes are located in the platform and contact the feet. This phase-sensitive 8-electrode technique enables a segmental impedance measurement of the trunk and of each limb. Specifically, impedances of the left and the right side of the body are measured separately and then averaged so that right-handers and left-handers are treated equally. The accuracy for measurements of both body sides at frequencies of 5 and 50 kHz is 5 Ω for Z and 0.5° for the PA (45). All measurements were conducted according to the manufacturer's instructions and as previously described in detail (19). In brief, participants were standing barefoot on the platform in a standing position with out-stretched arms, holding the handrail. The measurement started automatically as soon as both feet and hands contacted all electrodes correctly and lasted a total of 75 seconds. Compared to conventional BIA 4-electrode devices, the SMF-BIA device does not require a laying time of 10 minutes in a supine position to normalize body fluid distribution before testing. However, participants were asked to fast overnight, to refrain from strenuous physical activity within 12 hours and to avoid drinking alcohol within 24 hours prior to the measurement. Before measurement, participants were standing for at least 10 min to account for fluid shifts to lower body due to gravitational forces. All measurements were performed by trained personnel of the study center.

Statistical analysis

Data analyses were performed with R software, version 3.5.3 (R Foundation, Vienna, Austria), and SPSS, version 24.0 (SPSS Inc., Chicago, IL, USA). All descriptive data are presented as means ± standard deviation (SD) and ranges, where appropriate. Subgroup analyses were performed separately for the age groups: 65 – 75 years, 76 – 85 years and > 85 years to evaluate the effect of aging on the PA and BIVA. Anthropometric variables in men and women were compared using unpaired t-tests and, for not normally distributed data, by Mann-Whitney U

tests, respectively. To evaluate the effects of age and gender on the PA, a two-way ANOVA followed by Holm-Sidek *post-hoc* tests was conducted using the three age groups as factor 1 and the two genders as factor 2. The same analysis was applied to evaluate the effects of age and gender on the BIVA components R/h and Xc/h after logarithmic transformation to normalize data. Regression analyses were calculated to check for relationships between age, PA, R/h and Xc/h. For all analyses, a P value < 0.05 was considered statistically significant. PA-values as well as R- and Xc-values for the BIVA chart were calculated by using the mean of the right and the left body side measured at a frequency of 50 kHz. The tolerance ellipses for BIVA were determined according to Piccoli (23). For this purpose, the participants' R and Xc were normalized for body height (h) and for the group

Table 1. Descriptive characteristics of the study population stratified by gender and age groups.

