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Original article

Body composition assessment using bioelectrical impedance analysis (BIA) in a wide cohort of patients affected with mild to severe obesity

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SUMMARY

Background & aims: Obesity is characterized by fat mass excess (FM), extra cellular water increase (ECW) and, with ageing, decrease in fat free mass (FFM). The validity of body impedance analysis (BIA) in patients with mild to severe obesity is still debated. The purpose of this study is to describe the Resistance (Rz) and Reactance (Xc) values obtained by Body Impedance Analysis (BIA) in a wide cohort of Italian patients with mild to severe obesity. The secondary endpoint is to describe the resulting body composition values (as percentage and indexes) in this population.

Methods: The study enrolled adult in-patients with mild to severe obesity (classified with class I, II and III obesity) undergoing clinical care rehabilitation program for obesity complications and weight loss. BIA values were grouped by sex, BMI and age classes.

Results: A total of 8303 patients with obesity, aged 18 to 90 y, were studied. The Resistance (Rz) and Reactance (Xc) were reported by sex, age and BMI classes. In women and men both, the phase angle (PhA) decreases with increasing BMI (kg/m^2) and the resulting BIA vector was significantly shifted. The FM index (FMI) was higher ($p < 0.0001$) in women while FFM index (FFMI) was higher in men ($p < 0.0001$) and significantly associated with BMI. FFMI decreased with age in both sex ($p < 0.0001$). Skeletal mass (SM) presents a progressive reduction in relation to age and gender both.

Conclusions: The present BIA-based body composition analysis in a wide cohort of mild to severe obese patients revealed a significantly decreased Rz and Xc values with a consequent significant decrease of PhA in a BMI-dependent manner. The body compartments estimation with available equations was BMI, sex and age dependent. These observational results could be the basis for the development of new equations adapted for patients suffering from obesity.

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1. Introduction

The analysis of body composition is a fundamental part of nutritional status assessment especially in weight loss programs. Body weight and its composition are the result of genetics, metabolism, environment, behavior, and culture; furthermore, it is demonstrated that local fat accumulation has significant, adverse impact for morbidity (i.g. Cardiovascular diseases and diabetes), disability (i.g. overload of articular with functional reduction), emotional well-being and quality of life (i.g. work discrimination and reduction of social life). In patients with obesity the relative abundance and the functional relationships of fat mass (FM) and fat-free mass (FFM) result in important change of human energy control [1]. Evaluation of body compartments is important when considering that weight regain after weight loss frequently results in an increased amount of fat mass (FM) that can be greater than the FM initially lost [2]. The FFM is heterogeneous and includes skeletal muscle mass (SMM), organ mass, bone, body fluids and connective tissue associated with adipose tissue. The appendicular SMM is essential because its loss during weight loss can compromise physical function.

Bioelectrical impedance analysis (BIA) is a simple, noninvasive, rapid, portable, reproducible, and convenient method of measuring body composition and fluid distribution with fewer physical demands. The BIA is based upon the principle that the impedance of a geometrical system is related to conductor length and configuration, its cross-sectional area and the signal frequency. Using a constant signal frequency and a relatively constant conductor configuration, bioelectrical impedance to the flow of a current can be related to the volume of the conductor. Different BIA analyzers are available in the commerce. The analyzers can be classified basing on the used electrical current frequency into multi-frequency (MF-BIA) and single-frequency (SF-BIA) analyzers. MF-BIA uses different frequencies (0, 1, 5, 50, 100, 200, 500 kHz) to evaluate different body compartments. For the estimation of body composition, the frequency frequencies 50 kHz have been used. However, the most routinely used for the estimation is an alternating sinusoidal electric current of 400 lA at a single operating frequency of 50 kHz (SF-BIA) [3]. However, BIA does not measure body composition directly. It measures two bioelectrical parameters: body resistance (Rz) and reactance (Xc) and from these derives the Phase Angle (PhA). The PhA has been interpreted as an indicator of membrane integrity and water distribution between the intra- and extracellular spaces [4] and an indirect estimate of body cell mass [5–7]. It also been used as a nutritional status indicator in adults and children [8,9]. Considering that BIA-derived body compartments rely on a constant level of hydration, all subjects with fluid overload might be at risk for overestimation of FFM.

In patients with obesity, BIA has specific limitations mainly due to the assumptions of constant hydration and body fluids distribution to obtain a valid estimate of FFM [10]. Other factors, including body size and shape (cross-sectional areas in trunk and limbs) that depend on weight, body fat distribution, as well as age, gender and ethnicity, which are independent predictors of body composition, impact BIA-estimates of FFM. The designation of appropriate cut-offs ranges for FM, FFM and SMM in patients with BMI >35 kg/m^2 are not clearly defined, and routinely values established on normal weight subjects are used. The validity of BIA in obese and morbidly obese patients is then still debated [3,11,12].

The gold standard techniques for measuring body composition and total body water are isotope dilution (labelled deuterium), dual energy X-ray absorptiometry (DXA), underwater weighing and air-displacement plethysmography. Abdominal and visceral fat can be measured with computed tomography (CT) and magnetic resonance imaging (MRI). These techniques, however, require special facilities and cannot be used for daily bedside measurements. Hence since BMI cannot be a reliable predictor of FM on the single obese patient, and the cost/effectiveness of DXA, we tested the hypothesis of body composition analysis by BIA testing a wide cohort of patients with mild to severe obesity and of different age decades. For this aim, the main goal of this study was then to describe, in a large cohort of Caucasian (Italian) patients with mild to severe obesity, the body composition BIA parameters, the derived FM, FFM and SMM percentage and indexes by sex, age and BMI classes.

2. Materials and methods

2.1. Subjects

We enrolled patients from two specialized centers for obesity care and metabolic rehabilitation [Endocrinology and Nutrition Unit of IRCCS - Istituto Auxologico Italiano (San Giuseppe Hospital, Piancavallo, VB, Italy (recruitment center 1) and the Endocrinology and Nutrition Unit, Azienda di Servizi alla Persona, University of Pavia, Pavia, Italy (recruitment center 2)]. The inclusion criteria were (BMI) $\geq$30 kg/m^2 and age $\geq$18 yr. The exclusion criteria were: obese patients suffering from liver, heart, lung or kidney failure or peripheral vein thrombosis, patients with abnormal body geometry (such as arm or leg amputation) and patients with clinical condition suggesting peripheral fluid overload (= considered as body hydration $\geq$80%) (Fig. 1) [13]. The study was carried out in accordance with The Code of Ethics of the World Medical Association and performed under the approval of the Ethics Committee of IRCCS Istituto Auxologico Italiano (approval code #2017_05_16_09 and amendment code #2018_04_17_14). Participants gave their written consent prior to participation in this study.

