

Body composition assessment techniques in clinical and epidemiological settings: Development, validation and use in dietary programs, physical training and sports

Edited by

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Body composition assessment techniques in clinical and epidemiological settings: Development, validation and use in dietary programs, physical training and sports

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Editorial: Body composition assessment techniques in clinical and epidemiological settings: Development, validation and use in dietary programs, physical training and sports

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Editorial on the Research Topic

Body composition assessment techniques in clinical and epidemiological settings: Development, validation and use in dietary programs, physical training and sports

Body composition assessment is essential in both clinical and field settings to accurately describe and monitor nutritional status for a variety of medical conditions and physiological processes. Patients with cancer, osteoporosis, cardiovascular disease, diabetes, as well as sick and malnourished patients, pregnant women, nursing mothers, and the elderly, are a few examples among several other diseases that can be assessed by body composition. Body composition outcomes help evaluate the effectiveness of nutritional interventions, the alterations associated with growth and disease conditions, and it contributes to the development of personalized physical training programs (1–3).

There are several techniques for assessing body composition, from simple body indices based on anthropometric measurements to sophisticated laboratory methods such as magnetic resonance imaging (4), with the ability to assess different body compartments at different levels (5, 6). Thus, many studies have been conducted in order to develop and validate techniques, which can be extremely useful for health professionals to estimate body composition components such as fat mass, muscle mass, bone mass, and residual mass, or simply fat mass and fat-free mass (7–10).

The aim of this Research Topic is to address the most recent innovations in body composition assessment for its application in epidemiological studies, as well as in clinical practice, providing health professionals with concepts and evidence of its usefulness, while assisting them with the most appropriate selection of techniques according to the characteristics of the individuals or groups evaluated.

In this Research Topic, 22 papers were published, divided into three groups of studies: development of predictive models and validation of existing predictive models; cross-sectional descriptive studies; intervention studies; and a systematic review and meta-analysis.

All studies used anthropometry and/or bioelectrical impedance as a technique to assess body components, which can be explained by the relatively low cost and high applicability in clinical and field conditions (2, 11). However, studies that developed or tested the validity of predictive models of these techniques used mainly dual-energy X-ray absorptiometry (DXA) as the standard technique, while two studies used computed tomography and one study used air displacement plethysmography.

The most discussed aspect was the development of predictive models and the validation of existing models. Nine of the 22 (40.9%) published articles covered this topic, which represents a vital role for studies in body composition assessment, as the mathematical models developed to estimate body components are more specific with the source population, indicating that they cannot be generalized to several populations (12).

The study by Costa et al. first tested the validity of eight equations for estimating fat-free mass (FFM) by bioelectrical impedance analysis, developed for adolescents from different populations, verifying that none of them met the validity criteria in the sample of adolescents aged 10 to 19 years, from the northeastern region of Brazil. Thus, the authors developed and cross-validated a specific mathematical model for this population. Still, in the same region of the country, but for adults aged 20 to 59 years, Ribeiro da Costa et al. tested the validity of the body adiposity index (BAI) proposed by Bergman et al. (13), finding low validity for the studied sample. Then, the authors developed a regression equation that was included in the model, in addition to the BAI variables (height, waist circumference, and hip circumference), weight, gender, and age, to estimate the FFM and total body fat, using anthropometric measurements.

Likewise, or more important than the amount of body fat, is its distribution, as a higher concentration of fat in the abdominal region, especially visceral fat, is associated with non-communicable chronic diseases and increased morbidity and mortality (14, 15). However, measuring fat in this region demands high-cost laboratory techniques, such as magnetic resonance imaging or computed tomography (11, 16), indicating the need for valid predictive models for clinical or epidemiological screening. This aspect was contemplated in two articles by Lai et al., who developed an equation for the abdominal subcutaneous fat area using bioimpedance analysis (BIA) combined with a sagittal abdominal diameter, and of Ji et al., who developed formulas for calculating L3 skeletal muscle mass index and visceral fat area based on simple anthropometric measurements. Both studies used computed tomography as the standard technique.

Another aspect worth mentioning is that the validity of techniques for estimating the body composition of under 6 year-old children still needs to be clarified in the literature (17). Lyons-Reid et al., using air displacement plethysmography as a reference, developed empirical prediction equations to estimate FFM in childhood. The authors demonstrated that the inclusion of impedance in the equations instead of just anthropometric parameters improved performance in most cases, but the difference was slight. Further investigation was suggested before the routine use of BIA in childhood can be recommended.

Studies on changes in body composition due to aging have been highlighted, mainly due to the negative impact of sarcopenia on health in elderly populations, suggesting the need for valid clinical techniques to assess this condition (18). Of the four studies published in this issue which proposed testing techniques' validity, three included a sample composed of older adults, using bioimpedance and/or anthropometry. Cádiz-Ríos et al. verified the agreement between six bioimpedance equations and DXA to estimate the appendicular skeletal muscle mass; van den Helder et al. validated bioimpedance analysis to diagnose low appendicular lean mass; and Velázquez-Alva et al. evaluated the agreement between bioimpedance measurements and five anthropometric equations for estimating body fat, using DXA as a standard.

Another important aspect is the difficulty of assessing body composition in people with disabilities. Although there are mathematical models for estimating fat-free mass, by bioelectrical impedance analysis, in people with spinal cord injury, it needs to be clarified whether they can be generalized to people with this condition (19). Bauermann et al. demonstrated that using non-specific impedance measurement equations can lead to an erroneous interpretation of FFM values in male subjects with spinal cord injury, indicating the need to develop new predictive equations for this group.

Regarding the cross-sectional descriptive studies ($n = 7$), two articles addressed the Phase Angle, a variable obtained through bioelectrical impedance testing that has been widely used as a marker of cell membrane integrity and a prognostic factor in several diseases (20, 21). Mattiello et al., produced Phase Angle percentile curves in a healthy population covering most of the life cycle, stratified by sex and age, using generalized additive models for location, scale, and shape as a continuous function of age. de Moraes et al., studying adolescents, demonstrated that the variability in the phase angle is related to interindividual variation in sex, age, and maturation status, as well as differences in body size. The authors concluded that research with adolescents considering phase angle should use multilevel modeling with standardized parameters as default to adjust for the concurrent influence of sex, age, maturity status, and body size.

Using anthropometric measurements, such as body mass, height, body circumferences, and indices based on these and other measures derived from bioelectrical impedance analysis constitutes a tool for risk screening for adverse health conditions throughout life (13, 22). These measurements or indices may be associated with arterial properties and variations (Gómez-García et al.); the lipid and glucose profile of children and adolescents (Nogueira-de-Almeida et al.); malnutrition and its repercussions for all-cause mortality and cardiovascular mortality (Fan et al.); high blood pressure in adolescents (Borges et al.); and hypertensive disorders of pregnancy (Yuan et al.).

Many intervention studies with dietary and/or physical exercise programs seek to demonstrate their impact on changes in body composition (23, 24). In this Research Topic, five articles performed interventions to analyze different outcomes. Sheikholeslami-Vatani and Rostamzadeh investigated the effect of 8 weeks of high-intensity interval training and vitamin D3 supplementation on changes in appetite-dependent hormones and body composition in sedentary overweight men, finding satisfactory results. In the study by Lazzar et al., a 3-week multidisciplinary body weight reduction program with moderate energy restriction and regular physical activity was

sufficient to determine a 4–5% reduction in body mass, in addition to improving physical activity and induce beneficial changes in body composition in obese adolescents and adults. They carried out a randomized controlled trial to test the effects of aquatic resistance training and dietary education on health indicators in older women, including body composition. The results suggest that older women who practice regular and programmed underwater resistance training, among other benefits, have improved body composition variables (smaller fat compartments and greater muscle mass).

Another randomized controlled trial aimed to verify the impacts of water supplementation on body composition indices in young adults after a 12-h overnight fast to determine the ideal volume of water to improve body water composition. Among other findings, the authors concluded that 200 mL was the minimum volume capable of improving the distribution of water content among the participants of this study (Zhang et al.). And finally, studying preterm-born preschoolers with very low birth weight, Fernandes et al. verified the impact of a continuous early home-based intervention program on body composition. The study showed that an early intervention protocol from the newborn intensive care unit (NICU) to a home program performed by mothers of preterm with very low birth weight (VLBW) children from low-income families has a small effect on fat-free mass.

As mentioned, this Research Topic also published a systematic review and meta-analysis that surveyed diagnostic studies to identify the optimal cutoff value for the waist-to-height ratio (WHtR) to predict central obesity in children and adolescents. The 12 articles included in the meta-analysis led to the conclusion that 0.49 was the best cutoff point to predict abdominal obesity in children and adolescents of both sexes.

In summary, the results of the studies and the review in this volume bring a substantial amount of relevant data on body composition assessment techniques in their different uses.

Thus, these manuscripts contribute to a better understanding and better using different techniques for estimating body components in clinical and field situations to optimize dietary and physical exercise programs.

Author contributions

All authors participated in the elaboration, writing, revision and approval of the final document of this editorial.

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Changes in Appetite-Dependent Hormones and Body Composition After 8 Weeks of High-Intensity Interval Training and Vitamin D Supplementation in Sedentary Overweight Men

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Exercise and diet are important factors for energy balance and appetite regulation. The aim of this study was to investigate the effect of 8 weeks High Intensity Interval Training (HIIT) and vitamin D₃ supplementation in sedentary overweight men. Forty-eight participants were randomly assigned to one of the following four groups ($n = 12$): HIIT + VitD, HIIT + placebo (3 sessions per week, 10×1 min interval cycling at 90–100% VO_{2peak} separated by 1 min active recovery at 15% VO_{2peak} for 8 weeks), Vit D and control groups. Participants received 2,000 IU/day 25 (OH) D₃ or placebo. Measurements were taken pre and post training after 10 h overnight fasting. Insulin, weight, BMI and body fat percentage were significantly decreased, but PYY was significantly increased in the HIIT + Vit D and HIIT + placebo groups ($p = 0.001$ and $p = 0.001$, respectively) after 8 weeks of HIIT. Insulin ($p = 0.009$, $p = 0.001$), weight, BMI and body fat percentage ($p = 0.001$, $p = 0.001$) were significantly lower in the HIIT + Vit D and HIIT + placebo groups compared to the Vit D and control groups. However, PYY was significantly higher in the HIIT + Vit D group compared to the Vit D ($p = 0.025$) and control groups ($p = 0.007$) and also in the HIIT + placebo group compared to the Vit D ($p = 0.037$) and control groups ($p = 0.032$) after 8 weeks of HIIT. The combination of regular HIIT with vitamin D supplementation has a effect on appetite control and body composition.

Keywords: HIIT, appetite, vitamin D3, acylated ghrelin, PYY

INTRODUCTION

Today, controlling and preventing weight gain is one of the most important factors in preventing disease and mortality in the world (1). Control of appetite and energy intake is a complex issue and depend on a variety of hormonal-neurological, psychological and even cultural factors. At the physiological level, appetite regulating gut hormones play an important role in hunger and satiety (2). PYY is an anorexigenic peptide, synthesized from L-cells in the gastrointestinal tract and released into the bloodstream. In this connection, acylated ghrelin an orexigenic gut peptide, is also released in the stomach (3). It is now well-known that exercise reduces orexigenic peptide (acylated ghrelin) and increases anorexigenic peptides (i.e., PYY) (4, 5). Results of many studies

have shown a link between physical activity and physiological mechanisms of appetite control (4–6). As inactivity increases, a positive energy balance and subsequent weight gain occurs. However, physical activity plays a central role in the management of body weight by creating a negative energy balance and affecting the sensitivity of appetite-regulating hormones (7). One of the most essential aspects of exercise that might affect appetite regulation is the intensity of exercise (8).

The American college of sports medicine (ACSM) recently stated that high-intensity interval training (HIIT) is as effective as moderate-intensity continuous training (MICT) in improving body composition and insulin sensitivity in overweight and obese people (9). In this regard, high-intensity interval training\which involves short repetitive periods of activity with an intensity $>85\%$ of $\text{VO}_{2\text{max}}$ has been shown to have similar and in some cases more physiological and metabolic adaptations in comparison with moderate-intensity endurance work (10). Recent evidence suggests that HIIT alters appetite-regulating hormones, limits energy consumption (8) and leads to a significant reduction in body fat mass (11).

On the other hand, Vitamin D deficiency is a global public health problem (12). According to the some estimations, more than one billion people in the world suffer from a deficiency of this vitamin (13). Some studies have shown that there is a significant deficiency of vitamin D₃ in the adult population of different countries (35% in the United State, over 80% in Pakistan and Bangladesh, 90% in Turkey, 96% in India, and 67% in Iran) (13, 14). An inverse relationship has been found between the concentrations of 25 (OH) D and body fat mass (15). Excessive fat accumulation causes enzymatic disorders such as decreased activity of alpha-hydroxylase, the key enzyme in the conversion of 25-hydroxyvitamin D₃ to 1,25-dihydroxyvitamin D₃. This causes the accumulation of inactive forms and decreased bioavailability of vitamin D (16). As mentioned, vitamin D deficiency is associated with genesis of overweight and obesity (17). Reasons for decreased vitamin D levels during obesity include increased absorption of vitamin D by adipose tissue, decreased liver synthesis of vitamin D due to hepatosteatosis, and increased clearance of vitamin D during inflammation conditions (18, 19). In addition, vitamin D insufficiency increases parathyroid hormone concentration, activated lipogenesis, and results in greater accumulation of fat mass (20, 21). Vitamin D deficiency is associated with obesity and it has been reported that Vitamin D supplementation has similar effects of exercise on glucose metabolism and insulin sensitivity in overweight and obese individuals (22, 23).

It is believed that the hypothalamus will increase appetite and reduce energy expenditure due to low circulating levels of calcidiol due to vitamin D deficiency. These adjustments are made through the transcription pathways of the Neuropeptide Y (NPY) or Agouti related protein (AgRP) (24, 25). Vitamin D supplementation increases the expression of vitamin D receptor (VDR) gene in the pancreas (26), and VDR activation induces peptide YY transcription in pancreatic islets (an appetite suppression hormone that is produced in the pancreas in addition to intestinal L cells) (27). Daily intake of vitamin D supplements can maintain a sufficient serum level (above

30 ng/ml), but the daily dose varies according to age, gender, geographical location, skin pigmentation, physical activity and season. Research studies have illustrated that 2,000 IU/day is the minimum dose required to ensure a minimum goal (30 ng/ml) in the blood (28).

Independently, exercise and vitamin D₃ supplementation both directly and indirectly induce beneficial and adaptive responses to control obesity, appetite, and body fat loss. However, there is a lack of research on the simultaneous effect of vitamin D₃ supplementation and exercise on these variables. Therefore, the purpose of the present study was to investigate the simultaneous effect of HIIT and vitamin D₃ supplementation on appetite-dependent hormones and body composition in overweight sedentary men.

MATERIALS AND METHODS

Subjects and Experimental Design

Forty-eight Healthy and Overweight Male College Students (age: 21.7 ± 1.4 yr, Height: 175.3 ± 4.3 cm, Weight: 86.52 ± 3.92 kg and BMI: 27.28 ± 0.76 kg·m⁻²) Were Recruited From the University of Kurdistan to Participate in This Randomized Control Trial Study. Participants Were Randomly Assigned to four Groups: HIIT + Vitamin D, HIIT + Placebo, Vitamin D and Control Group. The Demographic Characteristics of the Subjects can be Seen in **Table 1**. The Sample Size Calculation Was Carried out Using G Powers Software (Heinrich-Heine-Universität, Düsseldorf, Germany). Questionnaires Covering Health History, Drug and Dietary Supplement Usage (3 months Prior to Study), Lacking Regular Exercise Training (in the Last 6 months) were completed by all subjects to determine eligibility. Having a Body Mass Index (BMI) Higher than 25 kg/m² and Vitamin D Deficiency (20 ng/ml) were the Inclusion Criteria and all Subjects Had Vitamin D Deficiency Before the Intervention (**Table 1**).

They were informed about the associated risks and potential benefits of participation before giving their written consent. This study was approved by the Ethical Review Board of the University of Kurdistan (IR. UOK.REC.1398.024) and was conducted in accordance with the principles stated in the Declaration of Helsinki. The study design was registered at the registry of clinical trials and assigned the following number: IRCT2017050917675N2.

A familiarization session was carried out in order to familiarize participants with HIIT protocol and study procedures. During this session high, body weight and BMI of each participant was assessed and body fat percentage (BF) was also measured using the Jackson and Pollock equation and SAEHAN calipers (made in South Korea) (29).

Dietary Records

In the present study, students living in the university dormitories with the same meals were used to relative control the nutritional status of the subjects. The daily meals of the university included three main meals of breakfast, lunch and dinner that ate at the central dining hall (without being able to choose different foods). Because it was possible for some subjects to consume other foods

TABLE 1 | Demographic characteristics of the participants.

Variable	Group	M ± SD		P-value
		Pre-intervention	Post-intervention	
Height (cm)	HIIT + Vit D	178 ± 3.92		
	HIIT + Placebo	177.9 ± 4.42		
	Vit D	177.5 ± 4.1		
	Control	177.3 ± 3.95		
Weight (kg)	HIIT + Vit D	87.25 ± 3.36	*α82.10 ± 2.91	¶ 0.001
	HIIT + Placebo	86.25 ± 2.93	*α82.40 ± 2.66	¶ 0.001
	Vit D	86.11 ± 2.92	86.02 ± 2.88	0.41
	Control	86.06 ± 3.19	86.11 ± 3.21	0.42
BMI (kg/m ²)	HIIT + Vit D	27.23 ± 0.36	*α25.48 ± 0.40	¶ 0.001
	HIIT + Placebo	27.25 ± 0.31	*α25.85 ± 0.44	¶ 0.001
	Vit D	27.32 ± 0.26	27.26 ± 0.25	
	Control	27.36 ± 0.35	27.37 ± 0.34	
BF (%)	HIIT + Vit D	35.18 ± 1.83	*α30.51 ± 1.88	¶ 0.001
	HIIT + Placebo	35.24 ± 1.74	*α31.08 ± 1.64	¶ 0.001
	Vit D	35.32 ± 1.63	35.17 ± 1.62	0.35
	Control	35.39 ± 1.65	35.41 ± 1.65	0.46
Vitamin D (ng/ml)	HIIT + Vit D	16.1 ± 1.01	*€34.6 ± 2.1	¶ 0.001
	HIIT + Placebo	15.86 ± 1.05	15.91 ± 0.98	0.35
	Vit D	15.99 ± 0.89	*€33.9 ± 2.47	¶ 0.001
	Control	15.78 ± 0.96	15.77 ± 1.03	0.65

HIIT, High-intensity interval training group; Vit D, Vitamin D group; BMI, Body mass index; BF, Body Fat percent; ¶Significant difference compared to the pre-test; *Significant difference compared to the control group; αSignificant difference compared to the Vitamin D group; €Significant difference compared to the HIIT+Placebo group.

outside the university's diet program, the 24-h dietary recall form was used before the start of each blood sampling stages. The calorie intake from the four groups in the pre and posttest was analyzed by nutrition software (Table 2).

In addition, mental perception of appetite (desire to eat and fullness) 24 h before the start of the training session and 48 h after the last training session was assessed in the fasting state using the Visual Analog Scale (VAS) in a continuum of zero to 100 Score (30) (Tables 3, 4).

Training and Supplementation Protocols

A familiarization session was used to help participants understand how to carry out training protocol a week before the start of the training protocol at exercise physiology lab in university of kurdistan. During this session, body composition indices including height, body weight, body mass index, and body fat percentage was also measured. The training program was conducted entirely under the supervision of a member of research team. The experimental groups (HIIT + Vit D and HIIT + placebo groups) performed their HIIT program for 8 weeks, 3 sessions per week and each session lasted ~40 min. The exercise protocol involved 10 min warm-up, and 30 min main HIIT phase (10 × 1 min intervals cycling at 90% VO_{2peak} separated by 1 min active recovery at 15% VO_{2peak} for the first to fourth weeks and 10 × 1 min intervals cycling at 100% VO_{2peak}

TABLE 2 | The mean calorie intake of the subjects in the HIIT + Vitamin D, HIIT + Placebo, Vitamin D, and Control groups.

Variable	Group	Pre-intervention	Post-intervention	Within-group p-value
CHO (kcal)	HIIT + Vit D	1,384 ± 47	1,360 ± 45*α	0.001 ¶
	HIIT + Placebo	1,385 ± 48	1,363 ± 40*α	0.001 ¶
	Vit D	1,382 ± 50	1,388 ± 42	0.623
	Control	1,388 ± 46	1,387 ± 48	0.825
Fat (kcal)	HIIT + Vit D	485 ± 40	415 ± 35*α	0.001 ¶
	HIIT + Placebo	483 ± 38	415 ± 35*α	0.001 ¶
	Vit D	488 ± 42	485 ± 40	0.535
	Control	482 ± 40	484 ± 38	0.750
Protein (kcal)	HIIT + Vit D	265 ± 12	300 ± 12*α	0.001 ¶
	HIIT + Placebo	264 ± 14	301 ± 14α	0.001 ¶
	Vit D	266 ± 10	268 ± 10	0.812
	Control	265 ± 12	267 ± 12	0.835
Total calorie (kcal)	HIIT + Vit D	2,134 ± 50	2,075 ± 38*α	0.001 ¶
	HIIT + Placebo	2,132 ± 55	2,079 ± 42*α	0.001 ¶
	Vit D	2,136 ± 48	2,141 ± 45	0.125
	Control	2,135 ± 52	2,136 ± 55	0.536

Data are presented as means ± SD. Vit D, Vitamin D group; CHO, carbohydrates; HIIT, High-intensity interval training group; ¶Significant difference compared to the pre-test; *Significant difference compared to the control group; αSignificant difference compared to the Vitamin D group.

separated by 1 min active recovery at 15% VO_{2peak} for the fifth to eighth weeks) followed by 10 min cool-down (or recovery) in each session (31). The maximum heart rate was calculated by the Caronen formula and polar heart rate monitor model RS 400 (made in Finland) was used to control heart rate. The Vit D and control groups did not have any regular training program throughout the study period. HIIT + Vit D and Vit D group received 2,000 IU/day Vitamin D₃ supplementation in capsule form and the HIIT + placebo group received placebo (Maltodextrin) capsules daily (28). Due to the fact that the subjects in the placebo group were deficient in vitamin D, at the end of the research protocol, they were also supplemented with vitamin D for 1 month according to the groups receiving the supplements.

Blood Sample Analyses

In the first blood sampling (baseline), 8 ml of blood was drawn from a cubital vein under fasting conditions (10 h overnight fasting) at 8 am and the second blood sample was drawn 48 h after the last training session under the same conditions. Blood samples (8 mL) were transferred into tubes containing EDTA and a protease inhibitor [4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF)] to prevent the degradation of acylated ghrelin. The tubes containing EDTA were immediately centrifuged at 3,000 rpm at 4°C for 10 min. Then, samples were pipetted into micro tubes and immediately frozen at -80°C for later analysis. The acylated ghrelin, PYY and insulin plasma levels were determined using commercial kits (Human ELISA,

TABLE 3 | Appetite assess using visual analog scale (VAS).

Variable	Group	M ± SD		Within-group p-value
		Pre-intervention	Post-intervention	
Hunger (mmVAS)	HIIT + Vit D	40.8 ± 10.5	27.4 ± 8.64	0.001*
	HIIT + Placebo	41.1 ± 10.9	26.9 ± 9.15	0.001*
	Vit D	41.5 ± 11	40.9 ± 11.85	0.655
	Control	40.7 ± 11.25	40.2 ± 11.5	0.718
Desire to eat (mmVAS)	HIIT + Vit D	42.5 ± 12.17	25.5 ± 7.64	0.001*
	HIIT + Placebo	41.9 ± 11.90	26.5 ± 7.15	0.001*
	Vit D	42 ± 13	41.7 ± 11.85	0.735
	Control	42.8 ± 12.25	42.1 ± 11.5	0.412
Satiety (mmVAS)	HIIT + Vit D	59.2 ± 11.2	72.6 ± 14.5	0.001*
	HIIT + Placebo	58.9 ± 11	73.1 ± 15.8	0.001*
	Vit D	58.5 ± 11.5	59.1 ± 14.5	0.752
Fullness (mmVAS)	Control	59.3 ± 11.15	59.8 ± 14.17	0.812
	HIIT + Vit D	57.5 ± 14.15	74.5 ± 16.65	0.001*
	HIIT + Placebo	58.1 ± 14.30	73.5 ± 16.5	0.001*
	Vit D	58 ± 13.90	58.3 ± 14.5	0.823
	Control	57.2 ± 14	57.9 ± 14.17	0.593

*Significant difference at the level of $p < 0.05$. Vit D, Vitamin D group; HIIT, High-intensity interval training group.

HANGZHOU EASTBIOPHARM Co., LTD, CHINA) according to the manufacturer's protocol with a lower detection limit of 2.6, 2.53 ng/ml, and 0.25 μ IU/ml, respectively. The intra-assay and inter-assay coefficients of variation were <10% and <12% for acylated ghrelin, <10% and <12% for PYY, and <6.45% and <6.45% for insulin, respectively. Fasting blood glucose (FBG) samples were taken in a sitting position following 10 h overnight fasting before and after the intervention. FBG was measured by biochemical autoanalyzer A15 with Biosystem kit (made by Spain).

Statistical Analyses

Results are expressed as Mean \pm SD. The normal distribution of data and homogeneity of variances were assessed using the Shapiro-Wilk and Levene tests, respectively. Then, dependent *t*-test was used to analyze within-group changes. Analysis of variance with repeated measure and Bonferroni *post-hoc* test were used to evaluate within-group, between-group, and interaction (time \times group) (Table 5). Data was analyzed using SPSS for Windows version 23 (IBM Corp., Armonk, N.Y., USA). The significance level was set at $P \leq 0.05$.

RESULTS

Prior to exercise intervention, no intergroup differences were observed in any of the study variables (acylated ghrelin, PYY, insulin, appetite, weight, BMI, body fat percentage, and plasma vitamin D level) ($p > 0.05$).

TABLE 4 | Comparison of appetite between groups after the interventions.

Variable	Post intervention	P
Hunger (mmVAS)	HIIT + Vit D vs. HIIT+ Placebo	0.064
	HIIT + Vit D vs. Vit D	0.001*
	HIIT + Vit D vs. Control	0.001*
	HIIT + Placebo vs. Vit D	0.001*
	HIIT + Placebo vs. Control	0.001*
Desire to eat (mmVAS)	Vit D vs. Control	0.63
	HIIT + Vit D vs. HIIT+ Placebo	0.065
	HIIT + Vit D vs. Vit D	0.001*
	HIIT + Vit D vs. Control	0.001*
	HIIT + Placebo vs. Vit D	0.001*
Satiety (mmVAS)	HIIT + Placebo vs. Control	0.001*
	Vit D vs. Control	0.72
	HIIT + Vit D vs. HIIT + Placebo	0.078
	HIIT + Vit D vs. Vit D	0.001*
	HIIT + Vit D vs. Control	0.001*
Fullness (mmVAS)	HIIT + Placebo vs. Vit D	0.001*
	HIIT + Placebo vs. Control	0.001*
	Vit D vs. Control	0.71
	HIIT + Vit D vs. HIIT+ Placebo	0.75
	HIIT + Vit D vs. Vit D	0.001*
	HIIT + Vit D vs. Control	0.001*
	HIIT + Placebo vs. Vit D	0.001*
	HIIT + Placebo vs. Control	0.001*
	Vit D vs. Control	0.55

Values are mean \pm SD. Vit D, Vitamin D group; HIIT, High-intensity interval training group.

*Significant difference at the level of $p < 0.05$.

Serum Concentrations of Acylated Ghrelin, PYY, Insulin, Glucose, and Vitamin D

Circulating levels of acylated ghrelin (Figure 1A) and glucose (Figure 1D) were not affected by any of the HIIT or vitamin D supplementation.

For the PYY hormone, there was a significant group \times time interaction ($F = 36.5$, $P = 0.001$). The present results illustrated that PYY increased only in HIIT + Vit D ($P = 0.001$) and HIIT + placebo ($P = 0.001$) groups after 8 weeks of intervention. It was also revealed that PYY was higher in the post-intervention in HIIT + Vit D group compared to Vit D ($P = 0.025$), control ($P = 0.007$) and HIIT + placebo ($P = 0.036$) groups. PYY was also higher in the post-test in HIIT + placebo group compared to Vit D ($P = 0.037$) and control ($P = 0.032$) groups (Figure 1B).

There was a significant group \times time interaction for insulin ($F = 110.53$, $P = 0.001$). Regarding this variable it was observed that insulin decreased in HIIT + Vit D ($P = 0.001$), HIIT + placebo ($P = 0.001$) and Vit D ($P = 0.043$) groups after interventions. In addition, it was demonstrated that insulin was lower in the post-intervention in the HIIT + Vit D group compared to Vit D ($P = 0.001$) and control ($P = 0.001$) groups. Furthermore, insulin was noticeably lower in the post-test in HIIT + placebo group

TABLE 5 | The ANOVA test results regarding research variables.

Variable	ANOVA	F	P
Weight (kg)	Time	975.77	*0.001
	group × time	332.89	*0.001
	Group	58.12	*0.001
BMI (kg/m ²)	Time	560.33	*0.001
	group × time	181.49	*0.001
	Group	60.31	*0.001
BF (%)	Time	932.3	*0.001
	group × time	412.28	*0.001
	Group	46.69	*0.001
Accylated ghrelin (pg/ml)	Time	1.66	0.203
	group × time	1.004	0.40
	Group	0.30	0.99
PYY (pg/ml)	Time	95.34	*0.001
	group × time	36.56	*0.001
	Group	6.12	*0.001
Insulin (ng/dl)	Time	339.28	*0.001
	group × time	110.53	*0.001
	Group	4.04	*0.013
Vitamin D (ng/ml)	Time	720.7	*0.001
	group × time	635.2	*0.001
	Group	84.89	*0.013
Glucose (mg/dl)	Time	1.02	0.283
	group × time	1.13	0.345
	Group	1.42	0.243

BMI, Body mass index; BF, Body Fat percent; PYY, Peptide YY. *Significant difference at the level of $p < 0.05$.

compared to Vit D ($P = 0.001$) and control ($P = 0.001$) groups (Figure 1C).

There was significant group × time interaction ($F = 635.2$, $P = 0.001$) for serum concentrations of Vitamin D. In connection with this, it was shown that serum concentrations of Vitamin D increased in HIIT + Vit D ($P = 0.001$) and Vit D ($P = 0.001$) groups after 8 weeks. Serum concentrations of Vitamin D in HIIT + Vit D group compared to HIIT + placebo ($P = 0.001$) and control ($P = 0.001$) groups were higher. In addition, serum concentrations of Vitamin D were also higher in the post-intervention in Vit D group compared to HIIT + placebo ($P = 0.001$) and control ($P = 0.001$) groups (Figure 1H).

Appetite Ratings (Hunger, Desire to eat, Satiety and Fullness) and Total Calorie Intake

There was a significant group × time interaction for total calorie intake ($F = 10.86$, $P = 0.001$). Current results showed that total calorie intake decreased in HIIT + Vit D ($P = 0.001$) and HIIT + placebo ($P = 0.001$) groups after 8 weeks (Table 2). Aftermore, total calorie intake in HIIT + Vit D and HIIT + placebo groups compared to Vit D ($P = 0.001$) and control ($P = 0.001$) groups were lower in the post-intervention (Table 2).

Findings on appetite ratings indicated that there were significant group × time interaction for hunger ($F = 323.55$, $P = 0.001$), desire to eat ($F = 347.34$, $P = 0.001$), satiety ($F = 313.27$, $P = 0.001$) and fullness ($F = 267.44$, $P = 0.001$). Ratings of hunger and desire to eat decreased in HIIT + Vit D ($P = 0.001$) and HIIT + placebo ($P = 0.001$) groups after 8 weeks of intervention (Table 3). Ratings of hunger and desire to eat in HIIT + Vit D group compared to Vit D ($P = 0.001$) and control ($P = 0.001$) groups, as well as in the HIIT + placebo group compared to Vit D ($P = 0.001$) and control ($P = 0.001$) groups were lower in the post-intervention (Table 4). Ratings of satiety and fullness increased in HIIT + Vit D ($P = 0.001$) and HIIT + placebo ($P = 0.001$) groups after 8 weeks (Table 3). It is confirmed that ratings of satiety and fullness in HIIT + Vit D group compared to Vit D ($P = 0.001$) and control ($P = 0.001$) groups, and in HIIT + placebo group compared to Vit D ($P = 0.001$) and control ($P = 0.001$) groups were notably higher in the post-intervention (Table 4).

Body Weight, BMI and Body Fat Percent

There were significant group × time interaction for body weight ($F = 332.89$, $P = 0.001$), BMI ($F = 181.49$, $P = 0.001$) and body fat percentage ($F = 412.28$, $P = 0.001$) (Table 5). Body weight decreased in HIIT + Vit D ($P = 0.001$) and HIIT + placebo ($P = 0.001$) groups after 8 weeks of intervention. In the HIIT + Vit D group compared to Vit D ($P = 0.012$) and control ($P = 0.010$) groups body weight were lower in the post-intervention. The same result was observed for body weight in the HIIT + placebo group compared to Vit D ($P = 0.025$) and control ($P = 0.020$) groups (Figure 1E).

In regards BMI, it was decreased in HIIT + Vit D ($P = 0.001$) and HIIT + placebo ($P = 0.001$) groups after 8 weeks (Table 5). BMI in the HIIT + Vit D group compared to Vit D ($P = 0.001$) and control ($P = 0.001$) groups were lower in the post-test. A noticeable decrease in BMI was observed in the post-test for the HIIT + placebo group compared to Vit D ($P = 0.001$) and control ($P = 0.001$) groups (Figure 1F).

Body fat percentage also decreased in HIIT + Vit D ($P = 0.001$) and HIIT + placebo ($P = 0.001$) groups after 8 weeks of intervention (Table 5). In relation to this variable, it revealed that body fat percentage in the HIIT + Vit D group was greatly reduced compared to the Vit D ($P = 0.001$) and control ($P = 0.001$) groups in the post-intervention. The same result was obtained for the HIIT + placebo group compared to Vit D ($P = 0.001$) and control ($P = 0.001$) groups (Figure 1G).

DISCUSSION

The combination of exercise and diet affects energy balance and appetite regulation in individuals with obesity and is potentially a major mechanism in weight control. This study is the first to evaluate the effect of HIIT and vitamin D supplementation simultaneously on appetite, appetite-regulated hormones and body composition in sedentary overweight men.

The results of present study showed that 8 weeks of HIIT with an intake of 2,000 IU/day of vitamin D₃ supplementation

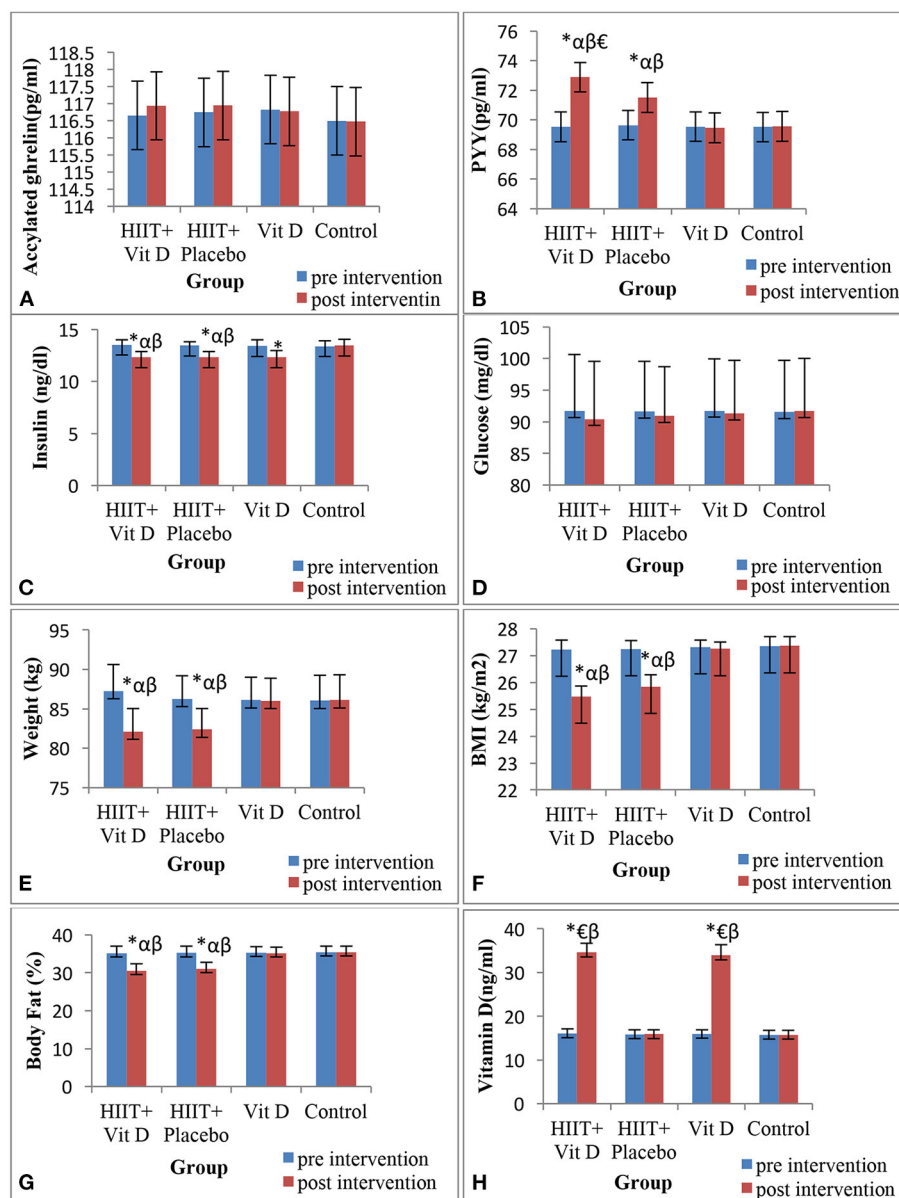


FIGURE 1 | Changes in acylated ghrelin (A), peptide YY (PYY) (B), insulin (C), glucose (D), body weight (E), BMI (F), body fat percentage (G) and vitamin D (H) during 8 weeks of HIIT protocol in overweight sedentary men. *Significant difference compared to the pre-test; αSignificant difference compared to the Vitamin D group; βSignificant difference compared to the control group; €Significant difference compared to the HIIT + Placebo group.

significantly reduced the serum levels of PYY and insulin, but no change was observed in the serum levels of acylated ghrelin. Numerous studies have evaluated the effect of HIIT on appetite-related hormones. In this regard, Liao et al. in a study proved that serum levels of orexin in people with obesity after 6 weeks of HIIT activity significantly decreased while acylated ghrelin did not change (32). Little et al. and Racil et al. also observed a significant decrease in serum insulin levels through an increase in GLUT4 on individuals with obesity after a 12 weeks of HIIT activity (33, 34). Although the reason for the change in appetite hormones after HIIT activity are still unknown, researchers

believe that one of the reasons for these changes is due to the redistribution of blood flow from splanchnic areas to active skeletal muscle (35). Changes in insulin and glucose after HIIT might also be due to increased GLUT4 transporter protein, increased glycogen synthase and hexokinase activity in appetite and related hormones (36). Increased IL6 after exercise is one of the factors affecting appetite suppression (increased PYY) (37) which unfortunately was not evaluated in this study. On the other hand, Martins et al. showed that various exercises such as HIIT did not significantly change the acylated ghrelin and PYY of the subjects in the exercise groups (38). Larsen et al.

also showed that HIIT reduced acylated ghrelin compared to moderate-intensity exercise in overweight sedentary men (39). Duration of exercise (one session vs. 8 weeks) as well as individual differences between subjects, including age and gender are likely reasons for the contradictory results (40). Sim et al. also did not observe a significant change in appetite-dependent hormones (acylated ghrelin and PYY) in individuals with overweight and obesity after 12 weeks of HIIT (8). The reason for the difference might be due to the training protocol, particularly the intensity of training.

The results of the present study demonstrated that overweight sedentary men, in addition to changes in appetite-dependent hormones experienced a significant decrease in weight, BMI, body fat percentage and mental perception of appetite after 8 weeks of HIIT activity. In confirmation of these results, Sim et al. showed that high-intensity exercise reduced the feeling of hunger and energy intake in overweight sedentary individuals up to 24 h after activity compared to moderate-intensity exercise (41). Liao et al. observed a significant reduction in body weight and BMI in individuals with obesity after 6 weeks of HIIT (32). Dupuit et al. also showed that HIIT reduced body fat percentage, body weight and BMI in women with overweight (42). One of the metabolic adaptations due to HIIT is an increase in fat oxidation by changing the metabolism pathway [decreasing the fatty acid synthase (FAS) enzyme and increasing fat oxidation] which lead to changes in body composition (43). Weight loss might also be due to decreased energy intake and increased energy expenditure during exercise as recent evidence suggests that high-intensity activity could be regulating downstream signaling pathways of hunger hormones and upstream signaling pathways of satiety hormones (PYY) alter appetite (by altering appetite-regulating hormones, including PYY) and limit energy intake as an exercise-induced anorexia (41, 44). On the other hand, the findings of Larsen et al. illustrated that HIIT did not cause significant change in the subjects' appetite and energy intake compared to moderate-intensity exercise despite changes in appetite-dependent hormones (39). Changes in appetite-dependent hormones are not always consistent with the mental perception of appetite and the amount of calorie expenditure due to activity, and this indicates the complexity in regulating appetite and the impact of various physiological and psychological factors (45).

The results of the present study showed that vitamin D supplementation with HIIT caused significant changes in appetite-dependent hormones (PYY and insulin), body weight, BMI, body fat percentage and mental perception of appetite. Studies have also evaluated the effect of vitamin D supplementation on appetite-dependent hormones. In this regard, Bhatt et al. showed that taking vitamin D supplements in overweight women decreased insulin resistance and fasting blood glucose (FBG) (46). Vitamin D regulates insulin and increases insulin sensitivity directly and indirectly in pancreatic β cells

(47). The direct effect of vitamin D on glucose metabolism might be through its binding to vitamin D receptors (VDR) in pancreatic β cells and activation of intracellular signaling pathways (47). Choi et al. showed that vitamin D indirectly increased the expression of the PYY hormone gene in the pancreas of mice. They showed that vitamin D supplementation increases the expression of vitamin D receptor genes in the pancreas and VDR activation induces peptide YY transcription in pancreatic islets (27). Regarding the effect of vitamin D supplementation on weight loss, BMI and body fat percentage, there is no research to show that vitamin D alone causes weight loss, decreased BMI and body fat percentage, but researchers have found a link between serum vitamin D levels and these variables. In this regard, Saliba et al. demonstrated that there is an inverse relationship between vitamin D supplementation with weight and BMI (48). Caron et al. also found that taking vitamin D supplements and reaching levels above 25 ng/dl was directly related to reducing body fat (49). Vitamin D can indirectly increase metabolism and reduce fat mass by increasing muscle mass, stimulating sympathetic nerves and reducing insulin (50). Vitamin D might also have a direct effect on adipogenesis and differentiation of fat cells and reduce the absorption of fatty acids in the intestine (51). Vitamin D increases fat oxidation by regulating genes involved in fatty acid oxidation and mitochondrial metabolism, thus limiting weight gain (52).

Overall, the findings of this study showed that HIIT cause changes in appetite-dependent hormones, decrease appetite, weight, body fat percentage and BMI, and if these exercises are accompanied with an intake of 2,000 IU/day of vitamin D₃, changes in the variables are more perceptible.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethical Review Board of the University of Kurdistan (IR.UOK.REC.1398.024). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

DS-V performed the analytic calculations and supervised the project. All authors contributed to the article and approved the submitted version.

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Development and Cross-Validation of a Predictive Equation for Fat-Free Mass in Brazilian Adolescents by Bioelectrical Impedance

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The bioelectrical impedance analysis (BIA) is one of the most commonly used techniques for assessing body composition in a clinical setting and in field approaches, as it has the advantages of easy application, fast, and non-invasive, in addition to its relatively low cost. However, the available predictive equations need to be valid for the evaluated subjects. The aim of this study was to verify the validity of several published BIA equations in estimating fat-free mass (FFM) among Brazilian adolescents, in addition to developing and cross-validating a BIA equation to estimate FFM appropriate for Brazilian adolescents. This is a cross-sectional study with 257 adolescents (128 girls) aged 10–19 years, randomly divided into two groups, namely, development ($n = 172$) and cross-validation ($n = 85$). The standard technique for assessing FFM was dual X-ray absorptiometry (DXA). The paired t -test, multiple regression, and the Bland-Altman plots were used to test the validity of the proposed models and to perform cross-validation of the model. The equation derived in this study was as follows: $\text{FFM} = -17.189 + 0.498 (\text{Height}^2/\text{Resistance}) + 0.226 \text{ Weight} + 0.071 \text{ Reactance} - 2.378 \text{ Sex} + 0.097 \text{ Height} + 0.222 \text{ Age}$; $r^2 = 0.92$; standard error of the estimate = 2.49 kg; the new equation for FFM showed better agreement when compared with that of the equations developed in other countries. In conclusion, the newly developed equations provide a valid FFM estimation and are recommended for Brazilian adolescents with similar characteristics.

Keywords: body composition, bioelectrical impedance analysis (BIA), fat-free mass (FFM), fat mass, equations, mathematical models, cross-validation

INTRODUCTION

Body composition is an important component of the health-related physical fitness of children and adolescents (1, 2) and, therefore, deserves prominence in the prescription and monitoring of dietary and physical exercise programs. In addition, it is fundamental for the identification and monitoring of nutritional deviations (3–5).

The World Health Organization (WHO) defines adolescents as young people aged 10–19 years (6). The growth and development processes that occur in adolescence cause profound changes in the quantities and distribution of the different body components and, hence, the need to monitor these changes for the assessment of health status (4, 7). Furthermore, body composition variables in adolescents are inherently challenging because of the rapid growth-related changes in height, weight, fat-free mass (FFM), and fat mass (FM), but they are essential for the quality of the clinical follow-up (4).

Several techniques have been used to assess the body composition of children and adolescents, and dual X-ray absorptiometry (DXA) is one of the standard reference methods and non-invasive measurements for FFM and FM (8–10). However, its use requires high-cost equipment and specialized technical personnel, which makes it unfeasible in clinical and field situations (11). Although DXA cannot be considered the gold standard for the determination of FFM at the molecular level, and the four-compartment model (4C) is the most suitable reference method to assess FM and FFM at the molecular level (12), due to the complexity of the technique (13), the use of DXA to derive BIA equations has been widely accepted (14, 15). In addition, in Brazil, two previous studies have developed equations to estimate the FFM of men (16) and women (17) aged 20–59 years, with high validity, using DXA as a reference technique.

Whole-body-based techniques, such as anthropometry and bioelectrical impedance analysis (BIA), used as predictors in regression equations, developed and validated using DXA as the reference method, are a viable alternative for clinical evaluation since they are non-invasive, ideal for quantitative estimates of FFM and FM based on mathematical models, portable, and relatively low cost (9, 18, 19). However, it is essential that predictive equations are selected for subjects with similar characteristics regarding gender, age group, pubertal stage, ethnicity, and nutritional status (20, 21). These characteristics make BIA the most used tool to assess body composition worldwide (22).

In Brazil, no studies have developed predictive equations of FFM by bioelectrical impedance for adolescents using DXA as a reference method, and equations developed in other countries (15, 23, 24) are frequently used, which may limit the validity of the results obtained.

Thus, the objectives of this study were: (a) to verify the validity of several BIA equations, published in different countries, in order to estimate FFM in Brazilian adolescents, and (b) to develop and cross-validate BIA equations to estimate FFM in Brazilian adolescents using DXA as a reference method for assessing body composition.

MATERIALS AND METHODS

This is an observational study with a cross-sectional design for the development and cross-validation of a regression equation to estimate body composition, carried out between January 2018 and April 2019, in Natal, which has an estimated population of

884,122 inhabitants according to the IBGE (Brazilian Institute of Geography and Statistics) (25).