65 – 75 years	**Women n = 147**	**Men n = 163**	**All n = 310**
Age (years)	69.6 ± 3.2 (65 – 75)	69.6 ± 3.3 (65 – 75)	69.6 ± 3.3 (65 – 75)
Weight (kg)	66.7 ± 11.6 (45.8 – 112.6)	81.5 ± 12.6 (55.4 – 132.3)***	74.5 ± 14.2 (45.8 – 132.3)
Height (cm)	162.5 ± 5.5 (149 – 179)	175.7 ± 6.6 (160 – 195)***	169.5 ± 9.0 (149 – 195)
BMI (kg/m²)	25.3 ± 4.2 (17.2 – 39.9)	26.4 ± 3.7 (18.9 – 42.7)***	25.8 ± 4.0 (17.2 – 42.7)
WC (cm)	87.4 ± 10.7 (57 – 124)	97.3 ± 10.6 (78 – 136)***	92.6 ± 11.7 (57 – 136)
$R_{50\ kHz}$ (Ω)	648 ± 65 (460 – 858)	532 ± 52 (373 – 696)***	587 ± 82 (373 – 858)
$Xc_{50\ kHz}$ (Ω)	51 ± 7 (35 – 71)	47 ± 6 (30 – 66)***	49 ± 7 (30 – 71)
76 – 85 years	**n = 107**	**n = 108**	**n =215**
Age (years)	79.1 ± 2.7 (76 – 85)	78.8 ± 2.4 (76 – 85)	78.9 ± 2.5 (76 – 85)
Weight (kg)	66.6 ± 12.2 (46.0 – 111.5)	78.6 ± 10.2 (56.3 – 102.1)***	72.6 ± 12.7 (46.0 – 111.5)
Height (cm)	161.1 ± 6.6 (143 – 177)	173.6 ± 7.1 (153 – 200)***	167.4 ± 9.2 (143 – 200)
BMI (kg/m²)	25.7 ± 4.6 (18.2 – 42.0)	26.1 ± 2.8 (19.8 – 33.5)	25.9 ± 3.8 (18.2 – 42.0)
WC (cm)	89.1 ± 11.4 (66 – 126)	98.5 ± 9.5 (76 – 122)***	93.8 ± 11.5 (66 – 126)
$R_{50\ kHz}$ (Ω)	633 ± 67 (480 – 820)	537 ± 54 (376 – 666)***	585 ± 77 (376 – 820)
$Xc_{50\ kHz}$ (Ω)	46 ± 6 (32 – 60)	42 ± 6 (24 – 63)***	44 ± 6 (24 – 63)
> 85 years	**n = 27**	**n = 15**	**n = 42**
Age (years)	89.6 ± 3.2 (86 – 96)	89.5 ± 3.2 (86 – 97)	89.6 ± 3.2 (86 – 97)
Weight (kg)	60.7 ± 10.2 (47.7 – 87.6)	73.3 ± 9.5 (55.2 – 96.5)***	65.2 ± 11.6 (47.7 – 96.5)
Height (cm)	159.2 ± 7.9 (145 – 178)	168.7 ± 3.2 (160 – 174)***	162.6 ± 11.8 (145 – 178)
BMI (kg/m²)	23.9 ± 3.2 (17.6 – 30.3)	25.7 ± 3.0 (20.9 – 32.6)	24.5 ± 3.2 (17.6 – 32.6)
WC (cm)	90.3 ± 9.6 (74 – 110)	97.1 ± 8.7 (82 – 117)^{a}	92.7 ± 9.7 (74 – 117)
$R_{50\ kHz}$ (Ω)	648 ± 83 (522 – 861)	562 ± 82 (379 – 783)^{b}	618 ± 92 (379 – 861)
$Xc_{50\ kHz}$ (Ω)	38 ± 7 (27 – 55)	33 ± 7 (20 – 49)^{c}	36 ± 7 (20 – 55)

Data are presented as mean values ± SD and ranges; BMI, body mass index; WC, waist circumference; R, resistance and Xc, reactance, measured at 50 kHz and averaged for the left and the right side of the body; ^{a}(P = 0.028), ^{b}(P = 0.003), ^{c}(P = 0.039), and ***(all P-values < 0.001) = significantly different compared to women.

Table 2. Mean values, ranges and percentiles for resistance (R) and reactance (Xc) standardized to height, and phase angle (PA) of the study population stratified by gender and age groups.