2.2. Weight and height assessment

Body weight (kg) and body height (m) were measured to the nearest 0.1 kg and 0.5 cm respectively, using a mechanical column scale (Scale-Tronix, Wheaton, IL) and a stadiometer (Scale-Tronix, Wheaton, IL), and BMI was calculated as body weight/height squared (kg/m^2). The two recruitment centers were provided with the same equipment.

2.3. Body composition measurements

The BIA measurements were performed in the early morning, after a 12-h overnight fast, using a phase-sensitive, single-frequency bioimpedance analyzer (BIA 101, Akern, Pisa, Italy), which applies an alternating-current of 400 μA at 50 kHz. The instrumentation used in the two recruitment centres was the same (*i.e.* BIA 101, Akern, Pisa, Italy), checked by the manufacturer, and with the same procedure of calibration and controls. Before each testing session, the external calibration of the instrument was checked with a calibration circuit of known impedance value (Rz = 470 Ohm and Xc = 90 Ohm, 1% error). The mean coefficient of variation was 1% for within-day and 3% for intra-individual measurements in the steady state condition and 2% for the inter-operator variability in both centres. Before the measurement, each subject removed clothing and metal jewellery and rested in a supine position for 5 min, to equilibrate body fluids. The impedance measurements were made with subjects with a leg opening of approximately 45° compared to the median line of the body and the upper limbs positioned about 30° away from the trunk [14]. After cleaning the skin with alcohol, two Ag/AgCl low-impedance electrodes (Biatrodes, Akern Srl, Florence, Italy) were placed on the back of the right hand and two electrodes on the corresponding foot, with a

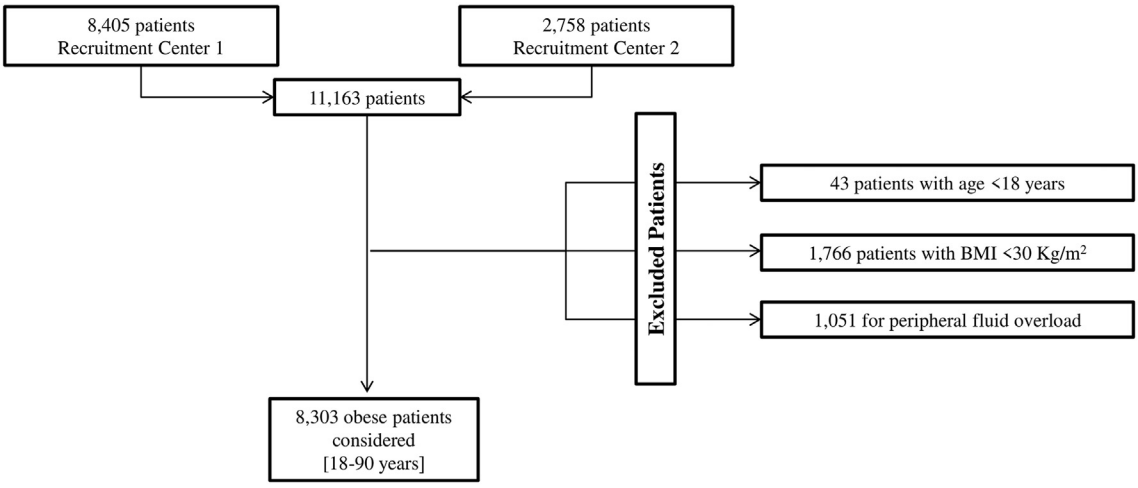

Fig. 1. Flow chart of enrolled/excluded patients with brief indication of selection criteria.

distance of 5 cm between each other [14]. Vector length (VL) was calculated as $(R^2+Xc^2)^{0.5}$ and PhA as the arctangent of [Xc/R x 180/π]. We normalized the raw data of impedance measurements of Rz and Xc by the height (H, m) of an individual patient [*e.g.,* Rz/H and Xc/H in Ω/m] to illustrate individual and group values on the RzXc graph. Patients with water or electrolyte imbalances (*i.e.* edema or ascites) were identified with vectors outside of the 70% tolerance ellipses (hyperhydrated subjects) using the Rz/Xc graph (BIVA software) by Piccoli et al. [15]. The FFM (kg) was calculated by using the population-based prediction model of Sun S. et al. [16]. The value of FFM divided by H^2 (squared meters) was the FFM index (FFMI = FFM/H^2) [17]. The FM was calculated by difference between body weight and FFM. The value of FM divided by squared meter height was the fat mass index (FMI = FM Kg/H^2) [17]. Total SMM (kg) was calculated using the prediction equation of Janssen et al. [18]. As for FFMI, also the SMM was normalized by body surface as index following the equation (SMI) = SMM (kg)/H^2 (m). The equations used are validated for Caucasian population ranging from 18 to 86 yr with BMI 16–48 kg/m^2 [19,20]. The sex and age specific percentiles for SMI (kg/m^2) were calculated considering the whole sample. The cut-off values for SMI, previously reported by a Jansen I et al. [19] were used to define SM amount (kg) and to check whether there was a decreased in muscle mass as needed parameter for the diagnosis of sarcopenia (*i.e.* SMI <8.5 kg/m^2 for men and <5.75 kg/m^2 for women with body weight in a normal range).