Sample

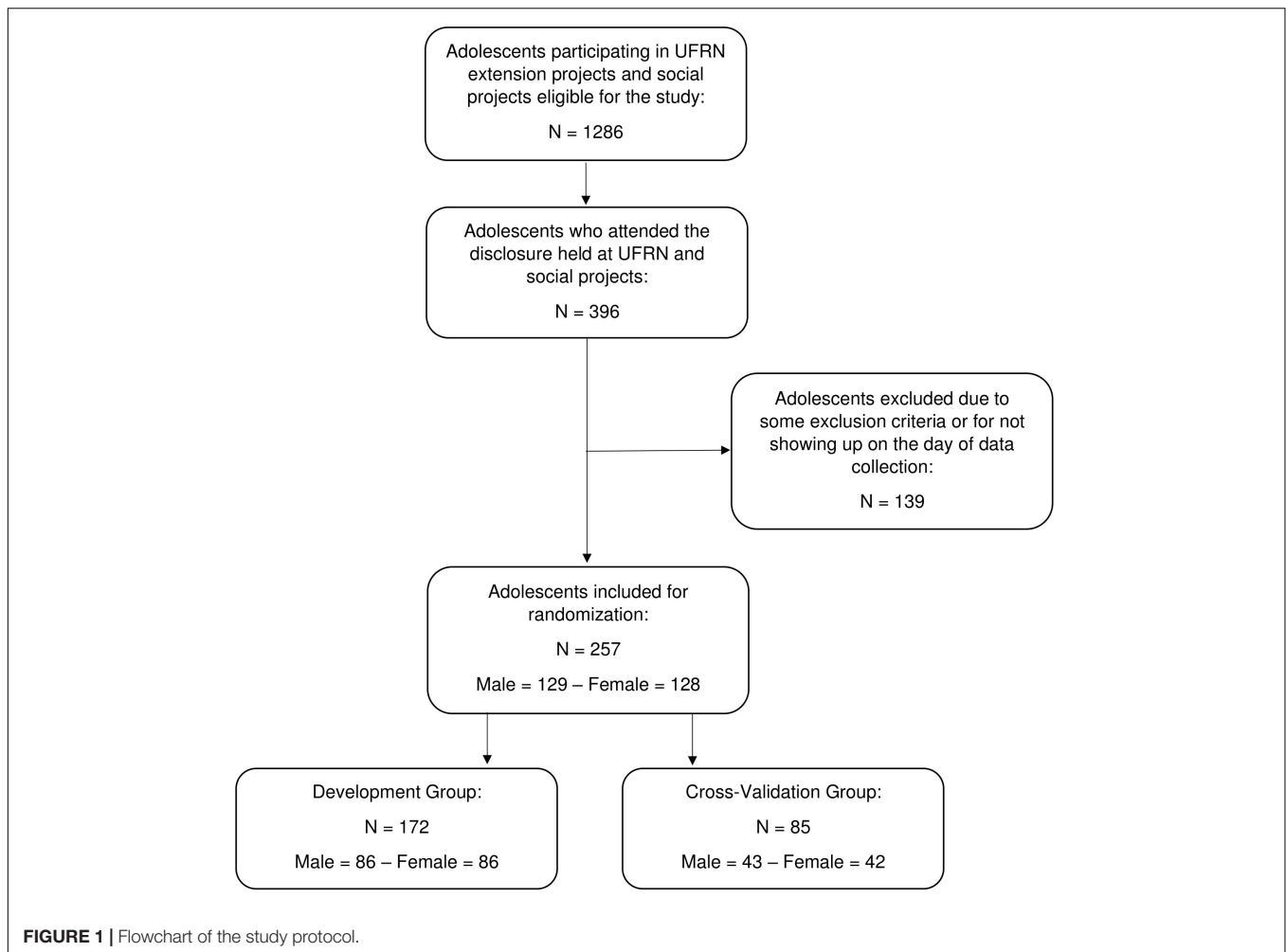
The convenience sample consisted of 257 adolescents (128 girls), aged 10–19 years, from the northeast region of Brazil, who were recruited through dissemination among the participants of university extension projects from the Physical Education Department of the Federal University of Rio Grande do Norte (UFRN) and two social projects maintained by the federal government (**Figure 1**). After their inclusion in the study, the sample was randomly divided into two groups, namely, the development of a predictive equation for FFM ($n = 172$) and cross-validation ($n = 85$). For the sample size calculation, using FFM as a primary outcome, we considered a medium to small effect size (0.12) with five predictors (independent variables), with a type I error of 5% and a power of 95%. Using these parameters, a total of 171 participants were required.

The inclusion criteria were non-athlete adolescents of both sexes aged 10–19 years, regardless of nutritional status, without any medical condition that could interfere with body composition results. The exclusion criteria were pregnancy; hypovolemic or hypervolemic conditions, including diet, diuretic, or corticoid use; edema; individuals with any physical disability or chronic disease; or individuals who had a prosthesis that could alter the results of the body composition assessment. It should be noted that the option for non-athletes is based on possible differences in body composition found in athletes, which can be defined as people in competitive sporting events individually or in engaged teams, with high physical performance and specific training methods (26, 27). Although there are people who, despite not being athletes, may present body composition characteristics similar to those of athletes (28), they could be included in the study because they are conceptually recognized as non-athletes.

All data collections were conducted in a single visit by each participant to the laboratory to perform, sequentially, anthropometric measurements, BIA and DXA assessments, in addition to assessing the pubertal stage. All participants and their parents or legal guardians were informed about the study protocol and signed a free and informed assent/consent form (FICF). The overarching protocol was approved by the Research Ethics Committee of the University Hospital Onofre Lopes—HUOL/UFRN (#34804414.7.0000.5292).

Anthropometric Measurements

Anthropometric measurements were performed by one physical education professional, who was properly trained in accordance with international recommendations (29). Weight was measured using a digital scale with 0.1 kg resolution from Sanny®, model BL200PP (American Medical do Brasil, São Bernardo do Campo, Brazil), with the participants being barefoot and wearing light clothes. In addition, all jewelry and metals were removed for this and all subsequent measurements. Height was measured using a stadiometer from Sanny® with a resolution of 0.1 cm, Caprice model (American Medical do Brasil, São Bernardo do Campo, Brazil), with the participants being barefoot and in orthostatic



position. Body mass index (BMI) was calculated by dividing body mass (kg) by the square of height (m), and adolescents were classified as underweight, normal weight, overweight, or obese using growth charts proposed by the WHO (30).

Bioelectrical Impedance Analysis

The assessment by BIA, for the determination of resistance (R), reactance (Xc), and phase angle (PhA), was conducted with single-frequency tetrapolar equipment (50 kHz) at a current of 800 μ A, in equipment from Sanny®, BIA1010 model (American Medical do Brasil, São Bernardo do Campo, Brazil). The BIA equipment validity measurement was periodically measured with an electrical resistor and capacitor. Calibration values were considered normal if the R was not higher than 500 ± 5 ohm (Ω) and Xc was not higher than 52 ± 0.5 Ω , according to the manufacturer's instructions. The Sanny bioimpedance equipment was chosen because it is the only one manufactured in Brazil, which implies easy access in the country and relatively low operating cost.

To verify the quality of the measurements obtained by the equipment, reproducibility was calculated in a previous study with 46 women from the northeast region of Brazil. The results

obtained were a coefficient of variation (CV) of 0.17 and 0.72% for R and Xc, respectively, and a technical error of measurement (TEM) of 0.76 Ω (0.22%) and 0.35 Ω (0.92%) for R and Xc, respectively (17).

Participants were evaluated after lying down for 10 min in the supine position on a non-conductive stretcher. Arms and legs were abducted 30° from the midline of the body. To avoid short-circuiting in obese participants, a foam device was used between the lower limbs. The skin was cleaned with 70% alcohol before placing the electrodes, which were positioned on the dorsal surface of the wrist, hand, ankle, and foot, in the right hemibody. The evaluated individuals were asked to fast for at least 4 h before the assessment, not to perform any strenuous physical exercise in the previous 24 h, and not to consume alcohol in the previous 48 h. In addition, they were asked to empty their bladder 30 min before the assessment. The resistance index (Ht^2/R) was calculated by dividing the square of height (m) by R (Ω).

Dual X-Ray Absorptiometry

Dual X-ray absorptiometry was performed with Lunar Prodigy equipment, NRL 41990 model (GE Lunar, Madison, WI, United States), by a laboratory technician experienced in

radiological evaluation. The scan was conducted with the participants lying in the supine position along the longitudinal axis of the midline of the table. Feet were positioned together and stuck at the level of the fingers to immobilize the legs, while the hands were held in the prone position within the scanning region of the equipment. The participants remained still during the digitalization process. Measurements were performed following the recommendations proposed by Nanna et al. (31). Body composition was determined using the enCore™ 2011 version 13.6 software (GE Health Lunar). As described elsewhere, CV for FM, bone mineral content (BMC), and lean soft tissue (LST) using the current equipment were 0.74, 0.28, and 0.26%, respectively. TEM were 0.25, 0.02, and 0.25 kg to FM, BMC, and LST, respectively (32). The FFM was obtained by the sum of BMC and LST ($FFM = BMC + LST$).

Pubertal Stage

For the identification of the pubertal stage, the self-assessment technique (33) was used, based on the classification proposed by Tanner (34), which uses five levels to classify the development of the breasts (i.e., M1, M2, M3, M4, and M5) for girls and the development of the genitalia (i.e., G1, G2, G3, G4, and G5) for boys, with them being considered prepubescent adolescents than those who report being in M1 and G1, pubescent from M2 to M4 or G2 to G4, and postpubescent M5 and G5. After the anthropometric assessment, the adolescents were taken individually to a room where the researcher explained the importance of this assessment and presented boards with images of breasts/genitalia and pubic hair. This procedure was carried out with great professionalism and rigor to avoid causing embarrassment or discomfort to the adolescents, as well as any inappropriate representation on the boards. For the data analysis, we chose to use organ development for both sexes, since pubic hair alone can be influenced by ethnic characteristics, as previously described (35).

Statistical Analysis

The Kolmogorov-Smirnov test was applied to verify the normal distribution of data. The descriptive analysis consisted of mean and standard deviation of all study variables, and the comparisons between groups were performed by Student's *t*-test for independent samples. The stepwise multiple regression analysis was used to propose the predictive equation for FFM. The stepwise regression analysis was conducted using FFM obtained by DXA as a dependent variable and age, weight, height, BMI, R, Xc, PhA, R index, and pubertal stage as possible independent variables. During model development, normality of residuals and homogeneity of variance were tested. Significance at $p < 0.05$ was established as the criterion for inclusion of a predictor, whereas removal criteria were set at $p > 0.1$. If more than one variable remained in the model, and to assess multicollinearity, a variance inflation factor (VIF) and the tolerance (reciprocal of VIF) were calculated for each independent variable, and a VIF < 10 or tolerance higher than 0.1 was considered appropriate (36, 37). To verify the validity of the proposed equation, the estimated mean results were compared with the mean results measured in DXA by the paired *t*-test.

In addition, the Pearson's correlation coefficient (r), coefficient of determination (r^2), and standard error of the estimate (SEE) were calculated.

The approach proposed by Lin (38) was used for the concordance correlation coefficient (CCC) analysis to verify the validity (Cb) and accuracy (ρ) between estimated and measured FFM values. For the cross-validation of the equation proposed in this study, a multiple regression analysis was performed.

In turn, the new BIA equation accuracy was evaluated using pure error (PE), which was calculated as the squared root of the mean of the sum of squared differences between the measurement and estimate of FFM (15). The Bland-Altman (39) plots were used to verify bias and concordance between FFM measurement and estimate, in which the limits of agreement (LOAs) were defined as the mean of differences ± 1.96 standard deviations, including the analysis of the correlation between the mean and the difference of the methods. Additionally, the same procedures were used to test the validity of the other eight equations proposed for estimating FFM in adolescents (15, 23, 24, 40–44). Analyses were carried out with the statistical package SPSS v.20.0 (SPSS Inc., IBM Corp., Armonk, New York, NY, United States) and MedCalc version 12.5.0. Statistical significance of $p < 0.05$ was considered for all tests.

RESULTS

Table 1 presents the physical characteristics and body composition variables for the developmental and cross-validation groups, as well as for the whole sample with no differences observed between the two groups (i.e., developmental and cross-validation) ($p > 0.05$). The characteristics of the samples from the eight equations tested in this study are shown in **Table 2**.

The analysis of the validity of the eight equations (**Table 3**), developed in other countries, showed that, only for three equations (23, 41, 44), no association was found between the mean and the difference of the BIA and DXA methods ($p > 0.05$). The mean difference in the Bland-Altman plot was not different from zero in just two equations (15, 42; $p > 0.05$). All equations showed high LOA, indicating poor agreement with the reference method (**Figure 2**). These results justified the need to develop and validate a specific equation for our population.

In preliminary analyses, we found no significant interaction with sex for any of the main independent predictor, and, thus, girls and boys were combined for the development of the prediction models.

Table 4 shows the regression model for the prediction of FFM (kg). A preliminary model was developed to estimate FFM, including anthropometric and BIA variables, that is, age, weight (W), height (H), BMI, R, Xc, PhA, resistance index (Ht^2/R), and pubertal stage. Only variables contributing to the estimates using a backward stepwise approach were used in the model. The performance of the developed model can be observed by high coefficients of determination ($r^2 = 0.95$) and low SEE ($SEE = 2.5$ kg).

TABLE 1 | Descriptive [mean \pm sd or *n* (%)] characteristics and body composition of development and cross-validation groups.

	Development group (DG)			Cross-validation group (CVG)		
	Male (<i>n</i> = 86)	Female (<i>n</i> = 86)	Total sample (<i>n</i> = 172)	Male (<i>n</i> = 43)	Female (<i>n</i> = 42)	Total sample (<i>n</i> = 85)
Age (years)	13.6 \pm 2.9	14.5 \pm 3.6	14.1 \pm 3.3	13.7 \pm 3.1	14.2 \pm 3.1	14.0 \pm 3.1
Pubertal stage - <i>n</i> (%)						
Prepubertal	11 (12.8)	7 (8.1)	18 (10.5)	8 (18.6)	2 (4.8)	10 (11.8)
Pubescent	52 (60.5)	48 (55.8)	100 (58.1)	23 (53.5)	29 (69.0)	52 (61.2)
Postpubertal	23 (26.7)	31 (36.0)	54 (31.4)	12 (27.9)	11 (26.2)	23 (27.1)
BMI status (30) - <i>n</i> (%)						
Low weight	8 (9.3)	4 (4.7)	12 (7.0)	1 (2.3)	5 (11.9)	6 (7.1)
Normal weight	61 (70.9)	58 (67.4)	119 (69.2)	32 (74.4)	27 (64.3)	59 (69.4)
Overweight	15 (17.4)	18 (20.9)	33 (19.2)	9 (20.9)	6 (14.3)	15 (17.6)
Obesity	2 (2.3)	6 (7.0)	8 (4.7)	1 (2.3)	4 (9.5)	5 (5.9)
Weight (kg)	47.7 \pm 16.5	50.9 \pm 15.1	49.3 \pm 15.9	49.0 \pm 16.0	48.1 \pm 12.4	48.6 \pm 14.3
Height (cm)	156.4 \pm 14.1	157.2 \pm 10.8	156.8 \pm 12.5	157.1 \pm 14.9	156.1 \pm 9.0	156.6 \pm 12.3
BMI (kg/m ²)	18.9 \pm 3.8	20.2 \pm 4.2	19.6 \pm 4.1	19.3 \pm 3.6	19.7 \pm 4.8	19.5 \pm 4.2
FM (kg)	10.9 \pm 6.0	17.3 \pm 7.7	14.1 \pm 7.6	11.5 \pm 5.4	16.5 \pm 6.9	14.0 \pm 6.6
FM (%)	22.7 \pm 7.2	32.9 \pm 7.0	27.8 \pm 8.9	23.7 \pm 8.1	33.4 \pm 7.0	28.5 \pm 9.0
FFM (kg)	36.7 \pm 12.8	33.7 \pm 8.8	35.2 \pm 11.1	37.3 \pm 13.4	31.6 \pm 7.0	34.6 \pm 11.1
Resistance (Ω)	634.1 \pm 112.7	684.8 \pm 101.5	659.4 \pm 109.9	634.7 \pm 119.4	714.0 \pm 108.6	673.9 \pm 120.3
Reactance (Ω)	63.6 \pm 10.6	65.9 \pm 10.3	64.8 \pm 10.5	67.1 \pm 12.6	67.7 \pm 9.6	67.2 \pm 11.2
Phase angle (°)	5.8 \pm 1.0	5.6 \pm 0.9	5.7 \pm 0.9	6.2 \pm 1.5	5.5 \pm 1.0	5.8 \pm 1.3
Resistance Index (Ht ² /R)	41.2 \pm 14.4	37.4 \pm 9.2	39.3 \pm 12.2	41.7 \pm 14.7	35.1 \pm 7.3	38.5 \pm 12.1

BMI, body mass index; FM, fat mass; FFM, fat-free mass.

TABLE 2 | Sample characteristics of the bioelectrical impedance analysis (BIA) equations for the prediction of fat-free mass (FFM) in adolescents.

	Age (years)	<i>n</i> M/F	Ethnicity	Reference methods
Deurenberg et al. (23)	7–25	130 M, 116 F	C	UW
Houtkooper et al. (24)	10–19	225 M/F	C	UW and DD
Sun et al. (15)	12–94	669 M, 944 F	C and As	DD, UW, and DXA
Boileau (40)	8–16	NR	C	UW and DD
Horlick et al. (41)	4–18	645 M, 602 F C, AA, Af, and As	DD and DXA	
Schaefer et al. (42)	3–19	59 M, 53 F	C	TBK
Suprasongsin et al. (43)	10–22	21 M, 21 F	C	ID
Wang et al. (44)	9–19	127 M, 128 F	As	DXA

M, male; F, female; NR, no report; C, Caucasian; As, Asian; AA, American African; Af, African; UW, underwater weighing; DD, deuterium dilution; DXA, dual-energy X-ray absorptiometry; TBK, total body potassium; ID, isotope dilution, heavy water tracer.

The resulting prediction model included is presented below:

$$\text{FFM} = -17.189 + 0.498 (\text{Height}^2/\text{Resistance}) + 0.226 \text{Weight} + 0.071 \text{Reactance} - 2.378 \text{Sex} + 0.097 \text{Height} + 0.222 \text{Age}$$

$$\text{Sex : male} = 0; \text{female} = 1$$

Estimated FFM by the specific equation developed in this study did not present significant differences in comparison with the value determined by DXA for both the development and cross-validation groups. All parameters used for proposing and

TABLE 3 | Cross-validation of fat-free mass predictive new equation, and validation of other published equations.

	FFM (kg)	<i>p</i> -value*	CCC analysis				
			CCC	ρ	<i>C_b</i>	<i>r</i> ²	PE (kg)
New equation	34.8 \pm 10.6	0.322	0.984	0.985	0.999	0.97	1.95
Deurenberg et al. (23)	36.6 \pm 10.9	<0.001	0.932	0.949	0.983	0.90	4.05
Houtkooper et al. (24)	36.9 \pm 10.6	<0.001	0.955	0.978	0.977	0.96	3.27
Sun et al. (15)	34.3 \pm 12.9	0.732	0.847	0.857	0.988	0.73	6.63
Boileau (40)	37.2 \pm 10.0	<0.001	0.946	0.980	0.965	0.96	3.50
Horlick et al. (41)	36.6 \pm 11.6	<0.001	0.964	0.981	0.983	0.96	3.05
Schaefer et al. (42)	34.5 \pm 9.3	0.715	0.960	0.974	0.985	0.95	2.88
Suprasongsin et al. (43)	40.5 \pm 11.8	<0.001	0.870	0.969	0.898	0.94	6.13
Wang et al. (44)	37.3 \pm 10.8	<0.001	0.949	0.979	0.969	0.96	3.51

FFM, fat-free mass; CCC, concordance correlation coefficient; ρ , accuracy; *C_b*, validity; PE, pure error. *Differences between predictive equations and reference method by paired *t* test.

validating the equation confirmed their validity. Additionally, no association was found between the mean and the difference of the methods ($r = 0.113$; $p = 0.141$).

The performance of the cross-validation of the new equation and the validity of the eight equations developed for adolescents in other countries are shown in **Table 2**, and the LOA for each of the equations is shown in **Figure 2**.

Figure 3 presents the LOA for FFM between the standard method (DXA) and the BIA equation derived in this study.

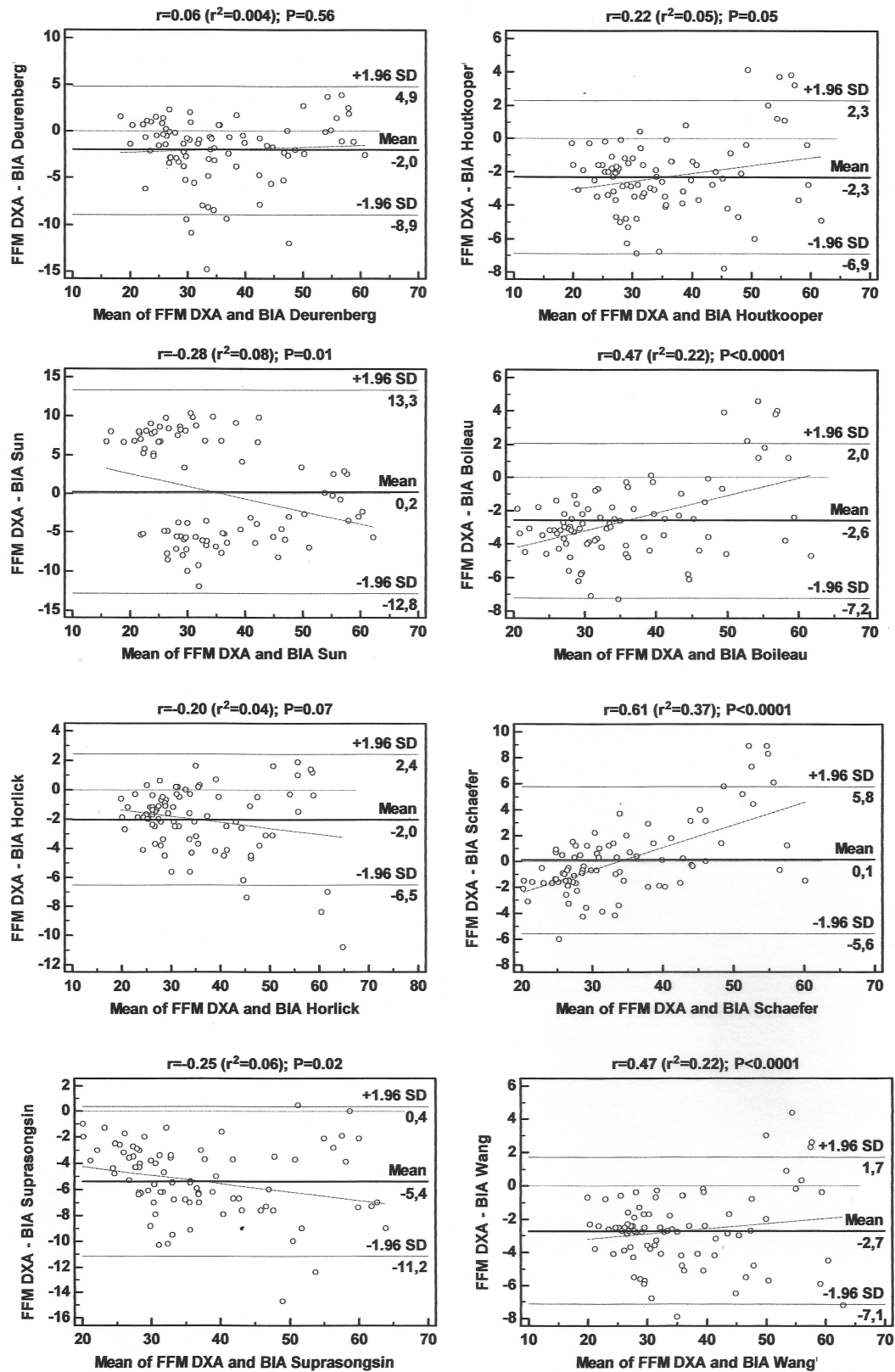
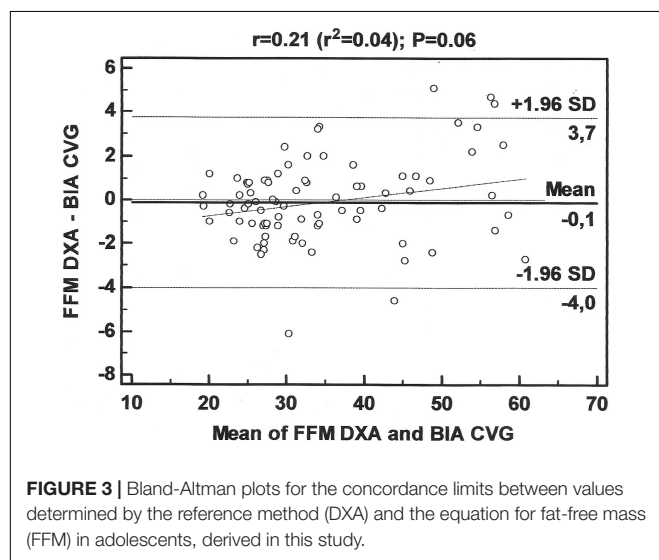


FIGURE 2 | Bland-Altman plots for the concordance limits between values determined by the reference method (DXA) and eight equations for fat-free mass (FFM) in adolescents.

TABLE 4 | Regression model for the prediction of fat-free mass (kg).

Variables included in the model	Regression coefficient	r^2	SEE	p -value	Collinearity statistics	
					Tolerance	VIF
Constant	-17.189			<0.001		
Ht ² /R	+0.498	0.916 ^a	3.214	<0.001	0.144	6.961
Weight	+0.226	0.935 ^b	2.850	<0.001	0.175	5.713
Reactance	+0.071	0.942 ^c	2.689	<0.001	0.639	1.565
Sex	-2.378	0.947 ^d	2.579	<0.001	0.693	1.443
Height	+0.097	0.949 ^e	2.528	0.002	0.533	1.625
Age	+0.222	0.951 ^f	2.498	0.027	0.355	2.817

SEE, standard error of the estimate; VIF, variance inflation factor. Predictors: ^a(Constant), Ht²/R. ^b(Constant), Ht²/R, weight. ^c(Constant), Ht²/R, weight, and reactance; ^d(Constant), Ht²/R, weight, reactance, and sex. ^e(Constant), Ht²/R, weight, reactance, sex, and height; ^f(Constant), Ht²/R, weight, reactance, sex, and age. The r^2 change was significant for a, b, c, d, e, and, f.



The mean difference in the Bland-Altman plot was not different from zero in the cross-validation group ($p = 0.322$). The LOA ranged between -4.0 and 3.7 kg to the cross-validation group, indicating a good agreement between the developed equation and the reference method.

DISCUSSION

The BIA is one of the most convenient techniques for assessing body composition in a clinical setting and in epidemiological approaches, mainly, because it is easy to apply, fast, and non-invasive, in addition to having a relatively low cost (5, 45, 46). The use of predictive equations that have been developed and cross-validated in groups with similar characteristics to those of the subjects that we intend to evaluate can reduce discrepancies among studies (7, 20, 47). Observing the lack of predictive equations for FFM in Brazilian adolescents, developed using DXA as a reference technique, this study aimed at developing and cross-validating an equation in a sample of Brazilian adolescents

of both sexes, in addition to testing the validity of equations developed in other countries, used frequently in our country.

In this study, the most relevant predictor was the resistance index (Ht²/R), which explained alone 92% of the variability of our equation. The electrical properties of the human body may explain the use of BIA to estimate FFM by the resistance index. R of the conductor is expressed by $R = \rho L^2/V$, so $V = \rho L^2/R$, wherein ρ is the conductor resistivity, L is the length, and V is volume (48, 49). Thus, as the lean body mass contains a large amount of water, it presents low R to the flow of electric current, while the FM has greater R to the passage of current. Therefore, the R associated with height can be a good estimator of these body compartments.

The other variables that entered the model were weight, Xc, sex, and age. Considering that adolescence is a phase of profound morphological changes, which include body composition (4, 50, 51), and that the age at which each stage of puberty occurs can vary considerably (52), we listed the pubertal stage among the possible independent variables; however, this variable was not included in the model by the stepwise regression. The same was verified in the other studies that proposed predictive equations of FFM by BIA in adolescents, which we tested for validity in the sample of this study (15, 23, 24, 40–44).

In the sample of this study, mean BMI in boys (18.9 kg/m^2) and girls (20.2 kg/m^2) was similar to the mean BMI of a representative sample of 12- to 17-year-old boys (21.0 kg/m^2) and girls (21.3 kg/m^2) from the Brazilian cities with more than one hundred thousand inhabitants (53). Thus, although the sample was not representative of adolescents across Brazil, the relative body size of these adolescents seems to resemble that of other adolescents of the country living in larger cities. However, it is important that the techniques for body composition assessment that are used have been validated for the target population (37, 54, 55).

Although the use of predictive equations in subjects with different characteristics from those presented by the group of origin of the equations is questionable (15), we did not always have mathematical models validated for similar groups to the ones we wanted to evaluate. In addition, many BIA devices do not refer to the predictive equations available in their software (17). Thus, it is possible that health professionals are using inadequate equations for their patients, which indicates the need for studies to validate predictive equations that already exist in different population groups, as well as the development of new equations.

The equations developed for adolescents from other countries, tested in this study, did not prove to be valid for our sample. When comparing the predicted mean FFM values with those obtained by the reference technique, only two equations showed no significant difference (15, 42). However, all equations, including these two (15, 42), showed high limits of agreement, limiting their use at the individual level, for the subjects of this study.

A study carried out by Koury et al. (50) in Brazil, with a sample of 368 adolescent athletes aged 11–16 years, tested the validity of three equations (23, 41, 56), concluding that none of them was adequate for the evaluated sample. The authors developed gender-specific equations for the study sample, including skeletal maturity for boys and menarche status for girls as

dependent variables, demonstrating good performance; however, there was no cross-validation of the new equations. We did not test the validity of these equations in our sample because we did not evaluate athletes and did not collect information about skeletal maturity.

A systematic review was carried out by Silva et al. (7) to identify predictive equations for assessing FM and FFM in healthy children and adolescents using the multicomponent molecular models as a reference method. Similar to this study, most of the equations were developed using DXA as a reference method, but a limited number of studies provided cross-validation results. The authors of the systematic review identified that, of the 33 equations analyzed, only seven were cross-validated, two studies examined the PE in the FFM estimate, and none of the studies examined the CCC, while the agreement between the methods was included only in three studies. Based on the limitations found in other studies, we sought to use the most recommended methodological practices for the development and cross-validation of predictive models of body composition assessment.

This study has several strengths. To our knowledge, this is the first study to develop and cross-validate a predictive equation of FFM by BIA, using DXA as a reference method, in Brazilian non-athlete adolescents. The equation developed in our study showed a high coefficient of determination and good limits of agreement in relation to the reference method, and all parameters used for the proposition and cross-validation of the model confirmed their validity for the studied population (15, 37–39, 57), which can be used to monitor changes in FFM resulting from growth, dietary programs, and physical exercises (14, 58, 59).

However, some limitations must also be addressed. This study included a sample of adolescents from only one region of the country, and ethnicity was not assessed. Other studies carried out in Brazil for the development of predictive equations by anthropometry (60) and BIA (32) also used ethnically mixed samples, miscegenation, and ethnic differences, which suggest the need to validate the equation proposed in the study in other regions of the country and with subjects of different ethnic origins. Another important issue concerns the standard technique used. The 4C model is the most appropriate reference method for assessing FM and FFM at the molecular level (12). However, due to the complexity of the technique (13), the use of DXA to derive BIA equations has been widely accepted (14, 15). Hydration status was not determined to ensure a euhydration state prior to body composition measures, although self-reported water intake was within the normal range, and pale morning urine was reported. It should be noted that the new equations are only useful for Brazilian adolescents with similar characteristics.

In addition, further research should be conducted to test the accuracy of the new model in tracking FFM.

In conclusion, based on the results obtained, the equation developed in this study met the validation criteria to estimate FFM, while the equations developed in other countries were not considered valid for the studied sample. Thus, this new equation can be considered a good alternative for assessing the body composition of adolescents with similar characteristics by BIA due to the good validity presented.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Research Ethics Committee of the University Hospital Onofre Lopes – HUOL/UFRN (#34804414.7.0000.5292). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

RC and KM: conceptualization. RC, KM, and TC: data curation. RC: formal analysis and writing—original draft. PD: funding acquisition and project administration. RC, KM, TC, BC, and PD: investigation. RC, AS, KM, BC, GF, and PD: writing—review and editing. All authors contributed to the article and approved the submitted version.

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Association of Four Nutritional Scores With All-Cause and Cardiovascular Mortality in the General Population

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Background and Aims: Malnutrition is a well known risk factor for adverse outcomes in patients with cancer, cardiovascular disease (CVD) and chronic kidney disease, but epidemiological evidence on its relationship with the long-term risk of all-cause mortality and cardiovascular death is limited.

Methods: A total of 20,116 adults from the United States National Health and Nutrition Examination Survey 2007–2014 were enrolled. The Geriatric Nutritional Risk Index (GNRI), Prognostic Nutritional Index (PNI), Controlling Nutritional Status (CONUT) score, and Triglycerides (TG) × Total Cholesterol (TC) × Body Weight (BW) Index (TCBI) were calculated at baseline. Cox regression and the Kaplan–Meier analysis were conducted when participants were divided into three groups according to the tertiles of objective nutritional scores. Restricted cubic spline was performed to further explore the shape of the relationship between all-cause mortality, cardiovascular death, and nutritional scores. In addition, the area under the curve (AUC), continuous net reclassification improvement (NRI), and integrated discrimination improvement (IDI) were conducted to assess which nutritional scores have the greatest predictive value for all-cause death and cardiovascular death in the general population.

Results: The cumulative incidence of all-cause death and cardiovascular death was significantly higher in participants with a higher CONUT score, lower GNRI, and lower PNI. TCBI showed the worst performance on grading and risk assessment. After adjusting confounding factors, the lowest PNI and GNRI tertile and highest CONUT score were independently and significantly associated with increased risk of all-cause death (all $P < 0.01$) and cardiovascular death (all $P < 0.05$) analyzed by a multivariate Cox regression model. An L-shaped association between the HR (hazard ratio) of all-cause mortality and nutritional scores (GNRI, PNI and TCBI) was observed in the overall populations. In addition, the PNI had the highest predictive value for all-cause mortality [AUC: 0.684, 95% confidence interval (CI): 0.667–0.701] and cardiovascular death (AUC: 0.710, 95% CI: 0.672–0.749) in the general population compared with other nutritional scores.

Conclusion: The poorer the nutritional status of the general population, the higher the all-cause mortality and cardiovascular mortality. The PNI score may provide more useful predictive values than other nutritional scores.

Keywords: malnutrition, nutritional scores, all-cause death, cardiovascular death, general population

INTRODUCTION

Malnutrition is a prevalent problem in patients with chronic diseases, such as cancer, end-stage renal diseases (ESRD), coronary chronic total occlusion (CTO) (1–3). It is associated with higher complications, increased mortality, and length of hospitalization (4, 5). In addition, malnutrition can interfere with wound healing by delaying the healing response and was associated with poor cognitive development (6, 7). Therefore, nutrition management and assessment are essential for patients at risk of malnutrition.

However, it is difficult to comprehensively evaluate the nutritional status because malnutrition is affected by many factors (8). At present, four objective nutritional scores have been used in previous studies, including Geriatric Nutritional Risk Index (GNRI) (9), prognostic Nutritional Index (PNI) (10), Triglycerides (TG) \times Total Cholesterol (TC) \times Body Weight (BW) Index (TCBI) (11), controlling nutritional status (CONUT) score (12). These scores include two or three of the following elements: albumin, lymphocytes count, TC, TG, and body weight. Previous studies have demonstrated that the GNRI, PNI, COUNT, and TCBI have significant prognostic value for the mortality or adverse events of patients with a wide range of cardiovascular disease (CVD) (11, 13–15). The poor nutritional status assessed by these nutritional indexes is significantly associated with the poor clinical outcome of patients. According to a recent study, compared with PNI and TCBI, GNRI had the greatest incremental value in predicting mortality after acute myocardial infarction (16).

However, epidemiological evidence on which score is more effective in predicting all-cause mortality and cardiovascular death is limited. In addition, the relationship between these four nutritional scores and all-cause and cardiovascular mortality remains elusive in the general population. Therefore, we used the data from the National Health and Examination Survey (NHANES) to address the knowledge gap.

MATERIALS AND METHODS

Study Population

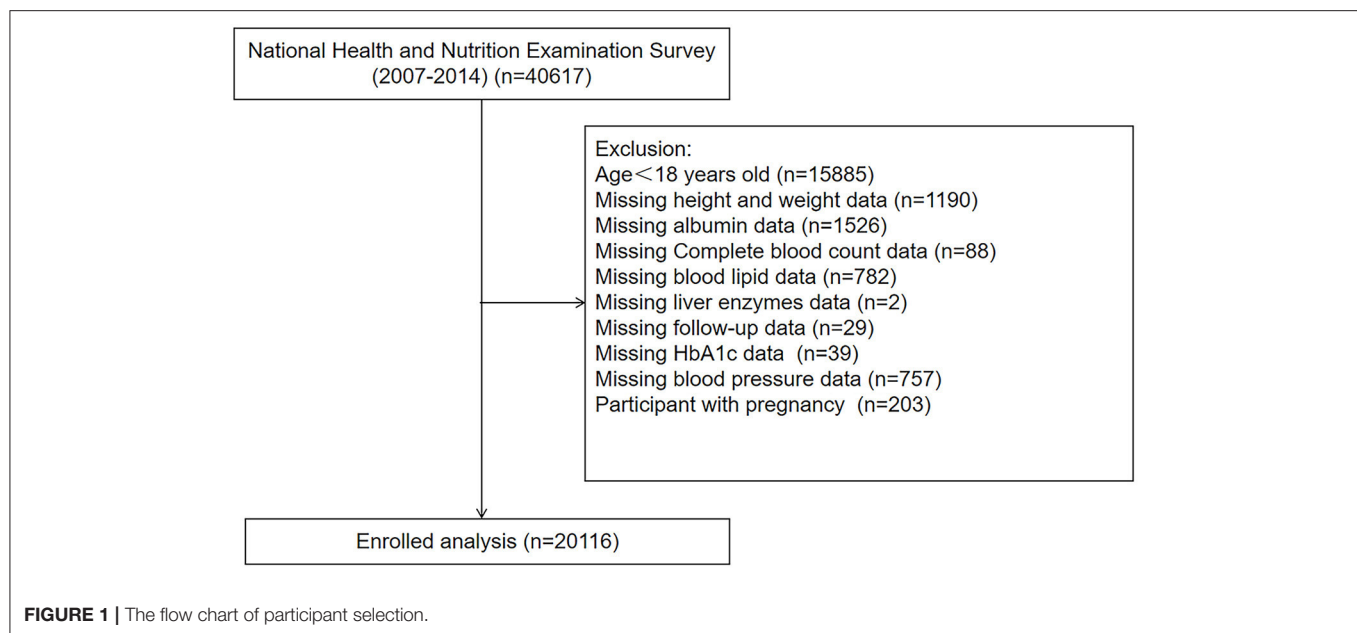
We analyzed data from the National Health and Nutrition Examination Survey (NHANES) between the period of 2007–2014, a nationwide cross-sectional survey conducted by the Center for Disease Control and Prevention (CDC) in the United States to assess the health status of US citizens (17). Participants with age < 18, pregnancy, and those without complete medical records were excluded. Finally, a total of 20,116 participants were enrolled in our study (Figure 1).

Exposure

Serum albumin levels were measured using the bichromatic digital endpoint method on a DxC800. Lymphocyte counts were obtained from the whole blood using the Coulter method. The measurement of serum TG and TC was performed with enzymatic assays. Height and Weight were measured at the Mobile Examination Center (MEC) examination. If TG was ≤ 400 mg/dL, missing low-density lipoprotein cholesterol (LDL-C) data was computed by the Friedewald formula (18). The GNRI was calculated by using the following formula: $GNRI = [1.489 \times \text{serum albumin (g/l)}] + [41.7 \times \text{weight (kg)/ideal body weight (kg)}]$. The calculation of the ideal body was as follows: $22 \times \text{square of height}$ because of its validity. The ratio of weight-to-ideal body weight was set to 1 if the actual body weight exceeded the ideal body weight (3). The PNI was defined by the following formula: $PNI = \text{serum albumin (g/L)} + 5 \times \text{total lymphocyte count (10}^9\text{/L)}$. The TCBI was calculated using the formula: $\text{serum level of TG (mg/dL)} \times \text{TC (mg/dL)} \times \text{body weight (kg)/1000}$. The CONUT score was described in **Supplementary Table 1**. Nutritional scores (including GNRI, PNI, TCBI) were divided into three groups according to the tertiles: low (GNRI < 102.75), intermediate (GNRI: 102.75–107.22), and high (GNRI > 107.22) GNRI groups; low (PNI < 51), intermediate (PNI: 51–55), and high (PNI > 55) PNI groups; and low (TCBI < 1,211.19), intermediate (TCBI: 1,211.19–2,421.71), and high (TCBI > 2,421.71) TCBI groups. For the COUNT score, a score of 0 was considered normal nutritional status, scores of 1 to 2 were considered mild to moderate malnutrition, and scores of ≥ 3 were considered severe malnutrition.

Covariates

With the use of standardized questionnaires, participants provided information on age, gender, race, smoking, drinking, medical history (hypertension, diabetes, and CVD), and medication use. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), uric acid (UA), glycosylated hemoglobin (HbA1c), and creatinine (CRE) were measured by standard methods. The details of laboratory methodology are available at https://wwwn.cdc.gov/Nchs/Nhanes/2013-2014/BIOPRO_H.htm. Body mass index (BMI) was calculated using the following equation: $\text{body weight (kg)/the square of height (m}^2\text{)}$. The estimated glomerular filtration rate (eGFR) was computed by Modification of Diet in Renal Disease formula (19). The race was classified as non-Hispanic white, non-Hispanic black, Mexican American, other Hispanic, or others. CVD history was defined as self-reported congestive heart failure, coronary heart disease, angina pectoris, heart attack, and stroke. Participants were considered as hypertensives if they were taking antihypertensive medications, had the average systolic blood pressure (SBP)



exceeding 140 mmHg, or the average diastolic blood pressure (DBP) exceeding 90 mmHg. The mean systolic and diastolic blood pressures were calculated from up to four readings obtained in a seated position and using sphygmomanometers. Diabetes was defined as fasting glucose > 7 mmol/L or glycated hemoglobin A1c $\geq 6.5\%$ or the usage of hypoglycemic drugs or history of diabetes.

Outcomes

Outcomes of our study mainly were all-cause and cardiovascular mortality. Mortality status was obtained by linkage to the National Death Index by December 31, 2015. These mortality files are available for online access (<https://www.cdc.gov/nchs/datalinkage/mortality-public.htm>). Cardiovascular mortality in our study was defined according to the International Classification of Diseases, 10th Clinical Modification (ICD-10) System codes (I00–I09, I11, I13, I20–I51, I60–I69) (20).

Statistical Analysis

Continuous variables are expressed as median with interquartile range. Categorical variables are expressed as frequencies and percentages. Depending on the nature of data, Chi-square, ANOVA, or Kruskal-Wallis H-test were performed to detect subgroup differences. The initial confounding factors were selected based on previous studies, data availability, and established associations (20–25). If these factors changed the estimates of PNI on all-cause mortality or cardiovascular death by more than 10% or were significantly associated with all-cause mortality or cardiovascular death after adjustment for sociodemographic factors (age, gender, and race), they were included as the covariates in the Cox regression analysis (26). Finally, we excluded platelet count and lipid-lowering drugs. Three sets of Cox regression models were constructed to evaluate the association of nutritional scores with all-cause and

cardiovascular mortality. Model 1 only included nutritional scores. Model 2 was adjusted for age, sex, and race. Model 3 was adjusted for age, sex, race, BMI, Smoking, Drinking, hypertension, diabetes, HDL-C, LDL-C, SBP, DBP, eGFR, AST, ALT, UA, HbA1c, CVD, hypotensive drugs, and hypoglycemic drugs. Kaplan–Meier method was used to perform the analysis of the time-to-event data, and the log-rank test was used to compare the differences between each group. Restricted cubic splines (RCS) were applied using the R package “rms” based on the Cox proportional hazards models further to explore the relationship between the nutritional scores and endpoints. Receiver operating characteristic (ROC) curve analyses were performed using the R package “pROC” to compare these four nutritional indexes in predicting all-cause and cardiovascular mortality. The differences in the area under the curve (AUC) between two ROC curves were analyzed using DeLong test. Moreover, the continuous net reclassification improvement (NRI) and integrated discrimination improvement (IDI) were calculated using the R package “PredictABEL.” Subgroup analyses were conducted to evaluate whether the results between mortality and PNI were modified by age, gender, diabetes, hypertension, and cardiovascular diseases based on the fully adjusted multivariable regression model with interactions between PNI and stratified covariates. A P value < 0.05 was considered statistically significant. All statistical analyses were conducted using R software (version 4.1).

RESULTS

Baseline Characteristics

The baseline characteristics of the study population according to PNI tertiles are shown in **Table 1**. A total of 20,116 participants were enrolled in this study, 1,188 patients died for various reasons, and 18,928 survived. The lowest tertile (PNI < 51)

TABLE 1 | Baseline characteristics.

Variables	PNI			P-value
	<51 (n = 6,074)	51–55 (n = 7,704)	>55 (n = 6,338)	
Age (years)	57.0 (42.0–71.0)	47.0 (32.0–62.0)	39.0 (26.0–55.0)	<0.001
Gender, male, n (%)	2,585 (42.6%)	3,663 (47.5%)	3,598 (56.8%)	<0.001
Hypertension, n (%)	3,019 (49.7%)	2,966 (38.5%)	2,059 (32.5%)	<0.001
Diabetes, n (%)	1,260 (20.7%)	1,125 (14.6%)	766 (12.1%)	<0.001
Race, n (%)				<0.001
Mexican American	702 (11.6%)	1,248 (16.2%)	1,090 (17.2%)	
Non-Hispanic white	2,843 (46.8%)	3,332 (43.3%)	2,731 (43.1%)	
Non-Hispanic black	1,518 (25.0%)	1,540 (20.0%)	1,097 (17.3%)	
Other Hispanic	555 (9.1%)	831 (10.8%)	675 (10.7%)	
Other races	456 (7.5%)	753 (9.8%)	745 (11.8%)	
Hypotensive drugs, n (%)	2,129 (35.1%)	1,915 (24.9%)	1,161 (18.3%)	<0.001
Hypoglycemic drugs, n (%)	870 (14.3%)	773 (10.0%)	491 (7.7%)	<0.001
Cardiovascular disease, n (%)	954 (15.7%)	677 (8.8%)	383 (6.0%)	<0.001
Smoking, n (%)	2,665 (43.8%)	3,342 (43.4%)	2,965 (46.8%)	<0.001
Drinking, n (%)	3,816 (62.8%)	5,090 (66.1%)	4,223 (66.6%)	<0.001
BMI, kg/m ²	28.6 (24.7–33.5)	27.5 (23.9–31.9)	26.9 (23.4–31.1)	<0.001
SBP, mmHg	123.0 (111.0–136.0)	119.0 (110.0–131.0)	119.0 (110.0–129.0)	<0.001
DBP, mmHg	69.0 (61.0–77.0)	70.0 (63.0–77.0)	71.0 (63.0–77.0)	<0.001
Lymphocyte count (10 ⁹ /L)	1.6 (1.3–1.9)	2.3 (2.0–2.7)	3.1 (2.6–3.6)	<0.001
Total cholesterol, mg/dL	184.0 (159.0–211.0)	188.0 (163.0–216.0)	191.0 (164.0–220.0)	<0.001
Triglyceride, mg/dL	108.0 (74.0–159.0)	113.0 (76.0–173.0)	127.0 (83.0–195.0)	<0.001
HDL, mg/dL	52.0 (43.0–63.0)	51.0 (42.0–62.0)	49.0 (41.0–59.0)	<0.001
LDL, mg/dL	105.1 (83.0–128.0)	108.0 (86.0–132.4)	109.6 (87.0–134.9)	<0.001
AST, U/L	22.0 (19.0–27.0)	23.0 (20.0–27.0)	24.0 (20.0–28.0)	<0.001
ALT, U/L	19.0 (15.0–25.0)	21.0 (16.0–28.0)	22.0 (17.0–30.0)	<0.001
Albumin, g/L	40.0 (38.0–42.0)	43.0 (41.0–44.0)	45.0 (43.0–47.0)	<0.001
UA, umol/L	315.2 (261.7–374.7)	315.2 (261.7–368.8)	327.1 (273.6–386.6)	<0.001
eGFR, mg/min/1.73 m ²	81.1 (65.0–97.9)	86.9 (71.9–104.3)	89.4 (75.4–104.9)	<0.001
HbA1c, %	5.6 (5.3–6.0)	5.5 (5.2–5.8)	5.4 (5.2–5.8)	<0.001
GNRI	101.3 (98.3–102.7)	105.7 (102.7–107.2)	108.7 (105.7–111.7)	<0.001
TCBI	1,604.8 (964.7–2,638.6)	1,698.9 (987.6–2,889.5)	1,916.0 (1,060.3–3,314.9)	<0.001
COUNT score, ≥3, n (%)	859 (14.1%)	137 (1.8%)	20 (0.3%)	<0.001

Values are given as median and interquartile range or numbers and percentages.