65 – 75 years	**Women n = 147**	**Men n = 163**	**All n = 310**
PA (°)	4.56 ± 0.46 (3.18 – 5.63)	5.07 ± 0.50 (3.18 – 6.45)***	4.83 ± 0.54 (3.18 – 6.45)
5th P	3.76	4.23	3.97
50th P	4.54	5.09	4.80
95th P	5.41	5.87	5.69
R/h$_{50\ kHz}$ (Ω/m)	399 ± 51 (293 – 526)	303 ± 32 (212 – 407)***	349 ± 60 (212 – 526)
5th P	340	251	264
50th P	396	304	339
95th P	467	352	446
Xc/h$_{50\ kHz}$ (Ω/m)	32 ± 4 (21 – 44)	27 ± 4 (17 – 39)***	29 ± 5 (17 – 44)
5th P	26	21	22
50th P	32	27	29
95th P	39	33	37
76 – 85 years	**n = 107**	**n = 108**	**n =215**
PA (°)	4.18 ± 0.41 (3.17 – 5.27)	4.50 ± 0.52 (3.18 – 6.09)***	4.34 ± 0.49 (3.17 – 6.09)†††
5th P	3.55	3.56	3.57
50th P	4.13	4.47	4.36
95th P	4.79	5.39	5.17
R/h$_{50\ kHz}$ (Ω/m)	394 ± 42 (306 – 515)	310 ± 34 (225 – 411)***	351 ± 57 (225 – 515)
5th P	332	262	270
50th P	393	305	350
95th P	472	367	457
Xc/h$_{50\ kHz}$ (Ω/m)	29 ± 3 (21 – 38)	24 ± 4 (15 – 37)***	27 ± 4 (15 – 38)†††
5th P	22	19	20
50th P	29	24	26
95th P	35	30	34
> 85 years	**n = 27**	**n = 15**	**n = 42**
PA (°)	3.40 ± 0.64 (2.17 – 5.07)	3.41 ± 0.67 (2.19 – 4.95)	3.41 ± 0.64 (2.17 – 5.07)$^{+++}$
5th P	2.50	2.32	2.41
50th P	3.29	3.38	3.32
95th P	4.46	4.71	4.59
R/h$_{50\ kHz}$ (Ω/m)	408 ± 53 (311 – 538)	334 ± 50 (220 – 458)***	381 ± 63 (220 – 538)$^{\dagger\dagger, \S\S}$
5th P	322	242	307
50th P	401	325	380
95th P	489	439	478
Xc/h$_{50\ kHz}$ (Ω/m)	24 ± 5 (16 – 38)	20 ± 5 (11 – 30)***	23 ± 5 (11 – 38)$^{+++}$
5th P	17	12	15
50th P	24	20	22
95th P	33	29	31

Data are presented as mean values ± SD, ranges and 5th, 50th and 95th percentiles (P); PA, R and Xc measured at 50 kHz and averaged for the left and the right side of the body; ***(all P-values < 0.001) = significantly different compared to women), ††(P = 0.003), †††(P < 0.001) = is significantly different compared to the age group 65 – 75 years, §§(P = 0.007) = significantly different compared to the age group 76 – 85 years, and $^{+++}$(all P-values < 0.001) = significantly different compared to the age groups 65 – 75 years and 76 – 85 years.

mean ± SD and transformed into bivariate Z-Scores. The Z-transformation was performed using the following equations:

Z(R/h) = (R/h – mean(R/h)) / SD(R/h) and *Z(Xc/h) = (Xc/h – mean(Xc/h)) / SD(Xc/h)*

RESULTS

In total, 567 (286 men, 281 women) healthy older adults (74.6 ± 6.8 years, range: 65 – 97 years) were examined in this study. Body weight ranged from 45.8 to 132.3 kg (73.1 ± 13.6

Table 3. Age dependency of phase angle and the BIVA components R/h and Xc/h for women and men.

Regression equation	R^2	SEE
PA_{women} = 0.0357 age – 0.00052 age² + 4.56	0.48	0.446
PA_{men} = 0.0332 age – 0.000602 age² + 5.62	0.62	0.479
R/h_{women} = –0.0182 age + 0.000166 age² + 4.38	0.03	0.407
R/h_{men} = -0.0288 age + 0.000272 age² + 3.69	0.09	0.317
Xc/h_{women} = 0.000961[a] age – 0.0000224 age² + 0.35	0.33	0.0428
Xc/h_{men} = –0.000896[a] age – 0.00000746[b] age² + 0.361	0.42	0.0397

PA, phase angle in; R/h and Xc/h, resistance/height and reactance/height, respectively, in Ω/cm, age in years, height in cm. All factors are significant with $P < 0.001$, except [a]$P < 0.05$ and [b] = not significant.