2.4. Statistical analysis

The continuous data are reported as mean ± standard deviation (SD) and 95% confidence interval (CI 95%) assuming a normal distribution of data (tested by means of Shapiro–Wilk). Gender-specific tolerance ellipse at 50%, 75% and 95% are built by plotting R/H and Xc/H and compared to tolerance ellipse built by Piccoli et al. [21]. Tolerance ellipse is a region that contains a given proportion of the population. ANOVA models are implemented to investigate the effect of three covariates sex, age, BMI (categorical variables) and their interactions on each of the following variables: Phase Angle (PhA), FFM, FFMI, FM, FMI, total body water (TBW), extracellular water (ECW) and SMI [16]. To perform post hoc comparisons among the different BMI categories considered versus the reference class (BMI 30–35 kg/m^2) the Dunnett's adjustment was applied to control the inflation of type I error. To describe the distribution of selected anthropometric variables in obese population we reported the 10th, 25th, 50th, 75th and 90th percentiles. A $p < 0.05$ was considered statistically significant. All statistical analyses were performed using Statistical Analysis System Software (SASS version 9.2; SAS Institute, Cary, NC) while tolerance ellipses were drawn using the Bodygram Plus™ software (Akern™).

3. Results

We obtained a total of 11,163 BIA assessments (8405 assessments from patients with diagnosis of obesity at center 1 and 2758 at recruitment center 2). Peripheral fluid overload was observed in 9.4% of the whole patients cohort. Patients that did not met the inclusion criteria were excluded, then resulting in a total of 8303 measures from patients with BMI [mean (SD)] 42.60 (7.19) Kg/m^2; age 57 (16) yr of which men were 3659 (44%) with BMI 41.68 (6.95) Kg/m^2 and age 57 (16) yr, and women were 4644 (56%) with BMI 43.32 (6.95) Kg/m^2 and age 56 (16) yr in the final dataset. In the whole group of patients (n = 8303) the mean impedance values (±SD) were 286.11 ± 54.58 Ω/m for Rz/H, 22.81 ± 7.51 Ω/m for Xc/H and 4.53 ± 1.15° for PhA. In men (N = 3659) the mean Rz/H (±SD) was 252.08 ± 40.70 Ω/m and the mean Xc/H (±SD) 20.43 ± 6.50 Ω/m for a resulting mean PhA (±SD) of 4.61 ± 1.18°, while in women group (N = 4644), the mean Rz/H (±SD) was 312.92 ± 48.89 Ω/m, the mean Xc/H 24.68 ± 7.72 Ω/m for a mean PhA (±SD) of 4.47 ± 1.12°.

Since Rz and Xc depend on sex, BMI and age, we sub-grouped the measured values by sex, BMI classes (30.0–34.9 kg/m^2 for Obesity class I, 35.0–39.9 kg/m^2 Obesity class II, 40–44.9 kg/m^2 -45-49.9 kg/m^2 and ≥50 kg/m^2 for Obesity Class III) as well as for age classes (following these decades intervals: 18–29, 30–39, 40–49, 50–59, 60–69 and ≥ 70 yr). Table 2 shows the mean values of Rz and Xc measured and corrected for H and the resulting PhA.

For the same BMI class, an age-dependent decrease in Rz/H and Xc/H values, together with a progressive decrease in PhA, was observed in men and women both. Since PhA reflects the ratio between intra and extracellular water, in obesity it may be affected by nutritional and hydration status. In healthy subjects, PhA ranges between 6° and 7° [22] and in athletes it may reach 8.5° [23] and low PhA values (such as less than 5°) may indicate cellular integrity loss. The PhA decrease observed in the studied cohort had a significant

Table 1
Bioimpendance values of reactance (Rz), resistance (Xc) corrected on height (H, meters) and phase angle (PhA) of 8303 obese patients (3659 men, panel A and 4644 women, panel B respectively) by gender, age and BMI classes. Mean value ± standard deviation (SD) and 95% confidence intervals (CI) are reported; Phase Angle, PhA, is expressed as degree, °.