PNI, Prognostic Nutritional Index; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; AST, aminotransferase; ALT, alanine aminotransferase; UA, uric acid; HbA1c, glycosylated hemoglobin; eGFR, estimated glomerular filtration rate; GNRI, Geriatric Nutritional Risk Index; COUNT score, Controlling Nutritional Status score; TCBI, Triglycerides × Total Cholesterol × Body Weight Index.

group had lower GNRI and TCBI and higher COUNT score ($p < 0.001$). In addition, hypertension, diabetes, and CVD were more common in the lowest tertile (PNI < 51) group ($p < 0.001$). All variables in the **Table 1** were statistically different across participants in different PNI tertiles (all $p < 0.001$). The distributions of the GNRI, PNI, TCBI, and COUNT score are shown in **Figure 2**.

The Relationship of Four Nutritional Scores With All-Cause and Cardiovascular Mortality

Kaplan–Meier curves illustrated the incidence of all-cause death and cardiovascular death in the general population (**Figure 3**).

Overall, the cumulative incidence of all-cause mortality and cardiovascular death was significantly higher in patients with a lower PNI and GNRI or higher COUNT score. TCBI showed the worst performance on grading and risk assessment for all-cause death (log-rank test, $p = 0.056$) and cardiovascular death (log-rank test, $p = 0.44$) in the general population (**Figures 3C,G**). In addition, PNI showed superior performance on grading and risk assessment to other nutritional scores.

According to the nutritional scores calculated by each equation as the categorical variable grouped previously mentioned, we performed the Cox regression hazard models. Cox regression analysis of nutritional scores with all-cause mortality and cardiovascular death in the overall population is shown in **Tables 2, 3**. In the univariate and multivariate Cox

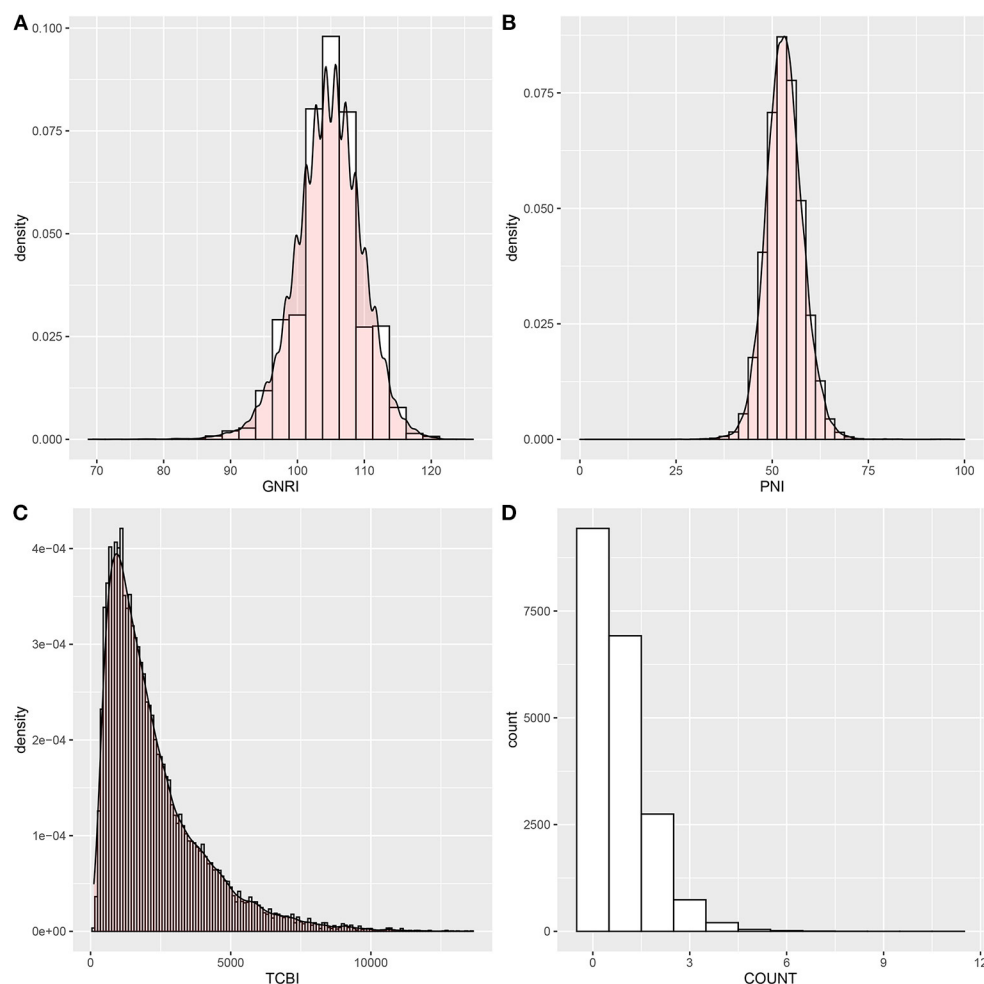


FIGURE 2 | Histograms show the population distribution of nutritional scores. **(A)** GNRI; **(B)** PNI; **(C)** TCBI; **(D)** COUNT score. PNI, Prognostic Nutritional Index; GNRI, Geriatric Nutritional Risk Index; CONUT score, Controlling Nutritional Status score; TCBI, Triglycerides \times Total Cholesterol \times Body Weight Index.

proportional hazards analysis, participants with the lowest GNRI (<102.75) and PNI (<51) had increased risks of all-cause death and cardiovascular death. In addition, the intermediate GNRI ($102.75\text{--}107.22$) and PNI ($51\text{--}55$) were also associated with the incidence of adverse events for all-cause death and cardiovascular death in univariate analysis. For COUNT score, the highest and intermediate groups were associated with increased risk for all-cause mortality and cardiovascular death in Model 1, 2, and 3. For TCBI, although the lowest score was associated with increased all-cause mortality in Model 2 and 3, the positive effect size was non-significant in Model 1. Conversely, the intermediate TCBI score was associated with increased all-cause mortality in Model 1. Furthermore, we also found that TCBI was non-significantly associated with cardiovascular death.

Restricted cubic splines were performed to further explore the associations of nutritional scores, which were treated as a continuous variable, with the HR (hazard ratio) of all-cause mortality and cardiovascular death after adjusting as Model 3

used in Cox analysis. An L-shaped relationship between the HR of all-cause mortality and nutritional scores (GNRI, PNI, and TCBI) was indicated in the overall populations (**Figure 4**). For GNRI, the HR sharply decreased until it reached ~ 104 ; thereafter, the HR tended to decrease slowly (**Figure 4A**). For PNI, the HR sharply decreased until it reached $\sim 52\text{--}53$; thereafter, the HR tended to the horizontal line with $\text{HR} = 1$ (**Figure 4B**). For TCBI, the HR sharply decreased until it reached $\sim 1,689$; thereafter, the HR tended to decrease slowly and the TCBI gradually showed a protective role but no statistical significance (**Figure 4C**). The higher the COUNT score, the worse the nutritional status of participants. For the COUNT score, the HR slowly increased until it reached ~ 2 ; thereafter, the HR tended to increase sharply (**Figure 4D**). We also found similar relationships between nutritional scores and cardiovascular death in the general population (**Figure 4**). However, the non-linear relationship between nutrition scores and cardiovascular death was weakened. In addition, no apparent correlation was found between the TCBI and cardiovascular death (**Figure 4G**).

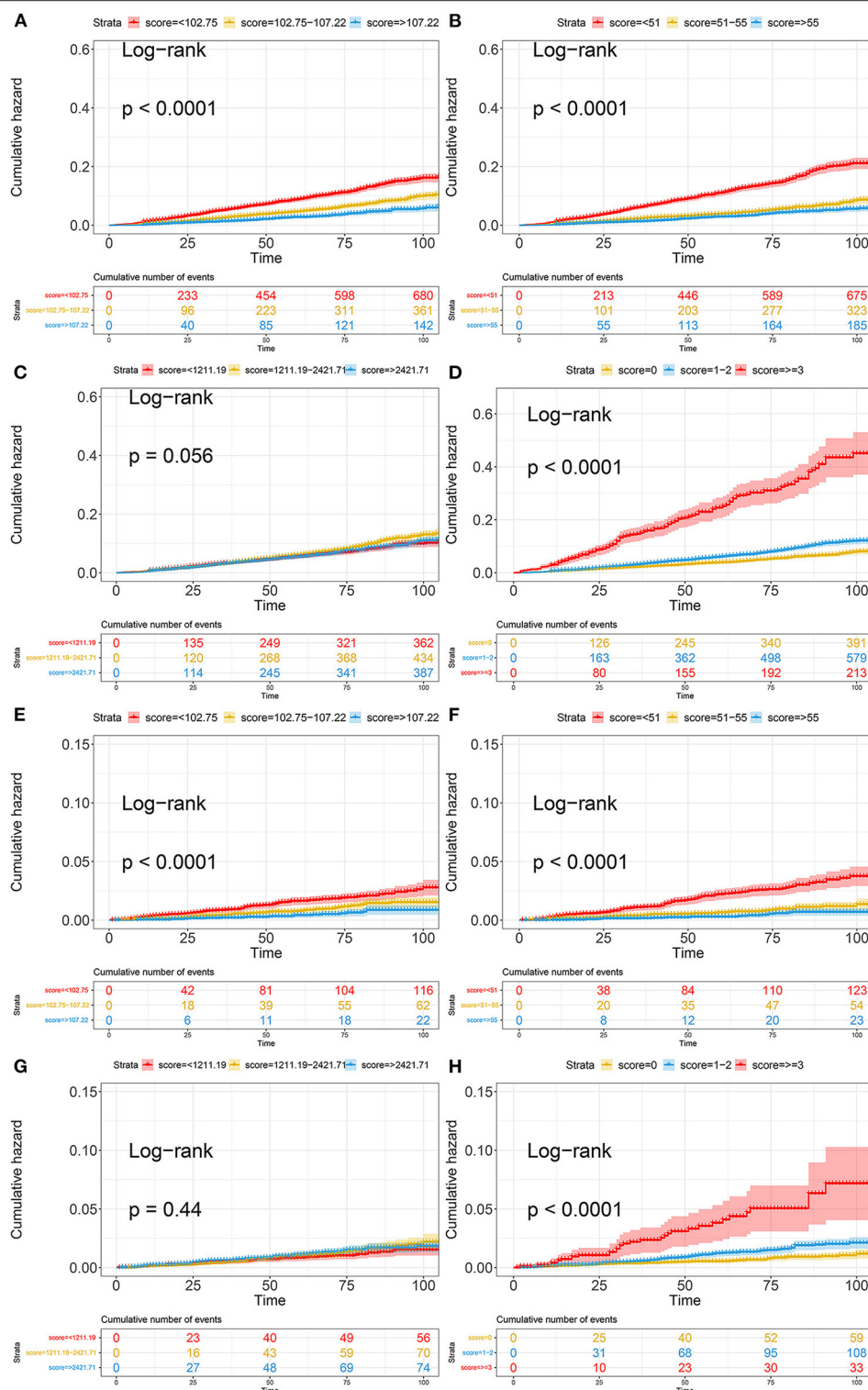


FIGURE 3 | Kaplan-Meier curves of all-cause death and cardiovascular death based on four nutritional scores. **(A)** Kaplan-Meier curves of all-cause death categorized by GNRI; **(B)** Kaplan-Meier curves of all-cause death categorized by PNI; **(C)** Kaplan-Meier curves of all-cause death categorized by TCBI; **(D)** Kaplan-Meier curves of all-cause death categorized by COUNT score; **(E)** Kaplan-Meier curves of cardiovascular death categorized by GNRI; **(F)** Kaplan-Meier curves of cardiovascular death categorized by PNI; **(G)** Kaplan-Meier curves of cardiovascular death categorized by TCBI; **(H)** Kaplan-Meier curves of cardiovascular death categorized by COUNT score. PNI, Prognostic Nutritional Index; GNRI, Geriatric Nutritional Risk Index; CONUT score, Controlling Nutritional Status score; TCBI, Triglycerides \times Total Cholesterol \times Body Weight Index.

TABLE 2 | Cox regression analysis of nutritional scores with all-cause mortality.

Nutritional score		Model 1		Model 2		Model 3	
		HR (95% CI)	<i>p</i>	HR (95% CI)	<i>p</i>	HR (95% CI)	<i>p</i>
GNRI	<102.75	3.09 (2.58–3.70)	<0.01	1.81 (1.51–2.17)	<0.01	1.81 (1.50–2.17)	<0.01
	102.75–107.22	1.72 (1.42–2.09)	<0.01	1.15 (0.95–1.39)	0.16	1.18 (0.97–1.44)	0.09
	>107.22	Reference		Reference		Reference	
PNI	<51	3.86 (3.28–4.54)	<0.01	1.69 (1.43–2.00)	<0.01	1.79 (1.52–2.12)	<0.01
	51–55	1.42 (1.20–1.70)	<0.01	1.00 (0.84–1.20)	0.96	1.06 (0.89–1.27)	0.52
	>55	Reference		Reference		Reference	
TCBI	<1,211.19	1.01 (0.87–1.16)	0.95	1.29 (1.12–1.49)	<0.01	1.42 (1.19–1.69)	<0.01
	1,211.19–2,421.71	1.16 (1.01–1.33)	0.04	1.04 (0.90–1.19)	0.60	1.09 (0.94–1.26)	0.24
	>2,421.71	Reference		Reference		Reference	
COUNT	0	Reference		Reference		Reference	
	1–2	1.54 (1.36–1.75)	<0.01	1.35 (1.19–1.54)	<0.01	1.32 (1.14–1.53)	<0.01
	≥3	6.33 (5.36–7.48)	<0.01	2.92 (2.45–3.48)	<0.01	2.71 (2.20–3.33)	<0.01

Data are presented as hazard ratios, 95% CI (confidence intervals), and *P*-value.

Model 1 adjusted for none.

Model 2 adjusted for age, sex, and race.

Model 3 adjusted for age, sex, race, BMI, Smoking, Drinking, hypertension, diabetes, HDL, LDL, SBP, DBP, eGFR, AST, ALT, UA, HbA1c, cardiovascular disease, hypotensive drugs, hypoglycemic drugs.

TABLE 3 | Cox regression analysis of nutritional scores with cardiovascular death.

Nutritional score		Model 1		Model 2		Model 3	
		HR (95% CI)	<i>p</i>	HR (95% CI)	<i>p</i>	HR (95% CI)	<i>p</i>
GNRI	<102.75	3.45 (2.19–5.44)	<0.01	1.83 (1.16–2.91)	<0.01	1.77 (1.11–2.83)	0.02
	102.75–107.22	1.93 (1.19–3.13)	<0.01	1.20 (0.74–1.96)	0.46	1.24 (0.76–2.02)	0.39
	>107.22	Reference		Reference		Reference	
PNI	<51	5.70 (3.65–8.89)	<0.01	2.11 (1.34–3.32)	<0.01	2.23 (1.41–3.52)	<0.01
	51–55	1.92 (1.18–3.13)	<0.01	1.27 (0.78–2.08)	0.33	1.36 (0.83–2.22)	0.22
	>55	Reference		Reference		Reference	
TCBI	<1,211.19	0.81 (0.57–1.15)	0.23	0.96 (0.67–1.36)	0.81	1.26 (0.82–1.95)	0.29
	1,211.19–2,421.71	0.98 (0.71–1.36)	0.89	0.82 (0.59–1.13)	0.23	0.94 (0.66–1.33)	0.72
	>2,421.71	Reference		Reference		Reference	
COUNT	0	Reference		Reference		Reference	
	1–2	1.89 (1.38–2.60)	<0.01	1.49 (1.08–2.05)	0.02	1.50 (1.04–2.15)	0.03
	≥3	6.41 (4.19–9.82)	<0.01	2.29 (1.46–3.58)	<0.01	2.31 (1.38–3.88)	<0.01

Data are presented as hazard ratios, 95% CI (confidence intervals), and *P*-value.

Model 1 adjusted for none.

Model 2 adjusted for age, sex, and race.

Model 3 adjusted for age, sex, race, BMI, Smoking, Drinking, hypertension, diabetes, HDL, LDL, SBP, DBP, eGFR, AST, ALT, UA, HbA1c, cardiovascular disease, antihypertensive drugs, hypoglycemic drugs.

Comparative Analysis of Four Nutritional Scores in Predicting All-Cause and Cardiovascular Mortality

We conducted ROC curve analysis of the predictive models for all-cause death and cardiovascular death with the GNRI, PNI, TCBI, and COUNT score (Figure 5; Table 4). In terms of the AUC for all-cause mortality, the PNI score had significantly higher AUC [0.684, 95% confidence intervals (CI): 0.667–0.701, reference] than the other nutritional scores, whereas the GNRI (AUC 0.642, 95% CI: 0.626–0.659, $p < 0.001$) and the COUNT

score (AUC 0.621, 95% CI: 0.604–0.638, $p < 0.001$) had similar AUC. The TCBI had the lowest AUC (0.508, 95% CI: 0.492–0.524, $p < 0.001$). We also obtained similar results in the AUC for cardiovascular death (Table 4).

Additionally, NRI and IDI in all-cause mortality and cardiovascular death were assessed by comparing GNRI, TCBI, and COUNT to PNI (reference). Overall, compared with PNI, the reclassification of other nutritional scores performed worse both on all-cause mortality and cardiovascular death (Table 4). The NRI and IDI of GNRI (−0.210, $p < 0.001$ and −0.007, $p < 0.001$,

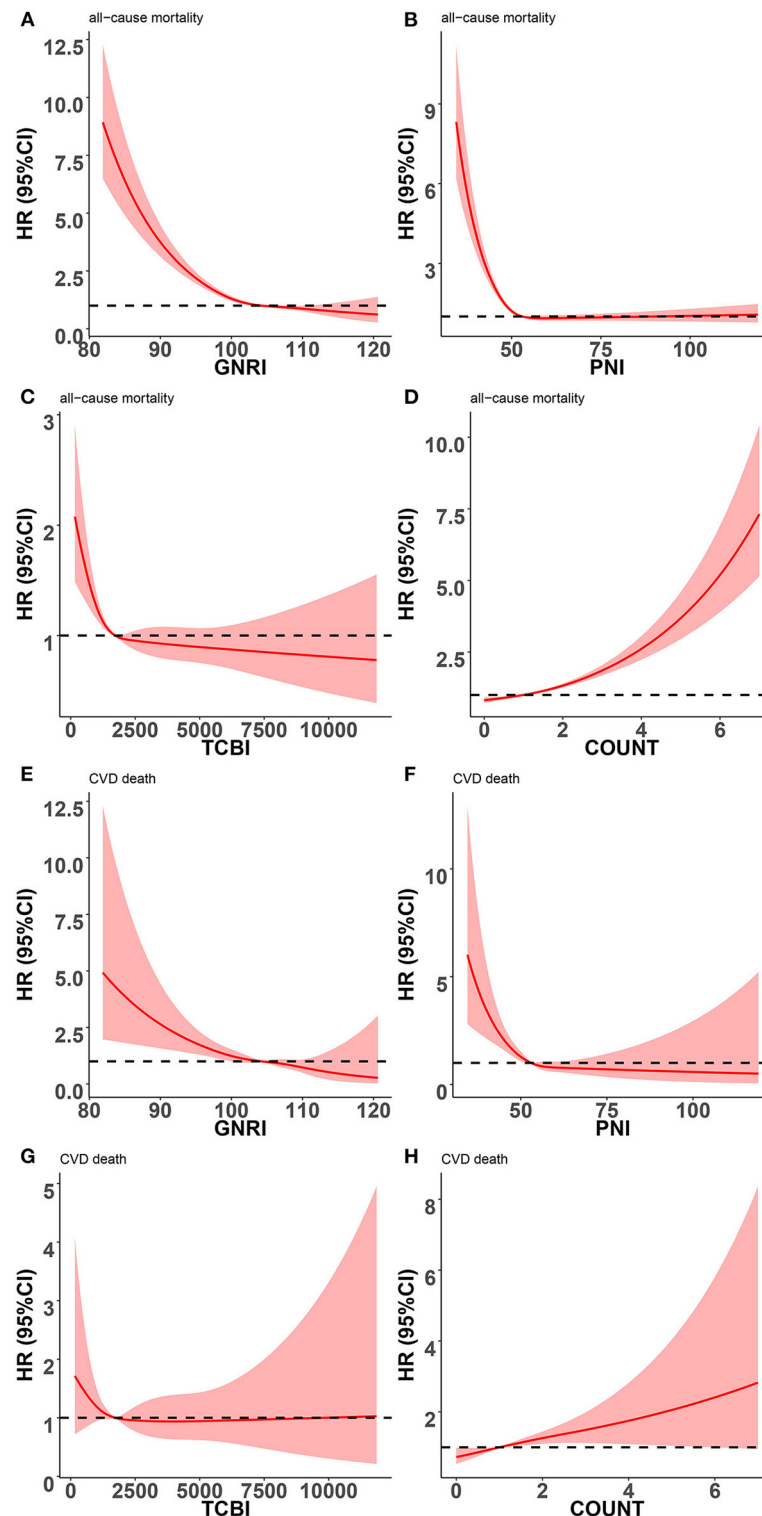


FIGURE 4 | Restricted spline curves for the associations between four nutritional scores and adverse events in general population. Red lines represent the HR (hazard ratio), and red transparent areas represent the 95% confidence intervals. HR (95% CI) were all adjusted according to Model 3 in Cox analysis. **(A)** Association between GNRI and all-cause mortality; **(B)** Association between PNI and all-cause mortality; **(C)** Association between TCBI and all-cause mortality; **(D)** Association between COUNT score and all-cause mortality; **(E)** Association between GNRI and cardiovascular death; **(F)** Association between PNI and cardiovascular death; **(G)** Association between TCBI and cardiovascular death; **(H)** Association between COUNT score and cardiovascular death; PNI, Prognostic Nutritional Index; GNRI, Geriatric Nutritional Risk Index; CONUT score, Controlling Nutritional Status score; TCBI, Triglycerides \times Total Cholesterol \times Body Weight Index.

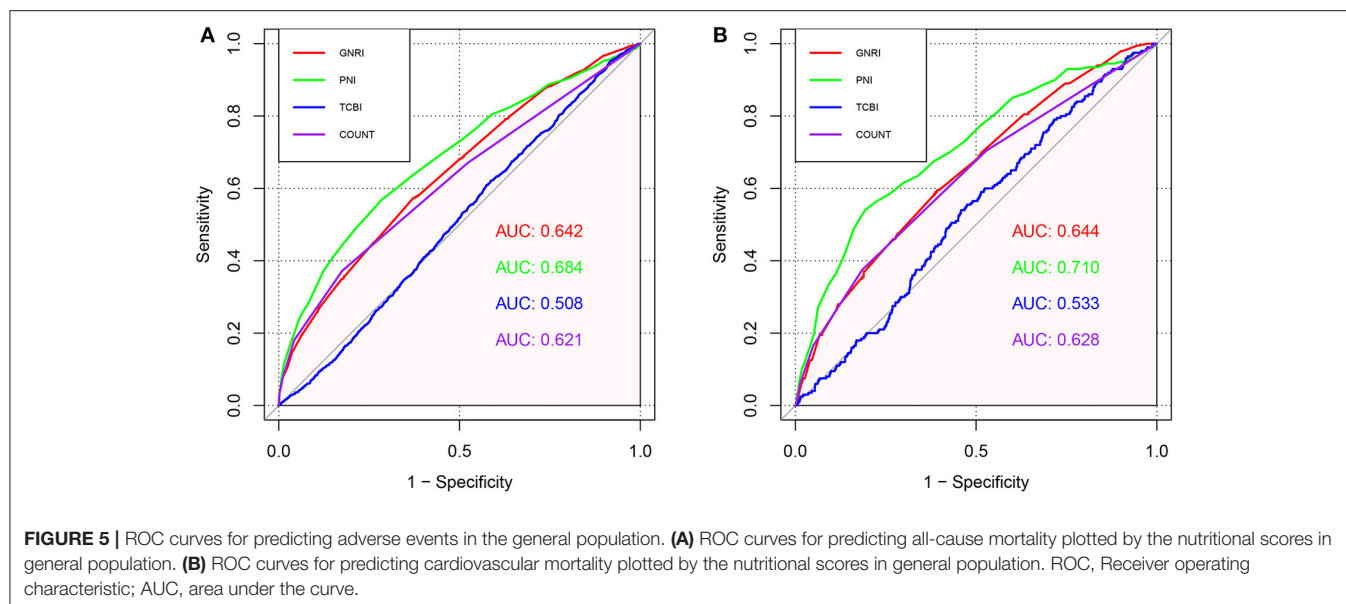


TABLE 4 | Comparisons of AUC, IDI, and NRI for GNRI, PNI, TCBI and COUNT.

Models	NRI (95% CI)	P-Value	IDI (95% CI)	P-Value	AUC (95% CI)	P-value
All-cause mortality						
GNRI	−0.210 (−0.267, −0.154)	<0.001	−0.007 (−0.010, −0.004)	<0.001	0.642(0.626–0.659)	<0.001
PNI	Reference		Reference		0.684 (0.667–0.701)	Reference
TCBI	−0.532 (−0.588, −0.476)	<0.001	−0.031 (−0.035, −0.028)	<0.001	0.508 (0.492–0.524)	<0.001
COUNT	−0.176 (−0.234, −0.118)	<0.001	−0.003 (−0.006, −2e-04)	0.036	0.621 (0.604–0.638)	<0.001
Cardiovascular death						
GNRI	−0.485 (−0.615, −0.356)	<0.001	−0.004 (−0.005, −0.003)	<0.001	0.644 (0.606–0.682)	0.016
PNI	Reference		Reference		0.710 (0.672–0.749)	Reference
TCBI	−0.601 (−0.733, −0.470)	<0.001	−0.007 (−0.009, −0.005)	<0.001	0.533 (0.495–0.570)	<0.001
CPUNT	−0.490 (−0.621, −0.360)	<0.001	−0.004 (−0.005, −0.003)	<0.001	0.628 (0.589–0.668)	<0.001

IDI, integrated discrimination improvement; NRI, net reclassification improvement.

NRI and IDI respectively), TCBI (−0.532, $p < 0.001$ and −0.031, $p < 0.001$, NRI and IDI respectively), and COUNT score (−0.176, $p < 0.001$ and −0.003, $p = 0.036$, NRI and IDI respectively) for the all-cause death were significantly inferior to PNI. In addition, the PNI remained incremental values for predicting the incidence of cardiovascular death.

Stratification Analysis

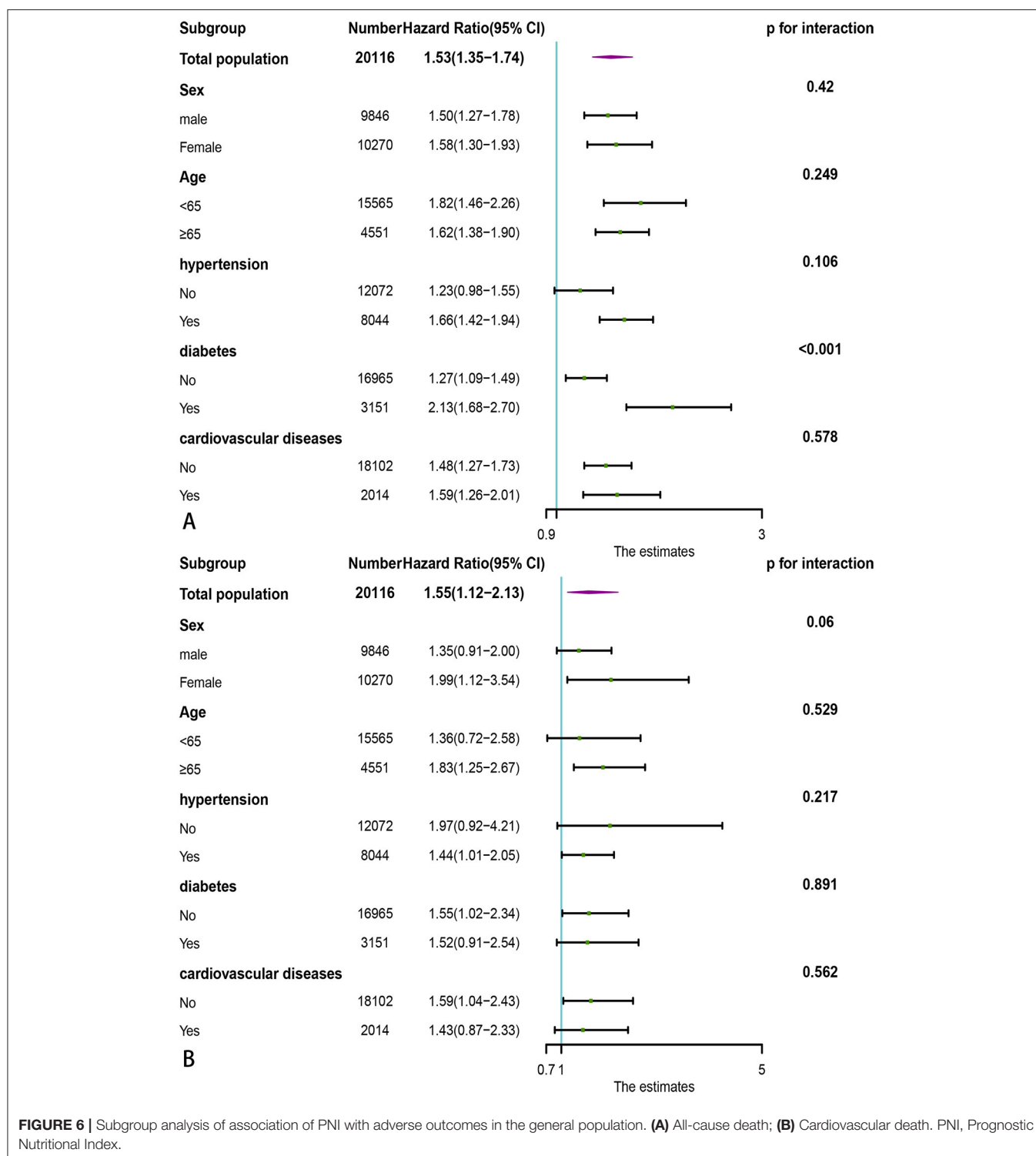
Since PNI had the highest predictive value for all-cause death and cardiovascular death in the general population, we stratified the individuals by age, gender, hypertension, diabetes, and cardiovascular diseases to further observe the association between PNI and all-cause mortality, cardiovascular death. The PNI (divided by the median of 53) was further treated as a dichotomous variable in subgroup analysis.

As shown in **Figure 6A**, lower PNI was found to be associated with increased risks of all-cause death in almost all the subgroups except for participants without hypertension (HR = 1.23, 95% CI: 0.98–1.55). In addition, lower PNI was also associated

significantly with increased risks of cardiovascular death in total population (HR = 1.55, 95% CI: 1.12–2.13) and half of subgroups (**Figure 6B**), except for male (HR = 1.35, 95% CI: 0.91–2.00), age < 65 (HR = 1.36, 95% CI: 0.72–2.58), participants without hypertension (HR = 1.97, 95% CI: 0.92–4.21), participants with diabetes (HR = 1.52, 95% CI: 0.91–2.54) and participants with CVD (HR = 1.43, 95% CI: 0.87–2.33). We also found that the association between lower PNI and all-cause mortality was more pronounced in the diabetic population [HR = 2.13, 95% CI: 1.68–2.70, p for interaction < 0.001].

DISCUSSION

In the present study, the nutritional status was evaluated by the GNRI, TCBI, PNI, and COUNT score. We explored their association with all-cause mortality and cardiovascular death in the general population recruited from a nationally representative sample of the United States. The major conclusions are as



follows: (1) Malnourished participants were at a higher risk of all-cause death and cardiovascular death. (2) We found an L-shaped relationship between nutritional scores (GNRI, PNI, and TCBI) and adverse outcomes in the general population. However, for COUNT score, the L-shaped association was the opposite.

(3) Compared with other nutritional scores, the PNI had the highest predictive value in the general population. However, TCBI showed the worst performance on risk assessment and prediction. (4) The association between lower PNI and all-cause mortality was more pronounced in the diabetic population.

The PNI, which consists of serum albumin level and the total lymphocyte count, has been widely applied to predict adverse outcomes in various patients (14, 27–29). The main advantages of PNI compared to other nutrition scores are as follows. First, besides being a nutritional indicator, there is evidence that serum albumin regulates the body's inflammatory response and negatively correlates with C-reactive protein (CRP) levels (30, 31). The stabilization of inflammatory cytokines and oxidative stress markers is also an important role of albumin (32). Meanwhile, it is well known that low lymphocyte count (LLC) can reflect a poorly regulated immune response. In addition, LLC is a common phenomenon during the inflammatory reaction (33). The above evidence suggested that PNI could not only assess the nutritional status but also effectively reflect the inflammation and immune status of the body. Second, the predictive value of low albumin for adverse events has been reported in many studies (34, 35), and LLC is also associated with poor prognosis in patients with a wide variety of diseases (33, 36). Therefore, participants with low PNI were at extremely high risk of mortality. Third, compared with COUNT score using categorical variables, albumin and lymphocyte count are used as continuous variables to calculate PNI, which minimizes the loss of information and better reflects the nutritional status of the general population. Fourth, lymphocyte count is a more stable indicator of body composition during long-term follow-up. In contrast, the indicators (body weight, TC, and TG) used to calculate GNRI and TCBI are more susceptible to some factors such as age, diet, drugs, smoking, drinking, and lifestyle habits. Therefore, our study suggested that PNI might be the most effective indicator for predicting the adverse events of the general population among the four nutritional scores. In the subgroup analysis, the risk of all-cause death in diabetic patients with lower PNI was significantly higher than that in non-diabetic patients, which showed that malnutrition might play a more critical role in the mortality of diabetic patients. One possible explanation is that malnutrition may aggravate systemic inflammation in diabetic patients, leading to increased all-cause mortality (37, 38).

The GNRI, which considers both serum albumin levels and body weight, is also used to assess the nutritional status of patients and shows the predictive value for adverse outcomes (2, 3). The unique advantage of the GNRI is that the ratio of body weight to ideal body weight allows for a better reflection of the extent to which malnourished participants deviate from normal BMI, which can help assess short-term nutritional status. However, the weight may change substantially during long-term follow-up, especially in young adults, which limits its predictive value in the general population. Furthermore, it is essential to emphasize that body weight is influenced by fluid distribution in the body, which may make the measured weight of participants with edema higher than their actual weight (39). Therefore, GNRI may overestimate the nutritional status of this population.

The TCBI index, a novel nutritional metabolism index, is calculated from variables reflecting lipid metabolism measured from blood tests. Some studies showed that TCBI was a useful prognostic indicator in patients with a wide range of CVD (11, 40, 41). The most significant advantage of the TCBI is the simplicity of the calculation, which saves time and effort in caring

for and treating ICU (intensive care unit) patients (40, 41). The calculation of TCBI simply requires multiplying three variables, while anyone who calculates GNRI or PNI needs to know the constant values and how to calculate ideal body weight. For CONUT, they need to know thresholds and scores for each indicator. In our study, TCBI was perhaps not an ideal tool in predicting adverse events in the general population. We think this may be caused by the reason that TC and TG cannot accurately reflect the nutritional status, inflammation level, and immune response of the body. On the one hand, the relationship between reduced TG and TC and poor nutritional status is currently not fully elucidated. On the other hand, as previously described, TC and TG are more prone to change due to various factors.

The COUNT score is also reported as an independent prognostic marker in patients with various malignancies (42, 43), acute heart failure (44), and coronary artery disease (45). The advantage of the COUNT score is that it incorporates the largest number of serum nutritional indicators. Compared with PNI, the COUNT score considers the influence of TC on nutritional status. Compared with GNRI and TCBI, lymphocyte count is included as a more stable indicator. However, our study demonstrated the COUNT score was lower than PNI in predicting all-cause mortality and cardiovascular death. We think this may be because the COUNT score treats serum albumin levels, total lymphocyte count, and TC as categorical variables, which is its greatest deficiency.

Malnutrition may decrease immunity and antioxidant capacity and increase inflammation and blood viscosity, which may lead to the occurrence of adverse outcomes. In our study, lower PNI (<51) and GNRI (<102.75) and higher COUNT score (>3) were significant independent predictors of all-cause mortality and cardiovascular death in the general population. The restricted cubic spline showed an L-shaped relationship between nutritional scores and adverse events, which also indicated that malnutrition significantly increased the risk of death. In addition, since PNI had the highest predictive value, we needed to focus more on the nutritional status assessed by PNI to reduce all-cause and cardiovascular mortality in the general population.

Despite the crucial findings being mentioned, our study has some limitations. First, the results were mainly applicable in the United States. Second, some covariates were self-reported using validated questionnaires, which might be affected by memory bias. Third, it is unclear whether nutritional status changes over time could influence the risk of all-cause mortality and cardiovascular death. Fourth, we only explored the association of nutritional scores with all-cause mortality and cardiovascular death but not with mortality related to other causes.

CONCLUSION

In summary, we reported an association between all-cause mortality, cardiovascular death and four objective nutritional scores (GNRI, PNI, TCBI, and COUNT). Meanwhile, our

study demonstrated that the PNI had the greatest predictive value for all-cause mortality and cardiovascular death in the general population.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by National Center for Health Statistics. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HF: conceptualization, project administration, writing—original draft, methodology, data analysis, and data collection. YH and

HZ: methodology, data analysis, and data collection. XF: data curation and data collection. ZY: resources, funding acquisition, and writing—review and editing. JZ: supervision, project administration, data curation, writing—review and editing, and methodology. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnut.2022.846659/full#supplementary-material>

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Effects of a 3-Week Inpatient Multidisciplinary Body Weight Reduction Program on Body Composition and Physical Capabilities in Adolescents and Adults With Obesity

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Background: The aim of the present study was to examine the short-term changes in body composition and physical capabilities in subjects with obesity during a multidisciplinary inpatient body weight reduction program (BWRP).

Methods: One hundred thirty-nine adolescents (56 boys and 83 girls; BMI: 37.1 ± 6.5 kg/m²; Fat Mass, FM: $45.3 \pm 7.2\%$) and 71 adults (27 males and 44 females; BMI: 44 ± 4.7 kg/m²; FM: $51.4 \pm 4.7\%$) followed a 3-week inpatient BWRP consisting of regular physical activity, moderate energy restriction, nutritional education and psychological counseling. Before (T0) and after the end of the BWRP (T21), body composition was assessed with an impedancemeter, lower limb muscle power with Margaria Stair Climbing Test (SCT), lower limb functionality with Short Physical Performance Battery (SPPB), and the capacity of performing activity of daily living (ADL) with Physical Performance Test (PPT).

Results: At T21, obese adolescents showed a 4% reduction in body mass (BM) ($p < 0.001$), associated with a FM reduction in boys (-10%) and girls (-6%) ($p < 0.001$) and with a 3% reduction in fat-free mass (FFM) recorded only in boys ($p = 0.013$). Obese adults showed a 5% BM reduction ($p < 0.001$), associated with a 2% FFM and 9% FM reduction ($p < 0.001$) in males, and 7% FM reduction in females ($p < 0.001$). Regarding physical capabilities, at T21 in obese adolescents, PPT score increased by 4% ($p < 0.001$), SCT decreased by -5% (boys) and -7% (girls) ($p < 0.001$), while SPPB score did not change significantly. In obese adults at T21, PPT score increased by 9% ($p < 0.001$), SCT decreased by -16% ($p < 0.001$) only in females, and SPPB score increased by 7% (males) and 10% (females) ($p < 0.01$).

Conclusion: In conclusion, moderate energy restriction and regular physical activity determine a 4-5% BM reduction during a 3-week inpatient BWRP, improve physical capabilities and induce beneficial changes in body composition in adolescents and adults with obesity.

Trial registration: This study was approved by the Ethical Committee of the Istituto Auxologico Italiano (Milan, Italy; research code: 01C124; acronym: PRORIPONATFIS). Registered 11 November 2020 - Retrospectively registered.

Keywords: physical capabilities, body composition, adolescents, adults, obesity, physical activity

INTRODUCTION

Over the past two decades, obesity has tripled in both children and adults in industrialized countries (1, 2), mainly due to the excess food-intake combined with an increase in time spent in sedentary activities, leading to a decrease in physical activity level (3–5). Excessive levels of body fat is associated with major health consequences associated with obesity, including hypertension (6–8), type 2 diabetes, and cardiovascular disease (9). In addition, reduced physical activity levels in adolescents and adults with obesity is reported to lead to a lower aerobic and anaerobic capacities, and lower limb muscle power output than their normal-weight counterparts (10–12). Consequently, obese subjects with high values of fat mass and poor physical abilities, usually suffer from lower limb functionality, higher perceived difficulty in performing physical exercise and activities of daily living (ADL) (13–16). Nevertheless, in obese subjects, short-term multidisciplinary inpatient body weight-reduction programs (BWRP) entailing moderate-intensity physical training, energy-restricted diets, and changes in food and behavioral habits have been reported to promote weight loss, reduce fat mass (FM) (17–19) and improve physical capacities. The improvements in body composition and physical capacities is reported to exert a positive effects on metabolic and cardiovascular risk profiles (20, 21), as well on both lower limb muscle power and functionality (17–19) and on performing ADL (13). However, the presence of a lower limb function and ability to perform ADL is often overlooked in adolescents and adults with obesity (22), even if their poor physical abilities actually worsen their quality of life, as it happens elderly obese (22).

Therefore, the aim of the present study was to determine whether a short-term (3-week) multidisciplinary BWRP entailing physical activity, moderate energy restriction, nutrition education, and psychological counseling, can positively affect body composition, lower limb muscle power and functionality, capacity of performing ADL in adolescents and adults with obesity.

Abbreviations: ADL, activities of daily living; BM, body mass; BMI, body mass index; BMI (SDS), body mass index standard deviation score; BP, blood pressure; BWRP, body weight-reduction programs; DBP, diastolic blood pressure; HR, heart rate; PPT, physical performance test; SBP, systolic blood pressure; SCT, stair climbing test; SPPB, short physical performance battery.

MATERIALS AND METHODS

Subjects

One hundred thirty-nine adolescents (56 boys and 83 girls; age range: 13–17 years) and 71 adults (27 males and 44 females; age range: 35–68 years) with obesity, participated in this study. Among adolescents, BMIs for gender and chronological age were above the 99th percentile (23), while for adults the BMIs were above 35 kg/m². Subjects were recruited as inpatients from the Division of Auxology (subjects aged <18 year) and from the Division of Metabolic Diseases (subjects aged >18 years), Istituto Auxologico Italiano, IRCCS, Piancavallo (VB), Italy. Before admission to the hospital for BWRP, none of the subjects had engaged in structured physical activity (i.e., regular activity of more than 60 min/week) evaluated with the validated International Physical Activity Questionnaire Short Form (IPAQ-SF) (24). All subjects had a complete medical history and physical examination. None of the adolescents or adults with obesity had signs or symptoms indicative of serious cardiovascular, respiratory, or orthopedic disease that could significantly interfere with the functional test used in the study.

Study Protocol

The study was approved by the Ethical Committee of the Istituto Auxologico Italiano (Milan, Italy; research code: 01C124; acronym: PRORIPONATFIS) and was in accordance with the Declaration of Helsinki 1975, as revised in 2008. For adolescents, the protocol was explained to parents and written informed consent was obtained from parents or legal representatives. Patients aged ≥18 gave written informed consent to participate in the study. Patients were hospitalized for a period of 3 weeks in the Division of Auxology (patients <18 years) or in the Division of Metabolic Diseases (patients >18 years), Istituto Auxologico Italiano, IRCCS, Piancavallo (VB). They followed a 3-week personalized BWRP consisting of moderate energy restriction, physical activity, nutrition education, and psychological counseling. Full testing sessions were conducted at the beginning (T0) and at completion of the 3-week BWRP (T21). Testing session included assessment of anthropometric characteristics, body composition, blood pressure (BP), lower limb muscle power, lower limb functionality, and ability to perform ADL (see below for detailed description).

Physical Activity

The physical activity program consisted of five training days per week, under the supervision of a physical trainer. Each training session included: (i) 45–60 min per day of aerobic activities (walking on a treadmill or cycling on an ergometer) under heart rate monitoring (HR) and medical supervision and (ii) 5–7 min of stretching before and after training. The intensity of aerobic activities was set at heart rate (HR) corresponding to 60 and 80% of the individual maximal HR estimated as $220 - \text{age (year)}$. The research assistant and the physical trainers verified that each subject participated in each training session, performed the exercises correctly, and completed at least 95% of the exercise session and program. In addition, subjects had 1 h/day of aerobic leisure activities at the institution on Saturday and Sunday.

Diet and Nutrition Education

A Mediterranean diet was prescribed based on the initial basal metabolic rate test and physical activity level for each patient, and the amount of energy to be given with diet was calculated by subtracting approximately ~25% from the estimated daily energy expenditure. In terms of macronutrients, the diet contained 21% proteins, 53% carbohydrates, and 26% lipids. The diet composition was formulated according to the Italian recommended daily allowance (25). Each patient was free to choose foods from a heterogeneous daily menu, although five daily servings of fruits and vegetables were mandatory. Foods to which the patient reported allergic reactions were eliminated from the menu. A fluid intake of at least 1.5 L/day was encouraged. In addition, the dietitian team checked that each subject had eaten every meal. On each day of the BWRP, the patients had dietetics classes consisting of lectures, demonstrations, and group discussions with and without a supervisor.

Psychological Counseling

Cognitive-behavioral therapy strategies, such as stimulus control procedures, problem-solving and stress management training, development of healthy eating habits, assertiveness and social skills training, cognitive restructuring of negative maladaptive thoughts, and relapse prevention training, were chosen for the psychological sessions, which were conducted by a clinical psychologist 2–3 times per week in individual or group sessions as previously reported (26). When possible (1 day per week), additional sessions were also conducted with parents of the obese adolescents aimed at improving motivation for lifestyle change and interpersonal communication.

Measurements

Physical Characteristics and Body Composition

Medical history was obtained and a baseline physical examination was performed. Stature and body mass (BM) were measured using a Harpenden stadiometer (Holtain Ltd., United Kingdom), and an electronic scale (Selus, Italy), respectively, with the subject wearing only light underwear. BMI (kg/m^2) was calculated. The standard deviation score (SDS) of BMI-SDS was calculated using the LMS method (23) on Italian reference values for children and adolescents (27). Body composition was measured using

a multifrequency tetrapolar impedancemeter (BIA, Human-IM Scan, DS-Medigroup, Milan, Italy) with a delivered current of 800 μA at a frequency of 50 kHz. To reduce measurement errors, care was taken to standardize the variables that affect the validity, reproducibility and precision of the measurement. The measurements were performed according to the method of Lukaski et al. (28) (after 20 min of rest in the supine position with arms and legs relaxed and without contact with other parts of the body) and under strictly controlled conditions according to NIH guidelines (29). Before measurements, technical accuracy has been validated by an external parallel circuit containing a high-precision resistor and capacitor. Low-impedance electrodes were used for reliable and accurate assessment of the raw bioimpedance parameters (e.g., R, Xc, and phase angle) (30). The within-day coefficient of variation for three repeated assessments of FFM in obese subjects (with repositioning of electrodes) has been previously assessed in our laboratory (2.4%).

All females were studied outside of the menstrual period in order to avoid any possible influence on fluid retention, as suggested by the NIH guidelines (29). For the adolescents, Fat-Free Mass (FFM) was calculated using the prediction equation previously developed by our group against DEXA (31):

$$FFM \text{ (kg)} = 0.87 \cdot ZI \text{ (cm}^2 \cdot \Omega) + 3 \cdot 1 \text{ (adjusted coefficient of variation} = 0.91)$$

where, ZI is the impedance index calculated as stature (cm^2) divided by whole-body impedance (Z) at 50 kHz (Ω). For the adults, we used the equation developed by Gray et al. (32):

$$FFM \text{ (kg)} = 0.00139 \cdot \text{stature (cm}^2) - 0.0801 \cdot Z(\Omega) + 0.187 \cdot BM \text{ (kg)}$$

FM (kg) was derived as the difference between BM (kg) and FFM (kg).

BP measurements were taken after the participants have rested in the sitting position for at least 5 min, the average of 2 BP readings was used for analysis.

Lower Limb Muscle Power

The Stairs Climbing Test (SCT) is a well-standardized procedure for measuring maximal anaerobic power in adolescents and adults with obesity (20, 33). Prior to administering the test, 2–3 practise trials were scheduled to allow subjects to gain sufficient confidence with the technique. Briefly, subjects were asked to climb an ordinary stair at the highest possible speed, according to their abilities. The stairs consisted of 13 steps of 15.3 cm each, so that a total vertical distance of 1.99 m was covered. An experimenter measured the time taken to complete the test using a digital stopwatch. SCT repeatability in obese subjects has been previously evaluated in our laboratory and the coefficient of variation between measurements was found to be lower than 5% (20).