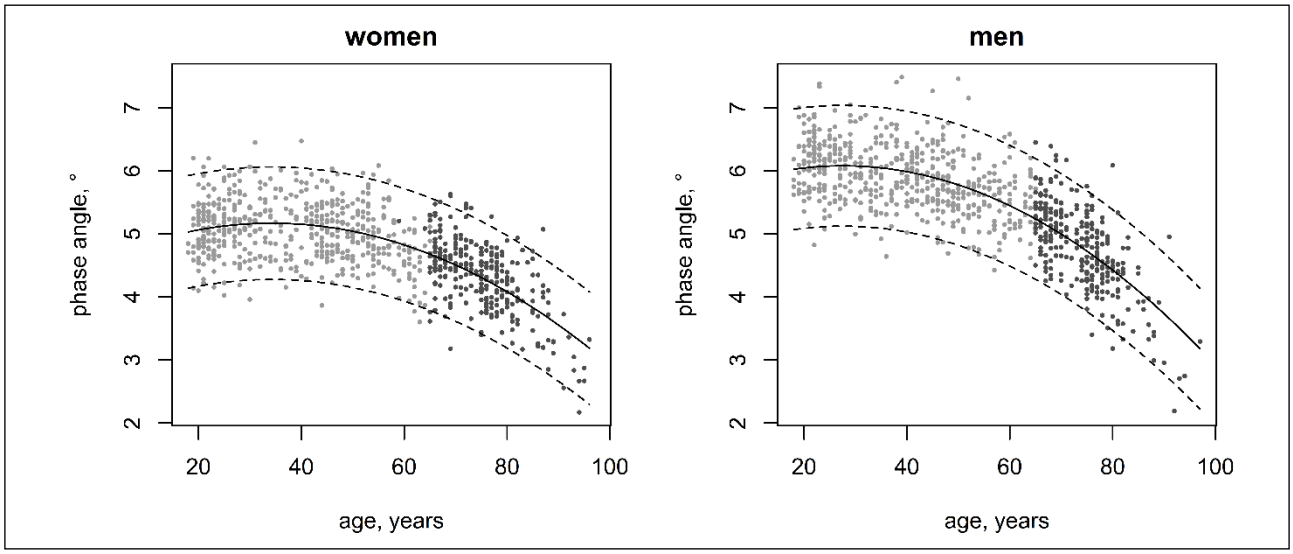

Fig. 1. Phase angle (measured at 50 kHz) of women and men with established percentile curves for the age range of 18 – 65 years (36), indicated as grey points, complemented by new percentile curves for older adults (≥ 65 years), indicated as black points. The solid line represents a quadratic regression versus age, while the dashed lines represent ± 2 SD.

kg), height from 143 to 200 cm (168.2 ± 9.2 cm), body mass index (BMI) from 17.2 to 42.7 kg/m² (25.8 ± 3.9 kg/m²) and WC from 57 to 136 cm (93.0 ± 11.5 cm). Based on the World Health Organization (WHO) BMI cut-off points (46), 6 individuals (1%) of the overall sample were classified as underweight (BMI < 18.5 kg/m²), 250 (44%) of normal weight (BMI < 25 kg/m²), 241 (42%) overweight (BMI < 30 kg/m²) and 70 (12%) obese (BMI ≥ 30 kg/m²). Across all age groups, men were significantly taller ($P < 0.001$) and had a higher weight ($P < 0.001$) and WC (age groups 65 – 75 and 76 – 85 years: $P < 0.001$, age group > 85 years: $P = 0.028$) when compared with women. Regarding BMI, there were only significant gender differences in the age group 65 – 75 years, where men showed greater mean values compared to women ($P < 0.001$). Body height ($P < 0.001$) and weight ($P < 0.001$) decreased with age in women and men. BMI remained rather stable in both genders, with only a tendency to decrease in the men of the oldest age group (> 85 years). Detailed characteristics of the study population stratified by gender and the three age groups are given in *Table 1*. *Table 2* shows the mean PA-, R/h- and Xc/h-values, ranges and the respective 5th, 50th and 95th percentiles for each gender and age group.