Panel A
Men

BMI (kg/m^2) class	Age (yr)	N	Rz/H (Ω/m)		Xc/H (Ω/m)		PhA (°)	
			Mean ± SD	95% CI	Mean ± SD	95% CI	Mean ± SD	95% CI
30–34.9	**All**	**576**	**289.98 ± 40.57**	**[278.66–285.30]**	**23.39 ± 6.91**	**[22.83–23.96]**	**4.73 ± 1.18**	**[4.63–4.83]**
	18–29	15	324.07 ± 74.37	[282.88–365.25]	32.24 ± 8.01	[27.81–36.68]	5.74 ± 0.85	[5.27–6.21]
	30–39	19	318.32 ± 53.78	[292.4–344.24]	27.97 ± 6.72	[24.73–31.21]	5.02 ± 0.87	[4.6–5.44]
	40–49	59	278.78 ± 40.67	[268.18–289.38]	26.47 ± 7.39	[24.54–28.39]	5.4 ± 1.17	[5.1–5.7]
	50–59	95	275.11 ± 36.59	[267.66–282.57]	24.06 ± 6.34	[22.77–25.36]	4.99 ± 1.16	[4.76–5.23]
	60–69	181	282.65 ± 39.56	[276.85–288.46]	23.63 ± 5.88	[22.76–24.49]	4.79 ± 1.06	[4.64–4.95]
	≥70	207	279.08 ± 35.18	[274.26–283.9]	20.94 ± 6.66	[20.03–21.85]	4.27 ± 1.16	[4.11–4.43]
35–39.9	**All**	**1075**	**263.12 ± 34.45**	**[261.05–265.18]**	**21.62 ± 6.56**	**[21.22–22.01]**	**4.68 ± 1.22**	**[4.61–4.75]**
	18–29	52	282.92 ± 41.1	[271.48–294.36]	27.01 ± 5.82	[25.39–28.63]	5.47 ± 0.97	[5.2–5.74]
	30–39	44	272.37 ± 39.51	[260.36–284.39]	25.37 ± 6.19	[23.49–27.25]	5.35 ± 1.12	[5.01–5.69]
	40–49	131	260.99 ± 30.9	[255.65–266.33]	23.44 ± 6.43	[22.33–24.55]	5.11 ± 1.23	[4.9–5.33]
	50–59	184	257.09 ± 36.88	[251.73–262.45]	23.25 ± 6.98	[22.24–24.26]	5.14 ± 1.24	[4.96–5.32]
	60–69	317	259.02 ± 31.95	[255.49–262.55]	20.93 ± 5.97	[20.27–21.59]	4.59 ± 1.08	[4.47–4.71]
	≥70	347	266.71 ± 33.34	[263.19–270.23]	19.4 ± 5.99	[18.77–20.03]	4.14 ± 1.11	[4.03–4.26]
40–44.9	**All**	**1024**	**247.73 ± 33.73**	**[245.66–249.79]**	**20.15 ± 5.80**	**[19.79–20.51]**	**4.64 ± 1.15**	**[4.55–4.64]**
	18–29	85	266.36 ± 29.64	[259.97–272.76]	24.06 ± 4.41	[23.11–25.02]	5.18 ± 0.87	[4.99–5.37]
	30–39	80	257.5 ± 44.03	[247.7–267.3]	23.66 ± 4.93	[22.56–24.75]	5.3 ± 0.93	[5.09–5.5]
	40–49	141	243.59 ± 29.29	[238.72–248.47]	22.09 ± 5.66	[21.15–23.03]	5.2 ± 1.25	[4.99–5.4]
	50–59	200	240.42 ± 31.16	[236.08–244.77]	20.19 ± 5.59	[19.41–20.97]	4.77 ± 1.05	[4.63–4.92]
	60–69	300	244.55 ± 33.04	[240.79–248.3]	19.24 ± 5.5	[18.61–19.86]	4.48 ± 1.05	[4.36–4.6]
	≥70	218	250.61 ± 33.38	[246.16–255.07]	17.29 ± 5.36	[16.58–18.01]	3.92 ± 1.01	[3.79–4.05]
45–49.9	**All**	**555**	**235.25 ± 36.31**	**[232.22–238.27]**	**18.76 ± 5.65**	**[18.29–19.23]**	**4.55 ± 1.13**	**[4.55–4.64]**
	18–29	79	262.44 ± 43.87	[252.62–272.27]	23.36 ± 5.06	[22.23–24.5]	5.14 ± 1	[4.91–5.36]
	30–39	69	230.6 ± 33.27	[222.61–238.6]	20.26 ± 4.66	[19.14–21.38]	5.03 ± 0.97	[4.8–5.26]
	40–49	88	229.2 ± 34.35	[221.92–236.48]	19.95 ± 5.58	[18.77–21.13]	4.97 ± 1.06	[4.74–5.19]
	50–59	106	224.95 ± 29.18	[219.33–230.57]	17.99 ± 4.86	[17.05–18.92]	4.55 ± 1.03	[4.36–4.75]
	60–69	131	232.4 ± 34.14	[226.5–238.3]	16.63 ± 5.71	[15.65–17.62]	4.06 ± 1.1	[3.87–4.25]
	≥70	82	237.29 ± 32.54	[230.14–244.44]	16.16 ± 4.42	[15.19–17.13]	3.9 ± 0.95	[3.69–4.11]
≥50	**All**	**429**	**216.51 ± 36.80**	**[213.02–220.00]**	**16.38 ± 5.69**	**[15.84–16.91]**	**4.29 ± 1.21**	**[4.18–4.41]**
	18–29	58	238.67 ± 42.03	[227.62–249.72]	20.22 ± 5.4	[18.8–21.64]	4.86 ± 1.14	[4.56–5.16]
	30–39	71	222.78 ± 42.47	[212.72–232.83]	18 ± 5.9	[16.6–19.4]	4.63 ± 1.34	[4.31–4.94]
	40–49	117	207.28 ± 29.53	[201.87–212.68]	16.34 ± 5.09	[15.41–17.27]	4.46 ± 1.08	[4.26–4.66]
	50–59	79	202.31 ± 32.6	[195–209.61]	14.18 ± 5.39	[12.97–15.38]	3.96 ± 1.19	[3.69–4.23]
	60–69	74	220.53 ± 34.47	[212.54–228.51]	15.44 ± 5.53	[14.16–16.72]	3.96 ± 1.08	[3.71–4.21]
	≥70	30	222.37 ± 27.91	[211.95–232.79]	13.33 ± 4.07	[11.81–14.85]	3.43 ± 0.99	[3.06–3.8]