Short Physical Performance Battery

A Short Physical Performance Battery (SPPB) (34, 35) was administered. The SPPB consists of the following three parts: (i)

tests of standing balance, included semi-tandem position, side-by-side stands and tandem position (each held for 10 s), (ii) walking a 4 m distance at normal gait speed, and (iii) rising from a chair and returning to the seated position five times. Scores for each item ranged from 0 to 4, for a maximum of 12 points. Performance categories were created for each set of performance measures to allow for analyses that included those unable to perform a task. The three tests of standing balance were considered hierarchical in difficulty by assigning a single score from 0 to 4 for standing balance (35). For 4 m walking and repeated chair stands, a score of 0 was assigned to those who could not complete the task. Those who were able to complete the task were assigned scores from 1 up to 4, corresponding to quartiles of time required for the task, with the fastest times scored as 4 (35). Higher scores were associated with better lower limb functionality (34).

Physical Performance Test

The ability to perform ADL was assessed using the Physical Performance Test (PPT) (36). The PPT test used in the present study includes 7 standardized tasks [(i) walk 15.2 m, (ii) put on and take off a coat, (iii) pick up a coin, (iv) lift a book, (v) simulate the act of eating, (vi) perform a 360° turn, and (vii) write a sentence]. The score for each item ranged from 0 to 4, with 0 corresponding to “unable to do” and 4 corresponding to “most able or quickest” (36). The maximum score was 28, and participants were classified as mildly to moderately frail if they scored between 19 and 24 (37).

Statistical Analyses

Statistical analyses were performed using Graph Pad Prism version 9.1.0-2021 software (GraphPad Software, Inc., San Diego, CA, United States) with a significance set at $p < 0.05$. All results were expressed as mean and standard deviation (SD). Normal distribution of the data was tested using the Kolmogorov–Smirnov test. The effects of gender, time, and the interaction between these variables on physical characteristics, body composition, lower limb muscle power, lower limb functionality and ability to perform ADL were tested using General Linear Model repeated measures. When significant differences were found, a Bonferroni *post hoc* test was evaluated implementing multiple comparisons. Relationships between the different factors were examined using Pearson or Spearman product–moment correlation coefficient.

RESULTS

Effects of the 3-Week BWRP in Adolescents

Anthropometric Characteristics and Body Composition

At T0, age, BM, BMI, and BMI-SDS were not significantly different between boys and girls, whereas stature was ~5% higher in boys than girls ($p < 0.001$, **Table 1**). At the end of BWRP (T21), BM and BMI decreased by ~4% ($p < 0.001$, **Table 1**) in

both genders, a greater reduction (~7%, $p < 0.001$, **Table 1**) being observed in BMI-SDS in both genders.

At T0, FFM (kg) and FFM (%) were 22 and 13% higher in boys than in girls, respectively ($p < 0.001$, **Table 1** and **Figure 1A**). Regarding FM (kg), no difference was found between boys and girls (**Table 1** and **Figure 1C**), while FM expressed as a percentage was ~15% ($p < 0.001$, **Table 1**) lower in boys than in girls. At T21, FFM (kg) decreased by 3% ($p = 0.013$, **Table 1**) in boys only (**Figure 1B**), while FFM (%) increased by ~3 and ~2% ($p < 0.005$, **Table 1**) in boys and girls, respectively. In contrast, FM (kg) decreased by ~9 and ~7% in boys and girls, respectively ($p < 0.001$, **Table 1** and **Figure 1D**), with a smaller decrease when FM was expressed as a percentage (~3 and ~2% in boys and girls, respectively, $p = 0.017$, **Table 1**).

At T0, systolic BP and diastolic BP were not significantly different between boys and girls (**Table 1**). At T21, systolic BP and diastolic BP decreased by ~7% in both genders ($p < 0.001$, **Table 1**).

Physical Capabilities

At T0, SCT and PPT scores were ~11 and ~3% lower in boys than in girls, respectively ($p < 0.05$, **Table 1**), while SPPB did not differ significantly between boys and girls (**Table 1**). At T21, SCT decreased by ~5 and ~7% ($p < 0.001$) in boys and girls, respectively. While PPT score increased by ~4% ($p < 0.001$, **Table 1**) in both boys and girls. The SPPB score did not change significantly in both genders (**Table 1**).

Changes in FM (Δ FM) (kg) were not related to changes in SCT (Δ SCT), SPPB (Δ SPPB), PPT (Δ PPT, score), systolic BP (Δ SBP), and diastolic BP (Δ DBP) in both boys and girls (**Table 2**).

Effects of the 3-Week BWRP on Adults

Anthropometric Characteristics and Body Composition

At T0, mean age and BMI did not differ significantly between males and females (**Table 3**), whereas stature and BM were significantly higher in males than females by ~7 and ~10%, respectively ($p < 0.005$, **Table 3**). At T21, BM and BMI decreased by ~5 and ~4% ($p < 0.001$, **Table 3**) in males and females, respectively.

At T0, FFM (kg) and FFM (%) were higher in males than females (+25% and +17%, respectively, $p < 0.001$), as shown in **Table 3** and **Figure 2A**. FM (kg) was not significantly different between males and females ($p = 0.240$, **Table 3** and **Figure 2C**), whereas males showed lower FM (%) than females (-16%, $p = 0.001$, **Table 3**). At T21, FFM (kg) decreased by ~2% in males and females with significant time interaction ($p = 0.007$) (**Table 3** and **Figure 2B**), while FFM (%) increased in males (~4%, $p < 0.001$) and females (~3%, $p = 0.012$) (**Table 3**). FM (kg) decreased by ~10 and ~6% in males and females, respectively ($p < 0.001$, **Table 3** and **Figure 2D**), although a decrease was also observed when FM was expressed as a percentage, in both males (~5%, $p = 0.002$) and females (~2%, $p = 0.012$) (**Table 3**).

At T0, systolic BP at rest was greater in males (+7%, $p = 0.001$, **Table 3**) than in females, while diastolic BP did not differ significantly between the two genders. At T21, systolic BP at rest reduced significantly in both males (~10%, $p = 0.001$) and

TABLE 1 | Physical characteristics of adolescents with obesity before (T0) and after (T21) the 3-week BWRP.

	Boys (n: 56)		Girls (n: 83)		P		
	T0	T21	T0	T21	G	T	G × T
Age (y)	14.9 ± 2.0		15.0 ± 2.1		0.841		
Stature (m)	1.68 ± 0.11		1.60 ± 0.07		0.001		
Body mass (kg)	103.3 ± 22.7	98.7 ± 21.8	96.8 ± 21.3	92.7 ± 20.2	0.095	0.001	0.154
BMI (kg/m ²)	36.4 ± 5.7	34.8 ± 5.6	37.5 ± 7.3	35.9 ± 6.9	0.338	0.001	0.906
BMI (SDS)	2.9 ± 0.6	2.7 ± 0.6	2.9 ± 0.6	2.4 ± 0.6	0.637	0.001	0.028
Fat-free mass (kg)	59.3 ± 11.6	57.4 ± 13.3	48.6 ± 7.6	47.7 ± 7.8	0.001	0.003	0.226
Fat-free mass (%)	57.8 ± 5.5	59.5 ± 6.7	51.1 ± 6.6	52.5 ± 7.2	0.001	0.001	0.635
Fat Mass (kg)	44.0 ± 13.4	40.1 ± 14.5	48.0 ± 16.1	44.8 ± 15.3	0.091	0.001	0.345
Fat Mass (%)	42.2 ± 7.7	40.5 ± 6.7	48.4 ± 6.7	47.4 ± 7.3	0.001	0.002	0.503
SBP (mmHg)	126.0 ± 11.0	117.6 ± 10.2	124.8 ± 10.6	116.0 ± 7.8	0.335	0.001	0.834
DBP (mmHg)	77.9 ± 7.2	74.2 ± 6.4	76.5 ± 7.0	72.6 ± 5.7	0.128	0.001	0.875
SCT (s)	2.9 ± 0.4	2.7 ± 0.4	3.5 ± 0.5	3.0 ± 0.4	0.001	0.001	0.112
SPPB score	11.9 ± 0.4	12.0 ± 0.1	11.9 ± 0.2	12.0 ± 0	0.124	0.027	0.465
PPT score	25.5 ± 1.6	26.6 ± 1.2	26.1 ± 1.3	27.1 ± 1.0	0.012	0.001	0.319

All values are mean and standard deviation (SD). BMI, body mass index; BMI (SDS), body mass index standard deviation score; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; SCT, stair climbing test; SPPB, short physical performance battery; PPT, physical performance test. Gender (G) and Time (T); Gender × time interaction (G × T).

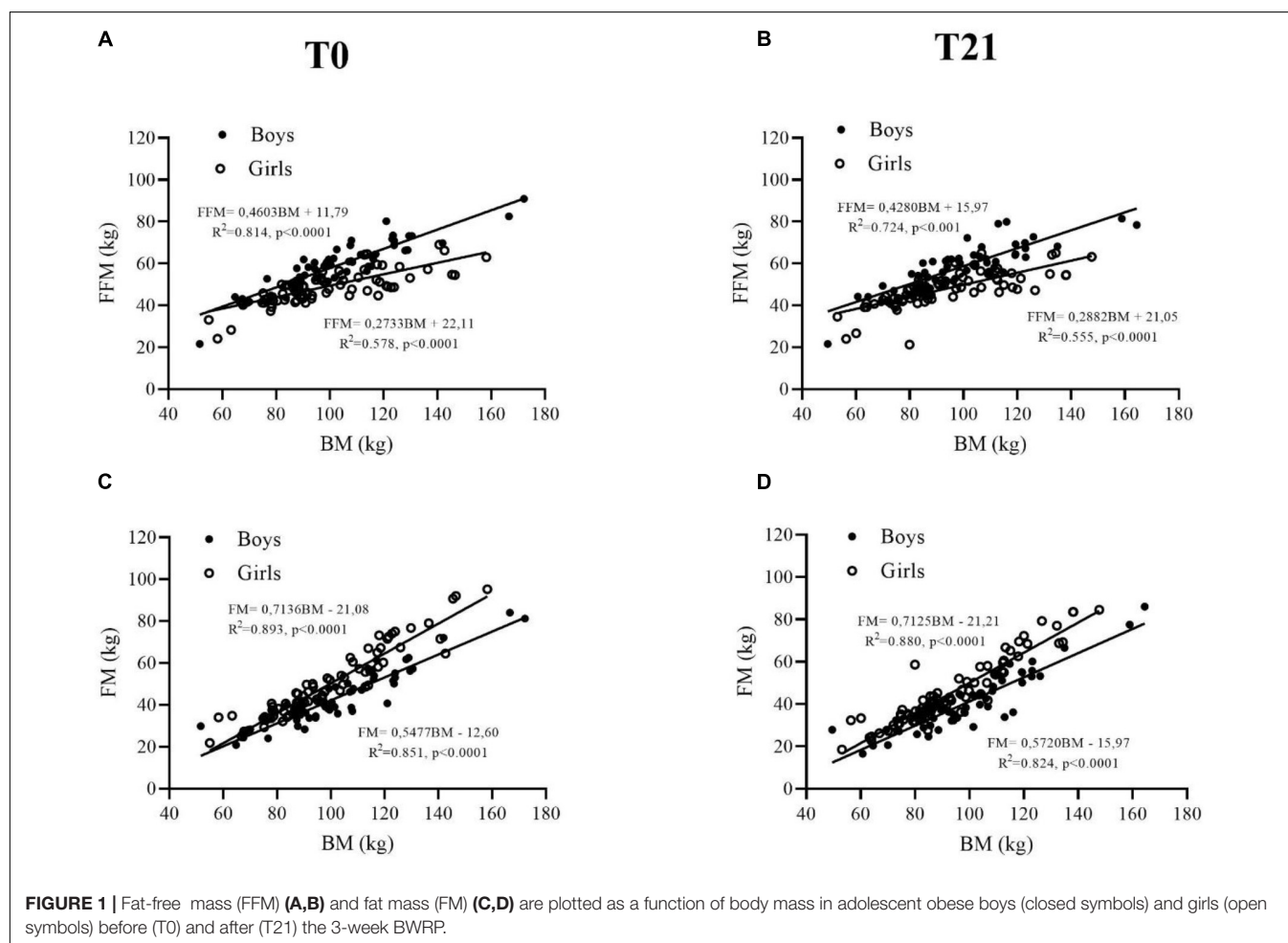


TABLE 2 | Linear regression between changes in Fat Mass (Δ FM) (kg) in adolescents with obesity and possible predictors.

	Boys (n: 56)		Girls (n: 83)	
	R ²	p-Value	R ²	p-Value
Δ SCT (s)	0.007	0.523	0.008	0.416
Δ SPPB (score)	0.057	0.076	0.032	0.103
Δ PPT (score)	0.002	0.719	0.015	0.271
Δ SBP (mmHg)	0.046	0.110	0.008	0.415
Δ DBP (mmHg)	0.013	0.523	0.043	0.059

SCT, stair climbing test; SPPB, short physical performance battery; PPT, physical performance test; SBP, systolic blood pressure; DBP, diastolic blood pressure; Δ is the difference of the values between the completing and the beginning of the weight reduction program.

females ($\sim 5\%$, $p = 0.010$, **Table 3**), while diastolic BP reduced significantly by $\sim 6\%$ in males only ($p = 0.012$, **Table 3**).

Physical Capabilities

At baseline, SCT was lower in males than females by $\sim 5\%$ ($p = 0.042$), whereas SPPB and PPT scores were not significantly different between the two genders (**Table 3**).

At T21, SCT decreased significantly only in females by $\sim 16\%$ ($p < 0.001$, **Table 3**). SPPB score increased by ~ 7 and $\sim 10\%$ ($p < 0.010$, **Table 3**) in males and females, respectively. Similarly, PPT score increased by mean 9% ($p < 0.001$, **Table 3**) in the two genders.

Changes in FM (Δ FM) (kg) were inversely related to changes in both systolic BP (Δ SBP) ($R^2 = 0.242$, $p = 0.009$; **Table 4**) and diastolic BP (Δ DBP) ($R^2 = 0.203$, $p = 0.009$; **Table 4**) in males. In obese females, changes in FM (Δ FM) (kg) were inversely related to changes in both SPPB (Δ SPPB) ($R^2 = 0.345$, $p < 0.001$; **Table 4**) and PPT (Δ PPT) ($R^2 = 0.187$, $p = 0.003$; **Table 4**).

DISCUSSION

The present study shows that a 3-week inpatient multidisciplinary BWRP, which includes physical training, moderate energy restriction, nutrition education, and psychological counseling, determines in adolescents and adults with obesity: (1) a significant reduction in BM and FM with a slight decrease in FFM, (2) improvements in SCT time, SPPB score in adults and PPT score in both groups, and (3) a positive relationship between reduction in FM and reduction in systolic BP and diastolic BP in obese male adults.

Our intervention was effective in reducing BM and FM for both adolescents and adults with obesity, in agreement with previous results reported in our laboratory after 3-week inpatient BWRP for patients aged 8–17 years (38, 39) and even for older obese male and females aged 61–75 year (40), confirming that body composition can be improved in obese individuals at any age. However, during the short-term BWRP adolescents and adults with obesity have shown a slight reduction in FFM, probably because no specific strength training was considered in this first phase of metabolic rehabilitation of patients with obesity, as previously observed (41).

After a 3-week of BWRP, SCT time was reduced after the BWRP in adolescents and in female, evaluated with the modified Margaria test (42) previously used to assess lower limb muscle power in obese subjects (43). Although there was no direct correlation between the BM reduction and the improvement in SCT time, as previously observed by our research group (17), it seems plausible to hypothesize that the reduction of BM may be associated with reduced body inertia (44) and the reduction of intramyocellular lipids may have played a role in the improvement of lower limb strength evaluated in our study by SCT (45). SPPB is a test useful for assessing lower limb functionality (static balance, 4 m walking and lower

TABLE 3 | Physical characteristics of adults with obesity before (T0) and after (T21) the 3-week BWRP.

	Males (n: 27)		Females (n: 44)		P		
	T0	T21	T0	T21	G	T	G \times T
Age (year)	56.8 \pm 11.2		50.9 \pm 15.1		0.060		
Stature (m)	1.71 \pm 0.06		1.60 \pm 0.06		0.001		
Body mass (kg)	125.8 \pm 16.0	119.0 \pm 14.9	113.9 \pm 16.5	109.2 \pm 15.7	0.007	0.001	0.001
BMI (kg/m ²)	43.1 \pm 3.6	40.8 \pm 3.3	44.9 \pm 5.8	43.0 \pm 6.0	0.101	0.001	0.001
Fat-free mass (kg)	66.5 \pm 7.4	65.3 \pm 6.6	49.6 \pm 4.1	48.8 \pm 3.5	0.001	0.007	0.650
Fat-free mass (%)	53.1 \pm 4.3	55.2 \pm 4.3	44.1 \pm 5.1	45.2 \pm 4.8	0.001	0.001	0.128
Fat mass (kg)	59.3 \pm 11.3	53.6 \pm 10.8	64.3 \pm 14.6	60.4 \pm 13.6	0.067	0.001	0.046
Fat Mass (%)	46.9 \pm 4.3	44.8 \pm 4.3	55.9 \pm 5.1	54.7 \pm 4.8	0.001	0.001	0.129
SBP (mmHg)	143.1 \pm 12.3	129.3 \pm 11.7	133.5 \pm 12.7	127.6 \pm 10.0	0.015	0.001	0.018
DBP (mmHg)	85.2 \pm 8.6	80.0 \pm 6.2	82.2 \pm 8.8	80.1 \pm 5.1	0.285	0.003	0.183
SCT (s)	4.3 \pm 1.2	3.8 \pm 0.9	6.5 \pm 5.3	5.6 \pm 3.9	0.035	0.001	0.230
SPPB (score)	11.1 \pm 1.3	11.8 \pm 0.5	10.2 \pm 2.5	11.2 \pm 1.6	0.085	<0.001	0.473
PPT (score)	24.4 \pm 2.4	26.7 \pm 1.2	24.2 \pm 4.4	26.4 \pm 3.4	0.787	<0.001	0.834

All values are mean and standard deviation (SD). BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; SCT, stair climbing test; SPPB, short physical performance battery; PPT, physical performance test. Gender (G) and Time (T); Gender \times time interaction (G \times T).

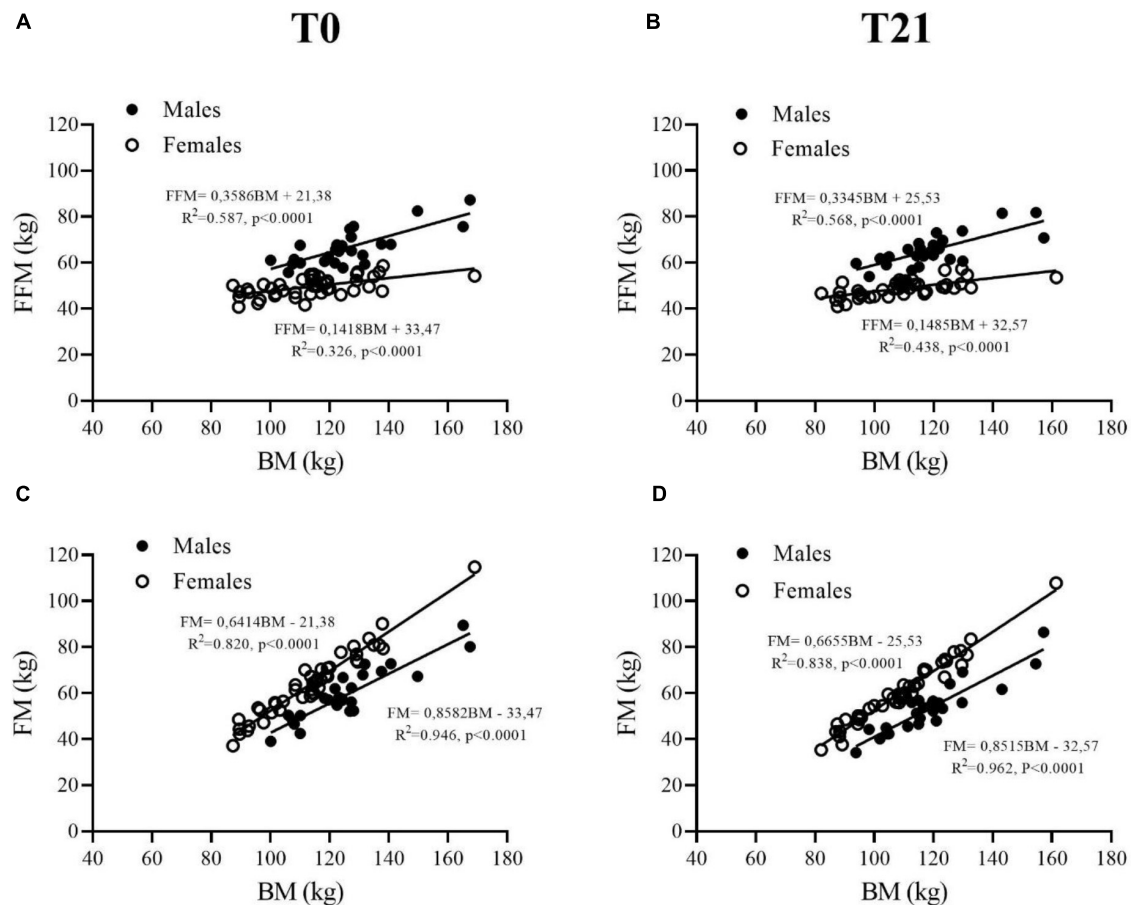


FIGURE 2 | Fat free mass (FFM) (A,B) and fat mass (FM) (C,D) are plotted as a function of body mass (BM) in adult obese males (closed symbols) and females (open symbols) before (T0) and after (T21) of the 3-week BWRP.

TABLE 4 | Linear regression between changes in Fat Mass (ΔFM) (kg) in adults with obesity and possible predictors.

	Males (n: 27)		Females (n: 44)	
	R ²	p-Value	R ²	p-Value
ΔSCT (s)	0.002	0.942	0.030	0.278
ΔSPPB (score)	0.002	0.837	0.346	0.001
ΔPPT (score)	0.002	0.816	0.187	0.003
ΔSBP (mmHg)	0.242	0.009	0.001	0.802
ΔDBP (mmHg)	0.203	0.018	0.025	0.297

SCT, stair climbing test; SPPB, short physical performance battery; PPT, physical performance test; SBP, systolic blood pressure; DBP, diastolic blood pressure; Δ is the difference of the values between the completing and the beginning of the weight reduction program.

limb strength) (46). In our study, although SPPB scores were at the upper limit of normality (i.e., in the range of 11–12 points) (34), improvements of ~ 0.8 and ~ 1 points were found for male and female obese adults after 3-week BWRP, whereas smaller improvements, albeit statistically significant, were found in obese adolescents. In this regard, our findings are relevant because multidisciplinary 3-week BWRP appears

to positively affect lower limb functionality in tasks such as walking and in those requiring getting up and down from a chair, which are typically impaired in obese patients (47–49), thus contributing to improve their quality of life. PPT is useful in assessing functional capabilities as it mimics ADL (36, 50–52). Previous studies showed that obese subjects had perceived difficulty in performing physical exercise and ADL (13–16). In the present study, the PPT score increased in both obese adolescents and adults, thus suggesting that a structured exercise program administered during a 3-week BWRP can improve the ability to perform ADL. Our findings are consistent with those recently reported by Wilson et al. (53), showing that regular physical activity and moderate energy restriction have positive effects on physical performance and quality of life. Furthermore, the improvements in SPPB and PPT scores in adult obese females were positively correlated with FM loss. At T0, mean SPPB and PPT scores in our obese females were lower than 11 and 28, respectively, and these two scores are considered signs of poor lower limb functionality (54, 55). Several factors have been considered to explain lower limb physical performance in females compared to males, such as lower muscle strength (56), higher body fat

(56), lower muscle mass (57), and greater muscle fat infiltration (58) with the greatest adipose tissue thickness in the lower limbs (59).

Finally, reduction of FM was positively related to a reduction in systolic BP and diastolic BP only in obese males. Indeed, visceral adiposity is generally higher in obese male adults than in female counterparts (45), and visceral fat has been linked to the secretion of adipocytokines, that contribute to the development and progression of hypertension (60). Thus, BWRP has proven to be effective in improvement cardio metabolic profile of obese males (61). However, since we did not directly measured visceral adiposity, this missing information can be considered a limitation of the present study. Future studies involving BWRP in the hospital environment should measure visceral adiposity not only in relation to cardiovascular risk profile, but also as a marker of quality of life improvement in adolescents and adults with obesity.

CONCLUSION

In conclusion, our study confirms that a 3-week inpatient multidisciplinary BWRP, even if it only detects a 4–5% reduction in BM, is able to induce a significant reduction in FM, improvements in lower limb functionality and lower limb muscle power, and improvements in performing ADL, all of which are positive changes highly relevant to improve quality of life of adolescents and adults with obesity.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

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ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethical Committee of the Istituto Auxologico Italiano (Milan, Italy). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

AS, SL, MD'A, and FV: conceptualization. AS, MD'A, SL, FV, GT, and RD: data curation. FV and MD'A: formal analysis. AS: funding acquisition, project administration, and supervision. GT and RD: investigation. MD'A: writing original draft. AS, MD'A, SL, and FV: writing, review and editing. All authors have read and agreed to the published version of the manuscript.

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Corrigendum: Effects of a 3-week inpatient multidisciplinary body weight reduction program on body composition and physical capabilities in adolescents and adults with obesity

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The First-Trimester Gestational Weight Gain Associated With *de novo* Hypertensive Disorders During Pregnancy: Mediated by Mean Arterial Pressure

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The relationship between first-trimester GWG (T_1 GWG) and risk of hypertensive disorders of pregnancy (HDP) remained uncertain. This study aimed to investigate the association between T_1 GWG and risk of *de novo* HDP. Meanwhile, we explored the mediated effect and constructed an early GWG category to evaluate the predictive capacity for HDP. T_1 GWG was defined as the weight difference between 13 ± 1 gestational weeks and pre-conception. HDP group was defined as having diagnosis of *de novo* HDP, including gestational hypertension or *de novo* pre-eclampsia (PE) during the current pregnancy. Early GWG category was constructed according to the risk of HDP within each pre-pregnancy body mass index (BMI) group. Cox regression model was utilized to check the association between the T_1 GWG and HDP. Serial mediation model was adopted to evaluate the potential mediators including mean arterial pressure (MAP) at 13th and 20th week. The logistic regression model with bootstrap was performed to assess the predictive capacity of Early GWG category and MAP for the risk of HDP. A total of 17,901 pregnant women (mean age, 29.0 years) were recruited from 2013 to 2017 at the Tongzhou Maternal and Child Health Hospital in Beijing, China. Compared to women in Class 1 of early GWG category, women in the Class 2, 3, 4 have increased risks of HDP by 1.42, 4.27, and 4.62 times, respectively (hazard ratio [HR] = 2.42, 95% CI: 2.11–2.77; HR = 5.27, 95% CI: 4.05–6.86; HR = 5.62, 95% CI: 4.05–7.79). The MAP measured at 13th and 20th week totally mediated 33.1 and 26.7% of association between T_1 GWG and HDP in total participants and overweight/obesity pregnancies, respectively. The area under receiver operator characteristic curve for predictive model utilizing early GWG category and MAP measured at 13th and 20th week for the risk of HDP is 0.760 (95% CI: 0.739–0.777).

The T_1 GWG was associated with *de novo* HDP, which was partially mediated by MAP measured at 13th and 20th week. Early GWG category showed a better predictive capacity for the risk of HDP compared to the National Academy of Medicine criteria for T_1 GWG.

Keywords: *de novo* hypertensive disorders of pregnancy, gestational weight gain, mean arterial pressure, overweight, obesity, national academy of medicine criteria

INTRODUCTION

Hypertensive disorders of pregnancy (HDP) are characterized by the abnormal elevation of blood pressure during pregnancy. It can be classified into four categories: chronic hypertension, gestational hypertension (GH), *de novo* and superimposed pre-eclampsia (PE), and eclampsia (1). GH and *de novo* PE are regarded as *de novo* HDP. Mean arterial pressure (MAP) was consisted of systolic blood pressure (SBP) and diastolic blood pressure (DBP) as a composite index to assess the blood pressure in clinical practice. Previous studies have inducted that the blood pressure was an important predictive factor to evaluate the risk of HDP before diagnosis (2, 3). The HDP are the leading causes of maternal and fetal morbidity and mortality globally. HDP is a dominant cause of maternal death in developing countries, accounting for one fifth of maternal deaths worldwide (4). The etiology of *de novo* HDP is still unknown. A recent review summarized the main modifiable risk factors, such as body mass index, anemia, lower education level; and non-modifiable risk factors, such as maternal age, primiparous, multiple pregnancy, HDP history, gestational diabetes mellitus, pre-existing type 2 diabetes mellitus, pre-existing urinary tract infection, single nucleotide polymorphism in the angiotensinogen gene, and a family history of HDP or type 2 diabetes mellitus (5).

Gestational weight gain (GWG) is a natural process during pregnancy. The change in maternal lifestyle and fetus, such as increasing nutrient intake, decreasing physical activity, and fetal growth, will both lead pregnant women to increase their body weight. Deviations from proper weight gain in either direction during pregnancy have been found to be associated with adverse pregnancy outcomes, such as small for gestational age (SGA), large for gestational age (LGA), macrosomia, cesarean delivery, gestational diabetes mellitus, PE, postpartum weight retention, and obesity of the offspring (6). The guideline for GWG from the National Academy of Medicine (NAM) have been applied to guide pregnancy weight management for over 30 years in the United States (7). The updated NAM guideline in 2009 provided a body mass index (BMI)-specific guideline (8), which provides a major set of recommendations for optimal total GWG during the entire pregnancy. HDP, usually diagnosed in the second and third trimester, was found to associate with the total GWG by several studies (9–11). Since the total GWG is calculated based on the subtraction between the pre-pregnancy and antepartum weights, the association between total GWG and HDP published previously could not provide evidence for a temporal relationship.

Gestational weight gain as a non-intrusive and easily attainable clinical indicator has been applied to estimate the

risk of adverse complications, such as GDM, SGA, and LGA. Due to the absence of temporal relationship, previous studies focusing on total GWG and HDP cannot provide risk assessment of HDP in terms of GWG. This study aimed to assess the association between first-trimester GWG (T_1 GWG) and *de novo* HDP. Meanwhile we explored the mediators and evaluated the predictive capacity of T_1 GWG and mediators for HDP.

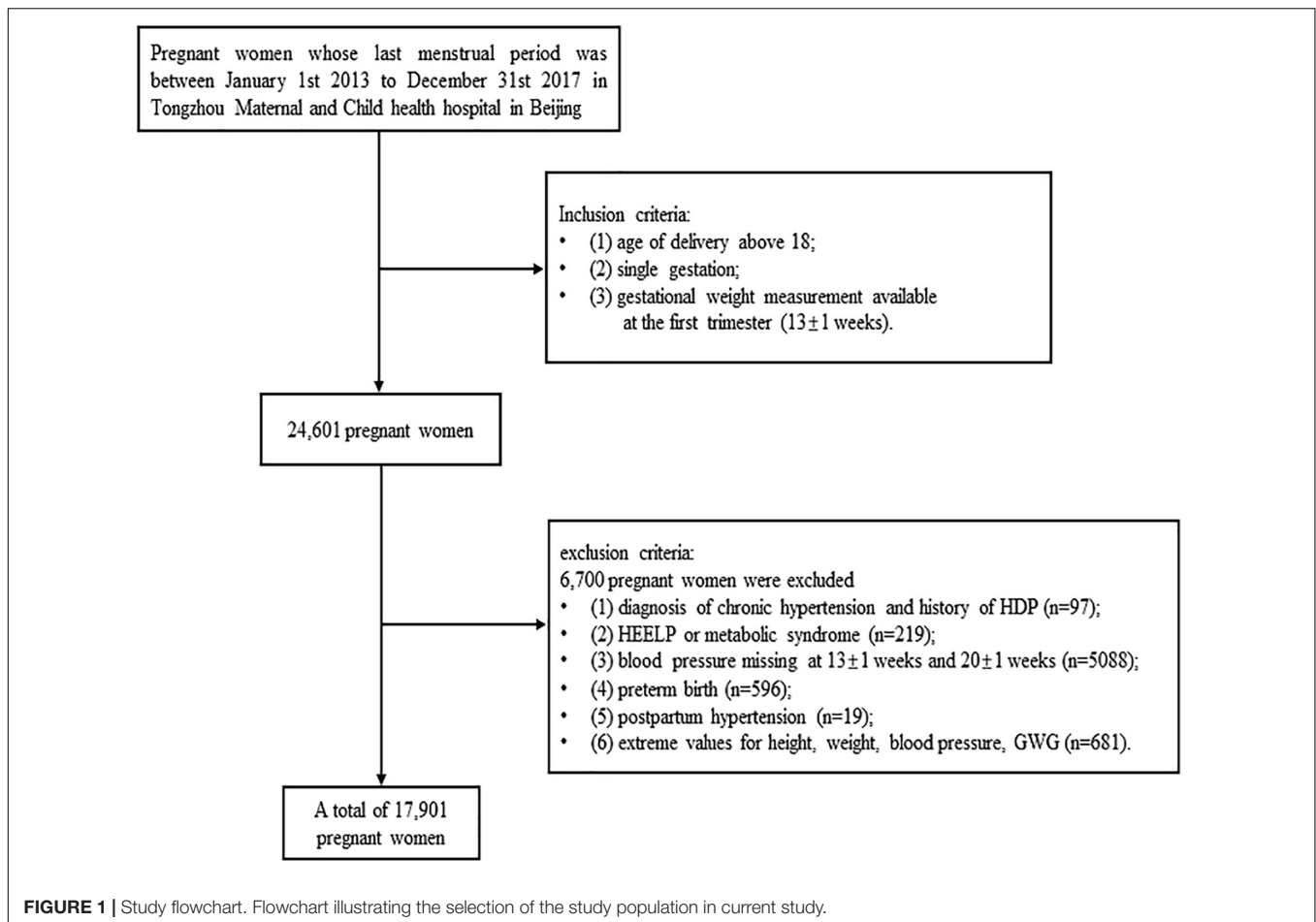
MATERIALS AND METHODS

Study Design and Participants

This study analyzed the data of a birth cohort, which were collected from 2013 to 2017 at Tongzhou Maternal and Child health hospital in Beijing, China. There are 17,901 pregnant women enrolled in the current study. The inclusion criteria of this cohort study were: (1) age of delivery above 18 years, (2) single gestation, (3) T_1 GWG available at the first trimester (13 ± 1 weeks). The exclusive criteria were: (1) diagnosis of chronic hypertension and history of HDP, (2) HEELP or metabolic syndrome, (3) blood pressure missing at 13 ± 1 and 20 ± 1 weeks, (4) preterm birth, (5) postpartum hypertension, (6) extreme values for height, weight, and blood pressure (height < 1 m or height > 2 m, weight < 30 kg or weight > 150 kg, SBP < 70 mmHg or SBP > 270 mmHg, DBP < 50 mmHg or DBP > 140 mmHg, T_1 GWG < -5.9 kg or T_1 GWG > 11 kg). The flow chart was presented as **Figure 1**.

Data Collection

The sociodemographic data of pregnant women were collected from the first antenatal clinical record, such as race, age, level of education, employment condition, elder gestation, gestational season, elder gestation, and pre-pregnant weight and height. The pre-pregnant weight was obtained from the pregnant women's self-reports. The gestational weight and blood pressure were measured by trained nurses at regular antenatal clinic. The GWG was defined as the difference between gestational weight and pre-pregnant weight. The MAP equaled to SBP plus 2/3 DBP. The pre-pregnant BMI was calculated using formula: BMI = (weight in kg) divided by (square of height in m). The pre-pregnant BMI class was identified according to the BMI criteria for Asian women (the BMI criteria of underweight, normal weight, overweight, and obesity were defined as < 18.5 , 18.5 – 23 , 23 – 27.5 , ≥ 27.5) (12). *De novo* HDP that includes GH and *de novo* PE was the primary outcome of this study. The diagnosis of GH or *de novo* PE was made by obstetricians according to the latest Chinese clinical practice guideline which was consistent with the diagnostic guidelines in the developed countries (13).



International Classification of Diseases 10 (ICD-10) codes were used to define GH and *de novo* PE (such as O13.01, O13.02 for GH; O11.01, O14.001, O14.101, O14.102, O14.901 for *de novo* PE). The text containing information related to GH and *de novo* PE in medical records was extracted to double confirm the disease and identify the week of disease diagnosis. The normotensive group was defined as free from any diagnosis of GH, PE, and without a history of hypertensive disorders.

Ethics

The study was approved by the Institutional Review Board of Peking University Health Science Center (No. IRB00001052-21023).

Statistics

Missing values of demographic variables, we imputed the missing data by the k-nearest neighbor (KNN) algorithm [Beretta and Santaniello (14)]. The Shapiro–Wilk test was applied to check the normality of the distribution for each variable. Normally distributed variables were presented as means with SD and analyzed with *t*-test for group comparisons between HDP and Normotensive groups. Non-normally distributed variables were shown as medians with interquartile range and analyzed with chi-square test or Mann–Whitney U-test.

An early GWG category (EwtGCat) in terms of the risk of HDP was constructed. The risk ratios (RR) for HDP were calculated using the multivariable Poisson regression model for each T_1 GWG interval within the particular pre-pregnant BMI class vs. all other women within that BMI class, borrowing the LifeCycle Project method (6). Considering the T_1 GWG was in a relatively small scale, we choose the 25th, 50th, 75th percentiles as the cut-off points for the category. The Class 1 of the EwtGCat included the women with underweight and normal weight pre-pregnant BMI. The women with overweight or obese during pregnancy were further grouped into class 2, 3, and 4. Class 2 was defined as the weight gain with significant negative association ($RR < 1$). Class 3 was the weight gain interval without statistical difference. Class 4 was the weight gain interval that showed remarkable positive association ($RR > 1$). The continuous T_1 GWG was also transformed into categorical variables using quartile method and NAM criteria. Multivariable Cox hazards regression model was utilized (15) to access the association between T_1 GWG (continuous, quartile, EwtGCat, and NAM criteria) and HDP after adjusting the covariables. The covariables include race, age, level of education, employment condition, elder gestation, and gestational season.

We constructed a serial mediation model to assess the effect of T_1 GWG on HDP with MAP at 13th and 20th weeks (MAP_{13 week}

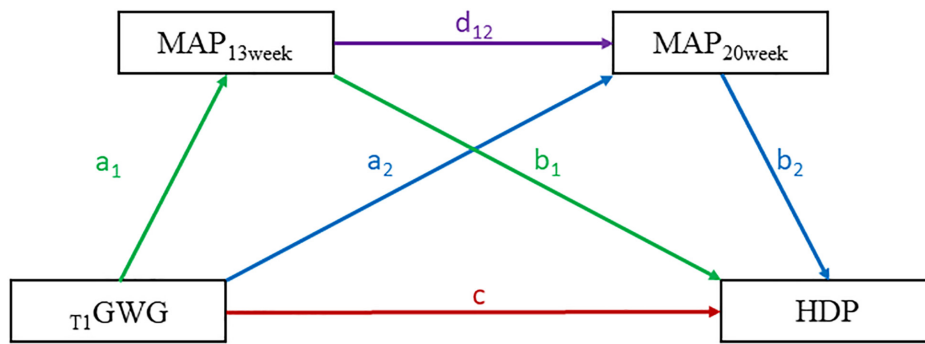


FIGURE 2 | The serial mediation pattern of the association between first-trimester GWG (T_1 GWG) and hypertensive disorders of pregnancy (HDP). The respective colorful lines represent the different pathways of the T_1 GWG effects on HDP. The direct effect (DE) of T_1 GWG on HDP was presented by red line. The indirect effect (IE) of T_1 GWG to $MAP_{13\text{ week}}$ to HDP was presented by green lines. The IE from T_1 GWG to $MAP_{13\text{ week}}$ to $MAP_{20\text{ week}}$ to HDP was presented by purple lines. The IE of T_1 GWG to $MAP_{20\text{ week}}$ to HDP was presented by blue lines. The serial mediation equation included three parts: (1). $MAP_{13\text{ week}} \sim a_1 * T_1\text{GWG} + \text{covariables}$. (2) $MAP_{20\text{ week}} \sim a_2 * T_1\text{GWG} + d_{12} * MAP_{13\text{ week}} + \text{covariables}$. (3) $HDP \sim c * T_1\text{GWG} + b_1 * MAP_{13\text{ week}} + b_2 * MAP_{20\text{ week}} + \text{covariables}$. And the c was defined as the DE from T_1 GWG to HDP, the mediation effects were expressed as follows: (1) IE_1 (T_1 GWG to $MAP_{13\text{ week}}$ to HDP) = $a_1 * b_1$. (2) IE_2 (T_1 GWG to $MAP_{20\text{ week}}$ to HDP) = $a_2 * b_2$. (3) IE_3 (T_1 GWG to $MAP_{13\text{ week}}$ to $MAP_{20\text{ week}}$ to HDP) = $a_1 * d_{12} * b_2$.

and $MAP_{20\text{ week}}$) as mediators by adjusting covariables. The direct effect (DE) defined as T_1 GWG to HDP. The indirect effects (IE) included three aspects: (1) IE_1 was defined as the pathway from T_1 GWG to $MAP_{13\text{ week}}$ to HDP, (2) IE_2 was the pathway from T_1 GWG to $MAP_{20\text{ week}}$ to HDP, (3) IE_3 was the pathway from T_1 GWG to $MAP_{13\text{ week}}$ to $MAP_{20\text{ week}}$ to HDP. All the estimated effects were validated by bootstrapping method for 5,000 loops. The serial mediation model pattern was presented as **Figure 2**. The bruceR package (version 0.7.0) was adopted in this part of analyses. The stratified analyses were conducted to explore the effect of EwtGCat on the risk of HDP across different subgroups, such as parity (unipara vs. multipara), maternal age (>35 age vs. ≤ 35 age), conceptional season (spring/winter vs. summer/autumn), education level (high vs. low). The sensitivity analyses were further performed to assess the association between T_1 GWG and onset time of HDP.

The adjusted logistic regression model was adopted to assess the prediction performance for HDP with 1,000 bootstrapping replications (16). Three different models were constructed and compared with each other by the Area Under Receiver Operator Characteristic Curve (AUC), sensitivity, and specificity. The models were as follow: model_{NAM} includes NAM criteria and covariables; model_{EwtGCat} included EwtGCat and covariables; model_{EwtGCat&MAP} included EwtGCat, $MAP_{13\text{ week}}$, $MAP_{20\text{ week}}$ and covariables. All the statistical analyses were performed on the R software (version 4.0.0). The p -value < 0.05 was considered as significant difference.

RESULTS

Characteristics of the Birth Cohort

Table 1 shows the maternal socio-demographical and first-trimester weight gain characteristics between *de novo* HDP and normotensive groups. The percentage of *de novo* HDP in our study was 5.31%. The 17,901 pregnant women included in this

study had the mean maternal age of 29.0 years ($SD = 3.8$). Women in the following strata had a significantly higher percentage of HDP diagnosis: educational level with high school or lower, overweight or obesity with pre-pregnant BMI class and unipara. The T_1 GWG was significantly higher in HDP group compared with normotensive women ($p = 0.001$). The detailed characteristics of the participants are listed in **Table 1**.

The Construction of Early Gestational Weight Gain Category

The Early GWG Category (EwtGCat) was constructed according to the **Supplementary Table 1**. The Class 1 of EwtGCat was identified as the women with underweight and normal weight pre-pregnant BMI classes, and there was no association between T_1 GWG and HDP. As for underweight and obesity, the significant negative association was obtained in Class 2 which the interval of T_1 GWG was below 0 kg. Class 3 was the interval of T_1 GWG from 0 to 2.5 kg which we failed to observe any statistical association. Class 4 was the interval of T_1 GWG above 2.5 kg which presented a significant positive association between T_1 GWG and HDP.

The Association Between T_1 GWG and *de novo* Hypertensive Disorders of Pregnancy

As the **Table 2** shown, T_1 GWG was significantly higher in the HDP pregnancies compared with normotensive women ($HR = 1.07$, 95% CI : 1.04–1.09, $p < 0.001$). In comparison with women in the lowest quartile of T_1 GWG, the hazards of HDP elevated in the women with Q3, Q4 of T_1 GWG ($HR = 1.29$, 95% CI : 1.08–1.55, $p = 0.005$; $HR = 1.43$, 95% CI : 1.21–1.69, $p < 0.001$). There were significant associations between T_1 GWG and HDP in the Class 2, 3, 4 compared with Class 1 under EGC criteria ($HR = 2.42$, 95% CI : 2.11–2.77, $p < 0.001$;

TABLE 1 | Maternal socio-demographical and first-trimester weight gain characteristics.

Characteristics	No HDP (N = 16950)	HDP (N = 951)	P-value
τ_1 GWG, mean(SD)	1.2 (2.4)	1.5 (2.5)	0.001
MAP _{13 week} , mean (SD)	83.2 (8.2)	88.9 (8.2)	<0.001
MAP _{20 week} , mean (SD)	80.6 (8.0)	86.9 (8.3)	<0.001
Age, mean(SD)	29.0 (3.8)	29.1 (3.8)	0.800
Education level, N(%)			
High school or lower	4269 (25.2)	286 (30.1)	<0.001
Vocational college	5440 (32.1)	322 (33.9)	
University or above	7241 (42.7)	343 (36.1)	
Employment condition, N(%)			
Unemployment	2220 (13.1)	128 (13.5)	0.700
Employment	14730 (86.9)	823 (86.5)	
Race, N(%)			
Minority	1031 (6.1)	44 (4.6)	0.066
Ethnic Han	15919 (93.9)	803(95.4)	
Maternal age (>35 age), N(%)			
No	15432 (91.0)	664 (89.5)	0.100
Yes	1518 (9.0)	189 (10.5)	
Parity, N(%)			
Unipara	12447 (73.4)	745 (78.3)	<0.001
Multipara	4503 (26.6)	206 (21.7)	
Pre-pregnant BMI class, N(%)			
Underweight	1927 (11.4)	45 (4.7)	<0.001
Normal weight	9596 (56.6)	370 (38.9)	
Overweight	4490 (26.5)	391 (41.1)	
Obesity	937 (5.5)	145 (15.2)	
Conceptional season, N(%)			
Spring	4044 (23.9)	311 (32.7)	<0.001
Summer	3696 (21.8)	189 (19.9)	
Autumn	4427 (26.1)	161 (16.9)	
Winter	4783 (28.2)	290 (30.5)	

T_1 GWG, first trimester gestational weight gain; HDP, hypertensive disorders of pregnancy; BMI, body mass index; N, number; MAP, mean arterial pressure.

$HR = 5.27$, 95% CI : 4.05–6.86, $p < 0.001$; $HR = 5.62$, 95% CI : 4.05–7.79, $p < 0.001$). However, there is no significant hazard difference between normal and abnormal groups under NAM criteria ($HR = 1.04$, 95% CI : 0.91–1.20, $p = 0.551$).

The Serial Mediation Effect of Mean Arterial Pressure on the Association Between T_1 GWG and Hypertensive Disorders of Pregnancy

The Table 3 indicated that MAP_{13 week} and MAP_{20 week} totally mediated 37.7% of association between T_1 GWG and HDP in all participants ($IE_{total} = 1.001 \times 10^{-3}$, $p < 0.001$; $DE = 2.654 \times 10^{-3}$, $p < 0.001$). The indirect mediation effect was consisted in 3 pathways, such as (1) T_1 GWG to MAP_{13 week} to HDP ($IE_1 = 3.330 \times 10^{-4}$, mediate proportion: 12.5%, $p < 0.001$); (2) T_1 GWG to MAP_{20 week} to HDP ($IE_2 = 3.970 \times 10^{-4}$, mediate proportion: 15.0%, $p < 0.001$); (3) T_1 GWG to MAP_{13 week} to MAP_{20 week} to HDP

($IE_3 = 2.710 \times 10^{-4}$, mediate proportion: 10.2%, $p < 0.001$). We reran the serial mediation model to test the IE of MAP in overweight/obesity women, which is similar to the results of all participants. The MAP_{13 week} and MAP_{14 week} mediated 26.7% of total effect of T_1 GWG on HDP. IE_1 , IE_2 , and IE_3 explained 6.1, 13.7, and 6.9%, respectively, of the total effect through 3 mediated pathways (all $p < 0.001$). However, we could not detect meaningful mediation effect in the underweight and normal weight women.

The Subgroup Analyses for the Early Gestational Weight Gain Category

The stratified analyses were conducted to assess the effect of EwtGCat on HDP under different variables. The Class 1 and Class 2 of EwtGCat were defined as low EwtGCat and the Class 3 and Class 4 were defined as high EwtGCat. The Figure 3 showed that high EwtGCat was elevated the risk of HDP especially in the women with multipara, maternal age above 35, spring/winter conception and low education level ($HR = 3.338$, 95% CI : 1.607–6.936; $HR = 3.004$, 95% CI : 1.119–8.064; $HR = 2.313$, 95% CI : 1.447–3.696; $HR = 2.704$, 95% CI : 1.431–5.016, respectively). In the sensitivity analyses, we failed to observe a significant association between T_1 GWG and onset time of HDP ($p = 0.677$).