There was a significant main effect of gender ($P < 0.001$) and age ($P < 0.001$) on PA and a significant ($P = 0.003$) age-by-gender interaction, indicating that the effect of gender on PA differs by age, and vice versa. PA was significantly ($P < 0.001$) greater in men than in women, except for the age group > 85 years. In both genders, PA decreased significantly with increasing age ($P < 0.001$). Moreover, there was a significant age-related increase of the R/h-values (main effect $P = 0.013$), while the Xc/h-values decreased with age (main effect $P < 0.001$). Mean R/h- and Xc-values were significantly higher in women compared to men (main effect $P < 0.001$). Regression analyses using the data generated from adults in the age range of 18 – 65 (36) and from the older adults (≥ 65 years) of the present study revealed a significant dependency of age on PA and the BIVA components R/h and Xc/h for women and men (*Table 3*).

Fig. 1 displays the new PA percentile curves for women and men aged ≥ 65 years, complementary to existing curves previously established for adults in the age range of 18 – 65 years (36). It became evident that the decline in PA in older adults from the age of 65 years onwards was steeper in men compared to women.

Fig. 2 shows a graphic representation of the BIVA vectors and respective tolerance ellipses (50%, 75%, 95%) for the examined population of older adults (≥ 65 years) compared to a younger population (18 – 65 years) (36). Due to the observed significant dependency of age on Xc/h (*Table 3*), the equation for the Z-transformation was subsequently modified by the addition of the factor age, as follows:

Z (Xc/h) = (Xc/h – f(age)) / SEE.

Using the data of a 65-year old male participant as an example, *Fig. 3* demonstrates how the normal ranges and the