Panel B
Women

BMI (kg/m^2) class	Age (yr) class	N	Rz/H (Ω/m)		Xc/H (Ω/m)		PhA (°)	
			Mean ± SD	95% CI	Mean ± SD	95% CI	Mean ± SD	95% CI
30–34.9	**All**	**498**	**354.38 ± 50.04**	**[349.97–358.79]**	**29.84 ± 7.97**	**[29.14–30.54]**	**4.80 ± 1.09**	**[4.71–4.90]**
	18–29	49	388.92 ± 62.41	[370.99–406.84]	35.72 ± 8.1	[33.4–38.05]	5.27 ± 1.05	[4.97–5.57]
	30–39	44	340.8 ± 40.7	[328.43–353.17]	29.46 ± 7.55	[27.16–31.76]	4.94 ± 1.08	[4.61–5.27]
	40–49	47	350.99 ± 42.06	[338.64–363.34]	29.92 ± 7.67	[27.67–32.17]	4.9 ± 1.18	[4.55–5.24]
	50–59	96	350.9 ± 39.05	[342.99–358.81]	31.07 ± 7.03	[29.65–32.5]	5.05 ± 0.97	[4.85–5.25]
	60–69	98	348.99 ± 51.1	[338.75–359.24]	29.15 ± 8.53	[27.44–30.86]	4.75 ± 1.1	[4.53–4.97]
	≥70	164	353.93 ± 51.7	[345.96–361.9]	27.84 ± 7.43	[26.7–28.98]	4.49 ± 1.04	[4.33–4.65]
35–39.9	**All**	**1103**	**331.10 ± 42.51**	**[328.59–333.61]**	**27.07 ± 7.54**	**[26.62–27.51]**	**4.65 ± 1.08**	**[4.59–4.71]**
	18–29	97	350.71 ± 55.01	[339.63–361.8]	30.77 ± 6.43	[29.47–32.07]	5.04 ± 0.85	[4.87–5.21]
	30–39	71	336.72 ± 44.31	[326.23–347.21]	30.09 ± 5.91	[28.69–31.48]	5.12 ± 0.83	[4.92–5.31]
	40–49	144	325.29 ± 40.21	[318.67–331.92]	27.88 ± 7.13	[26.71–29.06]	4.88 ± 1	[4.71–5.04]
	50–59	236	325.79 ± 37.97	[320.92–330.66]	27.26 ± 7.16	[26.35–28.18]	4.75 ± 1.05	[4.62–4.89]
	60–69	265	334.83 ± 38.46	[330.18–339.49]	28.01 ± 8.06	[27.04–28.99]	4.76 ± 1.18	[4.62–4.91]
	≥70	290	326.95 ± 43.36	[321.94–331.96]	23.64 ± 6.99	[22.84–24.45]	4.1 ± 0.97	[3.99–4.21]
40–44.9	**All**	**1420**	**315.73 ± 38.76**	**[313.71–317.75]**	**25.26 ± 6.83**	**[24.91–25.62]**	**4.56 ± 1.08**	**[4.51–4.62]**
	18–29	108	330.36 ± 50.02	[320.82–339.91]	29.02 ± 5.64	[27.95–30.1]	5.04 ± 0.78	[4.89–5.19]
	30–39	96	320.41 ± 33.37	[313.65–327.17]	27.95 ± 5.64	[26.81–29.1]	5.02 ± 1.07	[4.8–5.23]
	40–49	221	308.32 ± 36.3	[303.51–313.14]	26.56 ± 6.93	[25.64–27.48]	4.91 ± 1.11	[4.77–5.06]
	50–59	320	312.71 ± 34.62	[308.9–316.51]	26.34 ± 6.36	[25.64–27.03]	4.8 ± 0.99	[4.69–4.91]
	60–69	369	318.54 ± 39.19	[314.52–322.55]	24.55 ± 6.57	[23.87–25.22]	4.39 ± 0.97	[4.29–4.49]
	≥70	306	314.21 ± 39.55	[309.76–318.66]	21.9 ± 6.73	[21.15–22.66]	3.96 ± 1.04	[3.84–4.07]
45–49.9	**All**	**862**	**299.99 ± 43.75**	**[297.06–302.91]**	**22.89 ± 6.79**	**[22.44–23.35]**	**4.36 ± 1.13**	**[4.28–4.43]**
	18–29	57	318.26 ± 60.5	[302.2–334.31]	27.5 ± 6.87	[25.67–29.32]	4.94 ± 0.86	[4.71–5.16]
	30–39	72	312.66 ± 57.5	[299.15–326.18]	24.14 ± 7.57	[22.36–25.92]	4.48 ± 1.38	[4.15–4.8]
	40–49	144	294.63 ± 46.16	[287.02–302.23]	23.8 ± 6.61	[22.71–24.89]	4.63 ± 1.18	[4.44–4.83]
	50–59	198	295.27 ± 38.79	[289.84–300.71]	23.92 ± 6.03	[23.07–24.76]	4.61 ± 0.97	[4.48–4.75]
	60–69	226	300.73 ± 38.57	[295.67–305.79]	21.9 ± 6.4	[21.06–22.74]	4.16 ± 1.06	[4.02–4.3]
	≥70	165	297.45 ± 37.62	[291.67–303.24]	20.09 ± 6.59	[19.08–21.1]	3.84 ± 1.08	[3.67–4.01]

Table 1 (*continued*)

Panel B Women								
BMI (kg/m^2) class	Age (yr) class	N	Rz/H (Ω/m)		Xc/H (Ω/m)		PhA (°)	
			Mean ± SD	95% CI	Mean ± SD	95% CI	Mean ± SD	95% CI
≥50	**All**	**761**	**268.84 ± 40.23**	**[265.98–271.71]**	**18.84 ± 6.10**	**[18.40–19.27]**	**4.36 ± 1.13**	**[4.28–4.44]**
	18–29	58	276.41 ± 39.82	[265.95–286.88]	20.62 ± 4.7	[19.38–21.85]	4.26 ± 0.77	[4.06–4.47]
	30–39	81	277.56 ± 41.41	[268.4–286.72]	19.29 ± 6.33	[17.89–20.69]	3.94 ± 1.05	[3.71–4.18]
	40–49	140	266.01 ± 38.52	[259.57–272.45]	19.71 ± 6.4	[18.64–20.78]	4.25 ± 1.35	[4.03–4.48]
	50–59	197	263.78 ± 40.55	[258.08–269.48]	18.83 ± 6.4	[17.93–19.73]	4.05 ± 1.13	[3.89–4.21]
	60–69	205	267.67 ± 39.02	[262.3–273.05]	18.01 ± 5.99	[17.18–18.83]	3.82 ± 1.05	[3.68–3.96]
	≥70	80	274.97 ± 42.79	[265.45–284.49]	17.71 ± 5.34	[16.52–18.9]	3.69 ± 0.92	[3.48–3.89]

Table 2
Percentiles of Skeletal muscle index (Kg/m^2) in men and women with mild to severe obesity.

Sex	Age	10th	25th	50th	75th	90th
Men	All	9.48	10.21	11.05	12.07	13.07
	≤65 yr	9.82	10.48	11.35	12.42	13.41
	≥65 yr	9.13	9.79	10.62	11.48	12.45
Female	All	7.26	7.90	8.67	9.55	10.53
	≤65 yr	7.53	8.08	8.87	9.72	10.71
	≥65 yr	6.85	7.49	8.27	9.12	9.94

reduction starting from a BMI of 40 kg/m^2 then becoming highly significant for BMI values higher than 50 kg/m^2 in men and women patients both, demonstrating the poor nutritional status and the low cellular integrity in case of extremely increased BMI (Fig. 2). Thus, the BIA vector ellipses for men and women patients with obesity differ from the reference population (Data not shown Fig. 3).

Hence the body compartments calculated as percentage of body weight (FFM%) and as indexes (fat-free mass index FFMI, Kg/m^2; skeletal muscle index, SMI, Kg/m^2) in each considered BMI classes were obtained and showed in Fig. 4. Globally, in each BMI class a similar trend was present with an age-related decrease in FFM% and an increase FM% (not shown) starting from the fifth decade of life. By sex analysis, men obesity affected showed, for the same BMI class, higher FFMI values than women ($p < 0.0001$; Fig. 4). The FFMI presented an increasing trend starting from 18years and peaking around 50–59 years, then having a progressive decline ($p < 0.0001$)