Predictive Performance of Early Gestational Weight Gain Category for Hypertensive Disorders of Pregnancy

We assessed the predictive capacity for HDP by model_{NAM}, model_{EwtGCat}, and model_{EwtGCat&MAP}. As shown in Figure 4,

TABLE 2 | Hazard ratios for the association between the first trimester gestational weight gain (T_1 GWG) and hypertensive disorders of pregnancy (HDP).

	No HDP (N)	HDP (N)	HR	95% CI	P-value
T_1 GWG	16950	951	1.07	1.04–1.09	<0.001
(continuous)					
T_1GWG quartile					
Q1	6055	298	ref		
Q2	3271	181	1.19	0.99–1.43	0.065
Q3	3510	208	1.29	1.08–1.55	0.005
Q4	4114	264	1.43	1.21–1.69	<0.001
EwtGCat (category, N)					
Class 1	11523	415	ref		
Class 2	4916	432	2.42	2.11–2.77	<0.001
Class 3	308	64	5.27	4.05–6.86	<0.001
Class 4	203	40	5.62	4.05–7.79	<0.001
NAM recommendation (category, N)					
Normal	11983	666	ref		
Abnormal	4967	285	1.04	0.91–1.20	0.551

T_1 GWG, the first trimester gestational weight gain; EwtGCat, early gestational weight gain categories; HDP, hypertensive disorders of pregnancy; NAM, national academy of medicine; HR, hazard ratios; CI, confidence interval. The HR was adjusted by race, age, education level, elderly maternal employment condition, parity. The cutoff points of Q1, Q2, Q3, Q4 were defined by the 25th, 50th, 75th quartiles of the distribution of T_1 GWG.

TABLE 3 | Mediation effect of mean arterial pressure (MAP) to the association between the T_1 GWG and HDP.

Participant	Mediation effect	Estimate	95% CI	Proportion	P-value
Total participants					
	Indirect effect	1.001×10^{-3}	$(7.020 \times 10^{-4}, 1.273 \times 10^{-3})$	37.7%	<0.001
	T_1 GWG-MAP _{13 week} -HDP	3.330×10^{-4}	$(2.060 \times 10^{-4}, 4.880 \times 10^{-4})$	12.5%	<0.001
	T_1 GWG-MAP _{20 week} -HDP	3.970×10^{-4}	$(2.260 \times 10^{-4}, 5.670 \times 10^{-4})$	15.0%	<0.001
	T_1 GWG-MAP _{13 week} -MAP _{20 week} -HDP	2.710×10^{-4}	$(1.700 \times 10^{-4}, 3.700 \times 10^{-4})$	10.2%	<0.001
	Direct effect				
	T_1 GWG-HDP	1.652×10^{-3}	$(1.860 \times 10^{-4}, 3.105 \times 10^{-3})$	62.3%	0.026
	Total effect	2.654×10^{-3}	$(1.157 \times 10^{-3}, 4.113 \times 10^{-3})$	100.0%	<0.001
Overweight/obesity participants					
	Indirect effect	1.853×10^{-3}	$(1.233 \times 10^{-3}, 2.563 \times 10^{-3})$	26.7%	<0.001
	T_1 GWG-MAP _{13 week} -HDP	4.200×10^{-4}	$(1.780 \times 10^{-4}, 7.120 \times 10^{-4})$	6.1%	<0.001
	T_1 GWG-MAP _{20 week} -HDP	9.500×10^{-4}	$(5.630 \times 10^{-4}, 1.386 \times 10^{-3})$	13.7%	<0.001
	T_1 GWG-MAP _{13 week} -MAP _{20 week} -HDP	4.810×10^{-4}	$(2.240 \times 10^{-4}, 7.680 \times 10^{-4})$	6.9%	<0.001
	Direct effect				
	T_1 GWG-HDP	5.088×10^{-3}	$(2.126 \times 10^{-3}, 7.987 \times 10^{-3})$	73.3%	<0.001
	Total effect	6.941×10^{-3}	$(4.035 \times 10^{-3}, 9.835 \times 10^{-3})$	100.0%	<0.001

T_1 GWG, the first trimester gestational weight gain; MAP, mean arterial pressure; HDP, hypertensive disorders of pregnancy. The serial mediation model was adjusted by race, age, level of education, employment condition, maternal age (>35 age), conceptional season. The pattern of serial mediation model was presented in **Figure 2**.

the AUC, sensitivity, and specificity of the model_{NAM}, which included NAM criteria and covariables, were 0.587 (95% CI: 0.561–0.611), 0.544 (95% CI: 0.463–0.606), and 0.578 (95% CI: 0.492–0.667). In terms of model_{EwtGCat}, the AUC was improved to 0.668 (95% CI: 0.649–0.688). The model_{EwtGCat&MAP} demonstrated the best predictive performance for HDP comparing to the other two model ($AUC = 0.760$, 95% CI: 0.739–0.777; sensitivity = 0.703, 95% CI: 0.677–0.719; specificity = 0.686, 95% CI: 0.635–0.726). The detail information of the predictive models was listed in **Supplementary Table 2**.

DISCUSSION

In this study, we observed a significant association between T_1 GWG and HDP. The MAP_{13 week} and MAP_{20 week} mediated the association between T_1 GWG and HDP. Meanwhile, we established a risk-specific EwtGCat to assess the risk of HDP according to the pre-pregnant BMI class and T_1 GWG of pregnant women. The combination of EwtGCat and MAP showed remarkable greater predictive capacity for HDP in comparison with NAM criteria only.

The previous studies mainly focus on the relationship between total GWG and adverse outcome during the total gestation (17–19). There are few studies investigating the effect of GWG during the first trimester on HDP. And the current tools such as NAM criteria did not access the GWG elevation during the first trimester in terms of HDP (20, 21). The GWG recommendation of NAM criteria provided a different recommended range of GWG per each pre-pregnant BMI class. According to the cut-off points from the NAM criteria, several studies had demonstrated an association between total GWG and HDP (22–24). However, edema and fluid retention that commonly occurred during later trimesters can potentially confound the accurate effect of GWG

on HDP (25). The NAM guideline was conducted to reduce the risk of multiple adverse outcomes (26), which failed to distinguish the risk of HDP during this period. In contrast, EGC were constructed by evaluating the risk of HDP per pre-pregnant BMI class. Our study constructed a novel specific-risk category to assess the effect of GWG on the risk of *de novo* HDP and discovered that excessive GWG elevated the risk of HDP in the first trimester, which filled the research gap in the early gestation period.

Abnormal elevated blood pressure was the core symptom of HDP. Numerous studies had demonstrated that the elevated blood pressure associated with the risk of HDP during pregnancy (27–29). However, there were few studies to explore the effect

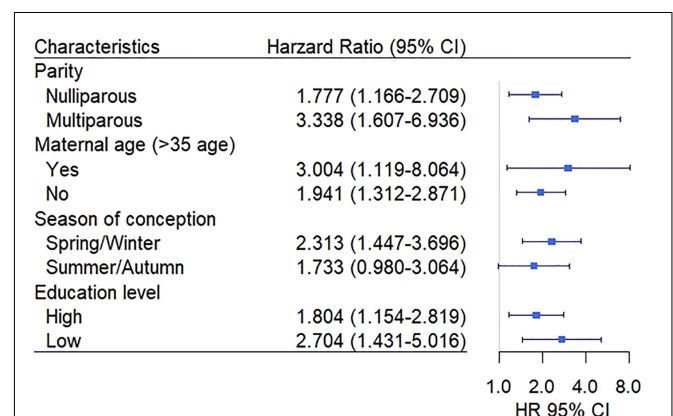


FIGURE 3 | The association between an early GWG category (EwtGCat) and HDP under different subgroup. The Class 1 and Class 2 were defined as low EwtGCat, and Class 3 and Class 4 were grouped into high EwtGCat. The low education level represents high school or below and the high education level includes vocational college and university or above.

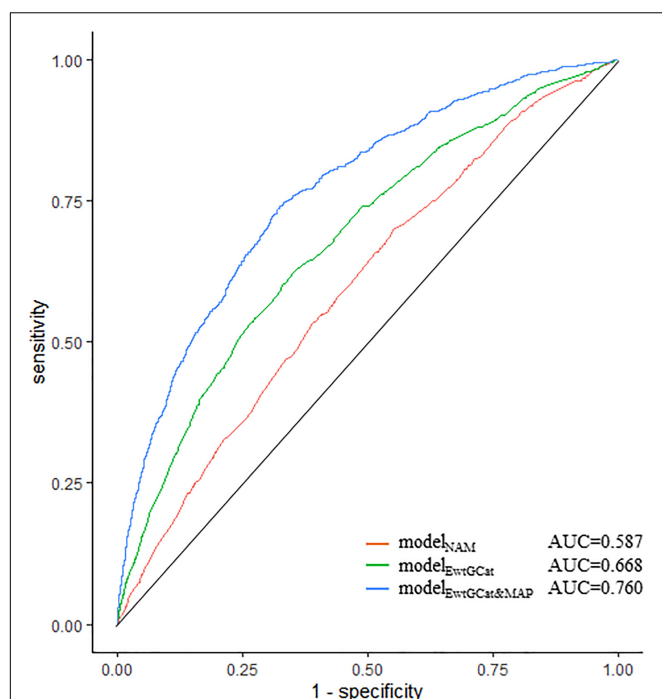


FIGURE 4 | The predictive capacity of prediction models for HDP. The ROC plot demonstrated the predictive capacity of prediction models. The model_{NAM}, model_{EwtGCat}, and model_{EwtGCat&MAP} were presented in red, green, and blue curves, respectively. The model_{NAM} included NAM criteria; model_{EwtGCat} included EwtGCat; model_{EwtGCat&MAP} was consisted of EwtGCat, MAP_{13 week}, MAP_{20 week}. All the models were adjusted by race, age, level of education, employment condition, maternal age (>35 age), gestational season.

of T_1 GWG on blood pressure before the HDP diagnosis. To our knowledge, our study is the first study to assess the serial mediation effects of blood pressure on the association between T_1 GWG and HDP before 20 weeks. Our mediation analyses indicated that the serial mediation effects were significant in the total participants and overweight/obesity pregnancies. Thus, the blood pressure before 20 weeks was an independent mediator involving in the association between T_1 GWG and HDP. Furthermore, we compared the capacities of EwtGCat and NAM criteria to predict the risk of HDP. The combination of EwtGCat, MAP_{13 week}, and MAP_{20 week} showed significantly higher capacity to predict HDP, compared to the NAM during the first trimester.

Hypertensive disorders of pregnancy is one of the most common gynecological diseases that affects 10% of pregnancies (30). An epidemiological study showed that 10–16% of maternal mortality worldwide was attributed to HDP and this disease was also associated with both a short- and long-term substantial disease burden (31, 32). Increasing number of studies supported that endothelial damage, vascular inflammation, and metabolic dysfunction participate the development of HDP, such as endothelial pathway, NF- κ b signaling pathway, abnormal glucose-metabolism, and dyslipidemia (33–36). However, the early identification of HDP remains significantly limited in the

clinical practice. GWG is a potential clinical indicator for the risk of HDP, which is non-intrusive and routinely measured in clinical practice. During the first trimester, weight gain came mainly from fat accumulation, while weight of the fetus, extravascular fluid, and maternal fat contributed to weight gain in the later trimesters. Our study focused on the weight gain during the first trimester, which was less likely to be affected by the above concern.

The potential mechanism underlying the association between T_1 GWG and HDP remained elusive. In the previous studies, maternal obesity was considered as an important risk factor for HDP. A multicenter Chinese retrospective study showed that overweight and obesity were a risk factor for HDP (37). Another Japanese study obtained a similar result that obese pregnant women were significantly associated with an increased risk of HDP (38). Current theory believes that obesity is a chronic inflammation and accumulating studies have found abnormal immune cells and cytokines in pregnant women with obesity such as CD4 + T cells, macrophages, IL-6, and TNF- α (39–42). Endothelial damage and vascular inflammation are the underlining pathological modifications at every stage of HDP development.

Some strengths were presented in this study. First, the gestational weight gain by the end of first trimester was used to study the temporal relationship between GWG and *de novo* HDP. Second, we constructed a risk-specific EwtGCat which showed greater capacity to identify the risk of *de novo* HDP. Third, this is the first study to explore the mediated mechanism underlying the association between T_1 GWG and HDP. Fourth, we illustrated a great potential for using EwtGCat and MAP for the prediction of HDP risk. Meanwhile, there are some limitations to our present study. First, the study subjects were all from the Tongzhou Maternal and Child Health Hospital (Beijing, China), which represent the northern Chinese population. Second, we did not collect the information of maternal lifestyle, such as the physical exercise and stress condition. These factors may be the potential confounding bias on the present study.

CONCLUSION

The GWG during the first trimester was associated with the risk of *de novo* HDP. MAP_{13 week} and MAP_{20 week} partially mediated the association between T_1 GWG and HDP. Early GWG category showed a better predictive capacity for the risk of HDP compared to the NAM criteria for first-trimester GWG. Therefore, the pregnancies were supposed to keep the gestational weight gain in an appropriated range to avoid the hazards of HDP. The overweight and obese women especially need to pay more attention on their blood pressure during pregnancy.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board of Peking University Health Science Center. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YJ and ZY: conceptualization, formal analysis, and methodology. ZY, JC, YP, YJ, SZ, HB, HW, SL, JY, and JL: data curation. H-JW: funding acquisition and supervision. ZY, JC, YP, YJ, SZ, HB, HW, and SL: investigation. H-JW, NH, and TS: project administration. ZY: visualization and writing – original draft. YJ and H-JW: writing – review and editing. All authors contributed to the article and approved the submitted version.

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Fat-Free Mass Index, Visceral Fat Level, and Muscle Mass Percentage Better Explain Deviations From the Expected Value of Aortic Pressure and Structural and Functional Arterial Properties Than Body Fat Indexes

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Bioelectrical impedance analysis (BIA)-derived indexes [e.g., fat (FMI) and fat-free mass indexes (FFMI), visceral fat level (VFL)] are used to characterize obesity as a cardiovascular risk factor (CRF). The BIA-derived index that better predicts arterial variability is still discussed.

Aims: To determine: (1) the association of classical [weight, height, body mass index (BMI), basal metabolic rate (BMR)] and BIA-derived indexes, with arterial properties deviations from expected values (arterial z-scores); (2) maximum arterial variations attributable to BIA-derived indexes; (3) whether the composition of total body, trunk and/or limbs is most closely associated with arterial variations.

Methods: Hemodynamic, structural, and functional parameters of different histological types of arteries were assessed ($n = 538$, 7–85 years). Classical and BIA-derived indexes [fat mass and percentage, FMI, VFL, muscle mass percentage (PMM), FFMI, and percentage] were measured (mono- and multi-segmental devices). Arterial z-scores were obtained using age-related equations derived from individuals not-exposed to CRFs ($n = 1,688$).

Results: First, regardless of the classical index considered, the associations with the arterial properties showed a specific hierarchy order: diameters and local stiffness > aortic and brachial blood pressure (BP) > regional stiffness. Second, all the associations of FMI and FFMI with z-scores were positive. Third, FFMI exceeded the association obtained with BMI and BMR, considering structural z-scores. In contrast, FMI did not exceed the association with z-scores achieved by BMI and BMR. Fourth, regardless of CRFs and classical indexes, arterial z-scores would be mainly explained by FFMI, VFL,

and PMM. Fifth, regardless of the body-segment considered, the levels of association between FMI and z-scores did not exceed those found for classic and FFMI. Total fat mass and trunk indexes showed a greater strength of association with z-scores than the FMI of limbs. Sixth, compared to lower limb FFMI indexes, total and upper limbs FFMI showed higher levels of association with z-scores.

Conclusions: FFMI (but not FMI) exceeded the strength of association seen between BMI or BMR and structural z-scores. Regardless of the body segment analyzed, the associations between FMI and z-scores did not exceed those found with classic and FFMI. Arterial z-scores could be independently explained by FFMI, VFL, and PMM.

Keywords: aortic pressure, arterial stiffness, bioelectrical impedance analysis, body composition assessment techniques, cardiovascular diagnosis, cardiovascular research, epidemiological research, intima-media thickness

INTRODUCTION

The rate of progression of structural and functional arterial disease is directly associated with the exposure to an increasing number of cardiovascular risk factors (CRFs) (1, 2). In Latin America, as well as in all western societies, overweight and obesity represent a major public health concern, which affects virtually all age groups of different socioeconomic status (3, 4). Obesity has the particularity to increase the risk of other CRFs such as diabetes and hypertension, all of which further accelerate the development of arterial disease (5). Over the past years, there has been significant efforts of the medical community to reduce the obesity, which resulted in different countries to introduce policies aimed at obesity prevention, intensification of the control of associated CRFs, and early arterial disease screening (i.e., using non-invasive arterial evaluation) (6). Furthermore, there has been growing interest in trying to better understand the association between body composition and early arterial changes and/or disease by investigating, which is the best approach to characterize both body composition indexes [using fast, simple, non-invasive, and easily repeatable analyses such as bioelectrical impedance analysis (BIA)] and arterial impairment among subjects (7). Despite the fact that overweight and obesity have been associated with worse cardiovascular status and disease prognosis, classic indexes used to characterize abnormal or

excessive fat accumulation [e.g., body weight (BW) or body mass index (BMI)] have shown predictive limitations (7). For instance, BMI does not consider fat distribution and may not indicate adequately fat content. In this regard, it could overestimate the degree of adiposity in both individuals who are overweight but very muscular (e.g., athletes) and in older patients who have lost body height (BH) secondary to spine osteoporosis. On the other hand, BMI may underestimate adiposity in elder individuals who have lost muscle mass in association with aging (8). Undoubtedly, this could result in inaccurate cardiovascular risk prediction at the patient level.

Recently, BIA-derived body fat distribution and the use of new indexes, such as fat mass and fat-free mass indexes (FMI and FFMI) have been used to characterize overweight and obesity. These parameters have shown an acceptable accuracy in estimating several health outcomes compared with classical anthropometric indexes (9, 10). Moreover, several studies have reported that FMI and FFMI are trustworthy obesity markers and are associated with cardiovascular abnormalities such as increased arterial stiffness, carotid wall thickness, and blood pressure (BP) (9, 11, 12). Yet, it remains unclear which BIA-derived body composition parameter better predicts structural, functional, and hemodynamic arterial changes. Additionally, the whole-body BIA (total or mono-segmental) aimed at measuring body composition could not be sensitive enough to detect specific segmental changes [i.e., increase in central (trunk), but not in lower limb adiposity]. Consequently, the measurement of both, the total body composition (mono-segmental BIA) and different body segments (trunk, lower and upper limbs; multi-segmental BIA) could theoretically provide complementary information about the link between body composition characteristics and cardiovascular properties.

Recently, our group has shown that traditional and non-traditional CRFs present different levels of association with parameters of arterial structure and function of different arterial territories [i.e., different histological type of arteries: elastic (carotids), transitional (brachial), and muscular (femoral)], as well as with different hemodynamic properties (13–19). We found that the impact of different CRFs, including time in sedentary behavior and sleep time (13), low birth weight and catch-up growth (14), physical activity level (e.g., assessed using

Abbreviations: aoBP, Aortic blood pressure; aoDBP, Aortic diastolic blood pressure; aoSBP, Aortic systolic blood pressure; BA, Brachial artery; baDBP, Brachial artery diastolic blood pressure; baMBP, Brachial artery mean blood pressure; baSBP, Brachial artery systolic blood pressure; BH, Body height; BIA, Bioelectrical impedance analysis; BMI, Body mass index; BMR, Basal metabolic rate; BP, Blood pressure; BW, Body weight; CCA, Common carotid artery; CFA, Common femoral artery; cfPWV, Carotid-femoral pulse wave velocity; CRFs, Cardiovascular risk factors; crPWV, Carotid-radial pulse wave velocity; DD, End-diastolic arterial diameter; EM, Elastic modulus; FFMI, Fat-free mass index; FMI, Fat mass index; IB, In body [multi-frequency (20 kHz and 100 kHz) and multi-segmental bioelectrical impedance device (InBody-120, InBody Co., Seoul, Korea)]; IMT, Intima-media thickness; MV, Mean value; OM, Omron [mono-frequency (50 kHz) and mono-segmental bioelectrical impedance device (HBF-514C (OM), Omron Healthcare, Inc., Illinois, USA)]; PBF, Body fat percentage; PWV, Pulse wave velocity; PWV Ratio, Pulse wave velocity ratio (cfPWV/crPWV quotient); SCOR, SphygmoCor-CvMS device; SD, Standard deviation; SysD, Peak systolic arterial diameter; VFL, Visceral fat level; z-, z-score; β , Beta (stiffness) index.

hip- and wrist-worn accelerometers) (15), high BP (17) and z-BMI (as a continuous variable) or obesity (18, 19) could differ, depending on the arterial parameter (structural vs. functional) or territory (central vs. peripheral, elastic, muscular or transitional) considered. To our knowledge, there are no studies to date that have comprehensively analyzed the association between classical anthropometric and BIA-derived body composition indexes (i.e., fat mass, fat-free mass) and arterial properties, considering (i) central and peripheral BP levels, (ii) carotid, femoral, and brachial diameters, and wall thickness, and (iii) regional and local arterial stiffness of different vascular territories. On the other hand, an important issue would be to identify to what extent BIA-derived indexes are associated with arterial properties, independently of the exposure to other CRFs (including classical anthropometric indexes). BIA-derived indexes showing an independent association could be the most useful to indicate the expected values of arterial parameters regardless of other individuals' characteristics (e.g., age, sex, high BP, BMI).

As in previous studies, for each analysis that included the arterial system, we analyzed the levels at which each arterial parameter deviates from the expected "optimal" value, accounting for the subject age (z-score) (13–15). The z-score describes the position of a subject-specific raw score in terms of its "distance" from the mean value in standard deviation units. For instance, a z-score for arterial stiffness equal to +2 or –2 indicates that a particular individual has stiffness levels of two standard deviations above or below the expected value, respectively, for an age-matched healthy individual not exposed to traditional CRFs (13–15). Thus, our analysis focuses on identifying the extent to which classical anthropometric and BIA-derived indexes would explain the level of deviation of the arterial system from the values considered "optimal," independently of other factors. In this way, it is possible to analyze whether the levels of the body composition indexes are able to explain the "deviations from normality," and not simply whether they are associated with the levels of the cardiovascular variables, which are expected to vary with age.

In this context, the aims of this work were (in healthy children, adolescents, and adults):

- First (Aim 1), to characterize the level of association between (i) classical [BW, BH, BMI, basal metabolic rate (BMR)], (ii) fat mass, and (iii) fat-free mass indexes, and cardiovascular z-scores (considering hemodynamic, structural, and functional parameters; central and peripheral arteries).
- Second (Aim 2), to evaluate and compare classical anthropometric variables with fat mass and fat-free mass indexes (mono-segmental BIA-derived), as potential explanatory variables of cardiovascular z-scores levels.
- Third (Aim 3), to quantify the maximum variations in cardiovascular variables (*effect size*), which can be attributed to variations in BIA-derived indexes.
- Finally (Aim 4), to analyze whether fat and fat-free mass distribution analysis (multi-segmental BIA) is able to identify specific body regions (e.g., total body vs. trunk vs. upper limbs vs. lower limbs) that are significantly associated with cardiovascular z-scores.

MATERIALS AND METHODS

Study Population

This study was carried out in the context of the Centro Universitario de Investigación, Innovación y Diagnóstico Arterial (CUiDARTE) project (13–27). This includes data derived from community-based studies on demographic and anthropometric variables, exposure to CRFs, personal and family history of cardiovascular disease and data on hemodynamic, structural, and functional vascular parameters. From this database, 538 subjects with body composition measurements with single-frequency mono-segmental BIA device were selected (110 of whom were also evaluated with multi-frequency multi-segmental BIA) (Table 1). Additionally, a "Reference Group" ($n = 1,688$) was selected from the CUiDARTE project database ($n = 3,619$) in order to quantify cardiovascular z-scores (15, 21–25). All procedures were conducted in agreement with the Declaration of Helsinki (1975 and reviewed in 1983), and the study protocol was approved by the Institution's Ethics Committee. In adults, written informed consent was obtained prior to the evaluation. In children and adolescents (<18 y), parents' written consent and children's assent were provided before the study.

Anthropometric and Clinical Evaluation

The participants were asked to avoid exercise, tobacco, alcohol, caffeine, and food intake 4 h before the evaluation, and not to perform strenuous physical activity in the previous 24 h. Additionally, the participants should empty their bladder 30 min before the anthropometric and body composition assessment. A clinical interview and the anthropometric evaluation enabled us to assess CRFs exposure, defined according to the criteria described below (data analysis). A family history of cardiovascular disease was defined by the presence of at least one first-degree (for all the subjects) or second-degree (for subjects ≤ 18 y) relatives with early (<55 y in males; <65 y in females) cardiovascular disease.

Body weight and BH were measured with the participants wearing light clothing and no shoes. BH was measured using a portable stadiometer and recorded to the nearest 0.1 cm. BW, fat mass, fat-free mass, muscle mass, BMR, and visceral fat level (VFL) were measured with two validated BIA devices: (i) mono-frequency (50 kHz) and mono-segmental [Omron HBF-514C (OM), Omron Healthcare, Inc., Illinois, USA]) and (ii) multi-frequency (20 kHz and 100 kHz) multi-segmental [InBody-120 (IB), InBody Co., Seoul, Korea]. To minimize variations due to fluid shifts in the body, the different BIA devices were placed side by side so that the subject could move from unit to unit without wasting time and too much movement. FMI and FFMI were calculated by dividing fat mass and fat-free mass by the square of the BH, respectively. Specific variables per segment (i.e., FMI and FFMI of trunk, upper, and lower limbs) were also calculated using the fat mass and fat-free mass of each segment and the same BH. BMI was calculated as BW divided by the square of BH. Body fat percentage (PBF) was calculated, dividing body fat mass by BW and multiplied by 100 (Figure 1). Detailed information on the validity of BIA measurements using InBody and OMRON technology,

TABLE 1 | Characteristics of subjects evaluated by bioelectrical impedance analysis (OMRON HBF-514C device).

	All (n = 538)				Male (n = 286)				Female (n = 252)			
	MV	SD	Min	Max	MV	SD	Min	Max	MV	SD	Min	Max
Cardiovascular risk factors												
Age (years)	29.70	18.17	7.00	85.79	32.55	19.02	7.00	75.00	26.46	16.61	11.00	85.79
Current smoker (%)		9				9.2				8.9		
Hypertension (%)		13				17.9				8.1		
Dyslipidemia (%)		16				17.1				13.8		
Diabetes (%)		2				3.2				1.6		
Obesity (%)		13				14.8				9.9		
History of CVD (%)		0				0				0		
Family History of CVD (%)		8				7.2				9.0		
On anti-hypertensive drug (%)		11				15.3				5.7		
On anti-HLD drug (%)		9				12.1				4.9		
On anti-diabetic drug (%)		3				3.9				2.0		
Anthropometric indexes												
Body Height (OM) (m)	1.68	0.10	1.21	1.96	1.74	0.08	1.21	1.96	1.60	0.06	1.32	1.75
Body Weight (OM) (kg)	69.53	17.09	27.80	134.7	76.88	16.88	27.80	134.7	61.34	13.17	40.20	120.0
BMI (OM) (kg/m ²)	24.56	4.85	15.10	48.20	25.24	4.71	15.10	40.60	23.82	4.90	16.50	48.20
BMR (OM) (kcal)	1,544	276	1,044	2,392	1,736	209	1,178	2,392	1,323	152	1,044	1,994
Body fat mass indexes												
BFM (OM) (kg)	20.00	10.15	2.60	65.40	18.32	10.18	2.60	52.50	21.86	9.81	5.42	65.40
PBF (OM) (%)	27.97	10.34	5.40	68.00	22.21	8.60	5.40	68.00	34.35	8.13	11.00	59.40
FMI (OM) (kg/m ²)	7.19	3.80	0.93	28.60	5.99	3.24	0.93	17.25	8.54	3.94	2.07	28.60
VFL (OM) (range: 1–30)	7.18	4.94	1.00	27.00	8.96	5.47	1.00	27.00	5.13	3.21	1.00	23.20
Body fat-free mass indexes												
FFM (OM) (kg)	49.88	12.22	24.70	97.25	59.39	8.52	24.70	97.25	39.33	4.72	27.97	62.43
PMM (OM) (%)	32.32	7.12	16.90	49.20	37.11	5.75	23.20	49.20	26.80	3.73	16.90	46.00
FFMI (OM) (kg/m ²)	17.51	2.72	7.62	25.93	19.49	1.97	7.62	25.93	15.29	1.41	12.91	21.56
PFFM (OM) (%)	72.03	10.34	32.00	94.60	77.79	8.60	32.00	94.60	65.65	8.13	40.60	89.00
Arterial structural parameters												
L-CCA DD (mm)	7.11	0.90	5.43	10.81	7.29	0.87	5.55	10.81	6.55	0.79	5.43	8.65
R-CCA DD (mm)	7.13	0.84	5.37	10.40	7.31	0.83	5.62	10.40	6.57	0.60	5.37	7.85
L-CFA DD (mm)	8.28	1.38	5.21	11.86	8.71	1.22	5.51	11.86	6.93	0.94	5.21	8.91
R-CFA DD (mm)	8.33	1.39	5.04	12.79	8.72	1.24	5.33	12.79	7.12	1.12	5.04	9.19
BA DD (mm)	4.20	0.71	2.67	5.72	4.39	0.64	2.67	5.72	3.50	0.46	2.90	4.38
Arterial functional parameters												
L-CCA IMT (mm)	0.72	0.20	0.29	1.24	0.75	0.20	0.29	1.24	0.65	0.19	0.41	1.17
R-CCA IMT (mm)	0.72	0.20	0.36	1.56	0.74	0.20	0.36	1.56	0.64	0.16	0.41	0.98
L-CCA EM (mmHg)	992	393	315	2,129	1,042	397	316	2,129	833	345	348	1,482
L- CCA Beta	9.93	3.72	3.51	21.87	10.37	3.83	3.51	21.87	8.56	3.05	4.36	14.07
R-CCA EM (mmHg)	943	387	279	2,291	984	379	397	2,291	815	390	279	1,632
R- CCA Beta	9.53	3.73	3.67	22.28	9.88	3.74	4.37	22.28	8.40	3.54	3.67	16.27
L-CFA EM (mmHg)	1,234	476	417	2,494	1,293	485	462	2,494	1,052	407	417	2,103
L- CFA Beta	12.61	4.64	4.41	27.16	13.07	4.78	4.41	27.16	11.18	3.93	5.68	19.69
R-CFA EM (mmHg)	1,215	502	416	2,990	1,263	516	458	2,990	1,066	434	416	2,181
R- CFA Beta	12.32	4.69	5.09	26.90	12.68	4.87	5.09	26.90	11.20	3.99	5.46	21.77
BA ME (mmHg)	1,471	827	346	3,792	1,592	833	408	3,792	1,025	641	346	2,902
BA Beta	14.96	8.15	3.75	40.36	16.02	8.20	4.93	40.36	11.05	6.85	3.75	30.86
cfPWV (m/s)	8.24	1.73	4.42	15.57	8.34	1.75	4.95	15.57	7.90	1.65	4.42	10.69
crPWV (m/s)	10.80	1.35	7.70	13.80	10.68	1.36	7.70	13.80	11.21	1.28	8.70	13.00
PWV Ratio	0.77	0.18	0.37	1.42	0.79	0.19	0.37	1.42	0.70	0.11	0.48	0.96

(Continued)

TABLE 1 | Continued

	All (n = 538)				Male (n = 286)				Female (n = 252)			
	MV	SD	Min	Max	MV	SD	Min	Max	MV	SD	Min	Max
Arterial blood pressure												
aoSBP (mmHg)	111	11	83	131	113	10	87	131	104	10	83	120
aoDBP (mmHg)	75	8	53	94	76	8	53	94	72	8	53	85
baSBP (mmHg)	125	11	102	152	127	11	102	152	119	9	102	138
baDBP (mmHg)	74	7	55	90	75	7	55	90	70	7	56	82

MV, mean value; SD, standard deviation; Min, Max., minimum and maximum; R, right; L, left; BMI, body mass index; HLD, hyperlipidemic; OM, Omron bioelectrical impedance device; PBF, body fat percentage; PMM, muscle mass percentage; BMR, basal metabolic rate; VFL, visceral fat level (30 levels); PFFM, fat-free mass percentage; FFM, fat-free mass index; FMI, fat mass index; SBP, DBP, systolic and diastolic blood pressure (suffix: ao: aortic, ba: brachial artery); CCA, CFA, BA, common carotid, common femoral and brachial artery; DD, diastolic diameter. IMT, intima-media thickness; EM, elastic modulus; cfPWV, crPWV, carotid-femoral and carotid-radial pulse wave velocity.

and technical characteristics of both devices can be found in **Supplementary File 1**.

Cardiovascular Evaluation

All measurements were performed in a temperature-controlled environment (21–23°C), with the subject in supine position and after resting for at least 10–15 min. Cardiovascular evaluation in the CUiiDARTE project included assessing hemodynamic, structural, and functional parameters (21–27). In this study, we focused on central and peripheral BP levels, a beat-to-beat arterial diameter, intima-media thickness (IMT), and regional and local arterial stiffness indexes.

Peripheral and Central Blood Pressure

Using a validated oscillometric device (HEM-433INT; Omron Healthcare Inc., Lake Forest, IL, USA), heart rate and brachial systolic and diastolic BP (baSBP, baDBP) were recorded simultaneously and/or immediately before or after each non-invasive echographic, tonometric, and oscillometric record. Brachial mean BP (baMBP) was quantified as $baDBP + (baSBP - baDBP)/3$.

Systolic and diastolic aortic BPs (aoSBP, aoDBP) were non-invasively obtained by means of applanation tonometry [SphygmoCor-CvMS (SCOR), v.9, AtCor-Medical, Australia] (20, 22). Briefly, radial BP waveform was obtained by tonometry, and the aortic BP (aoBP) waveform was then derived indirectly from the calibration of the acquired radial waveforms and application of a general transfer function. Radial waveforms were calibrated with baDBP and baMBP (**Figure 1**).

Regional Arterial Stiffness and Central-to-Peripheral Stiffness Gradient

Carotid-femoral (cfPWV, a marker of aortic stiffness) and carotid-radial pulse wave velocity (crPWV, a marker of upper arm arteries stiffness) were obtained by tonometry (SCOR) (23, 28). cfPWV and crPWV were obtained as the median of three recordings. The pulse wave velocity (PWV) ratio (a marker of a central-peripheral stiffness gradient) was quantified: $cfPWV/crPWV$ (23, 29, 30) (**Figure 1**).

Local Arterial Stiffness, Diameter, and Intima-Media Thickness

Left (L-) and right (R-) common carotid arteries (CCA), common femoral artery (CFA), and left brachial artery (BA) were analyzed

using ultrasound (6–13 MHz, M-Turbo, Sonosite Inc., WA, USA). Sequences of images (30 s, B-Mode, longitudinal views) were stored for off-line analysis. A beat-to-beat diameter and IMT waves were obtained using border detection software (HemoDyn 4-M, Dinap s.r.l., Bs.As., Argentina). Peak systolic (SysD) and end-diastolic (DD) diameters and IMT (far wall, end diastole) values were obtained by averaging at least 20 beats. The CCA diameter and IMT were measured a centimeter proximal to the carotid bulb. The CFA diameter was measured in the penultimate centimeter proximal to the bifurcation. BA measurements were acquired at the elbow level in a straight segment of at least one-centimeter long (26) (**Figure 1**).

Local arterial stiffness was quantified by the elastic modulus (EM) and the beta index (β). The EM measures the ability of the artery to change its dimensions in response to the BP caused by cardiac ejection [BP change required for (theoretic) 100% increase in diameter]: $EM = (SBP - DBP) / ((SysD - DD) / DD)$. To minimize the impact that BP levels have on stiffness, the β was quantified: $\beta = \ln(SBP/DBP) / [(SysD - DD) / DD]$. The baSBP and baDBP were used to quantify CFA and BA EMs and β s; aoSBP and aoDBP were used to quantify CCA EM and β (**Figure 1**).

Data Analysis

Standardized Cardiovascular Variables (z-Scores)

Considering specific inclusion and exclusion criteria, the subjects to be included in the reference group were identified to get standardized cardiovascular variables expressed as z-scores (**Supplementary File 2: Table S1**). As in previous works, the reference group was determined by selecting a healthy sub-population from the CUiiDARTE database ($n = 1,688$) that included children, adolescents, and adults who did not meet any of the following exclusion criteria: (i) history of cardiovascular disease; (ii) use of BP-, lipid- or glucose-lowering drugs; (iii) arterial hypertension (≥ 18 y: baSBP ≥ 140 mmHg or baDBP ≥ 90 mmHg; < 18 y: baSBP and baDBP > 95 th percentile for sex, age, and BH); (iv) current smoking; (v) diabetes, defined as self-reported or fasting plasma glucose ≥ 126 mg/dL (if available); (vi) dyslipidemia, defined as self-reported or total cholesterol ≥ 240 mg/dL or HDL cholesterol < 40 mg/dL (if available); (vii) obesity (≥ 18 y: BMI ≥ 30 kg/m²; < 18 y: z-BMI ≥ 2.0) (23–25). None of the subjects had congenital or chronic conditions, infectious diseases, or significant cardiac arrhythmias.

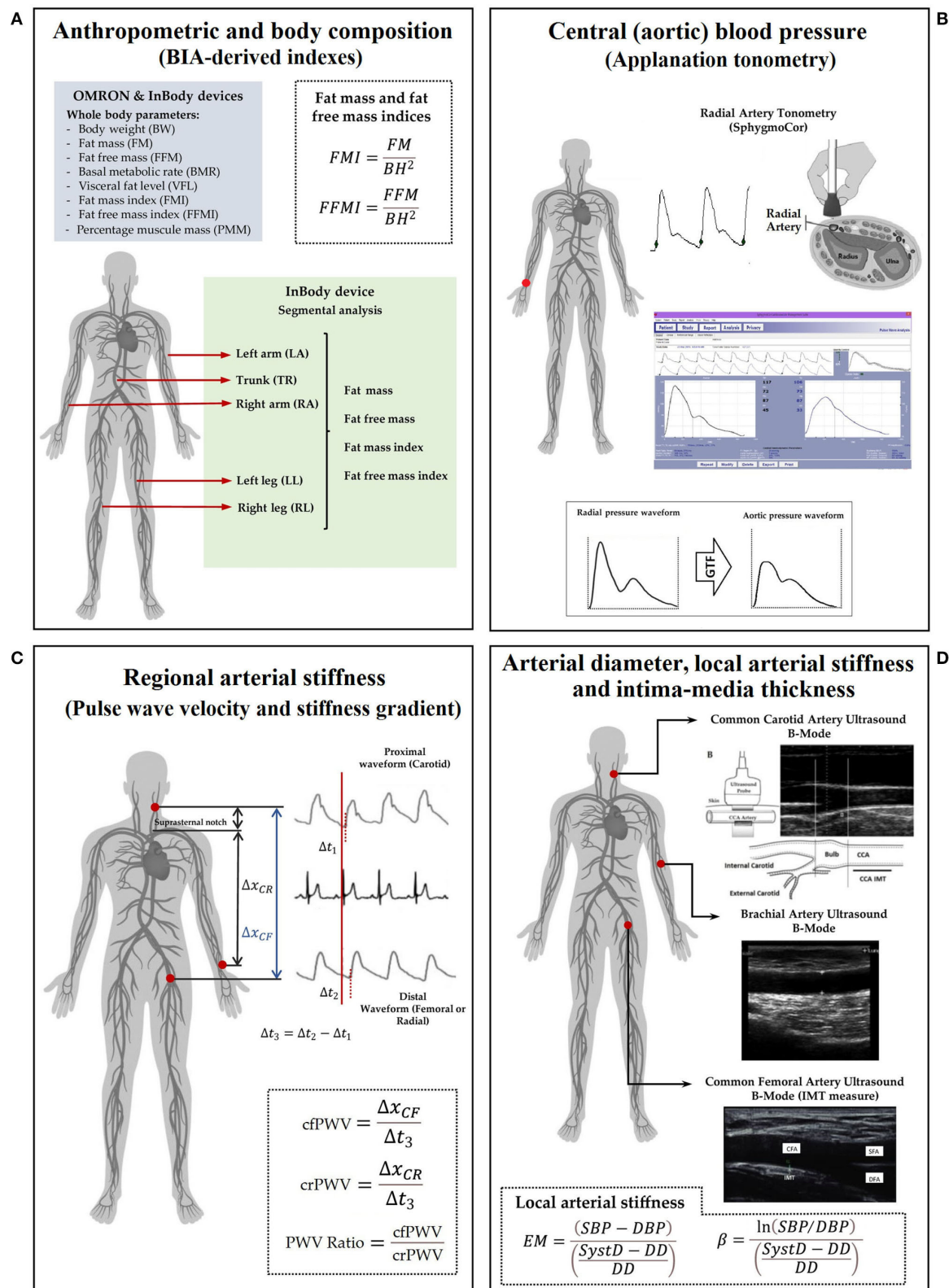
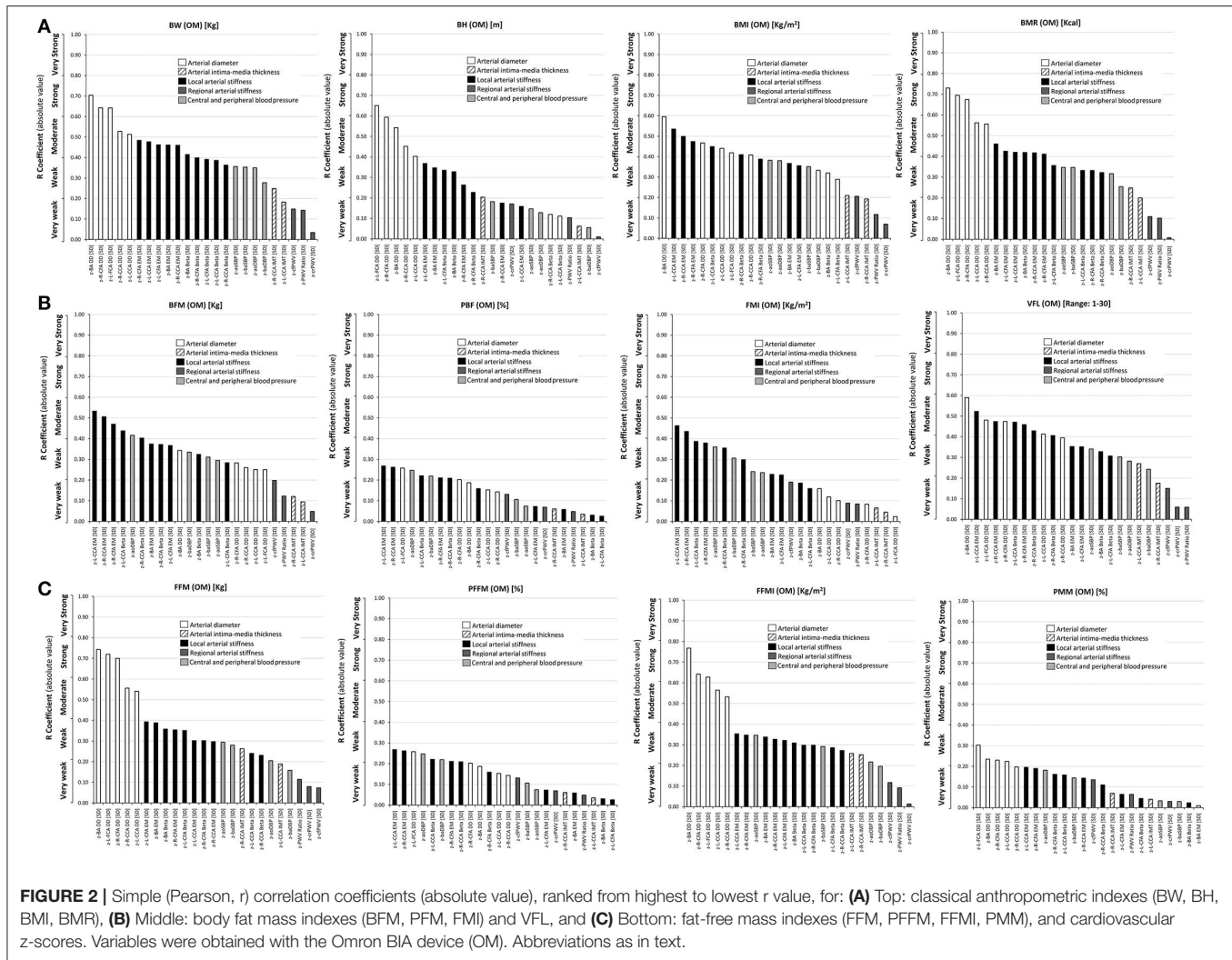


FIGURE 1 | A measurement scheme. Left to right, top to bottom. **(A)** anthropometric evaluation using OMRON HBF-514C (OM) and InBody-120 (IB); **(B)** central and peripheral blood pressure evaluation; **(C)** regional arterial stiffness: PWV (cfPWV and crPWV) and PWV ratio; **(D)** arterial diameter, IMT, and local arterial stiffness. Abbreviations as in text.



Once the reference group was built, age-related equations were obtained for mean value (MV) and standard deviation (SD). To this end, we implemented parametric regression methods based on various types of models (fractional polynomials, polynomial, ratios of polynomials) (23–25, 31, 32).

Figure S1, in **Supplementary File 3**, exemplifies (for a baSBP variable) the fractional polynomial models used to obtain these equations. This procedure provides different age-related equations for each model to calculate z-scores, and then the most adjusted model is chosen to calculate an individual's cardiovascular z-scores (**Supplementary File 2: Table S2**). Subsequently, by using these equations, we were able to quantify the z-score levels of each arterial variable in the subjects who had BIA-derived measurements (**Supplementary File 2: Tables S3, S6; Supplementary File 3: Figure S1**).

Mono-Segmental BIA-Derived Body Composition Indexes: Correlation and Regression Models

Two-tailed simple bivariate correlations were performed to quantify the strength of association between exposure to CRFs and classical anthropometric and mono-segmental

BIA-derived indexes, and cardiovascular z-scores (**Figures 2, 3; Supplementary File 2: Table S4**). Multiple linear regression models (Input: stepwise) were constructed, considering the cardiovascular z-scores as dependent variables and CRFs, classical anthropometric and mono-segmental BIA-derived indexes as independent variables (**Table 2**). In addition, by using: (i) multiple linear regression-derived non-standardized B coefficients, (ii) MV and SD data (the reference group), and (iii) the minimum and maximum values (range) of each mono-segmental BIA-derived indexes, it was possible to quantify for each arterial variable (in the respective units): (i) the maximum variation that could be associated (attributed) to the different values obtained on body composition indexes and (ii) the variations that could be (theoretically) expected, considering the inter-individual variations on BIA-derived body composition indexes (**Table 3, Figure 4**).