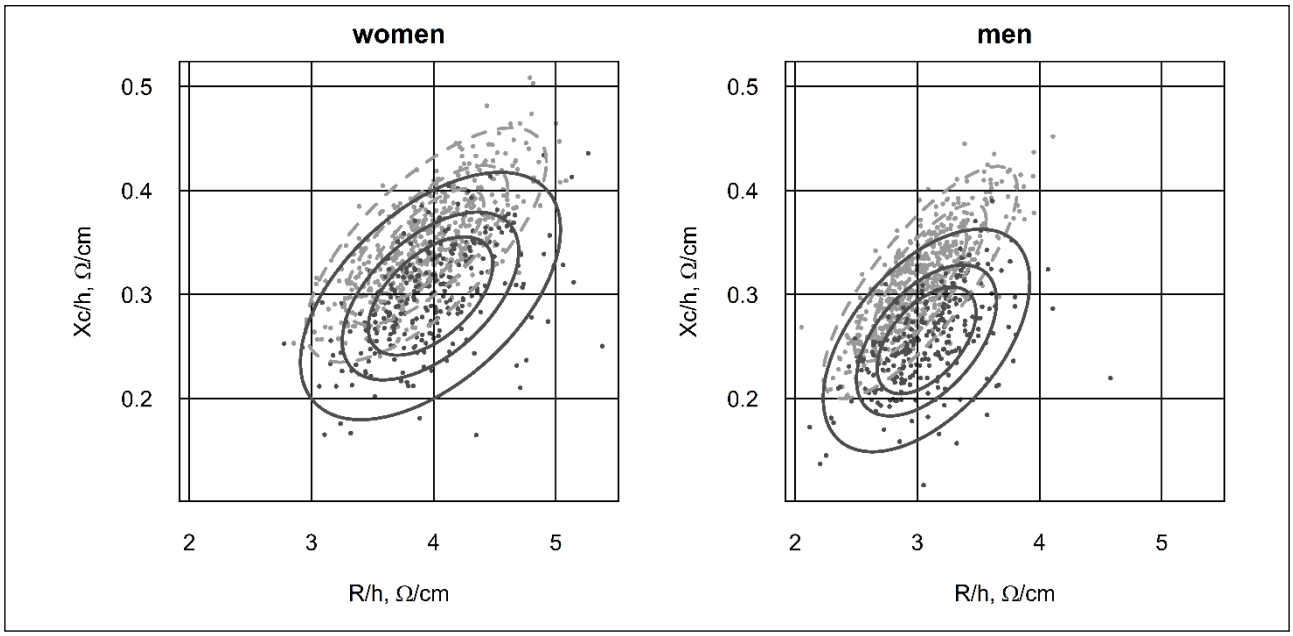

Fig. 2. Bioelectrical impedance vector analysis (BIVA) and 50%, 75% and 95% tolerance ellipses (according to Piccoli *et al*. (23)) of older women and men (≥ 65 years), indicated as black points and full lines, compared to women and men in the age range of 18 – 65 years (36), indicated as grey points and dashed lines.

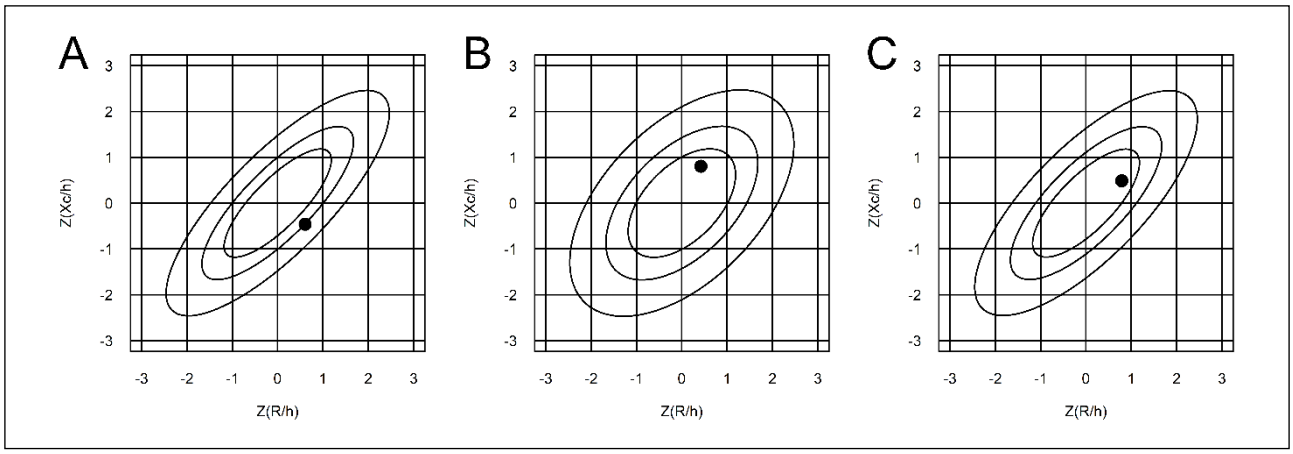

Fig. 3. Vector point position and 50%, 75% and 95% tolerance ellipses after Z-transformation according to the data established for adults in the age range of 18 – 65 years (36) (A), according to the new data obtained from older adults (≥ 65 years) of the present study (B), and according to the combined new data of both datasets and the age dependent Z-transformation (C). Data are illustrated for a 65-year old male participant.

position of the vector point within the Z-transformed BIVA-graph vary, depending on whether the Z-transformation was conducted by means of the population data of (A) adults in the age range of 18 – 65 years as previously established (36) (*Fig. 3A*); (B), the new data obtained from the older adults (≥ 65 years) of the present study (*Fig. 3B*) and (C), by using the combined data of both datasets and the age dependent Z-transformation (*Fig. 3C*).