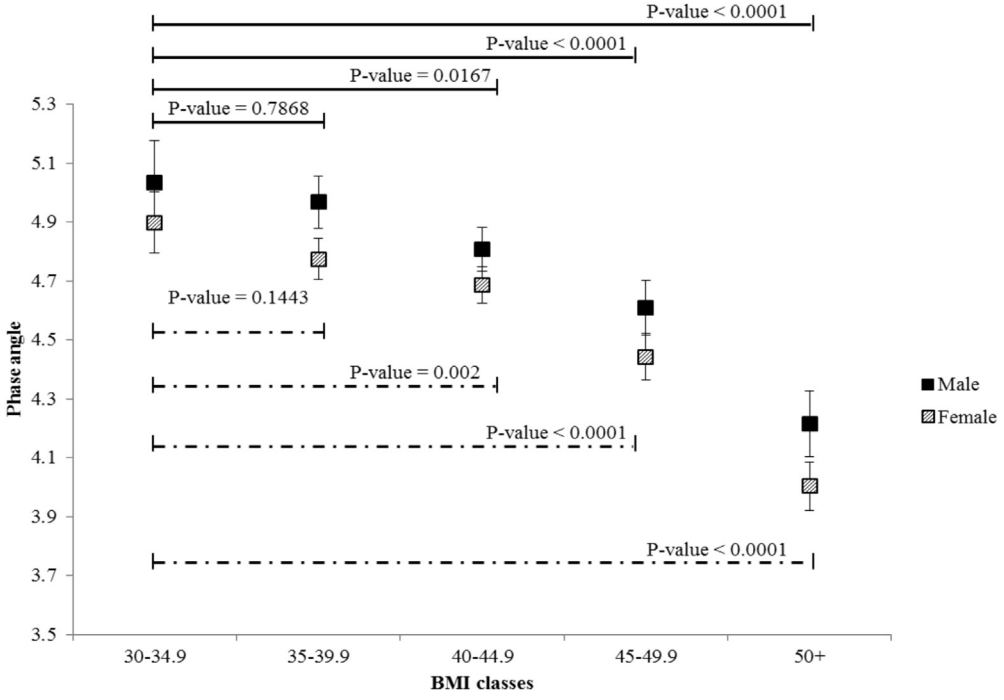

Fig. 2. Phase angle *decrease* with BMI (Kg/m^2) increase in *the studied cohort. Comparisons were made by sex (back box are men and gray box women) versus BMI class 30-34.*9 kg/m^2 *as reference.* Least Square means and their 95% confidence intervals and p-values are represented *(p-values with back represent men comparisons and with women comparisons).*

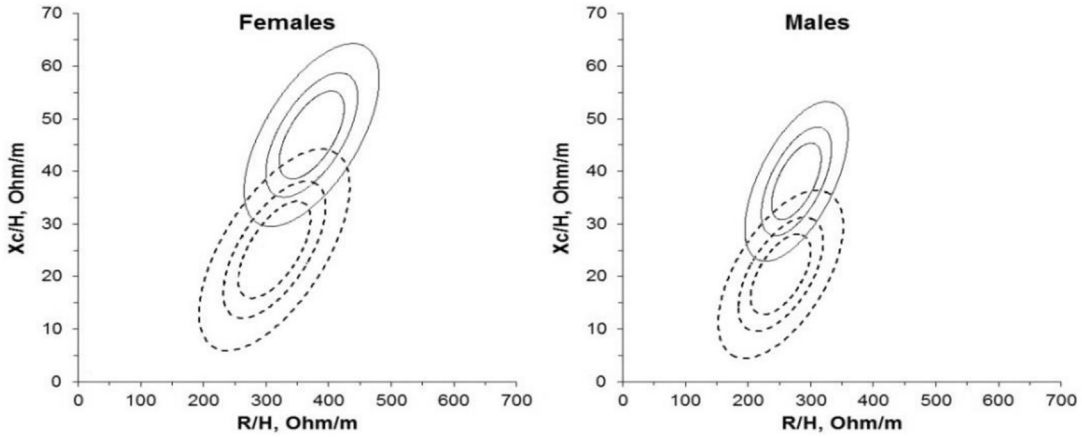

Fig. 3. Comparison of confidence intervals for BIA vector distributions in normal weight subjects (continuous lines, derived from Piccoli et al. [24]) and obesity patients (dotted liens). Confidence intervals are plotted on the RXc graph to represent the 50%, 75% and 95% centiles for the population studied.

in men and women patients both. Age was the major predictor factor of the observed decline in FFM, in both sex ($p < 0.0001$). Considering the large range of BMI and the large sample size studied, we calculated the percentiles for SMI (kg/m^2) by age and gender for all obese patients (Fig. 5). The cut-off values for SMI, used to define SM amount (kg) and to check whether there was a decreased in muscle mass, a necessary parameter for the diagnosis of sarcopenia [19] were used. Based on these cut-offs only 4% of morbidly obese patients presented value that fell below these limits, suggestive for a decreased muscle mass.

The observed percentile value for SMI (kg/m^2) in men and women patients were then subdivided by age less than 65 years, representing possibly the effect of obesity alone, and over 65 years, when a possible combined effect of both obesity and ageing occurs (see Table 2). In the whole studied cohort values of SMI = 11.05 kg/m^2 for men and of SMI = 8.67 kg/m^2 for women with body weight in obesity range defined the 50th percentile of body skeletal muscle mass.