Agreement Between Mono-and Multi-Segmental BIA Devices

Lin's concordance correlation coefficient and Bland-Altman tests were performed to evaluate the agreement between BIA

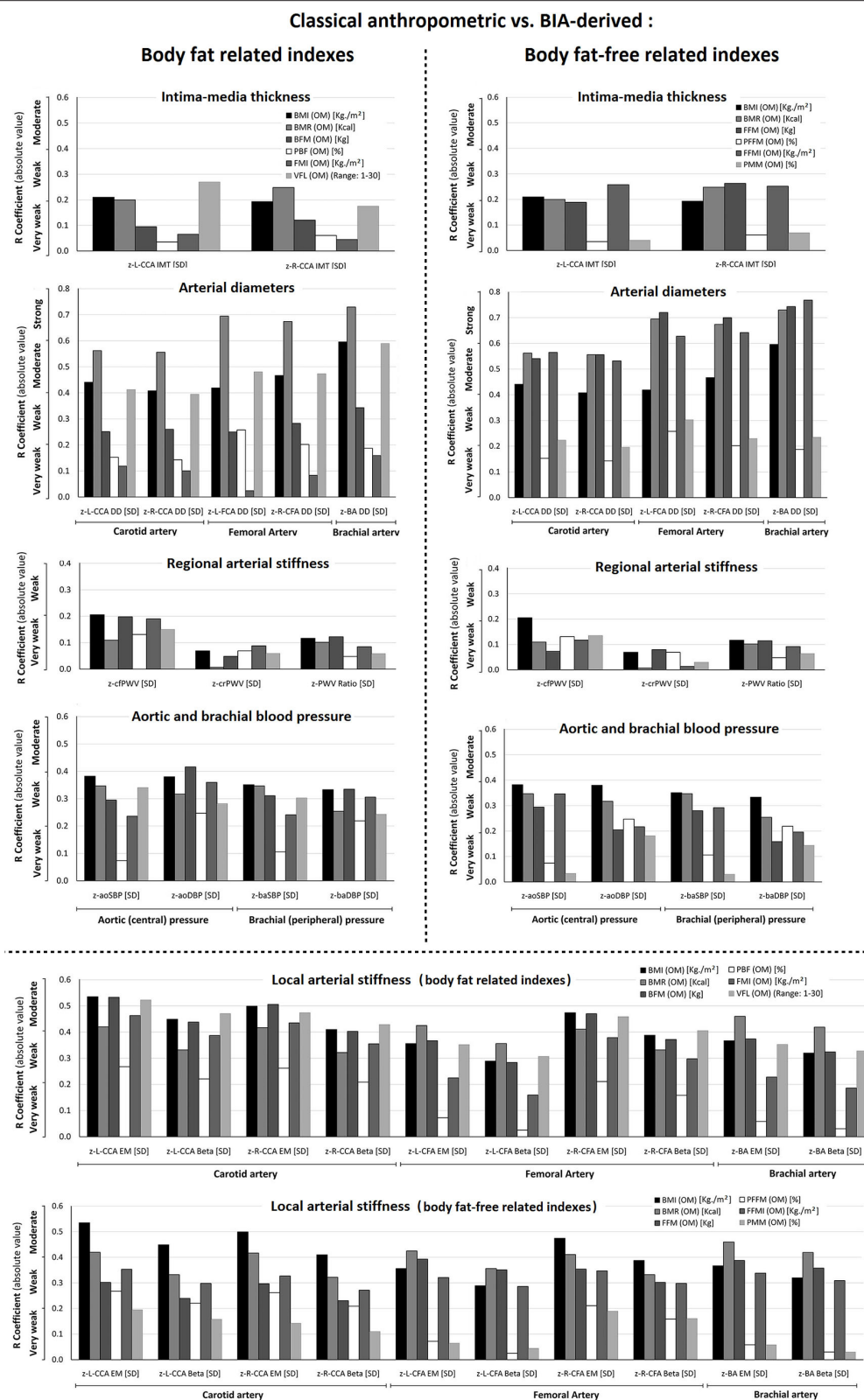


TABLE 2 | Association between cardiovascular z-scores (dependent variable) and cardiovascular risk factors, classical anthropometric and body composition indices (independent variables) (OMRON HBF-514C device).

Dependent variable	Independent variables	Bu	SE	95% CILL	95% CIUL	Bs	p	VIF	R	R ²	Adj R ²
Arterial structural parameters											
z-L-CCA DD (SD)	Constant	-2.331	0.397	-3.112	-1.550		<0.001		0.51	0.26	0.25
	FFMI (OM) (kg/m ²)	0.159	0.025	0.109	0.209	0.422	<0.001	2.16			
	Age (years)	-0.014	0.003	-0.021	-0.008	-0.265	<0.001	1.91			
	Diabetes	0.879	0.299	0.292	1.467	0.140	0.003	1.08			
	VFL (OM)	0.037	0.017	0.003	0.071	0.174	0.035	3.23			
z-R-CCA DD (SD)	Constant	-2.890	0.340	-3.558	-2.221		<0.001		0.43	0.18	0.18
	FFMI (OM) (kg/m ²)	0.172	0.019	0.135	0.210	0.435	<0.001	1.00			
z-L-FCA DD (SD)	Constant	-1.217	0.604	-2.407	-0.027		0.045		0.69	0.48	0.47
	Sex (1:Female; 0:Male)	-0.863	0.171	-1.201	-0.525	-0.443	<0.001	3.54			
	History of CVD	-0.586	0.265	-1.108	-0.064	-0.108	0.028	1.09			
	FFMI (OM) (kg/m ²)	0.108	0.032	0.045	0.171	0.318	0.001	4.11			
	Age (years)	-0.006	0.003	-0.012	-0.001	-0.130	0.021	1.44			
z-R-CFA DD (SD)	Constant	-2.110	0.647	-3.384	-0.836		0.001		0.66	0.44	0.44
	FFMI (OM) (kg/m ²)	0.141	0.032	0.078	0.204	0.374	<0.001	3.12			
	Sex (1:Female; 0:Male)	-0.695	0.182	-1.053	-0.337	-0.325	<0.001	3.12			
z-BA DD (SD)	Constant	-4.720	0.373	-5.457	-3.984		<0.001		0.72	0.52	0.52
	FFMI (OM) (kg/m ²)	0.258	0.020	0.219	0.296	0.727	<0.001	1.00			
z-L-CCA IMT (SD)	Constant	-1.002	0.361	-1.711	-0.292		0.006		0.22	0.05	0.04
	FFMI (OM) (kg/m ²)	0.069	0.020	0.029	0.109	0.181	0.001	1.04			
	Diabetes	0.678	0.337	0.016	1.340	0.107	0.045	1.04			
z-R-CCA IMT (SD)	Constant	-1.565	0.431	-2.413	-0.717		<0.001		0.34	0.12	0.11
	FFMI (OM) (kg/m ²)	0.102	0.024	0.055	0.150	0.217	<0.001	1.04			
	Diabetes	1.395	0.400	0.608	2.182	0.179	0.001	1.04			
	Family History CVD	0.689	0.223	0.250	1.129	0.155	0.002	1.00			
Arterial functional parameters											
z-L-CCA EM (SD)	Constant	-2.995	0.422	-3.824	-2.165		<0.001		0.39	0.15	0.14
	BMI (OM) (kg/m ²)	0.086	0.011	0.064	0.107	0.412	<0.001	1.13			
	PMM (OM) (%)	0.029	0.007	0.014	0.043	0.204	<0.001	1.13			
z-L-CCA Beta (SD)	Constant	-2.505	0.419	-3.329	-1.680		<0.001		0.3	0.10	0.09
	FMI (OM) (kg/m ²)	0.105	0.018	0.069	0.141	0.430	<0.001	2.18			
	PMM (OM) (%)	0.054	0.009	0.035	0.072	0.425	<0.001	2.18			
z-R-CCA EM (SD)	Constant	-0.917	0.148	-1.208	-0.626		<0.001		0.50	0.25	0.24
	VFL (OM)	0.086	0.013	0.060	0.113	0.422	<0.001	1.02			
	Diabetes	0.847	0.311	0.232	1.461	0.177	0.007	1.03			
	Family History CVD	0.477	0.203	0.076	0.878	0.151	0.020	1.00			
z-R-CCA Beta (SD)	Constant	-0.929	0.148	-1.221	-0.637		<0.001		0.43	0.18	0.17
	VFL (OM)	0.079	0.013	0.053	0.105	0.404	<0.001	1.00			
	Family History CVD	0.462	0.204	0.060	0.864	0.152	0.025	1.00			
z-L-CFA EM (SD)	Constant	-0.942	0.424	-1.777	-0.106		0.027		0.18	0.03	0.03
	FFMI (OM) (kg/m ²)	0.067	0.023	0.021	0.113	0.184	0.004	1.00			
z-L-CFA Beta (SD)	No variables were entered into the equation.										
z-R-CFA EM (SD)	Constant	-0.697	0.448	-1.579	0.185		0.121		0.23	0.05	0.04
	Hypertension	0.405	0.175	0.060	0.751	0.155	0.022	1.14			
	FFMI (OM) (kg/m ²)	0.050	0.025	0.000	0.099	0.133	0.050	1.14			
z-R-CFA Beta (SD)	Constant	0.158	0.069	0.023	0.293		0.022		0.14	0.02	0.01
	Hypertension	0.332	0.151	0.034	0.630	0.141	0.029	1.00			
z-BA EM (SD)	Constant	-1.898	0.835	-3.552	-0.244		0.025		0.36	0.13	0.11
	FFMI (OM) (kg/m ²)	0.153	0.041	0.072	0.234	0.330	<0.001	1.03			
	Age (years)	-0.019	0.008	-0.035	-0.003	-0.212	0.018	1.03			

(Continued)

TABLE 2 | Continued

Dependent variable	Independent variables	Bu	SE	95% CILL	95% CIUL	Bs	p	VIF	R	R ²	Adj R ²
z-BA Beta (SD)	Constant	−2.068	0.728	−3.510	−0.626		0.005		0.24	0.06	0.05
	FFMI (OM) (kg/m ²)	0.104	0.037	0.030	0.178	0.249	0.006	1.00			
z-cfPWV (SD)	Constant	0.056	0.063	−0.067	0.180		0.370		0.24	0.06	0.05
	Diabetes	1.097	0.341	0.427	1.767	0.168	0.001	1.03			
	Hypertension	0.430	0.156	0.123	0.738	0.144	0.006	1.03			
z-crPWV (SD)	No variables were entered into the equation										
z-PWV Ratio (SD)	No variables were entered into the equation										
Arterial Blood Pressure											
z-aoSBP (SD)	Constant	−2.444	0.351	−3.135	−1.753		<0.001		0.37	0.14	0.13
	FFMI (OM) (kg/m ²)	0.107	0.025	0.058	0.155	0.273	<0.001	1.65			
	BMI (OM) (kg/m ²)	0.031	0.014	0.004	0.059	0.140	0.027	1.65			
z-aoDBP (SD)	Constant	0.579	0.251	0.085	1.073		0.022		0.47	0.22	0.21
	VFL (OM)	0.055	0.011	0.033	0.077	0.261	<0.001	1.29			
	PMM (OM) (%)	−0.030	0.007	−0.043	−0.016	−0.202	<0.001	1.02			
	Diabetes	0.993	0.295	0.413	1.573	0.163	0.001	1.07			
	History of CVD	−0.845	0.331	−1.496	−0.195	−0.126	0.011	1.11			
	Hypertension	0.323	0.152	0.024	0.622	0.116	0.035	1.35			
z-baSBP (SD)	Constant	−2.456	0.584	−3.609	−1.302		<0.001		0.37	0.13	0.13
	BMI (OM) (kg/m ²)	0.103	0.021	0.061	0.144	0.373	<0.001	1.00			
z-baDBP (SD)	Constant	−2.036	0.591	−3.204	−0.867		0.001		0.32	0.10	0.10
	BMI (OM) (kg/m ²)	0.090	0.021	0.048	0.132	0.328	<0.001	1.00			

Bu y Bs: un- and standardized coefficients. R, Pearson coefficient; R², adjusted squared R; VIF, variance inflation factor (a VIF <5 was defined to evaluate (discard) multicollinearity). SE, standard error; LL, UL, lower and upper limits; CI, confidence interval; z: z-score. BW and BH, bodyweight and height; BMI, body mass index; OM, Omron bioelectrical impedance analysis device; PBF, body fat percentage; PMM, muscle mass percentage; VFL, visceral fat level; PFFM, fat-free mass percentage; FFMI, fat-free mass index; FMI, fat mass index; R, right. L, left; SBP, DBP, systolic and diastolic pressure (suffix: ao: aortic, ba: brachial artery). CCA, CFA, BA; common carotid, common femoral, and brachial artery; DD, diastolic diameter; IMT, intima-media thickness; EM, elastic modulus; cfPWV, crPWV, carotid-femoral and carotid-radial pulse wave velocity; PWV ratio, cfPWV/crPWV ratio; SD, standard deviation; CVD, cardiovascular disease. All anthropometric variables were included in the multiple linear regression. For diabetes, history of CVD, family history of CVD, hypertension: 1: Yes and 0: No. Only significant ($p < 0.05$) independent variables entered in the models (Stepwise) are shown.

devices (Supplementary File 2: Table S7; Supplementary File 3: Figure S2). Bland-Altman plots correspond to the mean of the methods considered (x-axis) against their difference (y-axis). Systematic error (bias) was considered present if mean error was significantly different from 0, whereas proportional error was considered present if the slope of the linear regression was statistically significant. Descriptive statistics obtained for the participants evaluated with multi-frequency BIA device (InBody-120) is shown in Supplementary File 2: Table S5.

Multi-Segmental BIA-Derived Body Composition Indexes: Correlation and Regression Models

Finally, using the information from the multi-segmental BIA device, an analysis similar to the one previously reported was performed. Correlation analyses were implemented to quantify the association between CRFs, classical anthropometric indexes, and multi-segmental BIA-derived indexes obtained for “total body,” “trunk,” “upper limb,” and “lower limb” segments and cardiovascular z-scores (Supplementary File 2: Table S8; Supplementary File 3: Figures S3–S8).

Statistical Analysis

According to the central limit theorem, a normal distribution was considered (taking into account Kurtosis and Skewness

coefficients distribution and number of studied subjects; sample size >30) (33). The number of the subjects included was much higher than the minimum required sample size, both to construct the reference group to obtain the MV and SD equations (included: 1,688, minimum required sample size: 377), and to perform the agreement and/or association analyses (included: 538 for OM and 110 for IB, a minimum required sample size: 103). Consequently, the number of the subjects studied was higher than the minimum number calculated for: $\alpha = 0.05$, $\beta = 0.20$, anticipated effect size = 0.15 (medium), and a total number of predictors in the multiple linear regression model = 7.

Even in this conservative context, when making associations, we performed Bootstrapping of the samples as a strategy to evaluate whether potential associations observed between cardiovascular z-scores and body composition indexes do maintain even after analyzing different random sampling settings (resampling with replacement from the original sample). In other words, with this mechanism, any initial $p < 0.05$ may no longer be significant after the “fictional random re-sampling” (i.e., bootstrapping). This type of test obligates the investigators to consider only those significant p values that replicate in both statistical scenarios (the actual sample and bootstrapping sampling). To this end, Bootstrap-derived 95% confidence intervals (1,000 samples) were obtained, applying bias-corrected

TABLE 3 | Impact of interindividual variations of body composition indices (independent variables) on cardiovascular properties (dependent variables) (OMRON HBF-514C device).

Dependent variable					Cardiovascular differences attributable to FFMI variations					
Cardiovascular variable	Age (y)	MV (RG)	SD RG)	Bu	5 units	10 units	15 units	20 units	Δ (Max-Min)	Δ%
Fat-free Mass Index (FFMI) (kg/m²)[MV: 17.51; SD: 2.72; Range: 7.62 - 25.93]										
L-CCA DD (mm)	10	4.71	0.52	0.16	0.41	0.83	1.24	1.65	1.51	32.05
	30	6.34	0.50		0.40	0.80	1.19	1.59	1.46	22.98
	50	6.72	0.66		0.52	1.04	1.57	2.09	1.91	28.42
	70	7.04	0.65		0.52	1.04	1.56	2.08	1.90	27.07
R-CCA DD (mm)	10	5.77	0.50	0.17	0.43	0.86	1.29	1.72	1.58	27.31
	30	6.45	0.51		0.44	0.88	1.32	1.76	1.61	24.94
	50	6.81	0.64		0.55	1.11	1.66	2.22	2.03	29.80
	70	7.19	0.63		0.54	1.08	1.62	2.15	1.97	27.43
L-FCA DD (mm)	10	5.51	0.68	0.11	0.37	0.73	1.10	1.47	1.34	24.42
	30	7.75	1.13		0.61	1.22	1.83	2.44	2.23	28.82
	50	8.33	1.47		0.79	1.59	2.38	3.18	2.91	34.92
	70	8.59	1.38		0.74	1.49	2.23	2.98	2.72	31.70
R-FCA DD (mm)	10	5.53	0.68	0.14	0.48	0.96	1.45	1.93	1.76	31.87
	30	7.77	1.07		0.75	1.50	2.25	3.00	2.74	35.30
	50	8.32	1.34		0.94	1.88	2.82	3.76	3.44	41.37
	70	8.33	1.35		0.95	1.90	2.85	3.80	3.48	41.74
BA DD (mm)	10	2.71	0.35	0.26	0.45	0.90	1.35	1.80	1.65	60.77
	30	3.70	0.62		0.80	1.60	2.40	3.20	2.93	79.08
	50	4.08	0.78		1.01	2.02	3.03	4.03	3.69	90.39
	70	4.21	0.64		0.82	1.65	2.47	3.30	3.02	71.61
L-CCA IMT (mm)	10	0.43	0.05	0.07	0.02	0.03	0.05	0.07	0.06	14.2
	30	0.55	0.08		0.03	0.06	0.09	0.12	0.11	19.3
	50	0.69	0.10		0.03	0.07	0.10	0.13	0.12	17.8
	70	0.85	0.19		0.06	0.13	0.19	0.26	0.24	27.8
R-CCA IMT (mm)	10	0.44	0.04	0.10	0.02	0.04	0.06	0.08	0.07	16.69
	30	0.54	0.10		0.05	0.10	0.15	0.19	0.18	32.74
	50	0.67	0.08		0.04	0.09	0.13	0.17	0.16	23.67
	70	0.83	0.15		0.08	0.16	0.23	0.31	0.29	34.24
L-CCA Beta	10	4.71	1.83	0.10	0.96	1.92	2.88	3.84	3.51	74.49
	30	7.19	1.90		0.99	1.99	2.98	3.98	3.64	50.64
	50	9.57	2.59		1.36	2.71	4.07	5.42	4.96	51.85
	70	11.79	4.01		2.10	4.20	6.30	8.40	7.68	65.14
z-L-CFA EM (mmHg)	10	829	339	0.07	114	227	341	454	416	50.14
	30	1,235	617		207	413	620	826	756	61.22
	50	1,243	579		194	388	581	775	709	57.09
	70	1,128	529		177	354	531	708	648	57.43
R-CFA EM (mmHg)	10	823	315	0.05	78	156	234	313	286	34
	30	1,185	520		129	258	387	516	472	39
	50	1,116	453		112	225	337	450	411	36
	70	919	329		81	163	245	326	298	32
BA EM (mmHg)	10	943	502	0.15	385	770	1,154	1,539	1,384	147
	30	1,265	690		529	1,059	1,560	2,080	1,904	151
	50	1,385	696		534	1,067	1,573	2,097	1,919	139
	70	1,475	733		562	1,125	1,657	2,210	2,022	137
aoSBP (mmHg)	10	91.7	8.31	0.11	4.4	8.8	13.2	17.7	16.2	17.6
	30	106.1	10.1		5.3	10.7	16.0	21.4	19.6	18.4
	50	109.6	9.3		4.9	9.9	14.8	19.8	18.1	16.5
	70	111.1	11.1		5.9	11.8	17.8	23.7	21.7	19.5

(Continued)

TABLE 3 | Continued

Dependent variable					Cardiovascular differences attributable to FFMI variations					
Cardiovascular variable	Age (y)	MV (RG)	SD RG)	Bu	5 units	10 units	15 units	20 units	Δ (Max-Min)	Δ %
Muscle Mass Percentage (PMM) (%) (MV: 32.32; SD: 7.12; Range: 16.9 - 49.2%)										
z-L-CCA EM (mmHg)	10	386	155	0.03	22	44	67	89	143	37
	30	676	179		26	52	77	103	166	25
	50	935	272		39	78	117	156	252	27
	70	1,143	395		57	113	170	227	366	32
z-L-CCA beta (SD)	10	4.71	1.83	0.05	0.49	0.99	1.48	1.97	3.18	68
	30	7.19	1.90		0.51	1.02	1.53	2.04	3.30	46
	50	9.57	2.59		0.70	1.39	2.09	2.79	4.50	47
	70	11.79	4.01		1.08	2.16	3.24	4.31	6.97	59
aoDBP (mmHg)	10	62.1	8.2	-0.03	-1.2	-2.4	-6	-4.8	-7.8	-12.7
	30	71.1	8.7		-1.2	-2.5	-3.8	-5.1	-8.3	-11.7
	50	75.0	7.5		-1.1	-2.2	-3.3	-4.4	-7.2	-9.6
	70	73.9	7.2		-1.0	-2.1	-3.2	-4.2	-6.9	-9.4
Dependent variable					Cardiovascular differences attributable to FFMI variations					
Cardiovascular Variable	Age (y)	MV (RG)	SD RG)	Bu	5 units	10 units	15 units	20 units	Δ (Max-Min)	Δ %
Visceral Fat Level (VFL) (MV: 7.18; SD: 4.94; Range: 1-27)										
L-CCA DD (mm)	10	4.71	0.52	0.04	0.09	0.19	0.28	0.38	0.49	10.46
	30	6.34	0.50		0.09	0.18	0.27	0.37	0.48	7.50
	50	6.72	0.66		0.12	0.24	0.36	0.48	0.62	9.27
	70	7.04	0.65		0.12	0.24	0.36	0.48	0.62	8.83
R-CCA EM (mmHg)	10	399	121	0.09	52	104	157	209	271	68
	30	661	166		72	143	215	286	372	56
	50	875	267		115	231	346	461	599	69
	70	1,060	368		159	317	476	635	825	78
z-R-CCA Beta	10	4.91	1.39	0.08	0.55	1.11	1.66	2.21	2.88	58.59
	30	7.06	1.77		0.70	1.40	2.10	2.81	3.65	51.68
	50	9.01	2.62		1.04	2.08	3.12	4.16	5.41	60.04
	70	10.93	3.15		1.25	2.50	3.75	5.00	6.50	59.51
aoDBP (mmHg)	10	62.2	8.2	0.05	2.2	4.5	6.7	9.0	11.7	18.8
	30	71.1	8.7		2.4	4.8	7.1	9.5	12.4	17.4
	50	75.1	7.5		2.1	4.1	6.2	8.2	10.7	14.3
	70	74.0	7.2		2.0	4.0	5.9	7.9	10.3	13.9

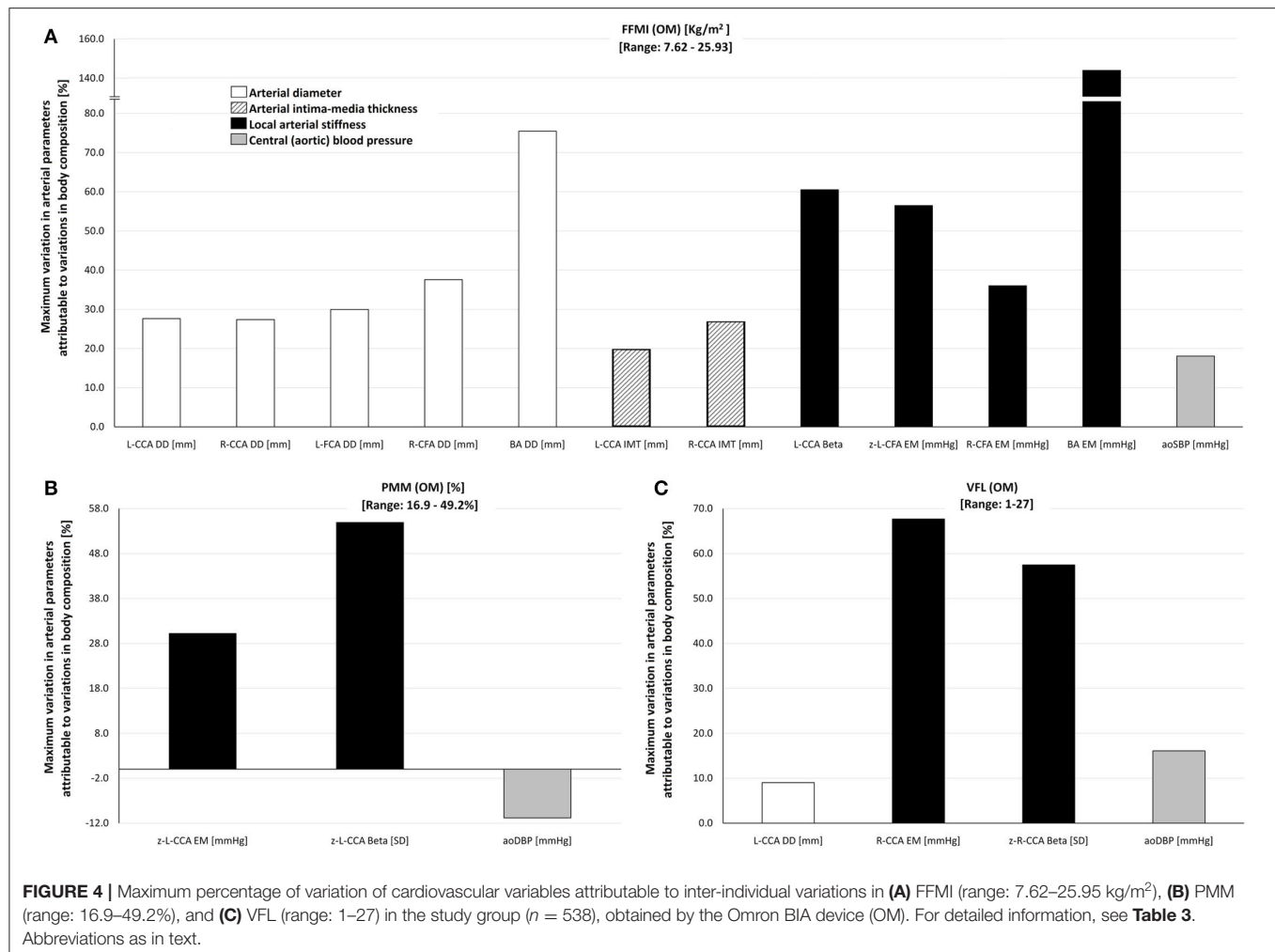
RG, reference group; MV, mean value; SD, standard deviation; CV, cardiovascular; Bu, beta un-standardized; Min, Max, minimum and maximum; R, right; L, left; SBP, DBP, systolic and diastolic pressure (suffix: ao: aortic, ba: brachial artery); CCA, CFA, BA, common carotid, common femoral, and brachial artery. DD: diastolic diameter. IMT, intima-media thickness; EM, elastic modulus. "Δ (Max-Min)" is expressed as absolute value. Δ% was quantified as: $[(\text{Max-Min})/\text{MV}] \times 100$.

and accelerated methods for computing confidence interval limits [lower and upper limits (LL and UL, respectively)]. The association was considered significant only if the 95% confidence interval of Pearson's coefficient, quantified by bootstrapping, did not contain the 0 value.

Evans's empirical classifications of interpreting correlation strength by using r were applied: $r < 0.20$, very weak; $r: 0.20-0.39$, weak; $r: 0.40-0.59$, moderate; $r: 0.60-0.79$, strong; $r \geq 0.80$, very strong (34). Analyses were done using SPSS (IBM-SPSS Inc., Chicago, IL, USA), MedCalc (MedCalc Inc., Ostend, Belgium) and NCSS 2020 (NCSS, Kaysville, UT; www.NCSS.com) software. A $p < 0.05$ was considered statistically significant.

RESULTS

The subjects' characteristics evaluated with mono-segmental BIA are shown in Table 1. There was a balanced distribution of sex (47% female), and a wide age range (7–85 years). The levels of CRFs exposure and drug use were similar to general population. There was a wide range of variation in fat mass and fat-free mass indexes. Inter-individual variations in fat mass indexes were for PBF (5.4–68.0%), FMI (0.9–28.6 kg/m²), VFL (1–27, scale between 1 and 30 levels), while, in fat-free mass, indexes were for PMM% (16.9–49.2%), FFMI (7.6–25.9 kg/m²), and PFFM (32.–94.6%).



Additionally, there was a wide inter-individual variation in arterial properties. For instance, while certain subjects presented z-scores lower than -2 , others had values higher than $+2$, or even than $+4$ (**Supplementary File 2: Tables S3, S6; Supplementary File 3: Figure S1**).

Classical Anthropometric, Body and Visceral Fat, and Fat-Free Mass Indexes (Mono-Segmental BIA-Derived): Association With Arterial Variations (Aim 1)

When comparing classical indexes (BW, BH, BMI, and BMR), those that showed the greatest association with cardiovascular z-scores varied, depending on the arterial parameter and segment, although BH never showed the greatest association with any of the arterial parameters. For BMR, the highest levels of association were obtained for CCA and CFA z-diameters (r : 0.56–0.73), z-R-CCA IMT (r : 0.25) and BA stiffness (r : 0.42–0.46). BMI showed the highest levels of association with z-L-IMT (r : 0.25), CCA stiffness (r : 0.41–0.55), and z-cfPWV (r : 0.21), whereas BW showed the highest level of association with CFA and BA z-stiffness (r : 0.39–0.48), the z-PWV ratio (r : 0.14), and

z-baSBP (r : 0.35). Therefore, except for BH, classical indexes had heterogeneous levels of association with arterial z-scores, but never these correlations reached strong association values (**Figure 2, Supplementary File 2: Table S4**).

Body weight, BMR, and BH showed the highest levels of association with z-diameters, followed by z-local stiffness. BMI showed a heterogeneous distribution, showing different levels of association between z-diameters and z-local stiffness. Regarding regional z-stiffness, the four indexes showed a “very weak” association (the lowest levels observed). Regardless of the classical index evaluated, from highest to lowest, a hierarchical order in the levels of association was observed: diameters or local stiffness > BP (aortic, brachial) > regional stiffness (**Figure 2, Supplementary File 2: Table S4**).

In general, higher mono-segmental BIA-derived fat mass indexes (i.e., BFM, PBF, FMI, VFL) were associated with (i) higher z-carotid, femoral and brachial local stiffness, and (ii) higher z-aoBP and z-baBP. BFM, FMI, and mainly VFL showed “moderate” levels of associations (r : 0.40–0.60) with cardiovascular z-scores, whereas the lowest levels were obtained for PBF (r < 0.3). Furthermore, z-arterial diameters and z-BP were mainly associated with VFL, FMI, and BFM, respectively.

VFL showed the greatest value of association with cardiovascular z-scores (**Figure 2, Supplementary File 2: Table S4**).

Considering each index individually, both BFM and FMI showed the highest levels of association with z-local stiffness, and only weak associations with z-diameters, whereas the VFL showed strong associations with both z-local stiffness and z-diameters (**Figure 2, Supplementary File 2: Table S4**). The levels of association with z-regional stiffness were very low. Additionally, considering the body fat mass indexes (BFM and FMI, except for visceral fat), there was the following hierarchical order in associations: local arterial stiffness > BP (aortic, brachial) > arterial diameters > CCA IMT and regional arterial stiffness (**Figure 2**). When considering visceral fat, the following order was shown: diameters and local stiffness > BP > CCA IMT and regional stiffness.

Higher fat-free mass indexes (especially FFM and FFMI) were associated with higher: (i) CCA, CFA, and BA z-diameter, (ii) CCA, CFA, and BA z-stiffness, and (iii) z-BP (aortic, brachial). Like fat mass indexes, the fat-free indexes showed a “very weak” level of association with z-regional stiffness. Both PFFM and PMM reported the lowest levels of association with cardiovascular z-scores ($r < 0.4$). The FFMI, followed by FFM, showed the highest number of associations with cardiovascular z-scores. Both indexes showed (i) “moderate” and “strong” association with z-diameters, followed by (ii) “weak or moderate” association with z-local stiffness. Unlike fat mass indexes, FFM and FFMI showed a marked difference between the levels of association with z-diameters and z-local stiffness (**Figure 2, Supplementary File 2: Table S4**).

The joint association analysis of fat (BFM, FMI, VFL) and fat-free mass indexes (FFM and FFMI) with cardiovascular z-scores showed that all significant associations were positive; besides, a higher index was associated with a higher cardiovascular z-score (**Supplementary File 2: Table S4**).

Comparative Analysis of Classical, Body, and Visceral Fat, and Fat-Free Mass Indexes (Mono-Segmental BIA-Derived) as Explanatory Variables of Arterial Variations (Aim 2)

Bivariate Analysis

The analysis of z-local stiffness was characterized by relatively strong associations with BMI and BMR, in which neither of the indexes of fat mass (especially VFL) nor fat-free mass exceeded these levels of association. Regarding regional stiffness, the associations with fat and fat-free mass indexes were comparatively “very weak,” being these values lower than BMI and BMR (**Figure 3, Supplementary File 2: Table S4**).

Association analyses between z-structural and fat-free mass indexes suggest that FFM and FFMI showed at least a stronger association than BMI and BMR. Conversely, although VFL showed the highest “ r ” value (a moderate level), BMI and BMR showed stronger associations with z-diameters (**Figure 3, Supplementary File 2: Table S4**). The associations between fat and fat-free mass indexes and z-IMT were very weak. Same weak associations were observed when considering BMI and BMR.

We found less or similar levels of association between fat and fat-free mass indexes (compared to BMI and BMR) with respect to z-BP (aortic, brachial) (**Figure 3, Supplementary File 2: Table S4**).

Multivariate Analyses

In general terms, regardless of age, sex, CRFs, and classical anthropometric indexes, variations in cardiovascular z-scores can be explained by variations in FFMI, VFL, and PMM (**Table 2**).

The z-structural variations (diameters, IMT) were mainly explained by variations in FFMI, regardless of the histological type of artery (**Table 2**). Always, (i) higher FFMI was associated with higher arterial z-structure, and, generally, (ii) FFMI was the explanatory variable with the highest relative weight [greatest explanatory ability evidenced by the B standardized (Bs) level]. Classical anthropometric indexes were not included in the explanatory z-structural models.

Variations in z-local stiffness were explained (i) by FMI (considering CFA and BA) and (ii) by PMM and VFL (considering CCA). With exception of left z-CCA EM, the BMR, BMI, BW, and BH were not included in local stiffness models. Variations in z-regional stiffness were not explained by anthropometric or body composition indexes (**Table 2**).

Considering aoBP and baBP, the z-BP-related parameters showed meaningful differences. Accordingly, variations in z-aoSBP were mainly explained by FFMI, but also by BMI. Variations in z-aoDBP were explained by VFL and PMM, while z-baBP was explained only by variations in BMI.

Effect Size Analyses: Maximal Inter-Individual Arterial Variations Explained by Mono-Segmental BIA-Derived Body Composition Indexes (Aim 3)

Table 3 shows cardiovascular variations, which could be explained (regardless of other cofactors) by variations in 5, 10, 15, and 20 units of: (i) FFMI, (ii) PMM, and (iii) VFL. The expected variations are presented according to different ages (10, 30, 50, 70 years). Also, **Table 3** shows the maximum cardiovascular variation (absolute and relative) that could be explained by variations in FFMI, PMM, or VFL. **Figure 4** summarizes these findings.

Variations in FFMI explain variations in arterial diameters. Their absolute levels (in mm) gradually increased when considering CCA, CFA, and BA (1.5–2.0, 1.5–3.8, and 1.8–4.0 mm, respectively). Additionally, FFMI levels explain absolute variations of 0.1 to 0.3 mm in CCA IMT (**Table 3**). In relative terms, FFMI variations are able to explain variations in CCA, CFA, and BA diameters (30, 40, and 75%, respectively) and IMT (20–30%).

Besides, FFMI-related variations in local stiffness were different between arterial segments. FFMI-associated variations in CCA and CFA EMs reached levels of 30–60%, while, in BA, EM reached maximum levels of 130–150% (**Figure 4, Table 3**). This seems to indicate that there is an arterial segment-dependent “sensitivity” (CCA vs. CFA vs. BA) to changes in FFMI.

Finally, FFMI explained variations in aoSBP, but not in baBP, indicating again an “arterial segment” dependency (central vs. peripheral). Accordingly, FFMI variations explained variations of 16–22 mmHg in aoSBP, representing 17–20% relating to the MV of the reference group (**Figure 4, Table 3**).

PMM and VFL variations were associated with structural and stiffness variations in CCA of 25–70% (but not in the CFA or BA), and with aoDBP variations (but not in baBP) of 10–20% (**Figure 4, Table 3**).

Mono- (Whole Body) and Multi-Segmental (Total, Trunk, Limbs) Fat and Fat-Free Mass Indexes: Association With Arterial Variations (Aim 4)

Supplementary File 2: Table S5 shows the subjects' characteristics assessed by multi- and mono-segmental BIA-derived approaches. It can be seen a wide age range (7–75 years), exposure to CRFs, and body composition levels (e.g., BMI: 17.1–44.6 kg/m²; FMI: 1.9–23.3 kg/m², FFMI: 13.4–25.0 kg/m²). Besides, this subgroup shows wide variation in cardiovascular parameters (e.g., average z-scores between −2.1 and +3.8) (**Supplementary File 2: Table S6; Supplementary File 3: Figure S1**).

High agreement between the two BIA devices (Omron HF-514 vs. InBody-120) was observed in concordance and Bland-Altman analysis when comparing “fat mass” and “fat-free mass” indexes (**Supplementary File 2: Table S7; Supplementary File 3: Figure S2**).

Supplementary File 2: Table S8 shows correlation analyses between demographic, clinical, anthropometric, body composition, and cardiovascular z-scores characteristics. **Supplementary File 3: Figures S3–S8** detail the association (“r” ranked from highest to lowest) between (i) cardiovascular z-scores and (i) FMI (**Supplementary File 3: Figures S3–S5**) or (ii) FFMI levels (**Supplementary File 3: Figures S6–S8**). In addition, information about the comparison of “total” marker [whole body; (IB)] and the five body segments, i.e., trunk (T), left and right arms (LA, RA), and left and right legs (LL, RL), are also provided.

For z-IMT and z-carotid diameters, the T-FMI showed the highest association, while, for z-CFA diameters, the T-FMI had the lowest level of association. The “total” (whole body) FMI achieved neither the highest nor the lowest level of association (**Supplementary File 3: Figure S3**). Similarly, the analysis of arterial z-stiffness showed that T-FMI followed by the “total” FMI showed the highest association regardless of the considered arterial segment (CCA, CFA, BA) (**Supplementary File 3: Figure S4**). Regarding the z-BP analysis (aoBP, baBP), T-FMI, and the “total” FMI had the strongest associations (**Supplementary File 3: Supplementary Figure S5**).

Regarding the CCA, CFA, and BA z-diameters, the “total” (whole body) and “upper limb” FFMI (RA or LA) showed the greatest levels of association (“moderate”), while the “trunk” and lower limb FFMI reached the lowest levels. Considering the z-IMT, upper limb FFMI achieved the highest degrees of association (**Supplementary File 3: Figure S6**).

Similarly, regarding z-local stiffness, “total” FFMI showed a greater association with CCA and CFA stiffness, while “upper limb” FFMI showed greater associations with BA z-stiffness (**Supplementary File 3: Figure S7**). For the z-BP, “total” FFMI (for DBP) and “upper limb” FFMI (for SBP) were found to have the highest association, while lower limb FFMI showed the lowest levels of association (**Supplementary File 3: Figure S8**).

DISCUSSION

To our knowledge, this is the first study to comprehensively evaluate the independent association (and effect size) of (i) mono- and (ii) multi-segmental BIA-derived body composition indexes (e.g., total fat, visceral fat, fat-free mass, and indexes) with the arterial system status. To this end, we have analyzed in a large sample of healthy children, adolescents, and adults (ii) several arterial pathways (elastic, transitional, and muscular; central and peripheral) and (iii) complementary hemodynamic, structural, and functional arterial parameters, using different non-invasive approaches (always considering the “gold standard” if available). The main findings can be summarized in six points, as follows:

- First, non-specific (classical) anthropometric indexes (i.e., BW, BMI, BMR) showed a high level of association with the structural, functional, or hemodynamic cardiovascular characteristics of the subject (z-scores). Furthermore, regardless of the classical index considered, the levels of association showed a specific hierarchy order: diameters and local arterial stiffness > BP (aortic and brachial) > regional arterial stiffness.
- Second, the joint association analysis of fat mass (i.e., BFM, PBF, FMI, VFL) and fat-free mass indexes (i.e., FFM, FFMI) with cardiovascular z-scores showed that all significant associations were positive. In other words, the higher the levels of body composition indexes, the higher levels of z-score (i.e., a greater starting point from the MV that is expected for a subject of similar age, who is not exposed to traditional CRFs).

Our study provides further evidence about the relationship between arterial characteristics (i.e., functional, structural, and hemodynamic properties) and body composition variables (i.e., FM and FFM indexes). Although there are no studies that jointly analyze BIA-derived body composition variables with arterial properties, some studies have analyzed them individually. Our results share similarities with the findings reported by Czernichow et al. (35) who reported an association between CCA IMT and body composition variables (i.e., BMI, PBF, FM, and FFM). Although, in our analysis, the structural arterial characteristics were the ones showing the highest levels of association, functional arterial parameters (mainly regional arterial stiffness) were not significantly explained by anthropometric or composition variables. These findings further support the aforementioned study, as no associations were observed by the authors between both FM and FFM and regional arterial stiffness after adjusting for covariates (35).

When analyzing the PBF and FMI individually, our results show that FMI had a stronger association with the arterial

parameters than PBF. This finding was in line with Ortega et al. (10) and confirms previous studies that reported that FMI was a better predictive index than PBF for both metabolic syndrome and cardiovascular mortality (10, 36). Furthermore, FMI was shown to be strongly associated with high BP and arterial stiffness in children, adolescents (11, 37), and adults (38).

The joint analysis of FM and FFM also showed that these indexes were meaningfully associated with arterial characteristics, and, in turn, increased levels of these indexes could be indicators of elevated cardiovascular risk. Indeed, Ortega et al. showed prospectively that higher levels of FM and FFM were also predictors of greater cardiovascular risk (10). Nevertheless, these findings differ from previous results reported in the literature, showing that higher levels FFM were protective, associated with a decreased mortality risk (39, 40).

- Third, simple correlation analysis showed that fat-free mass indexes exceed the association obtained with BMI and BMR, considering structural arterial z-scores. In contrast, fat mass indexes do not exceed the association with z-scores achieved by BMI and BMR.

This work adds further data to that reported by Ortega et al. (10), demonstrating that FFM and BMI may be complementary parameters. Interestingly, the independent association between both FFM and BMI and cardiovascular z-scores showed that the strength of these associations depended on the cardiovascular parameter considered. For instance, FFM exceeds BMI in z-aoSBP but not in z-baSBP. In addition, the investigators also reached similar conclusions in the sense that BMI increases according to an excess of FM plus FFM (10). This might confirm the strong association shown between BMI and cardiovascular properties, as well as its ability to predict cardiovascular mortality. Obese populations are also characterized by an increased FFM (that might be partially explained by higher blood volume) and might lead to the need of higher stroke volumes and cardiac outputs to match metabolic demands than non-obese peers. Those characteristics might represent an extra burden for the cardiovascular system, increasing the risk of heart disease (41, 42). In fact, not only the excess of FM is considered a CRF, but also FFM (43–45). In this regard, FFM has been considered a significant determinant of BP (44, 46), regional arterial stiffness (46), CCA IMT, and lumen area (35, 47). As previously mentioned, these findings are in contradiction with previous results, which did not show significant associations between FFM and structural parameters (e.g., IMT) and cardiovascular risk (39, 40, 48).

- Fourth, multivariate analysis indicated that, regardless of age, sex, CRFs, and classic anthropometric indexes (i.e., BMI, BMR, BW, BH), variations in cardiovascular z-scores can be explained by levels of FFMI, VFL, and PMM. Independently of both CRFs and classical indexes, FFMI explains mostly the inter-individual variations in (i) CCA IMT, (ii) diameters, and local arterial stiffness regardless of the arterial type and (iii) aoSBP.

It is worth mentioning that, in multiple linear regression models, variations in structural z-scores were mainly explained by either FFMI or z-aoSBP regardless of age, sex, presence of CRFs, and classical anthropometric indexes. In fact, variations in FFMI are able to explain variations in BP levels and CCA, CFA, and BA diameters. Our data point toward an association between FFMI and impaired arterial properties, which is in line with some but not all, recent reports. For instance, FFMI was strongly associated with cardiovascular conditions, such as hypertension, peripheral and coronary artery disease in adults aged 40–69 years (49). Interestingly, in a cross-sectional study of healthy Chinese children and adolescents $n = 1,609$, median age and interquartile range: 12.86 and 5.31 years, respectively (57.6% girls)], He et al. found that the effect size of the association between body composition and baBP differed in different age ranges (12). Accordingly, FFMI was positively associated with baSBP in 9–12 years and in 15–16 years age ranges but was not significantly associated with baDBP in any age range. Verma and Sinah. (50) reported similar results as previously mentioned in a randomized cross-sectional study in children and adolescents ($n = 733$; 10–18 years). In this study, FFMI and FMI were both positively correlated with BP, being FFMI the parameter that correlated most strongly with baBP.

Our findings significantly differ from previously published data, which showed a stronger association between FMI and baBP rather than with FFMI (51). It should be noted that, in this study, the anthropometric assessment differed from what was used by other investigators. More specifically, fat percentage, FMI, and FFMI were calculated from skinfolds thickness assessment rather than from bioelectrical impedance analysis (51).

- Fifth, regardless of the body segment considered (trunk, lower and upper limbs), levels of association between FMI and cardiovascular z-scores did not exceed those found with both classic anthropometric and fat-free mass indexes. However, total body fat mass and trunk indexes [T-FMI and FMI (IB)] showed a greater strength of association with cardiovascular z-scores than the FMI of upper and lower limbs.

Our data suggest that variations in total body fat mass or central fat mass (trunk) are associated (albeit weakly) with changes in arterial properties. Evidence has suggested that trunk fat mass as well as abdominal obesity should be considered as real CRFs (35, 37, 52, 53). Indeed, trunk fat mass and abdominal fat have shown a strong correlation between each other in adult women (54). Our results are also in line with previous findings where higher arterial stiffness was associated with high-trunk FMI in children, adolescents, and adults (11, 55, 56). Furthermore, it has been found that adolescents with higher fat trunk levels demonstrated a higher risk of developing cardiovascular disease at 26 and 36 years (57, 58). Considering that arterial stiffness is an early marker of atherosclerotic disease, the distribution of body fat (mainly in trunk and abdomen) becomes relevant in the stratification of cardiovascular risk (35, 52). Indeed, central fatness has been recognized as a primary risk

factor in cardiometabolic dysfunction (37) and an independent determinant of vascular health (55).

- Sixth, total (whole body) and upper limbs FFMI showed a higher level of association with z-diameters, z-IMT, z-local stiffness, and z-BP (surpassing almost all cardiovascular z-scores except for z-crPWV and PWV ratios) than lower limb FFMI indexes.

Although several studies have shown that fat distribution might be as relevant as total fat mass in stratifying the cardiovascular risk (11, 55), to our knowledge, no studies have found differences in FFMI of upper and lower limbs in relation to vascular properties. Accordingly, while increased levels of lower limb fat mass would work as a protective factor of cardiovascular disease in children, adolescents, and adults (53), increased arm fat mass was strongly associated with CRFs in women (59). Further studies, considering segmental body composition characteristics and cardiovascular properties are needed to further clarify these observations.

Importance of Results in Clinical and Epidemiological Settings

From data obtained in healthy children, adolescents and adults, our work provides evidence on which BIA-derived indexes have the highest independent levels of association with inter-subject hemodynamic, structural, and functional arterial variabilities (deviation from expected values). Knowing to what extent BIA-derived indexes are associated with arterial properties, with independence on other subjects' characteristics and exposure to CRFs (e.g., age or sex), would be useful to define the values of arterial properties expected in association with (explained by) data obtained on body composition. This information would be of value in both the research field (e.g., when selecting variables to assess in epidemiological studies aimed at evaluating the relationship between body composition and cardiovascular health) and clinical practice (e.g., to analyze the health impact of certain conditions and/or interventions on body composition). In this regard, it would be particularly important for professionals involved in physical activity and health (e.g., in the field of nutrition, exercise/sports, medicine) to know BIA-derived body-composition indexes and/or parameters with the greatest predictive capacity for cardiovascular status, since that would be useful in terms of assessment, diagnosis, definition of interventions' objectives and strategies (as well as in their evaluation and follow-up). In this regard, the following findings and contributions should be considered.

First, regardless of other subjects' characteristics, in children, adolescents, and adults, FFMI, VFL, and PMM were the BIA-derived indexes independently associated with arterial characteristics. This adds support to the proposal that, in healthy subjects, from the general population, fat-free mass-related indexes would be equally or even more valuable in terms of a predictive capacity when compared to classical anthropometric and fat mass indexes. Then, interventions (e.g., physical training) aimed at modifying (specifically) muscle mass levels and, consequently, FFMI and/or PMM could impact positively and

directly (independently) the cardiovascular system. Therefore, being aware of which and to what extent variations in body composition during actions aimed at improving physical fitness (e.g., physical activity and/or dietary programs) are associated with cardiovascular health could contribute to improved professional performance.

Second, structural characteristics of central arteries (i.e., CCA, IMT, and diameters) would be the most sensitive to variations (differences) in BIA-derived indexes (e.g., FFMI). Therefore, analysis of central (e.g., CCA) rather than peripheral (e.g., CFA and BA) arteries would be more valuable for tracking differences in arterial characteristics associated with body composition indexes. In turn, "local" arterial stiffness parameters (e.g., CCA EM) would be more sensitive than the "regional" ones in terms of association with variations in BIA-derived body composition indexes. In this regard, it is noteworthy that we found that regional arterial stiffness assessed (as in several clinical studies) through the cfPWV was not strongly associated with variations in body composition and would not be considered of choice when assessing the association of BIA-derived body composition indexes and cardiovascular status. The above add to the proposal that body composition would not homogeneously impact the arterial system but would differentially affect the arterial territories and properties. Consequently, when analyzing and discussing the impact of body composition on arterial function (e.g., arterial stiffness), it is necessary to specify the territory and parameter evaluated.