DISCUSSION

Age-related changes in body composition, such as muscle mass loss, are associated with various adverse health-outcomes, including a higher risk for disability, morbidity and early mortality (13, 14). Given that many industrialized countries worldwide are experiencing a shift in the age distribution of their populations, early preventive efforts and proper screening methods to detect clinically significant alterations in body composition (*e.g.* sarcopenia) in older adults are becoming increasingly important. In this context, BIA has gained widespread popularity because it is easy to use, non-invasive and relatively inexpensive. SMF-BIA is a novel, more precise approach compared to conventional whole-body BIA methods (16-21). However, to date, corresponding reference values for body composition parameters including the widely used PA and BIVA have only been established for adults aged from 18 – 65 years (36). The present study addressed this key limitation by determining PA and BIVA data in a representative sample of older adults (65+ years) using the novel SMF-BIA technique.

As already shown in previous studies using more conventional BIA-devices (37-44), we found a significant dependency of age on PA and the BIVA-components R/h and Xc/h. In both genders, the PA-values decreased with increasing age and men had higher PAs than women (except for the oldest age group > 85 years). Compared to a younger population (18-65 years) for which SMF-BIA-derived reference data have already been established (36), the mean PA-values of the older adults in the present investigation were 15% lower in women and 19% lower in men. In the oldest age-group (> 85 years), the PA-values were even 33% (women) and 42% (men), respectively, lower compared to the younger reference population. Likewise, we found an age-dependent downward migration of vector points in the BIVA chart and significant differences in the tolerance ellipses between the younger and older population. This shows that older adults cannot be compared to a younger population and underlines the importance of age-adapted PA- and BIVA-reference values for adults aged 65 years onwards.

The PA depends on several biological factors, including the integrity and functionality of the cell membrane, intracellular composition and the ratio of extracellular to intracellular water. A high amount of extracellular water reduces the PA, while a higher proportion of intracellular water is reflected by a higher PA. BIVA provides additional information on hydration and body cell mass. A displacement of the vector point along the longitudinal axis corresponds to a change in the R and thus an increase/decrease in body water, while a displacement along the transverse axis corresponds to a change in the Xc and thus an increase/decrease in the body cell mass (47). Hence, the progressive decrease of the PAs and the downward migration of the vector points observed in the older population with increasing age may be related to a reduction of cellular integrity and a loss of tissue mass, which typically occurs during the normal aging process (1, 2). Studies have shown, however, that older adults with lower PAs and significantly displaced vector points in relation to the healthy reference population are at increased risk for frailty, impaired functional performance and mortality (34, 35, 44). Thus, it has recently been postulated that the PA can be used as a global marker of health in aging (42). The gender differences in PA can be explained by the higher amount of muscle mass in men, reflected by the higher mean PA-values compared to women (22, 38, 40). It is to note, that the decline in PA was markedly steeper in men than in women aged 65+ years. This finding is in line with a previous study (43) and may be due to an age-related sexual dimorphism in muscle mass, leading to a faster rate of muscle atrophy in men than in women at a higher age (48).

The mean PA-reference values of older women and men were higher in previous studies using more conventional BIA-methods (mean values ranging from 5.07 to 6.3° in women and from 5.03 to 6.7° in men, respectively) (37, 38, 40-44) than in the present investigation (mean values 4.3° and 4.77° in women and men, respectively). These discrepancies could be explained by the measurement differences between the novel SMF-BIA technique used in this study compared to the more conventional BIA-methods, *i.e.* differences in the posture (standing versus supine) and different positioning of electrodes (49). In line with this, it has previously been reported that differences between BIA devices may contribute to differences in PA reference values obtained in different populations (38, 50, 51). This again underpins that the development of new SMF-BIA-specific reference data for older adults was urgently needed.

Accordingly, it may also be a limitation of our study that the developed reference data may not be applied to other SMF-BIA instruments. It is therefore recommended that analyzer-specific reference values should be developed for other SMF-BIA devices. Finally, given that the present reference data were derived from a Caucasian sample, further studies should be conducted to generate corresponding normal ranges for other ethnicities.

This study provides important new reference data for PA and BIVA in healthy older adults (65+ years) derived from the novel SMF-BIA method. These data can be used for clinical practice and research settings and may help to identify older adults at increased risk for adverse health events compared to a healthy older population.

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