4. Discussion

The present BIA-based body composition analysis in a wide cohort of patients suffering from mild to severe obesity when analyzed by sex, age and BMI class revealed a significantly decreased values for measured Rz and Xc with a consequent significant decrease of PhA in a BMI-dependent manner. By mean group vector analysis, the tolerance ellipses for patients with obesity had a dramatic difference from tolerance ellipses calculated in normal weight population and actually used as reference in vectorial analysis. Furthermore, the range for FFMI, FMI and SMI and the relative values as percentage of body weight when analyzed in relation to age and BMI showed a completely different trend in front of reference values of normal-weight subjects. Because we excluded patients with obesity and fluid overload, we conclude that the new ranges of body composition parameters represent more realistic body composition for diagnostic use in patients with obesity. Using impedance analysis two elements appear relevant: PhA and vector position on the RXc graph. Since PhA reflects the ratio between intra and extracellular water, in obesity it may be affected by nutritional and hydration status. In healthy subjects, PhA ranges between 6° and 7° [22] and in athletes it may reach 8.5° [23] and low PhA values (such as less than 5°) may indicate cellular integrity loss. Previous studies reported that PhA might change in relation to sex, age and BMI. Women present smaller PhA than men probably for less muscle mass [22] with lower PhA values seen in older people, probably for a reduction in Xc due to a loss of muscle mass and an increase in Rz related to an increase FM and reduction of body water [25]. The relationship with BMI is still not well defined and it appears affected directly by body mass increase in severely obese subjects (BMI>40 kg/m^2), an inversely by body fluid overload (ECW) [22]. Furthermore, different risk factors such as hyperglycemia, inflammatory cytokine increase, high leptin levels and insulin resistance were significantly higher in obese women with lower PhA [26] suggesting that change in cell health for different diseases appear relevant and we need reference values of PhA for different clinical condition. We cannot test, in our population, the presence of multimorbidity associated disease but, for clinical use, appear interesting the possibility to monitoring the progression of nutritional status or cardiovascular risk factors with PhA. The length of BIVA vector indicates hydration status of soft tissue [27]. Within the reference values, gender-specific, 75% tolerance ellipse indicate normal hydration; short vectors below this limit indicate overhydration and long vectors above the 75% tolerance ellipse indicate underhydration [15]. Significant vector displacement is seen in different disease severity such as obesity [28] previously reported that in severe obesity the vectors were shorter than those in the other groups and more frequently distributed on the left side of the graph. This indicate an increase in TBW with a proportional increase in ECW, due to increased soft tissue mass also with normal tissue hydration. However, it is difficult to evaluate the body compartments with conventional BIA prediction equations but it is reported that in most individuals with obesity, the increase in FM is associated with a parallel increase in FFM [29]. Thus, our data confirm that in adults with obesity, BIA analysis show a great number of normal-hydrated patients but with reduced values of PhA in relation to high BMI. The result is a shift of the mean vector, including the 95% tolerance ellipse, with new reference values for the consecutive BIA predictor equations of FFM, FM and SM.

We evaluated the reference range FFMI, FMI and SMI using the measured values of Rz and Xc obtained by BIA and the derived equations. Previous research [30–32] showed the importance of normalizing FFM data by height because FFMI is more representative than FFM alone of nutrition status and considering that a decreased height was associated with advancing age [22,33]. We investigated the trend of each parameter in relation to age and BMI. We found that FFMI differs for males and for females as previously described [34–36]; furthermore, significant increases in FFMI occurred between the ages of 15 and 23 years as expected due to adolescent growth and remained relatively stable between the

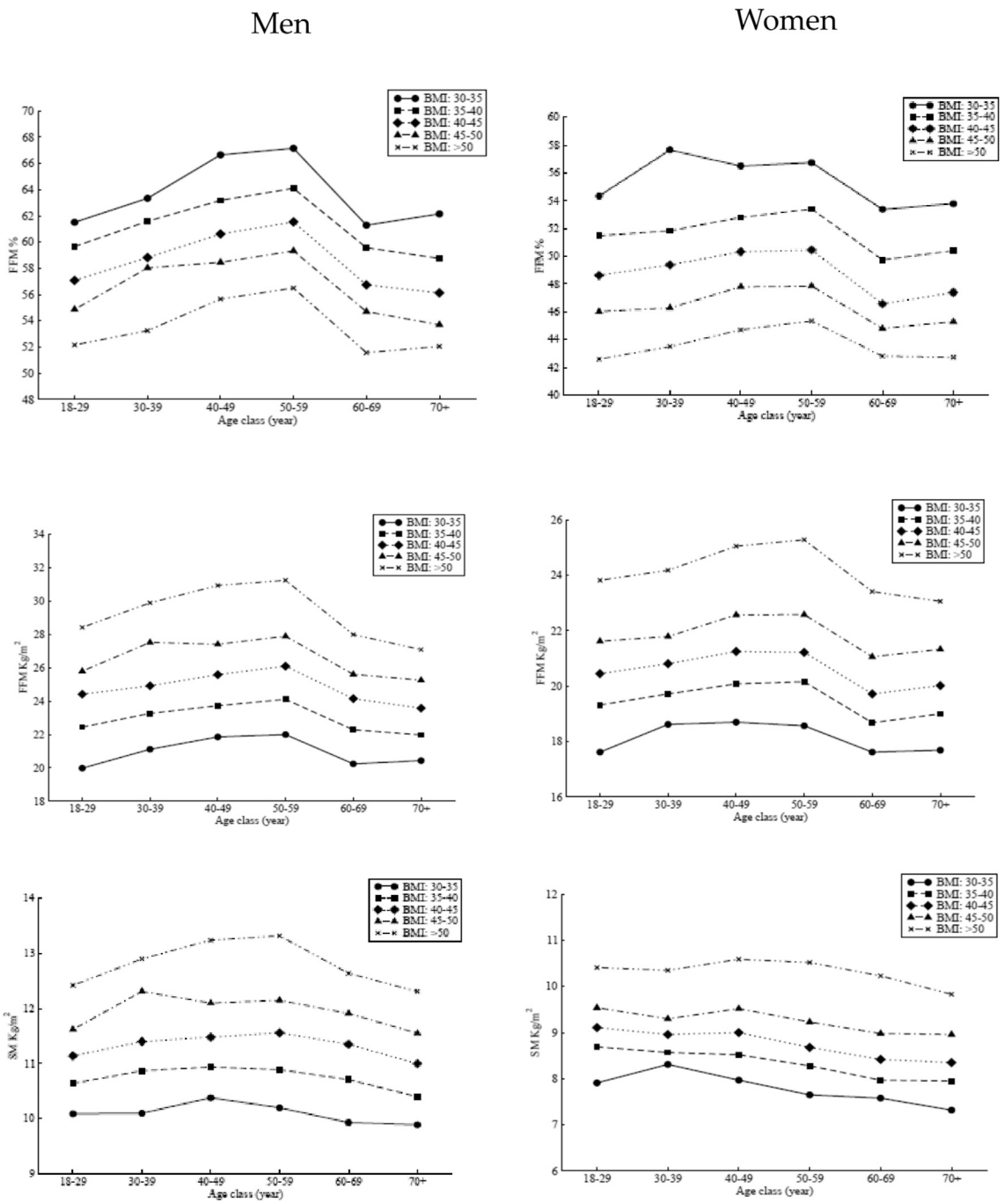

Fig. 4. Fat free Mass as body weight percentage (%, panel A), as index (Kg of FFM/H, panel B) and SM index (SMI, Kg/m^2) for each BMI class in age decades and subdivided by sex (men, M and women, W).

ages of 25 years with a peak between 40 and 50 years of age when a decline was registered until 80 years. In a previous study, that evaluated lean body mass index (LBMI) with DXA analysis in a large group of Italian population (large range of BMI and age) a significant increase was reported in both genders, and a significant and progressive decline of FFMI associated with aging was shown [34,37,38].