Finally, "classic" (e.g., BMI) and "new" indexes (e.g., BIA-derived FFMI) could provide complementary explanatory information. Thus, both types of indexes should not be seen in all cases as "competitors." The above reinforces the value of classical anthropometric measurements and BIA-derived recordings to comprehensively assess the association between body composition and arterial characteristics.

Strengths and Limitations

This work has strengths and limitations that should be considered. First, our study included a comprehensive non-invasive evaluation of arterial properties (including analysis of different histological types of arteries), obtained from a large population sample of children, adolescents, and adults. Second, the number of subjects and the statistical approach (e.g., a bootstrapping technique) were designed to increase the reliability and to analyze the association between BIA-derived body composition indexes and cardiovascular characteristics with independence of other CRFs, classical anthropometric indexes, and regardless of other body composition indexes. However, although we adjusted for several covariates, we cannot rule out the possibility of residual confounding factors that could have influenced our results. Third, having a reference group enabled us to determine through mathematical adjustments the MV, SD, and variations in cardiovascular z-scores. Since the reference group included Uruguayan children, adolescents, and adults non-exposed to CRFs, we avoided using bibliographical data from subjects who do not necessarily present characteristics similar to those of the Uruguayan population. Fourth, body composition data were corroborated using two validated BIA

devices (InBody-120; OMRON-HBF514C), which showed a good concordance correlation (60–62).

We are aware that our research may have limitations: First, it is a cross-sectional study, so the causal relationship between BIA-derived body composition indexes and cardiovascular properties could not be explored. Second, information on the waist-hip ratio and neck circumferences was not included since there was no reliable information for all the subjects. Third, the body composition assessment was performed by BIA devices, a technology which is not considered the “gold standard” method for measuring body composition such as dual-energy X-ray absorptiometry or magnetic resonance imaging. Yet, these advanced imaging modalities are more expensive and operator dependent. Nowadays, BIA devices are a low cost and reliable method widely used in clinical and epidemiological settings to measure FM and FFM parameters (63) (**Supplementary File 1**). Finally, despite the fact that two commercial BIA devices (validated and widely used) were used in the present study, it is worth noting that the equations that allowed BIA-derived indexes to be obtained were not derived from studies in the Uruguayan population. As the other authors have done for specific populations in South America (64, 65), further studies will allow the equations for obtaining BIA-derived indexes to be evaluated and validated in the Uruguayan population.

CONCLUSIONS

First, non-specific (classical) anthropometric indexes (BW, BMI, BMR) showed a high association with cardiovascular z-scores. Furthermore, regardless of the classical index considered, the levels of association showed a specific hierarchy order: diameters and local arterial stiffness > BP (aortic and brachial > regional arterial stiffness).

Second, the joint association analysis between both fat mass and fat-free mass indexes and cardiovascular z-scores showed that all significant associations were positive. The higher the levels of these indexes, the greater the deviation toward positive values of arterial characteristics (e.g., higher CCA IMT, DD, and/or local stiffness).

Third, fat-free mass indexes exceeded the association obtained with BMI and BMR, considering structural arterial z-scores. In contrast, fat mass indexes did not exceed the association with z-scores achieved by BMI and BMR.

Fourth, regardless of age, sex, classical CRFs and anthropometric indexes, variations in arterial z-scores can be mainly explained by levels of (i) FFMI, (ii) VFL, and (iii) PMM. FFMI explains mostly inter-individual variations in (i) CCA IMT, (ii) diameters and local arterial stiffness regardless of the arterial type, and (iii) aoSBP.

Fifth, regardless of the body segment considered (trunk, lower and upper limbs), levels of association between FMI and arterial z-scores did not exceed those found with both classic anthropometric and fat-free mass indexes. However, total body fat mass and trunk indexes showed a greater strength of

association with cardiovascular z-scores than the FMI of upper and lower limbs.

Sixth, total and upper limb FFMI showed a higher level of association with z-diameters, z-IMT, z-local stiffness, and z-BP than lower limb FFMI indexes.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comités de Ética del Hospital de Clínicas, Instituto Superior de Educación Física, and Centro Hospitalario Pereira-Rossell (Universidad de la República; Uruguay). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

MG-G, JT, DB, and YZ contributed to conception and design of the study, performed the anthropometric, body composition, and cardiovascular non-invasive recordings, constructed and organized the database, and performed the statistical analysis. MG-G, JT, MP, DB, and YZ wrote the first draft and final version of the manuscript, contributed to the manuscript revision, read, and approved the submitted version. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnut.2022.856198/full#supplementary-material>

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Bio-Electrical Impedance Analysis: A Valid Assessment Tool for Diagnosis of Low Appendicular Lean Mass in Older Adults?

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Background: The diagnosis of sarcopenia is essential for early treatment of sarcopenia in older adults, for which assessment of appendicular lean mass (ALM) is needed. Multi-frequency bio-electrical impedance analysis (MF-BIA) may be a valid assessment tool to assess ALM in older adults, but the evidences are limited. Therefore, we validated the BIA to diagnose low ALM in older adults.

Methods: ALM was assessed by a standing-posture 8 electrode MF-BIA (Tanita MC-780) in 202 community-dwelling older adults (age ≥ 55 years), and compared with dual-energy X-ray absorptiometry (DXA) (Hologic Inc., Marlborough, MA, United States; DXA). The validity for assessing the absolute values of ALM was evaluated by: (1) bias (mean difference), (2) percentage of accurate predictions (within 5% of DXA values), (3) the mean absolute error (MAE), and (4) limits of agreement (Bland–Altman analysis). The lowest quintile of ALM by DXA was used as proxy for low ALM (< 22.8 kg for men, < 16.1 kg for women). Sensitivity and specificity of diagnosing low ALM by BIA were assessed.

Results: The mean age of the subjects was 72.1 ± 6.4 years, with a BMI of 25.4 ± 3.6 kg/m², and 71% were women. BIA slightly underestimated ALM compared to DXA with a mean bias of -0.6 ± 1.2 kg. The percentage of accurate predictions was 54% with a MAE of 1.1 kg, and limits of agreement were -3.0 to $+1.8$ kg. The sensitivity for ALM was 80%, indicating that 80% of subjects who were diagnosed as low ALM according to DXA were also diagnosed low ALM by BIA. The specificity was 90%, indicating that 90% of subjects who were diagnosed as normal ALM by DXA were also diagnosed as normal ALM by the BIA.

Conclusion: This comparison showed a poor validity of MF-BIA to assess the absolute values of ALM, but a reasonable sensitivity and specificity to recognize the community-dwelling older adults with the lowest muscle mass.

Keywords: aging, lean body mass, muscle, nutritional assessment, sarcopenia

INTRODUCTION

Approximately 16% of the world population will be older than 65 years in 2050, and the number of persons older than 80 years will increase almost threefold between 2019 and 2050 (1). As society ages, the number of people facing physical disabilities due to co- and multi-morbidity will increase as well. Globally, over 45% of older adults aged 60 and over experience disabilities and physical limitations (2). A key contributor to these physical limitations is the reduction in skeletal muscle mass and strength, also referred to as sarcopenia (3, 4). Sarcopenia is defined as a skeletal muscle disorder that involves the accelerated loss of muscle mass and function (5). Sarcopenia also increases the risk for chronic diseases such as type II diabetes and obesity (6, 7) and is associated with fall incidence, institutionalization, dependence, and poor quality of life (8). Moreover, sarcopenia will increase the demand of our healthcare system and will result in tremendous healthcare costs (9).

To reduce these negative outcomes, screening for sarcopenia in clinical practice is of major importance (10). It allows the professional to identify those people at risk for negative outcomes and to intervene with nutritional and exercise strategies to prevent or counteract sarcopenia (11–14).

To assess sarcopenia, various criteria are proposed (3, 4, 8, 15–17). The majority of those criteria (8, 15–17) use dual-energy X-ray absorptiometry (DXA) to quantify appendicular lean mass (ALM). As such, DXA is considered as the reference standard to assess ALM. In practice, however, DXA is not often used as it is too expensive and needs safety precautions and proper training. In practice, bio-impedance analysis (BIA) is suggested as a practical alternative for DXA to diagnose sarcopenia (7, 8, 10, 16, 18). BIA is cheap, fast, and may provide an easy-to-use tool for professionals for diagnosis (19). The validity of BIA, however, is often discussed (7, 8, 10, 20, 21). Several studies assessed the validity of BIA against DXA only validating fat-free mass (FFM) (22–24). To date, only limited studies are available, which validate the ALM assessment by BIA against DXA (10, 25–29); and even few studies are available, which validate the diagnosis of low ALM by BIA against DXA (27, 30). The use of multi-frequency BIA (MF-BIA) including standing position is, furthermore, scarce with ALM validation (21, 31). Therefore, we aim to validate the assessment of ALM as well as the detection of low ALM by MF-BIA against DXA in older adults.

MATERIALS AND METHODS

Subjects

Baseline data of subjects in the VITAMIN (Vital Amsterdam older adults in the city) study (32, 33) were included in the present analysis. At baseline, the ALM and FFM of all subjects are assessed by both DXA and BIA. These subjects were recruited at community-based weekly exercise programs and by a mailing to community-dwelling inhabitants of Amsterdam and its surroundings. Subjects were included in the VITAMIN trial when they were 55 years or older, were able to understand the Dutch language, and were excluded when

they were cognitively impaired [mini-mental state examination (MMSE) < 15], had a knee or hip surgery in the past 6 months, or had current alcohol or drug abuse in the opinion of the investigator. A full description of the eligibility criteria is online available in the Dutch Trial Register (NL5472/NTR5888¹). The study was approved by the Medical Ethics Committee VUmc, Netherlands (Protocol ID: VUMC2016_025), and the written informed consent was obtained. All assessments were performed at the Amsterdam Nutritional Assessment Center (ANAC) in the Amsterdam University of Applied Sciences (AUAS) in Netherlands.

Anthropometry

Subjects were asked to come to the baseline visit in a semi-fasted state (5-h fasted, 2-h no drinks). Before the measurement routine, they went to changing room and removed their clothes (including jewelry and removable aids). This protocol provides high accuracy in follow-up measurements (19). Bodyweight was measured on a calibrated scale (Bodpod, Life Measurement Concord, United States). Height was measured to the nearest cm by using a wall-mounted stadiometer (Seca 222; Seca, Germany).

Bio-Impedance Analysis

The ALM and FFM both were assessed in a semi-fasted state by the Tanita MC-780MA (2015, Tanita Corporation, Japan). This Tanita MC-780MA is an 8 electrode multi-frequency (5 kHz/50 kHz/250 kHz) segmental body composition analyzer that predicts ALM and FFM from resistance and reactance (23). Some research is available on the validity of the device in healthy subjects and patients (34, 35). Impedance measurement includes whole body and limb segments, working with a constant current source (~90 A) with a high frequency (50 kHz). Anthropometry data concerning the subject (age, gender, and height) were entered by the accessor in the GMon Health Monitor software. By mounting the BIA scale, body weight was measured, and by holding the two handles with arms separated from the trunk, body composition analysis was performed in 1 min. All BIA measurements followed standard operating procedures. ALM-index (ALMi) and FFM-index (FFMi), our secondary parameters besides FFM, were calculated by dividing ALM and FFM by height in meters squared.

Dual Energy X-Ray Absorptiometry

The body composition was assessed by DXA (Hologic Inc., MA, United States) and the Apex software (version 5.5.3; Hologic Inc., Bedford, MA, United States). ALM and FFM were used for the analyses. ALM is the sum of the FFM minus bone mass in arms and legs; and FFM is the total fat-free mass including bone mass. The DXA was calibrated daily with a phantom. After lying down on the DXA table, the subject's feet were fixed to ensure a steady and correct position. Additionally, their arms were separated from the trunk by material which does not affect the DXA image and analysis. This procedure improves the segmentation analysis of the DXA image. Successively, the whole body DXA scan was performed in 3 min. If subjects didn't fit the DXA table because of

¹www.trialregister.nl

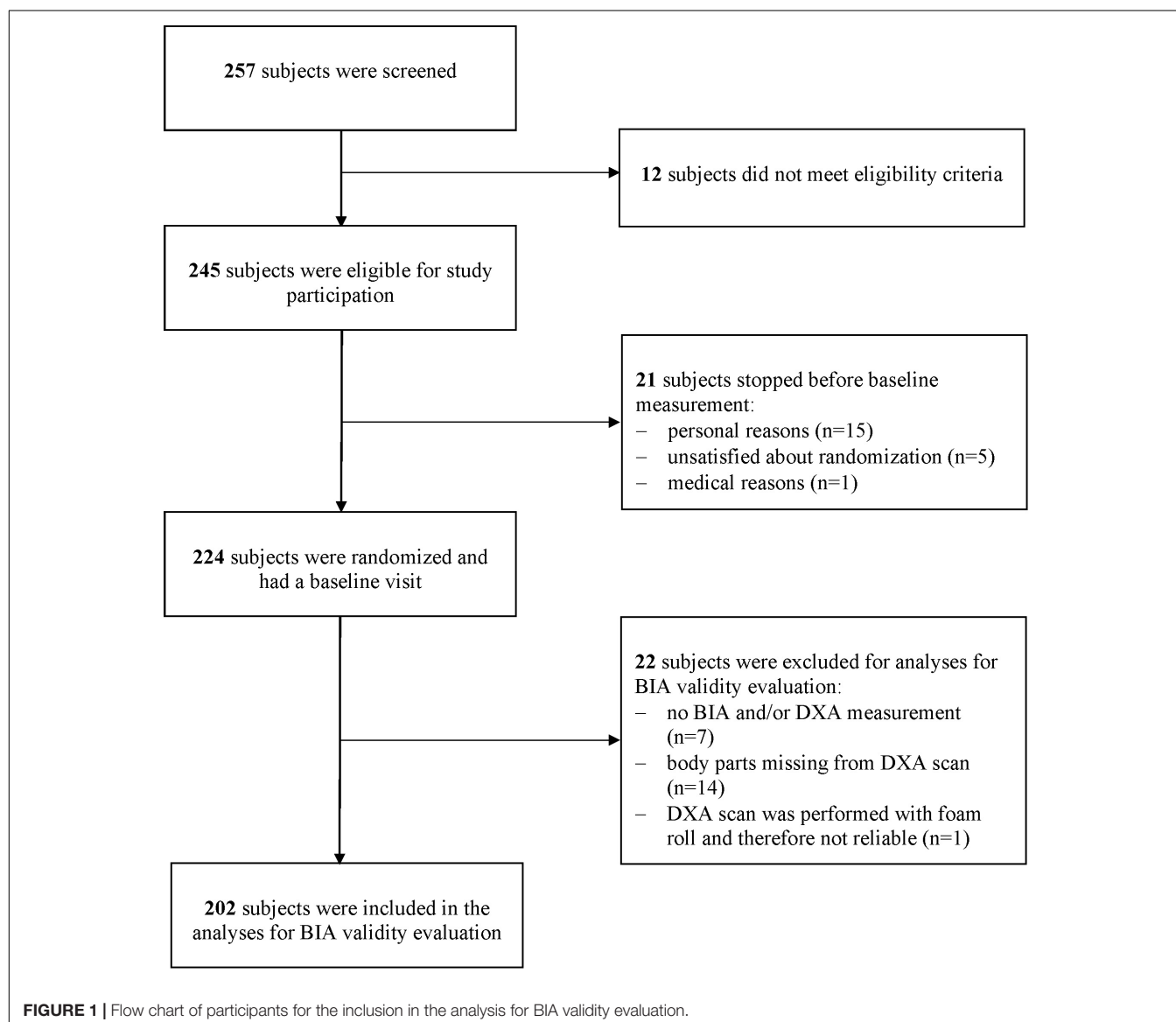
their body size, one side of the body was duplicated for analysis. The automatic segmentation of the whole body scan, thus arms and legs for ALM, was adjusted manually with the use of Hologic software (36). All the DXA measurements and segmentation analysis were performed by the same two trained and certified researchers [CD, JH] (37, 38).

Statistical Analysis

The double-data entry was performed and discrepancies were checked and adjusted. The primary parameter was ALM and the secondary parameters were ALMi, FFM, and FFMi. The validity for assessing absolute values of these parameters was evaluated by: (1) the bias, which is the mean difference between the two methods. (2) The percentage of accurate predictions, which is defined as a value within 5% of DXA values [this is consistent with technical measurement errors of 5% or less (39)]. If the BIA value was below 95% of the DXA value, the BIA value was defined

as an underestimation, and a value above 105% was defined as an overestimation. (3) The mean absolute error, which is presented to give insight on the average absolute deviation in kg (ALM and FFM) or kg/m² (ALMi and FFMi) between the two methods (40). (4) The limits of agreement analysis (Bland–Altman analysis) and proportional bias.

The lowest quintile (20%) of ALM (and the secondary parameters) by DXA was used as proxy for low ALM. Sensitivity and specificity of diagnosis a low ALM by BIA were assessed. The derived cut-offs from the lowest quintile were processed by cross tables to identify the Sensitivity (e.g., the true positive rate for both low ALM DXA and BIA) and Specificity (e.g., the true negative rate for both normal ALM DXA and BIA). In an additional analysis, subjects that were not classified as low ALM (whereas they were according to DXA) were compared to those that were correctly classified as low ALM to identify potential differences in subject characteristics.



The data analyses were performed for the total group and for men and women separately, since regression analysis with outcomes, FFM and FFMi by DXA showed a significant interaction between gender and FFM and FFMi by BIA ($p < 0.10$). No significant interaction was found between gender and ALM and ALMi, but for consistency reasons, all analyses are both presented for the total group and for men and women separately. Bland–Altman was checked for proportional bias, by evaluation of significant slopes for the regression lines. The statistical analyses were performed using the SPSS software (version 25.0, IBM). Mean absolute errors were calculated in MS Excel 2013.

RESULTS

Subject Characteristics

Of the 224 subjects included in the trial, 202 subjects were used in the present data analysis. Twenty-two subjects could not be included due to missing or poor quality of BIA and/or DXA assessments (Figure 1). The mean age of the subjects was 72.1 ± 6.4 year, with a BMI of 25.4 ± 3.6 kg/m² and 71% were women. Average time of the last drink until the assessment was 7.7 ± 4.9 h. Based on the EWGSOP2, ALMi-cutoffs (8) prevalence of sarcopenic low ALM (assessed by DXA) was 9% in men (5 out of 58) and 10% in women (15 out of 144) (Table 1).

Validity of Bio-Impedance Analysis

The evaluation of the validity of the BIA to assess ALM, ALMi, FFM, and FFMi is presented in Table 2. Overall, BIA slightly underestimated ALM compared to DXA with a mean bias of -0.60 ± 1.21 kg. The percentage of accurate predictions was 54% for all subjects.

Scatter plots and Bland–Altman plots in Figures 2, 3 visualize the agreement between BIA and DXA for all parameters. The limits of agreement for ALM were -3.0 to $+1.8$ kg. The Bland–Altman analysis shows proportional bias for women in ALM, with a significant negative β for BIA–DXA difference compared to the mean of BIA and DXA [ALM women $\beta = -0.16$ ($SE = 0.04$), $p < 0.001$]. This indicates a great underestimation by BIA for larger values of ALM in women, and overestimation appears in the smaller values of ALM. No significant proportional bias for men was present for ALM. See Figure 3 for additional results on the proportional bias.

Sensitivity and Specificity for Diagnosis of Sarcopenia

Sensitivity and specificity percentages for all parameters are presented in Table 2. In total 39 subjects (11 men and 28 women) were defined as low ALM by using the lowest quintile of ALM by DXA as cut-off. Sensitivity for detecting low ALM using by BIA was 80%, indicating that 80% of subjects that had low ALM according to DXA were also diagnosed as low ALM by BIA. Specificity was 90%, indicating that 90% of subjects who were diagnosed as normal ALM by DXA were also diagnosed as normal ALM by the BIA.

In an additional analysis, subjects that were incorrectly classified as low ALM were compared to those that were correctly classified as low ALM to identify potential differences in subject characteristics. A total of 7 out of 28 women were incorrectly diagnosed as normal ALM by BIA (while DXA diagnosed them as low ALM). These women had a significantly higher BMI (25.5 ± 3.9 vs. 22.4 ± 2.5 kg/m², $p = 0.026$) and fat percentage (37.6 ± 6.1 vs. $33.2 \pm 4.3\%$, $p = 0.044$) than women that were correctly diagnosed as low ALM by BIA (21 out of 28) (see Supplementary Figure 1). For men, this analysis could not be

TABLE 1 | Baseline characteristics of 202 older subjects of the VITAMIN-trial¹.

	All subjects (N = 202)		Males (N = 58)		Females (N = 144)	
	Mean \pm SD	Range ¹	Mean \pm SD	Range	Mean \pm SD	Range
Age (y)	72.1 \pm 6.4	55–89	72.0 \pm 6.0	60–89	72.1 \pm 6.6	55–88
Low education level (%) ²	21%		17%		22%	
BMI (kg/m ²)	25.4 \pm 3.6	16.8–39.2	25.2 \pm 3.0	20.2–32.8	25.6 \pm 3.8	16.8–39.2
Fat percentage (% , by DXA)	32.0 \pm 6.4	17.3–46.0	24.9 \pm 3.1	19.3–31.9	34.8 \pm 4.9	17.3–46.0
Handgrip strength (kg) ³	29.4 \pm 10.6	6.7–66.2	41.3 \pm 10.0	23.0–66.2	24.6 \pm 6.1	9.8–41.3
Gait speed (m/s) ⁴	1.34 \pm 0.36	0.48–2.08	1.47 \pm 0.43	0.75–2.80	1.29 \pm 0.32	0.48–2.13
Waist circumference (cm)	89.1 \pm 10.4	65–123	95.0 \pm 8.7	79–112	86.7 \pm 10.1	65–123
Waist/hip ratio	0.88 \pm 0.08	0.69–1.14	0.96 \pm 0.06	0.82–1.14	0.85 \pm 0.06	0.69–1.05
ALM (kg, by DXA)	20.4 \pm 4.2	13.5–35.3	25.6 \pm 3.1	19.1–35.3	18.3 \pm 2.4	13.5–25.4
FFM (kg, by DXA)	49.9 \pm 8.9	34.5–78.3	60.5 \pm 7.8	47.0–78.3	45.6 \pm 5.3	34.5–62.3
ALMi (kg/m ² , by DXA)	7.2 \pm 1.0	5.3–11.3	8.2 \pm 0.9	6.6–11.3	6.8 \pm 0.7	5.3–9.8
FFMi (kg/m ² , by DXA)	17.7 \pm 2.1	13.6–24.2	19.9 \pm 1.5	17.2–24.2	16.8 \pm 1.6	13.6–22.9
Sarcopenic low ALMi (%) ⁵			9%		10%	

¹Range is presented in minimum to maximum value.

²Low education level is defined as highest finished education is primary or secondary school.

³Average handgrip strength of the dominant hand.

⁴Measured by a 3-meter gait speed test, fastest of 2 repetitions.

⁵Based on the EWGSOP2 ALMi-cutoffs (male < 7.0 kg/m² | female < 6.0 kg/m²).

TABLE 2 | Evaluation of the validity of appendicular lean mass (ALM), fat free mass (FFM), ALM-index (ALMi), and FFM-index (FFMi), including sensitivity and specificity of diagnosing low muscle mass, assessed by BIA with DXA as reference¹ in 202 subjects of 55 years and older.

	ALM (kg)	ALMi (kg/m ²)	FFM (kg)	FFMi (kg/m ²)
All subjects (N = 202)				
Reference by DXA [mean (± SD)]	20.4 ± 4.2	7.2 ± 1.0	49.9 ± 8.9	17.7 ± 2.1
Mean (± SD)	19.8 ± 3.8	7.0 ± 0.9	50.1 ± 9.4	17.7 ± 2.1
Mean Bias ² (± SD)	−0.60 ± 1.21	−0.20 ± 0.43	0.24 ± 2.31	0.06 ± 0.80
Mean Bias in% (± SD)	−2.5 ± 5.8	−2.5 ± 5.8	0.4 ± 4.6	0.4 ± 4.6
Mean abs. error (kg or kg/m ²)	1.1	0.4	1.7	0.6
Accurate predictions ³ (%)	54.0	54.0	77.7	77.7
Under predictions ⁴ (%)	37.6	37.6	9.4	9.4
Over predictions ⁵ (%)	8.4	8.4	12.9	12.9
Sensitivity% ⁶	79.5%	74.4%	76.9%	59.0%
Specificity% ⁷	89.6%	84.0%	93.3%	92.6%
Males (N = 58)				
Reference by DXA [mean (± SD)]	25.6 ± 3.1	8.2 ± 0.9	60.5 ± 6.8	19.3 ± 1.9
Mean (SD)	24.5 ± 2.9	7.8 ± 0.8	62.5 ± 5.7	19.9 ± 1.5
Mean Bias ² (SD)	−1.1 ± 1.2	−0.4 ± 0.4	2.0 ± 2.1	0.6 ± 0.7
Mean Bias in% (SD)	−4.2 ± 4.5	−4.2 ± 4.5	3.6 ± 3.7	3.6 ± 3.7
Mean abs. error (kg or kg/m ²)	1.3	0.4	2.5	0.8
Accurate predictions ³ (%)	50.0	50.0	63.8	63.8
Under predictions ⁴ (%)	46.6	46.6	0	0
Over predictions ⁵ (%)	3.4	3.4	36.2	36.2
Sensitivity% ⁶	90.9%	100%	54.5%	36.4%
Specificity% ⁷	89.4%	83.0%	100%	100%
Cut-off DXA value ¹ (kg or kg/m ²)	22.8	7.4	55.0	17.5
Females (N = 144)				
Reference by DXA [mean (± SD)]	18.5 ± 2.6	6.9 ± 0.8	46.2 ± 6.1	17.1 ± 1.9
Mean (SD)	18.1 ± 2.3	6.7 ± 0.7	45.7 ± 5.7	16.9 ± 1.8
Mean Bias (SD) ²	−0.4 ± 1.2	−0.1 ± 1.4	−0.5 ± 2.1	−0.2 ± 0.8
Mean Bias in% (SD)	−1.8 ± 6.2	−1.8 ± 6.2	−0.9 ± 4.4	−0.9 ± 4.4
Mean abs. error (kg or kg/m ²)	1.0	0.4	1.5	0.5
Accurate predictions ³ (%)	56.9	56.9	83.3	83.3
Under predictions ⁴ (%)	32.6	32.6	13.2	13.2
Over predictions ⁵ (%)	10.4	10.4	3.5	3.5
Sensitivity% ⁶	75.0%	64.3%	85.7%	67.9%
Specificity% ⁷	89.7%	84.5%	90.5%	89.7%
Cut-off DXA value ¹ (kg or kg/m ²)	16.1	6.2	41.3	15.5

¹Low muscle mass diagnosed by ALM, FFM, ALMi or FFMi is defined as the lowest sex-specific quintile of each variable measured by DXA, N in the lowest quintile is 39 subjects: 11 for males and 28 for females.

²BIA value minus DXA value.

³The percentage of subjects predicted by this predictive equation within 5% of the DXA value.

⁴The percentage of subjects by this predictive equation < 5% of the DXA value.

⁵The percentage of subjects predicted by this predictive equation > 5% of the DXA value.

⁶Sensitivity is to be interpreted as the percentage of subjects with low ALM according to DXA that also had low ALM by BIA.

⁷Specificity is to be interpreted as the percentage of subjects with normal ALM according to DXA also had normal ALM (ALMi, FFM, or FFMi) with the BIA.

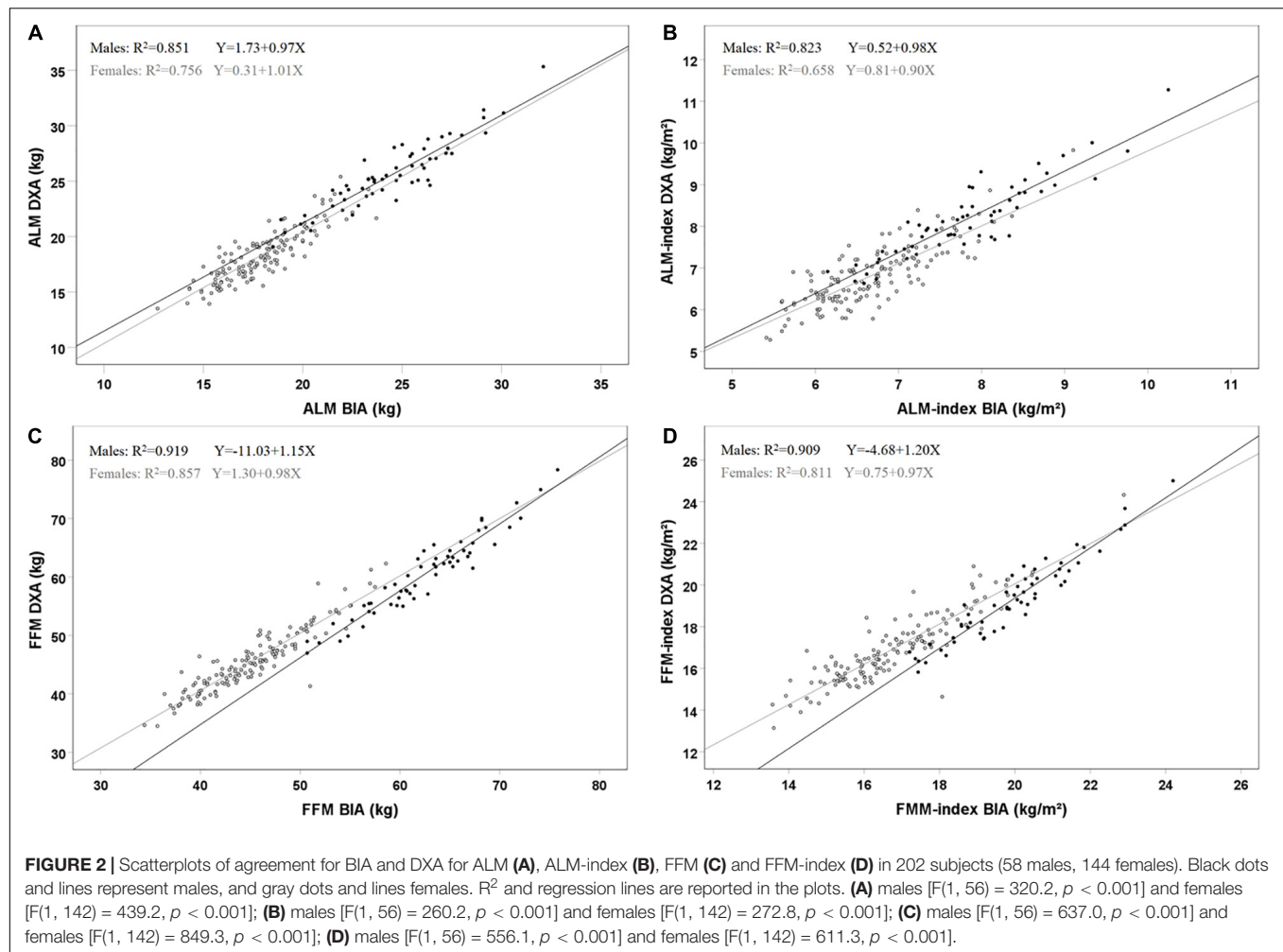
performed, since only 1 male (1 out of 11) was incorrectly diagnosed as low ALM by BIA.

DISCUSSION

In the present analyses, the validity of BIA against the DXA was assessed to quantify ALM and diagnose low ALM in community-dwelling older adults. Results showed a poor validity of BIA to assess absolute values of ALM, since the number of accurate predictions was low. Sensitivity and specificity for low ALM were reasonable and superior for ALM compared to ALMi, FFM, and FFMi. Additional analysis with a small sample revealed more overweight women as prone to be misdiagnosed as normal ALM while having low ALM, which is relevant for the assessment of sarcopenic obesity.

Sarcopenia, of which a low ALM is a key component, is known to markedly increase the risk of disability and loss of functional capacity and worsen clinical outcomes in older adults. To reduce these negative outcomes, screening for sarcopenia is of great importance (8), since the progress of sarcopenia can be slowed down or even reversed (41). ALM is needed to diagnose sarcopenia according to most definitions (4, 8, 15–17). Until now, several studies evaluated the validity of ALM by BIA in older adults (10, 24, 26–28). Contradictory to our study, most of these studies developed a new predictive equation by using the crude resistance and reactance values from the BIA device (10, 24, 26, 28). These studies did not evaluate the ability to diagnose sarcopenic low ALM in older adults. To our knowledge, only three studies are available that evaluated the sensitivity in diagnosing sarcopenia (27, 30, 42). Steihaug et al. (27) compared the validity of four BIA equations in estimating ALM in an older population 3 months after hip fracture. They showed sensitivity for estimating low ALM mass according to EWGSOP criteria (43) of 32–84% in men and 42–66% in women depending on the equation used. The sensitivity to assess sarcopenia in their study was lower for all equations compared to the sensitivity we found for the assessment of low ALM with the MF-BIA Tanita MC-780 (men 91%, women 75%). Both studies, Reiss et al. (30) and Sousa-Santos et al. (42) compared the DXA–BIA approaches with EWGSOP/EWGSOP2 cut-offs, and reported the accuracy in older adults. Although the sensitivity seemed comparable [55–70% in estimating reduced muscle mass, 69–92% in estimating sarcopenia (30), and 33–92% in diagnosing sarcopenia (42)], the methods differed. They handled with formulas and cut-offs as a reference, whereas we used the lowest quintile as reference. As well as gender was not taken into account. In general, the observed differences between studies might be attributed to the differences in cut-offs, device used (single/multi-frequency), position and the older population characteristics (42).

Regarding the device used, the study of Kim et al. (31) is the only comparable study with MF-BIA (InBody 720 vs. our Tanita MC-780MA) in older adults. Similar to our study, the authors found an overestimation of FFM and they found underestimation of whole-body LM. So far, our study is the only one that evaluates the validity of estimating absolute values



of ALM and the ability to diagnose low ALM by MF-BIA in community-dwelling healthy older adults.

As mentioned by Scafoglieri et al. and Walowski et al. (28, 44), BIA models have the tendency to overestimate ALM in sarcopenic older adults, and therefore, underestimate sarcopenia in obesity. Recent research confirms this overestimation of obese adults (45). Our study supports this, furthermore, our additional analysis revealed misdiagnosis among overweight women. This result needs to be interpreted with caution, because of the small sample. Nevertheless, a more extensive analysis of overweight older adults with a larger sample seems interesting for future research.

It should be noted that when comparing two methods, as is the case in all validation studies, there are always measurement errors in both methods. Not only measurement errors occur with the BIA, but the DXA has also measurement errors depending on the thickness of the tissue measured, the hydration status, and the calibration procedures (46). Furthermore, for measuring ALM with the DXA, a correct adjustment of the automatic segmentation has to be performed manually, which may introduce a measurement error. Since we use the DXA as the reference value, we can only demonstrate relative validity

compared to DXA. Although magnetic resonance imaging (MRI) and computed tomography (CT) are regarded as the golden standard, recent literature suggests that the DXA can be used as a reference to measure muscle mass (47).

Study Limitations

Some study limitations are important to mention: First, we studied a relatively healthy population of community-dwelling older adults. Subjects were recruited at community-based weekly exercise programs, so the population was performing exercise at least once a week. Therefore, our definition of sarcopenic low ALM based on the lowest quintile of ALM (or FFM, ALMi, or FFMi) was less strict compared to existing definitions for sarcopenia (4, 8, 15–17). Our cut-off DXA value vs. the EWGSOP2 (8) was slightly higher for men (22.8 vs. 20.0 kg) and for women (16.1 vs. 15.0 kg). At the lower end of ALM values, there were only a limited number of outliers and a fairly gradual increase across the whole ALM value range. Therefore, we do not expect a more strict 10% cut-off for low ALM to provide other results than this 20% cut-off. However, we can be more sure for women than men. Second, the overall sample size and the sample size for women are sufficient to perform the presented analysis,

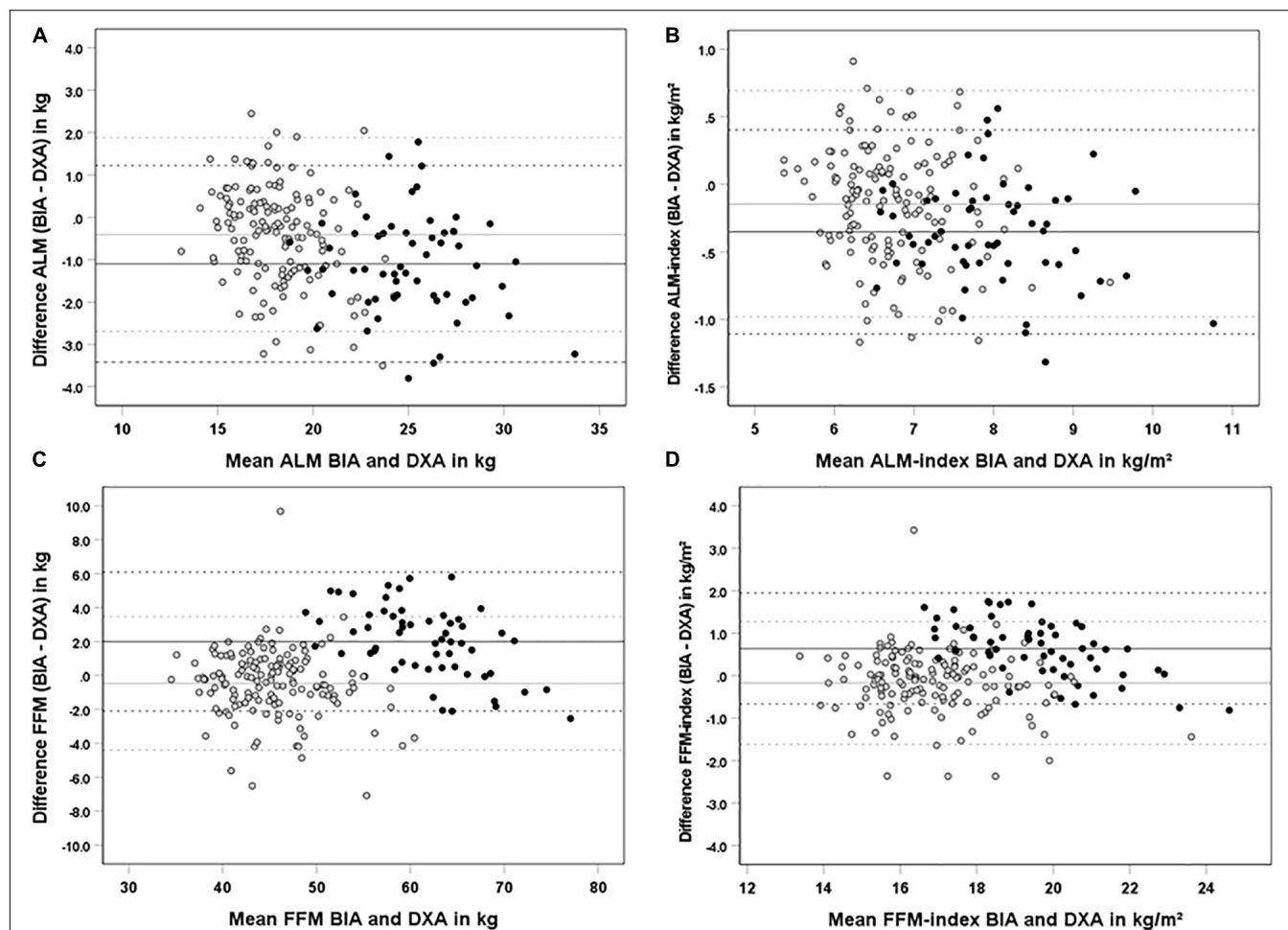


FIGURE 3 | Bland-and-Altman plots for agreement of BIA and DXA for ALM (A), ALM-index (B), FFM (C) and FFM-index in kg/m² (D) in 202 subjects (58 males, 144 females). Black dots and lines represent males, and gray dots and lines females. Continued lines represent the mean bias, dashed lines represent the limits of agreement [mean \pm (1.96 \times SD)]. Proportional bias is reported when the slope of the regression line was significant. (A) females $R^2 = 0.08$ | $Y = 2.41 - 0.16X$, $p < 0.001$; (B) females $R^2 = 0.03$ | $Y = 0.63 - 0.12X$, $p = 0.035$; (C) males $R^2 = 0.28$ | $Y = 13.1 - 0.18$, $p < 0.001$; (D) males $R^2 = 0.38$ | $Y = 5.29 - 0.24$, $p < 0.001$.

but the number of men in our study was low (48). Last, for this evaluation we used the formula of the Tanita MC-780MA itself, we did not use an existing validated equation based on measured resistance and reactance for a comparable population. Using the most appropriate equation among the equations available in the literature, taking into account nutritional, ethnic-related, and age-related characteristics of the sample in which the equation has been validated, might even improve the sensitivity of low ALM diagnosis (26).

Multi-frequency bio-electrical impedance analysis is a cheap and quick device and very easy to use in practical settings (19). However, our results show that the assessment of absolute values of ALM has to be interpreted with caution due to the low accuracy of prediction. Only just over 50% of our study population was correctly estimated. This study did not focus on the validity to detect changes over time. To evaluate the effectiveness of interventions aiming at preventing or counteracting sarcopenia, future studies should focus on the validity of the MF-BIA in detecting changes in ALM. For diagnosis of low ALM, the Tanita

MC-780MA MF-BIA seems reasonably accurate, but future studies need to confirm this in a population with lower muscle mass. Finally, future studies might include large samples of older adults in order to find optimal cut-offs for diagnosing low ALM by MF-BIA and support clinical practice.

In conclusion, our comparison showed a poor validity of this MF-BIA to assess absolute values of ALM, but a reasonable sensitivity and specificity to recognize the community-dwelling older adults with the lowest muscle mass. Regardless of reaching the sarcopenic cut-offs, the recognition of low ALM is an important step toward prevention of sarcopenia.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: DOI: 10.21943/auas.19165094.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by METc VU University Medical Center. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PW, MT, and JH designed the research (project conception, development of overall research plan, and study oversight). JH and CD conducted the research (hands-on conduct of the experiments and data collection). AV, JH, ME, PW, and MT analyzed the data or performed the statistical analysis. AV, JH, ME, PW, and MT wrote the manuscript. MT and PW had primary responsibility for the final content. All authors contributed to the article and approved the submitted version.

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Anthropometric Indicators of Body Composition Associated With Lipid and Glycemic Profiles in Overweight Brazilian Children and Adolescents From 2008 to 2020

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Background: Anthropometric indicators have been used to predict health problems. The objective was to determine which indicators present better correlation with dyslipidemia, hyperglycemia and peripheral insulin resistance, as well as the cutoff points capable of predicting lipid and glycemic alterations in Brazilian children and adolescents.

Methods: A cross-sectional study conducted with 568 overweight individuals, aged between 5 and 18 years, living in Southeast and South Brazilian regions, submitted to anthropometric and body composition evaluation by bioimpedance, in addition to fasting laboratory tests [total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), fasting glycemia, and homeostasis model assessment–insulin resistance (HOMA-IR)]. Pearson's correlation was used to evaluate the association between anthropometric indicators and serum biomarkers. The ROC curve with Youden's J index was used to suggest anthropometric cutoff points with better ability to predict or rule out lipid and glycemic changes.

Results: Cutoff points obtained for the z-score of body mass index (BMI), waist circumference (WC), and waist circumference for height (WC/H) showed high specificity (52 to 87%) and low sensitivity (23 to 59%), indicating greater ability to exclude changes in HDL-c, TG, and HOMA-IR levels. Cutoff points suggested for BMI ranged from +1.86 to +2.20 z-score. WC cutoff points ranged from +1.29 to +1.72, and, for the WC/H index, from +1.21 to +1.25. It was suggested the use of the following cutoff points to rule out changes in HDL-c, TG, and HOMA-IR values in clinical practice: BMI < z-score +2 and WC/H < z-score +1.29. In body fat percentage (BFP) analyses, the cutoff point < of 34% may be able to rule out changes in HDL-c (specificity of 70%), while the cutoff point > 36.6% may be able to predict changes in the HOMA-IR index (sensitivity of 76%).

Conclusion: It is not yet possible to state which anthropometric parameter has the best correlation with lipid and glycemic alterations in overweight children and adolescents. We suggest considering BMI, WC, and WC/H cutoff points together to rule out changes in HDL-c, TG, and HOMA-IR, and use the BFP cutoff point to predict changes in HOMA-IR.

Keywords: obesity, anthropometry, body composition, nutritional status, lipid profile, glycemic profile

INTRODUCTION

Obesity in childhood and adolescence is associated with several comorbidities, including dyslipidemia, peripheral insulin resistance, non-alcoholic fatty liver disease, polycystic ovary syndrome, psychosocial disorders, dermatitis, orthopedic problems, arterial hypertension, among others (1). Anthropometric and body composition parameters used for the diagnosis of obesity, such as waist circumference (WC) and body fat percentage (BFP), and indices, such as body mass index (BMI) and waist circumference for height (WC/H), have also been used to predict the coexistence of cardiometabolic risk factors (2–4). However, there is still no widely accepted definition of which of them is the best indicator, with the aggravating factor that different cut-off points have been used for each of them.

BMI can be assessed using reference curves published by the World Health Organization (WHO) and correlates with glucose and lipid profiles (5). For children over 5 years of age, BMI z-score values between +1 and +2 are used as the definition of overweight and obesity when $> +2$ (6). The data obtained in the WC measurement and WC/H index can be compared with age- and gender-specific cut-off points, using reference values defined by the National Health and Nutrition Examination Survey (NHANES) III (4), and values above z-score +2 can be considered high (4). Some authors, especially when WC is used as one of the components of the metabolic syndrome (MS), suggest the 90th percentile as a cut-off point for values that indicate the presence or absence of excess weight in an individual (7), which is approximately equivalent to the z-score of +1.29 (8), and this idea can also be expanded to the WC/H index.

In view of the above, the present study aims to determine which anthropometric indicator (BMI, WC, WC/H, and BFP) presents the best correlation with dyslipidemia, hyperglycemia, and peripheral insulin resistance, as well as the ideal cutoff point for anthropometric parameters capable of predicting or ruling out lipid and glycemic alterations in Brazilian children and adolescents.

METHODS

Study Design and Places

This is all cross-sectional study with data obtained from electronic medical records of patients from two health services located in two different regions of Brazil and with different socioeconomic profiles. Site 1: a clinic specialized in nutritional diseases and serving clients with health insurance in the southeast region of the country in the municipality of Ribeirão Preto/São Paulo. Site 2: patients from the public health system of the

reference clinic in obesity in a university hospital in the southern region of the country in the municipality of Porto Alegre/Rio Grande do Sul. This study was approved by the Research Ethics Committee of the Federal University of São Carlos (Nos. 4,133,407).

Study Population

Data collected at the first consultation during the years 2008 to 2020 and patients aged between 5 and 18 years and BMI above the z+1 score ($n = 1,296$ eligible) were included. Sampling was not performed because all the patients seen at the first visit in the time interval were initially eligible. Exclusion criteria were: presenting other diseases, such as type 1 diabetes mellitus ($n = 1$), hypothyroidism ($n = 6$), and inborn errors of metabolism ($n = 1$); unmeasured abdominal measure ($n = 89$); non-performing bioimpedance test ($n = 290$); and laboratory tests not available ($n = 341$). In the following criteria, 568 individuals participated in the study, 326 of which were attended in Locals 1 and 242 in Local 2. Therefore, a convenience sample was adopted.

Data Collection and Analysis

Anthropometric evaluation followed a standardized technique. Weight and height measurements followed WHO recommendations (6). All the patients underwent anthropometric and body composition evaluation by bioimpedance at the first visit, at which time they were asked to collect laboratory tests with 12 h of fasting, and an interval of up to 30 days was tolerated for return with the requested tests. The bioimpedance test was performed on Biodynamics® 310 equipment in both sites, and it was adequately validated (9).

BMI cutoff points for defining excessive adiposity were: (a) z-score $> +1$ and $\leq +2$, and (b) z-score $> +2$ (6). The WC cutoff points and the WC/H index for defining excessive adiposity were: a) z-score $\geq +1.29$ and $\leq +2$ (equivalent to 90 and 97th percentiles), and b) z-score $> +2$ (4). BFP was considered elevated when it was above the 85th percentile (10).

The laboratory tests of Site 1 were performed in one of the three laboratories in the city that were used under the free choice of the family, and all the laboratories constituted certified institutions that used similar methods and kits. The laboratory tests of Site 2 were performed in the laboratory of the university hospital. The serum biomarkers measured were fasting glucose, fasting insulin, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), and triglycerides (TG). The Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index was determined. Two tubes of 4 ml of blood were collected in a vial without additive and sent within 2 h to the laboratory for the processing of samples and

TABLE 1 | Correlation (r) between anthropometric indicators of excessive adiposity and serum biomarkers.