The FMI values were higher in females at all ages and increase progressively with age (25–35yrs), remain stable until 65yrs when decline [39]. When BMI increase, we registered an increase in FMI with higher values for women that was generally observed [31,34,40]. This gender difference, that is not completely explained, appears relevant as cardiovascular risk factor for female. As reported in several studies, BIA analysis is used to muscle mass assessment and sarcopenia diagnosis, but it is recommended the use of normative data of the study population, generated from appropriated equation/algorithm to be sure of reliable results [41]. Obesity might interfere in skeletal muscle mass assessment and few data are available on the effect of obesity "*per se*" in skeletal mass decline. Previous studies reported for the Italian population

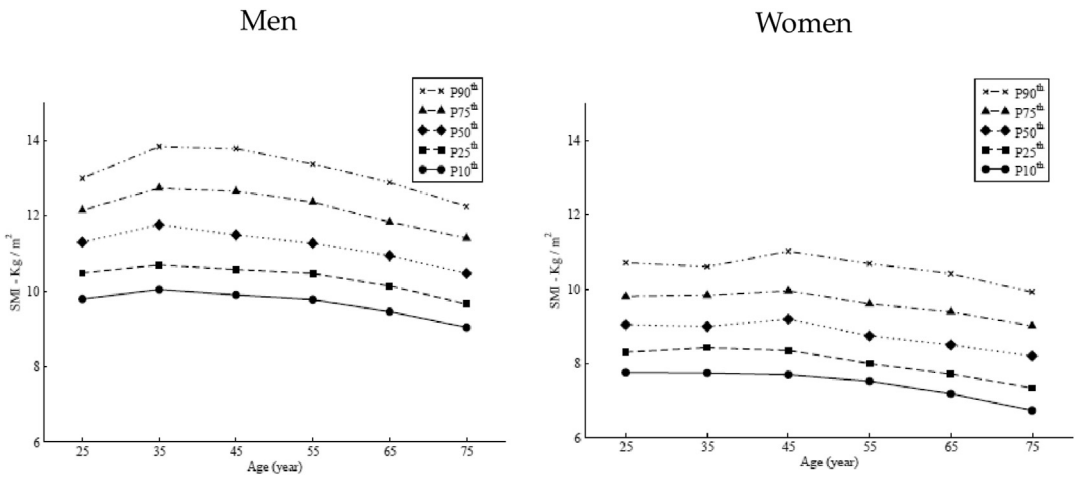

Fig. 5. Percentile distribution of SMI (Kg/m^2) in relation to age (years) in men (M) and women (W) affected by mild to morbid obesity.

cut-off values of SMI< 8.5/5.75 kg/m^2 (men and women respectively) for sarcopenia condition diagnosis in normal weight subjects associated with disability and mortality [42–44]. Our results demonstrated that the wide majority of patients with mild or severe degree of obesity have increased values of SMI in relation to BMI when compared to normal weight reference population. The trend toward a SMI decreased amount was observed principally in obese men after the fifth decade of life. We suggest new "normality ranges" for SMI in obese patients considering the percentile distribution observed in men and women. Our data however need to be validated in future studies and necessarily coupled with functional tests (such as handgrip, time up and go, 6 min walking test etc.) to better define the clinical relevance of these values and to establish the possibility of use them as new cut-off values for a proper sarcopenia diagnosis in patients with obesity.

Our study presents some possible limitations. The BIA is considered not satisfactory in patients with severe obesity and hyperhydration state. To avoid this bias, patients with known fluids imbalance were excluded considering as hyperhydration a total body water ≥80% (9.4% in the studied cohort). Then body composition assessment in these particular and category of patients remains to be determined and studied. Furthermore, our previous validation of phase sensitive tetrapolar SF-BIA against DXA in a group of obese showed no significant differences (*data not show*), although other BIA analyzers produce estimation of FFM values slightly higher than DXA values [45–47]. This probably indicates the need for new predictive equations adapted to men and women affected by mild to morbid obesity. Additionally, a possible bias due to data collection in two independent centers, despite performed by highly trained personnel, cannot be excluded.

At present, no studies are available and no specific equations have been validated in a population with BMI >35 kg/m^2. In this work, equations not validated for the obese population were used for the calculation of fat free mass and skeletal muscle mass. Our purpose was not to create an equation suitable for this population, but to evaluate the goodness of those already available. The present study, albeit descriptive, thanks to the large sample analyzed, could be in the future suitable to improve and create new equations adapted for patients with obesity.

5. Conclusions

In conclusion, with the present study we described BIA analysis in a wide cohort of patients with obesity defining a picture of the FFMI and SMI values in Italian population in relation to age and BMI classes. In particular, we demonstrated that PhA presented a decrease vs a normal representation that we need to consider for a correct evaluation of body composition in patients with obesity and suggesting poor nutritional status. An accurate assessment of nutritional status has three components (body composition, energy balance and functionality). Therefore, it is essential to set up an adequate treatment that must necessarily be of multidimensional type. The BIA assessment of body composition of normal hydrated subjects with obesity could represent a valid support to better characterize the nutritional status of patients with obesity and to plan a correct rehabilitation program aimed at reducing FM and maintaining/increasing FFM.

Author contributions

Conceptualization, A.B. and R.C.; methodology, A.B., R.C.; software, D.S.; formal analysis, D.S., A.Z.; investigation, A.B., S.P., M.R., S.B, C.V., P.C., R.C.; resources, A.B., M.R., S.B, P.C., C.V.; data curation, A.B, R.C., D.S.; writing—original draft preparation, A.B., R.C.; writing—review and editing, A.B., R.C., M.R., S.P.; supervision, H.L.; project administration, A.B., R.C. All authors have read and agreed to the published version of the manuscript.

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Conflicts of interest

The authors declare no conflict of interest.

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