	TC r (p-value) (95%CI)	LDL-c r (p-value) (95%CI)	HDL-c r (p-value) (95%CI)	TG r (p-value) (95%CI)	Blood glucose r (p-value) (95%CI)	HOMA-IR r (p-value) (95%CI)
BMI (z-score)	-0.041 (-0.123, 0.042)	-0.034 (-0.116, 0.049)	-0.159* (-0.238, -0.078)	0.108*** (0.026, 0.188)	-0.047 (-0.129, 0.035)	0.221* (0.141, 0.298)
WC (z-score)	0.030 (-0.053, 0.112)	0.015 (-0.067, 0.097)	-0.121** (-0.201, -0.039)	0.163* (0.082, 0.242)	0.034 (-0.048, 0.116)	0.131** (0.049, 0.211)
WC/H (z-score)	0.037 (-0.046, 0.118)	0.031 (-0.052, 0.113)	-0.147* (-0.226, -0.065)	0.151* (0.069, 0.230)	0.013 (-0.070, 0.095)	0.128** (0.046, 0.208)
BFP (percentile)	-0.067 (-0.149, 0.015)	-0.009 (-0.091, 0.073)	-0.165* (-0.244, -0.084)	0.028 (-0.054, 0.110)	0.006 (-0.077, 0.088)	0.408* (0.338, 0.475)

* $p < 0.001$. ** $p < 0.005$. *** $p < 0.05$.

TABLE 2 | Prevalence of dyslipidemia, hyperglycemia, and insulin resistance according to the cutoff points of different criteria for defining excessive adiposity.

	n	high TC	high LDL-c	lowHDL-c	high TG	Elevatedfastingblood glucose	high HOMA-IR
		n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Total population	568	229 (40.3)	192 (33.8)	316 (55.6)	318 (56.0)	52 (9.2)	362 (63.7)
Anthropometric indicators and cut-off points							
BMI (+1 < z score ≤ +2)	206	79 (38.3)	68 (33.0)	96 (46.6)	107 (51.9)	21 (10.2)	99 (48.1)
BMI (z score > +2)	362	150 (41.4)	123 (34.0)	220 (60.8)	211 (58.3)	31 (8.6)	263 (72.7)
P-value, chi-squared test		0.47	0.81	<0.01	0.14	0.52	<0.01
WC (z score > +2)	149	62 (41.6)	49 (32.9)	88 (59.1)	98 (65.8)	9 (6.0)	106 (71.1)
WC (+1.29 ≤ z score ≤ +2)	323	136 (42.1)	113 (35.0)	189 (58.5)	181 (56.0)	39 (12.1)	212 (65.6)
WC (z score < +1.29)	96	31 (32.3)	29 (30.2)	39 (40.6)	39 (40.6)	4 (4.2)	44 (45.8)
P-value, chi-squared test		0.21	0.67	<0.01	<0.01	0.02	<0.01
WC/H (z score > +2)	121	51 (42.1)	39 (32.2)	68 (56.2)	82 (67.8)	7 (5.8)	86 (71.0)
WC/H (+1.29 ≤ z score ≤ +2)	319	135 (42.3)	114 (35.7)	193 (60.5)	180 (56.4)	41 (12.9)	216 (67.7)
WC/H (z score < +1.29)	128	43 (33.6)	38 (29.7)	55 (43.0)	56 (43.8)	4 (3.1)	60 (46.9)
P-value, chi-squared test		0.21	0.44	<0.01	<0.01	<0.01	<0.01
BFP (percentile > 85)	538	218 (40.5)	178 (33.1)	301 (55.9)	303 (56.3)	51 (9.5)	352 (65.4)
BFP (percentile < 85)	30	11 (36.7)	13 (43.3)	15 (50.0)	15 (50.0)	1 (3.3)	10 (33.3)
P-value, chi-squared test		0.68	0.25	0.52	0.50	0.26	<0.01

biochemical and hormonal analyses. The biological material was separated into a Bio Eng® centrifuge model BE 4,000 for 5 min at 3,500 rpm between one and 2 and 1/2 h after collection (sufficient time for blood coagulation). The biochemical dosage of insulin was performed in one of the aliquots on the same day of the collection by the chemiluminescence method, with automation by the Immulitte DPC Medlab equipment®. Glycemia and lipidogram were evaluated by the enzymatic method with Cobas Mira Plus Roche automation equipment®.

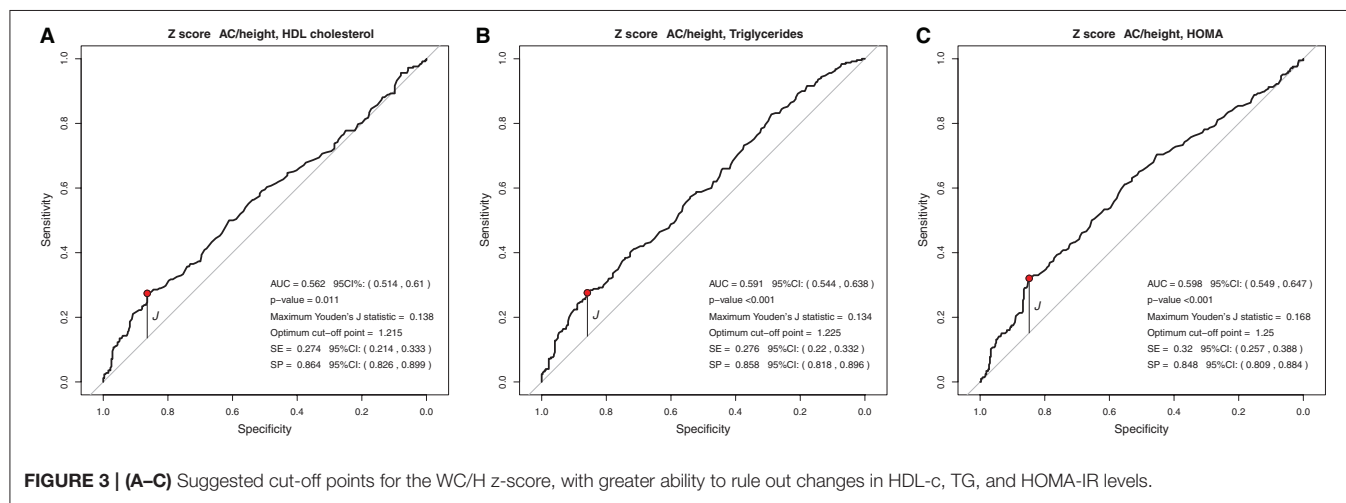
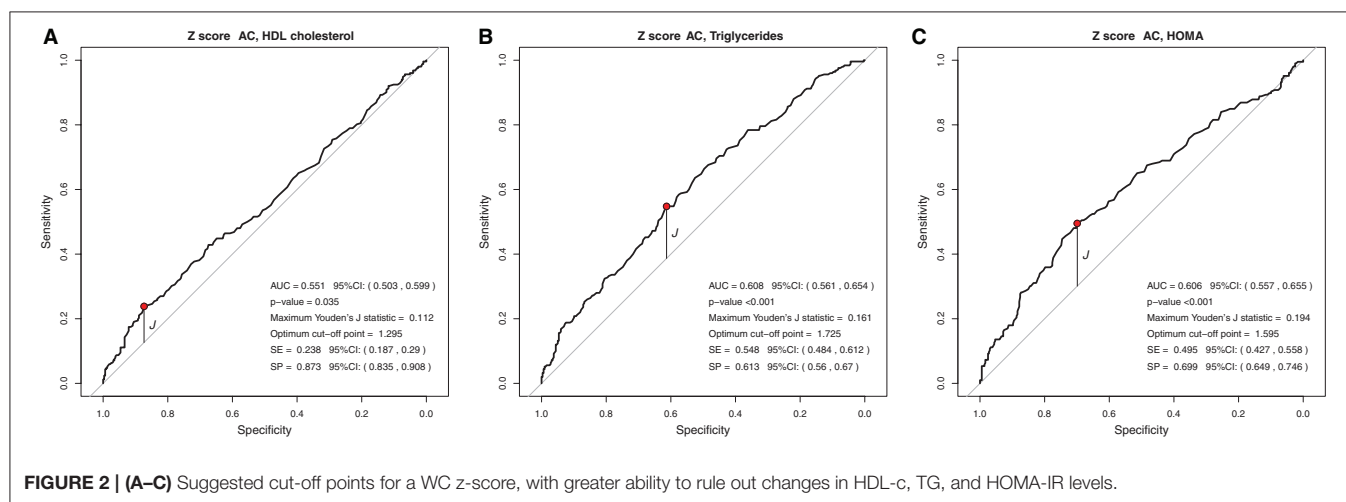
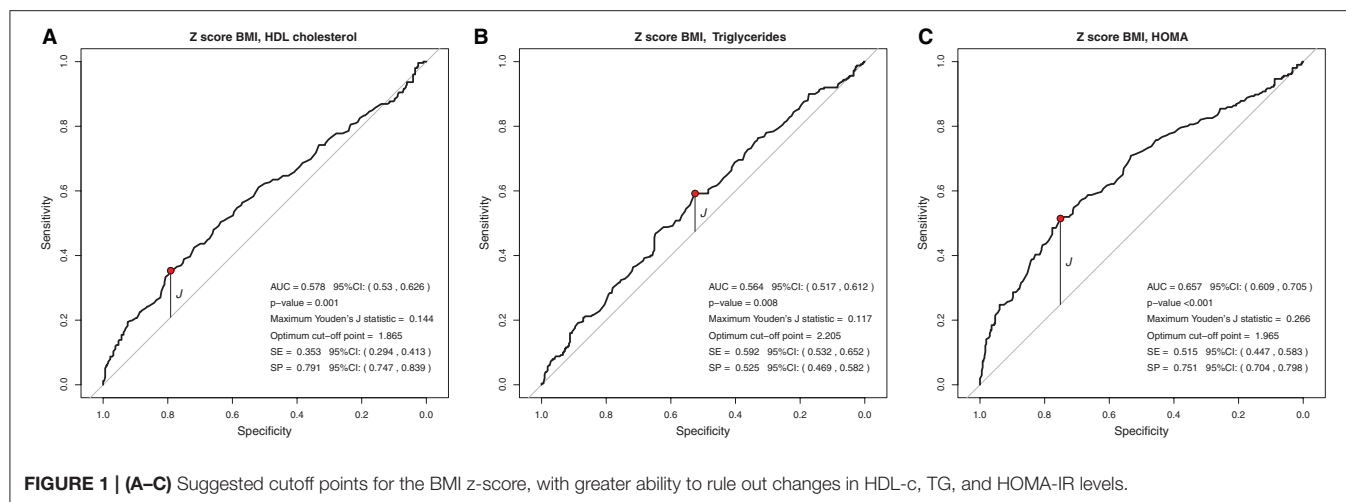
Hyperglycemia was considered when plasma glucose value was above 99 mg/dL and insulin resistance when HOMA-IR corrected for age and sex was above the cutoff points previously defined among Brazilian children and adolescents: 5 to 8.9 years: ≥1.76 (boys) and ≥1.39 (girls); 9 to 10.9 years: ≥1.97 (boys) and ≥2.62 (girls); 11 to 12.9 years: ≥2.65 (boys) and ≥3.02 (girls); 13 to 14.9 years: ≥3.21 (boys) and ≥3.46 (girls); 15 to 17.9 years: ≥2.39 (boys) and ≥2.89 (girls) (11, 12). The cutoff points for

defining dyslipidemia were: CT > 170 mg/dl; LDL-c > 110 mg/dl; HDL-c < 45 mg/dl; and TG > 75 mg/dl (1-9 years) or >90 (10 to 18 years) (13).

Statistical Analysis

The prevalence of individuals with elevated serum biomarkers was compared according to the cut-off points of different criteria for defining excessive adiposity using the Pearson chi-squared test. Pearson correlation coefficients (r) with corresponding 95% confidence intervals (95% CI) were used to assess the association between anthropometric indicators of excessive adiposity and serum biomarkers. The linearity assumption was visually checked by scatter plots of these variables.

Analyzes of the Receiver Operating Characteristic (ROC) curve with Youden's J index (14) were used to suggest the cut-off point for the z-score of BMI, WC and WC/H, and for the BFP, with greater ability to predict or rule out changes in lipids



and blood glucose. The Youden index is defined as $J = \max_c [\text{sensitivity}(c) + \text{specificity}(c) - 1]$ for given cut-off Point c in an ROC curve corresponds to the maximum distance from the diagonal line from (0, 0) to (1, 1). The ROC curves and outcome analyses were performed using the pROC package of R statistical software.

RESULTS

Population Characteristics

A total of 568 individuals were evaluated, 50.5% ($n = 287$) were female, and the mean age was 11.7 years (standard deviation, 2.7 years; range, 5.2 to 17.9 years). It was observed that 63.7% ($n = 362$) of the individuals were classified as obese (BMI > z score, +2). In the analysis of the prevalence of glycemic alterations, 63.7% ($n = 362$) of the individuals had high HOMA-IR values against only 9.2% ($n = 52$), with high fasting glucose. As for the lipid profile, the biomarker with the highest prevalence of serum inadequacy was TG (56%, $n = 318$).

Correlation Between Anthropometric Indicators and Lipid and Glycemic Changes

The raw values of the z-score of the anthropometric indicators BMI, WC, and WC/H showed a weak correlation with the serum levels of HDL-c, TG, and with the values of HOMA-IR. BFP was also correlated with serum HDL-c levels and with HOMA-IR values, showing a moderate correlation ($r = 0.408$, $p < 0.001$) (Table 1).

In addition, the prevalence of individuals with dyslipidemia, high blood glucose, and insulin resistance was analyzed according to different cutoff points already established in the scientific literature for BMI, WC, WC/H, and BFP (Table 2). The BMI analysis showed that the z-score > +2 cut-off point was able to identify a greater proportion of individuals with alterations in HDL-c and HOMA-IR, compared to the z-score cut-off > +1 and $\leq +2$. Analysis of WC and WC/H index showed that both cut-off points, z-score $\geq +1.29$ and z-score > +2, were able to identify a greater number of obese children with low HDL-c and elevated TG and HOMA-IR, compared to those with no alterations in these anthropometric parameters. On the other hand, values situated between the cut-off points z-score $\geq +1.29$ and $\leq +2$ for WC and WC/H were more suitable for identifying individuals with high fasting glucose. The BFP above the 85th percentile identified a higher percentage of individuals with alterations in HOMA-IR, compared to individuals classified below this percentile (Table 2).

Cut-Off Points for Anthropometric Indicators Capable of Predicting or Ruling Out Lipid and Glycemic Changes

In the analysis of the ROC curve, the cutoff points obtained by the Youden J index for the z-score of BMI, WC, and WC/H showed high specificity (52 to 87%) and low sensitivity (23 to 59%), indicating that individuals without lipid and glycemic alterations have a high probability of being below the cut-off

TABLE 3 | Cutoff points according to Youden's "J" index analysis to rule out or predict biochemical abnormalities or body fat percentage obtained from data from children and adolescents aged 5 to 18 years from two Brazilian overweight and obesity outpatient clinics.

Indicator	Biomarker	Cutoff point
Body mass index	HDL-c	< +1.86 z-score
	Triglycerides	< +2.20 z-score
	HOMA-IR	< +1.96 z-score
Waist circumference	HDL-c	< +1.29 z-score
	Triglycerides	< +1.72 z-score
	HOMA-IR	< +1.59 z-score
Waist circumference/Height	HDL-c	< +1.21 z-score
	Triglycerides	< +1.22 z-score
	HOMA-IR	< +1.25 z-score
Body fat percentage	HDL-c	<34.0%
	HOMA-IR	>36.6%

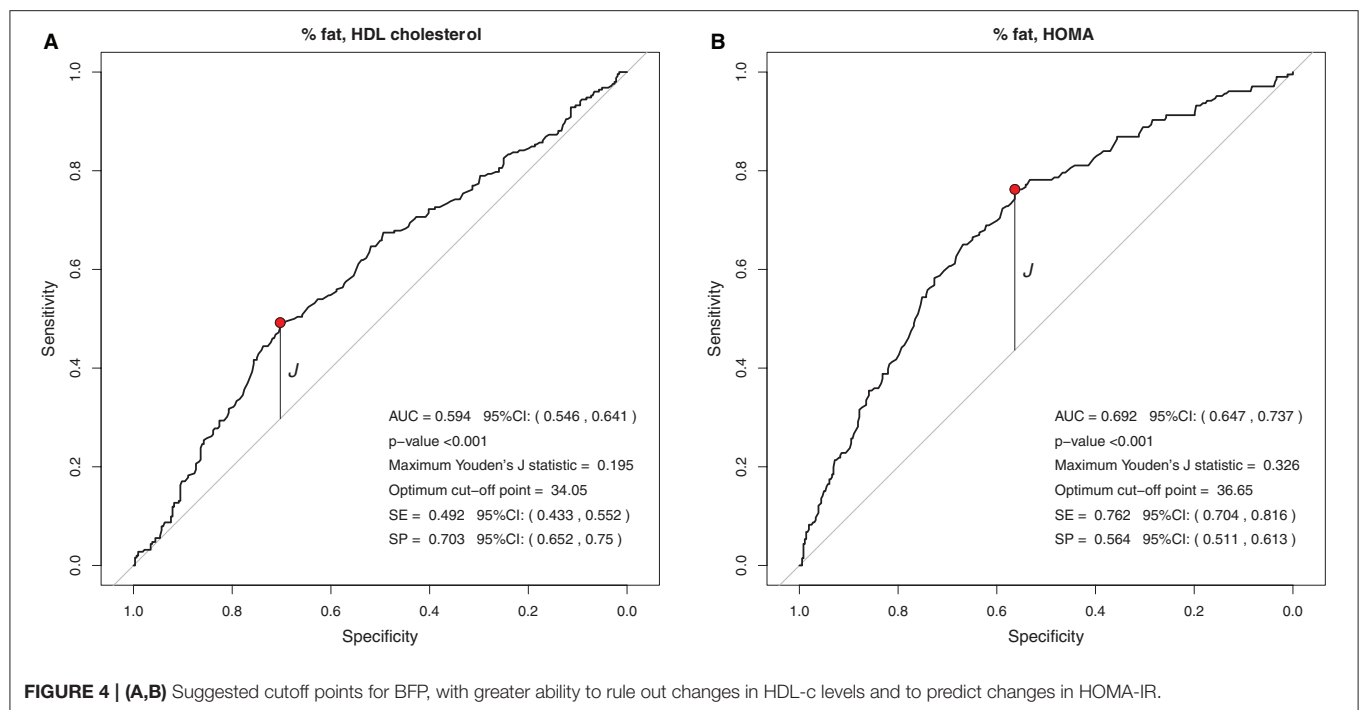
HDL-c, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance.

point found. The cutoff points suggested for the BMI in the population studied, capable of ruling out changes in HDL-c, TG, and HOMA-IR levels, were z-score < +1.86, z-score < +2.20, and z-score < +1.96, respectively (Figure 1). The cutoff points suggested for WC, capable of ruling out changes in HDL-c, TG, and HOMA-IR levels, were z-score < +1.29, z-score < +1.72, and z-score < +1.59, respectively (Figure 2). And the cutoff points suggested for the WC/H index, capable of ruling out changes in HDL-c, TG, and HOMA-IR levels, were z-score < +1.21, z-score < +1.22, and z-score < +1.25, respectively (Figure 3). These cut-off points are also shown in Table 3.

Regarding the BFP, the cut-off point obtained by the Youden J index showed low specificity and high sensitivity for HOMA-IR, indicating that individuals with insulin resistance have a high probability of being above the cut-off point. The suggested cutoff point for the BFP with the greatest ability to predict elevated HOMA-IR in Brazilian children and adolescents was 36.6% (Figure 4).

DISCUSSION

Obesity is a chronic nutraceutical-metabolic disease related to cardiometabolic events, (15) which may not be evident in some patients (16). Therefore, it is important to use anthropometric parameters as a low-cost tool, viable in clinical practice and of simple interpretation for cardiometabolic risk assessment in obese pediatric patients. BMI has been used by professionals worldwide as the main indicator of the presence of obesity and its comorbidities. Although it depends on two measures and a mathematical calculation, its use has become increasingly embedded in pediatric practice worldwide, especially since the publication of the WHO growth charts (6). Several countries adopt BMI curves in official childcare instruments, and professionals are accustomed to their use and interpretation. The WC, despite the extreme ease of measurement, still depends



on the use of curves or tables that are not widespread. The same can be said of the WC/H index, which still depends on the additional measurement of height. On the other hand, BFP, which should be the gold standard, considering that obesity is about excess adiposity, has its measurement even more complex, because it depends on equipment rarely presented in pediatric clinics and also depends on little known curves or tables. The present study aimed to identify, among the anthropometric and body composition parameters used in clinical practice and useful in the definition of obesity, the one that best correlates with alteration in biomarkers for dyslipidemia, hyperglycemia, and peripheral insulin resistance.

The z-score values for BMI, WC, and WC/H showed significant correlation with three serum biomarkers (HDL-c, TG, and HOMA-IR), while BFP correlated with two biomarkers (HDL-c and HOMA-IR). However, except for BFP, which showed moderate correlation with HOMA-IR, all other correlations were weak. Similar results were found by Faria et al. (17) in Brazilian adolescents and by Vásquez et al. (18) in a cohort of Chilean children aged 4, 7, and 10 years; however, in both studies, not all the participants were overweight. The observation of a moderate correlation between body fat and HOMA-IR was also observed in a study of overweight Saudi Arabian children and adolescents aged between 2 and 20 years (19). Excess body fat can lead to metabolic changes, including inflammatory mechanisms, which increases insulin resistance and may help to partially explain this finding.

A positive association between BMI and TG was also observed by Ejtahed et al. (20) among Iranian adolescents. In China, in a study carried out with 2,243 overweight individuals aged between 7 and 17 years, it was observed that BMI was the best

predictor of dyslipidemia in the group studied (21). In Brazil, it was observed that BMI was a significant predictor of dyslipidemia in a study with 874 children and adolescents aged 6 to 19 years in Belém (PA) (22). In our study, it was not possible to state that BMI is the best predictor of dyslipidemia and insulin resistance, suggesting that WC measurements and BFP analysis should also be considered in routine pediatric screening.

It is known that WC measurements are an effective indicator of central fat distribution, serving as a strong marker of cardiometabolic risk in the pediatric population (23, 24). In a multicenter study by da Silva et al. (25) in 2018, with 520 adolescents, it was also observed a positive association between WC and HOMA-IR. The study by Khoury et al. (26), carried out with more than 14,000 North American children and adolescents aged 5 to 18 years, also showed a positive association between the WC/H index and HDL-c and HOMA-IR levels. The authors also highlighted that individuals with high WC/H and BMI had higher cardiovascular risk compared to those with only high BMI and normal WC/H, confirming the importance of WC measurement in clinical practice.

Faria et al. (17) highlighted that BMI, WC, and WC/H were good predictors of excess body fat. Aristizabal et al. (27) found a moderate correlation of BMI, WC, and WC/H with HOMA-IR in Colombian children aged between 2 and 5 years. These data show the need to use several anthropometric parameters together for decision-making in clinical practice.

The attempt to find anthropometric indices of adiposity that can predict metabolic risk changes for the development of cardiovascular diseases has produced heterogeneous results. We observed that no anthropometric indicator was associated with serum levels of TC, LDL-c, and fasting blood glucose.

It should be noted that the CT and LDL-c tests do not compose the MS picture. These findings are different from those found by Olíosa et al. (3), who found a positive association between WC/H and BFP measurements with high CT values.

Regarding fasting blood glucose, this is an exam used in the diagnosis of MS, but this study did not find an association with the anthropometric indicators studied, as well as the study by de Quadros et al. (2). The glycemic alteration is unusual in obese Brazilian children, perhaps due to the so-called “metabolically healthy obese,” (28) which presents some normal exams and lower prevalence of changes in fasting blood glucose.

Our analyses of the prevalence of lipid and glycemic alterations according to anthropometric cutoffs already established in the literature positively evidenced the use of the z-score + 2 cutoff points for BMI and the 85th percentile for BFP in the detection of individuals with metabolic alterations. For WC and WC/H parameters, the cutoff z-score +1.29 was able to identify a greater proportion of individuals with biochemical alterations. Other studies have also used the same cutoff point for BMI (27) and WC (26, 29). As for the WC/H index, the authors still preferred to use the cutoff point of 0.5 (26, 30).

In the analysis of the ROC curves and Youden's J index, this study observed that the suggested cutoff points for the BMI varied between +1.86 and +2.20 z-scores, close to the cutoff point (z-score +2) already used by WHO for diagnosis of obesity. WC cutoff points ranged between +1.29 and +1.72, while, for the WC/H index, they ranged between +1.21 and +1.25, all also close to the z-score +1.29, used by some authors for predicting excess body fat. Therefore, for clinical practice, we suggest using the following cutoff points to rule out changes in HDL-c, TG, and HOMA-IR values: BMI < z-score +2 and WC and WC/H < z-score +1.29.

In BFP analyses, the cutoff point < 34% of body fat may be able to rule out changes in HDL-c (high specificity), while the cutoff point > 36.6% may be able to predict changes in the HOMA-IR index (high sensitivity). In the study by Abdelhamed et al. (19), a cut-off of 46.1% of BFP was shown to have the best sensitivity/specificity ratio for detecting obesity-related morbidities in Saudi children and adolescents. The joint analysis of several biochemical parameters (grouped as “obesity-related morbidities”) to estimate the accuracy of the BFP in predicting laboratory changes in overweight individuals may have contributed to the difference between the Saudi work and the present study.

It should be noted that, in an attempt to establish new anthropometric cutoffs capable of predicting or ruling out dyslipidemia and glycemic alterations, the interpretation of the area under the curve (AUC) value should be considered (31). While studying overweight individuals aged between 2 and 20 years in Saudi Arabia, Abdelhamed et al. (19) found AUC between 0.638 and 0.657 for the parameters BFP, WC, and BMI capable of predicting “obesity-related morbidities,” a condition that included, in addition to dyslipidemia, pre/hypertension, pre/diabetes, and hypovitaminosis D. In a study involving 3,327

overweight European children and adolescents aged 3 to 16 years, the authors found an AUC of 0.57, 0.58, and 0.59 when analyzing the BFP values capable of predicting changes in HDL-c, LDL-c, and TG, respectively; in this study, the authors found no differences between the AUCs of BFP and BMI (32).

Similar to the findings above, we observed in our study that BMI, WC, and WC/H had similar AUC values between 0.551 and 0.657, but with high specificity and low sensitivity, evidencing the ability to rule out changes in HDL-c, TG, and HOMA-IR. Thus, it seems that, when considered together, the cutoff points suggested for BMI, WC, and WC/H are capable of excluding, with moderate accuracy, alterations in the lipid profile and insulin resistance of Brazilian children and adolescents with excess of weight. If it is not possible to use these anthropometric indicators together in clinical practice, it is possible to use at least one of them (for example, BMI) in view of the similar ability of all of them to correlate with biochemical changes. As for the BFP, the AUC was 0.594 and 0.692 for HDL-c and HOMA-IR, respectively. The analyses between BFP and HOMA-IR showed high sensitivity, indicating a greater ability of the suggested cutoff point for BFP (>36.6%) to predict changes in HOMA-IR.

Among the strengths of the current study, the heterogeneity of our population stands out, which comprised individuals from a private clinic, which mainly serves middle- and upper-class populations, and from a public outpatient clinic, which serves low-income populations, in two different regions of Brazil. As for the limitations, it is noteworthy that the individuals studied came from reference services in the follow-up of children and adolescents with obesity, and these patients tend to have more severe disease, a fact that may influence the results. In addition, the level of physical activity was not analyzed. Complex metabolic interactions and other variables (e.g., the level of physical activity) that interfere with lipoprotein metabolism may help to explain the absence of a strong correlation between anthropometric parameters and the lipid profile in the current study. Finally, a dietary intake assessment would, perhaps, affect the data analysis as a cofactor, but our data set did not include this evaluation.

CONCLUSION

The z-score values for BMI, WC, and WC/H correlated with HDL-c, TG, and HOMA-IR, while BFP correlated with HDL-c and HOMA-IR. It is still not possible to say which anthropometric parameter has the best correlation with lipid and glycemic changes in obese Brazilian children and adolescents. The cutoff points for BMI, WC, and WC/H capable of ruling out alterations in HDL-c, TG, and HOMA-IR found in the present study were close to those already classically used in the scientific literature to define the indicator as altered or not. We suggest considering the BMI, WC, and WC/H cutoff points together to rule out changes in HDL-c, TG, and HOMA-IR, and using the BFP cut-off point to predict changes in HOMA-IR.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Research Ethics Committee of the Federal University of São Carlos (number 4,133,407). Written informed consent for participation was not provided by the participants' legal guardians/next of kin because: We used data base information with no patient identification.

AUTHOR CONTRIBUTIONS

CN-d-A: conception, design, acquisition, analysis, interpretation, drafting, final approval of the version to be published, figures, study design, data collection, data interpretation,

and data analyses. MF and AC: final approval of the version to be published, literature search, data analysis, and data interpretation. EDM: revising, final approval of the version to be published, literature search, data analysis, and data interpretation. RS: acquisition, analysis, or interpretation of data for the work, literature search, drafting, final approval of the version to be published, and data collection. IF: final approval of the version to be published, literature search, and drafting. MN-d-A: design of the work or the acquisition, analysis, drafting, and final approval. LD: conception or design of the work, drafting, final approval of the version to be published, data interpretation, and data analyses. EZM: analysis, or interpretation of data for the work, drafting, final approval of the version to be published, data interpretation, data analysis, and figures. FU: conception, interpretation, drafting, and final approval of the version to be published data interpretation. All authors contributed to the article and approved the submitted version.

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Development of Formulas for Calculating L3 Skeletal Muscle Mass Index and Visceral Fat Area Based on Anthropometric Parameters

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Background: The anthropometric index is not accurate but shows a great advantage in accessibility. Simple body composition formulas should be investigated before proceeding with the universal nutrition screening.

Materials and Methods: Clinical data of patients with a malignant tumor of the digestive system were collected. SliceOmatic 5.0 software (TOMOVISION, Canada) was used to analyze abdominal CT images and taken as references. A linear regression analysis was adopted to establish the formula for calculating skeletal muscle index (SMI) and visceral fat area (VFA). In addition, the relweights function was adopted to measure the contribution of each variable.

Results: In total, 344 patients were divided into the training set and 134 patients into the validation set. The selected formulas were $SMI_{pre} = 0.540 \times \text{weight (kg)} - 0.559 \times \text{height (cm)} - 13.877 \times \text{sex (male = 1, female = 2)} + 123.583$, and $VFA_{pre} = 5.146 \times \text{weight (kg)} - 2.666 \times \text{height (cm)} + 1.436 \times \text{age (year)} + 134.096$, of which the adjusted R^2 were 0.597 and 0.581, respectively. The “weight” explained more than 80% of R^2 in the prediction of VFA. In addition, “sex” occupied approximately 40% of R^2 in the prediction of SMI. The paired t -test showed no significant difference between the real measured indices and the predicting ones ($p = 0.123$ for SMI and $p = 0.299$ for VFA). The logistic regression analysis exhibited similar diagnostic efficacy of the real measured parameters and formulas.

Conclusion: The SMI and VFA formulas were developed through basic indices, such as weight, height, sex, and age. According to the contribution of each variable, weight should always be focused on preserving appropriate muscle and adipose tissue.

Keywords: cancer, nutrition, skeletal muscle mass, visceral fat area, formula

INTRODUCTION

Nutritional status is defined as “the condition of the body, resulting from the balance of intake, absorption, and utilization of nutrients and the influence of particular physiological and pathological status,” which is the foundation of all activities (1). Malnutrition, in all its forms, includes undernutrition (wasting, stunting, and underweight), inadequate vitamins or minerals,

overweight or obesity, and diet-related non-communicable diseases. Malnutrition results in disease and often includes cardiovascular diseases, certain types of cancer, and diabetes. Among these, cancer has been a leading cause of death worldwide, accounting for approximately one in six deaths. According to statistics from the World Health Organization (WHO), approximately one-third of deaths from cancer are due to tobacco use, high body mass index (BMI), alcohol consumption, low fruit and vegetable intake, and lack of physical activity, which are closely linked to malnutrition. Besides, undernutrition is common in cancer. The Investigation on Nutrition Status and its Clinical Outcome of Common Cancers (INSCOC) project reported that 40–80% of patients with cancer are diagnosed with undernutrition, and 20% of patients with cancer die due to undernutrition (2). Thus, the assessment of nutritional status should be universal and paid attention, which has major relevance to clinical practice for individual and public health (3).

There have been various tools for nutrition assessment. Body composition, which precisely distinguishes body components into categories, such as muscle tissue, adipose tissue, and bone, is demonstrated to be instructive in clinical nutrition assessment, drug dosage, adverse events management, and prognosis prediction. Anthropology is the most traditional way with indices, such as height, weight, waist circumference (WC), and BMI. A single anthropometric index is not accurate but shows a great advantage in accessibility. Thus, iconography is proposed and regarded as the most accurate and reliable technique by most guidelines, such as the Asian Working Group of Sarcopenia (AWGS) (4), the European Working Group on Sarcopenia in Older People (EWGSOP) (5), and the Foundation for the National Institutes of Health (FNIH) Sarcopenia Project (6). Muscle mass is a part of body compositions, and the loss of muscle mass indicates a worse quality of life and deteriorated clinical outcomes. Appendicular skeletal muscle mass (ASM) is the classical parameter of skeletal muscle mass and has been recommended by multiple guidelines. Martin et al. (7) proposed skeletal muscle index (SMI) at the third lumbar vertebra as a nutritional indicator, which behaved well in the detection of occult muscle depletion compared with traditional nutritional indicators, such as BMI and weight loss. Then SMI and visceral fat area (VFA) at the third lumbar vertebra have been demonstrated as substitutes for whole-body composition and have been associated with clinical outcomes, which were gradually adopted (8, 9). Another reason for the extensive use of the third lumbar vertebra is its inclusion in typical abdominal CTs. Zhuang et al. (10) reported that SMI is an independent predictor of severe postoperative complications [odds ratio (OR) = 3.010, $p < 0.001$] and long-term survival [hazard ratio (HR) = 1.653, $p < 0.001$] after radical gastrectomy for gastric cancer. Li et al. proposed cutoffs of VFA in sarcopenic obesity and demonstrated its unfavorable impact on survival (HR = 2.772, $p < 0.001$) (9). However, equipment and technical requirements are obvious drawbacks to iconography, which makes it seem unreasonable under the circumstance of universal and quick nutrition assessment. The updated 2019 AWGS consensus cited the formula of

calculating AMS simply by anthropometric indices, that is, $ASM\ (kg) = 0.193 \times weight\ (kg) + 0.107 \times height\ (cm) - 4.157 \times gender\ (male = 1, female = 2) - 0.037 \times age\ (year) - 2.631$, which was also demonstrated to be effective in the assessment of body composition (4). Sarcopenia diagnosed accordingly was an independent factor of 3-year mortality [HR = 2.49, 95% confidential interval (CI): 1.25–4.95] and readmission (HR = 1.81, 95% CI 1.17–2.80) in a population of elderly inpatients in acute care wards (11).

To shed light on this context, more simple body composition formulas should be explored to proceed with the universal nutrition screening, especially in rural districts where iconographic equipment or technology are unavailable. Herein, taking CT measured SMI and VFA as a reference, formulas of SMI and VFA by the simple anthropometric index were first established and verified in 478 patients with malignant tumors of the digestive system. In addition, the contribution of each variable involved was determined, which explicitly reminds health providers of the focus on multivariables.

PATIENTS AND METHODS

The study protocol adhered to the Declaration of Helsinki and was approved by the Ethics Committee of the First Hospital of Jilin University (2017-362).

Patients

The clinical data of patients with a malignant tumor of the digestive system who were admitted into the First Affiliated Hospital of Jilin University from November 2011 to December 2018 were collected. The inclusion criteria were as follows: (1) adults > 18 years old and (2) abdominal CT scans to be obtained. The exclusion criteria involved patients with incomplete data.

Clinical data were collected by trained personnel for each participant. (1) General characteristics include age, sex, smoking history, alcohol drinking, and comorbidities (diabetes and hypertension). (2) Anthropometric measurements include BMI: patients needed to empty their bladder and fast for 2 h before the measurement. During the measurement, patients took off their shoes and wore light clothing. Height and weight were measured accurately to 1 cm and 0.1 kg. BMI (kg/m^2) was calculated by $weight\ (kg)/height\ (m)^2$. For mid-arm circumference (MAC) and triceps skinfold thickness (TSF), MAC of the non-dominant side was measured to the nearest 0.5 cm using a non-elastic tape when patients were standing, and TSF at the same place was measured accurately to 1 mm using vernier calipers. For hand-grip strength (HGS), patients were seated with 90 degree elbow flexion and the grip strength of the dominant hand was measured to the nearest 1 kg at least two times with the Jamar dynamometer, and the maximum reading was recorded. For waist circumference (WC), the non-elastic tape was placed at the umbilicus and was encircled at the abdomen parallel to the ground, closing to the skin without squeezing the skin. WC was measured accurately to 0.5 cm. For maximum calf circumference (CC), the maximum circumference of the left calf was measured with a non-elastic tape when

standing, which was accurate to 0.5 cm. (3) Serum albumin concentration, serum C-reactive protein (CRP), and triglycerides (TGs) were assessed through laboratory examinations. (4) The patient-generated subjective global assessment (PG-SGA) was used as the evaluation scale.

Computer Tomography Indices

SliceOmatic 5.0 software (TOMOVISION, Canada) was used to analyze abdominal CT images. According to the voxel values, -29 to $+150$ Hu was identified as skeletal muscle mass and -190 to -30 Hu was identified as adipose tissue mass. Then, the skeletal muscle area (SMA) and VFA at the third lumbar vertebra were sketched. The SMA includes the psoas major, the erector spinae, the quadratus lumborum, the transverse abdominis, the external oblique, and the internal oblique. The VFA represents the intra-abdominal adipose tissue.

Figure 1 displays the diagrammatic sketch illustrating two patients with the same BMI but different body compositions. $\text{SMI} (\text{cm}^2/\text{m}^2) = \text{SMA}/\text{height}^2 (\text{m}^2)$.

Statistical Analysis

Data were analyzed by SPSS for Windows version 26.0 (IBM SPSS Statistics, IBM Corp., Armonk, NY) and R version 4.0 (R Foundation for Statistical Computing, Vienna, Austria).

The training set and validation set were separated randomly at a ratio of 7:3 (12). The Kolmogorov–Smirnov test was used to confirm normal distributions of continuous data. An independent *t*-test was used for normally distributed data. Counting data were examined by using the chi-square test. Pearson's correlation analysis was adopted. Multi-collinearity was tested by linear regression analysis, and the variance inflation factor (VIF) > 10 was considered as the existence of collinearity. The linear regression analysis was adopted to establish the

formula for calculating SMI and VFA. Then, the paired *t*-test was used to examine the accuracy of the regression formula in the training set and validation set. The logistic regression analysis was adopted to examine the efficacy of the formulas in malnutrition ($\text{PG-SGA} \geq 9$). The $p < 0.05$ was taken to indicate statistical significance.

RESULTS

Basic Characteristics of Involved Patients

In total, 478 patients were involved in the study. Out of which, 344 patients were separated into the training set and 134 patients into the validation set. All basic characteristics were consistent between the two sets ($p > 0.05$) (**Table 1**). The SMI was $45.40 \text{ cm}^2/\text{m}^2 \pm 9.17 \text{ cm}^2/\text{m}^2$ in the training set and $44.70 \text{ cm}^2/\text{m}^2 \pm 9.58 \text{ cm}^2/\text{m}^2$ in the validation set ($p = 0.451$). The VFA was $101.13 \text{ cm}^2 \pm 66.28 \text{ cm}^2$ in the training set and $102.41 \text{ cm}^2 \pm 69.15 \text{ cm}^2$ in the validation set ($p = 0.848$). The Pearson correlation coefficient was 0.725 between ASMI and SMI ($p < 0.001$).

Linear Regression Model of Skeletal Mass Index and Visceral Fat Area

After collinearity diagnosis, BMI was excluded ($\text{VIF} = 139.091$). Then, all subset regression was committed and the results are displayed in **Figure 2**. The most appropriate model was primarily selected according to the adjusted R^2 (which means how well the predictor variable explains the response variable) and the accessibility of the involved variables. Thus, the selected formula was $\text{SMI}_{\text{pre}} = 0.540 \times \text{weight (kg)} - 0.559 \times \text{height}$

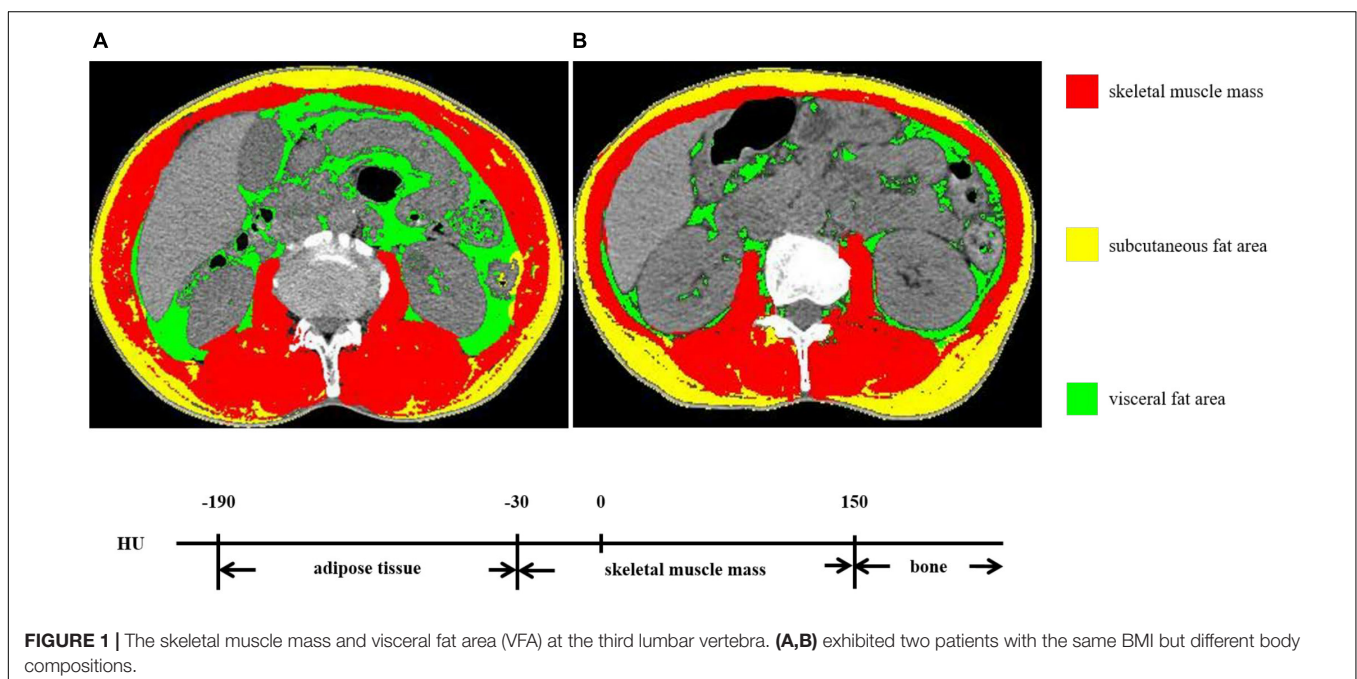


TABLE 1 | Basic characteristics of involved participants.

Characteristics	Training set	Validation set	t/χ^2	p
Age(year)	58.76 ± 10.53	59.46 ± 9.53	-0.680	0.497
Sex			0.755	0.385
Male	218(45.6)	88(18.4)		
Female	116(24.3)	56(11.7)		
Height(cm)	166.42 ± 8.37	165.42 ± 8.36	1.456	0.146
Weight(kg)	63.02 ± 11.76	62.06 ± 11.65	0.825	0.410
BMI(kg/m ²)	22.67 ± 3.45	22.62 ± 3.23	0.147	0.883
Smoking			2.416	0.120
Yes	165(34.5)	60(12.6)		
No	169(35.4)	84(17.6)		
Drinking			0.003	0.958
Yes	92(19.2)	40(8.4)		
No	242(50.6)	104(21.8)		
Comorbidities			1.817	0.969
Diabetes	18(3.8)	10(2.1)		
Hypertension	47(9.8)	20(4.2)		
MAC(cm)	26.36 ± 3.38	26.17 ± 3.09	0.582	0.561
TSF(mm)	16.24 ± 6.38	17.25 ± 10.73	-1.276	0.203
HGS(kg)	26.29 ± 10.62	24.86 ± 10.62	1.393	0.164
WC(cm)	82.31 ± 10.01	81.94 ± 10.09	0.302	0.763
CC(cm)	33.62 ± 4.28	33.15 ± 3.75	1.138	0.256
Albumin(g/L)	37.19 ± 5.34	37.81 ± 4.91	-1.187	0.236
CRP(mg/L)	20.51 ± 32.74	21.36 ± 41.34	-0.191	0.849
TG(moml/L)	1.37 ± 0.86	1.49 ± 1.05	-0.939	0.348
ASMI(kg/m ²)	6.96 ± 1.06	6.86 ± 1.14	0.845	0.398
SMI(cm ² /m ²)	45.40 ± 9.17	44.70 ± 9.58	0.754	0.451
VFA (cm ²)	101.13 ± 66.28	102.41 ± 69.15	-0.191	0.848

BMI: body mass index; MAC: mid-arm circumference; TSF: triceps skinfold thickness; HGS: hand grip strength; WC: waist circumference; CC: maximum calf circumference; CRP: C-reaction protein; TG: triglycerides; ASMI: appendicular skeletal muscle mass index; VFA: visceral fat area; SMI: skeletal mass index at the third lumbar vertebra; CT: computer tomography.

(cm) = $13.877 \times \text{sex}$ (male = 1, female = 2) + 123.583 and $\text{VFA.pre} = 5.146 \times \text{weight (kg)} - 2.666 \times \text{height (cm)} + 1.436 \times \text{age (year)} + 134.096$, of which the adjusted R^2 were 0.597 and 0.581, respectively. Then, the relweights function (13) was adopted to measure the importance of each variable. As shown in **Figure 3**, the most important variable was “weight,” both in the prediction of SMI and VFA. Especially, the “weight” explained more than 80% of R^2 in the prediction of VFA. In addition, “sex” should be paid attention to in the prediction of SMI, which occupied approximately 40% of R^2 . “Height” and “age” only occupied approximately 10% both in the formula of SMI and VFA.

Validation of Predicting Models of Skeletal Mass Index and Visceral Fat Area

Paired t -test indicated that there was no significant difference between the real measured SMI and SMI.pre (SMI calculated by the formula proposed above) in the training set ($p = 0.165$), and the paired sample correlation was 0.726 ($p < 0.001$). Additionally, the real measured VFA and VFA.pre (VFA calculated by the

formula proposed above) were consistent ($p = 0.388$) with the paired sample correlation of 0.770 in the training set. In the validation set, the paired sample correlation was 0.789 ($p < 0.001$) for SMI and 0.745 ($p < 0.001$) for VFA, and no significant difference was observed between the real measured indices and the predicting ones ($p = 0.123$ for SMI and $p = 0.299$ for VFA) (**Table 2**).

The Diagnostic Efficacy of the Established Models of Skeletal Mass Index and Visceral Fat Area

The thresholds of SMI and VFA were derived from previous studies. The cutoffs of SMI were 34.9cm²/m² for women and 40.8cm²/m² for men in sarcopenia (10), and the diagnostic consistency was 87.3%. The cutoffs of VFA were 61.2 cm² for women and 75.2 cm² for men (9) and the diagnostic consistency was 86.5%. The clinical events diagnosed by the real measured and predicted parameters are displayed in **Table 3**. No significant difference was detected between groups ($p > 0.05$). Then, the logistic regression analysis was adopted to examine the efficacy of the real parameters and established models for detecting severe undernutrition (PG-SGA ≥ 9). The ORs were 2.327 (95% CI 1.986–2.873, $p < 0.001$) for SMI and 2.106 (95% CI 1.735–2.494, $p < 0.001$) for SMI.pre. When combined with low SMI and high VFA, which is referred to as sarcopenic obesity, the ORs were 3.172 (95% CI 2.416–3.928, $p < 0.001$) for the real measured parameters and 2.743 (95% CI 2.016–3.748, $p < 0.001$) for the predicted parameters.

DISCUSSION

Undernutrition in patients with cancer is pretty common. The general prevalence of undernutrition is 40–80% in Chinese patients with cancer. Undernutrition could result from tumor-derived cytokine release, tumor mass effects, and side effects of cancer treatment. As a multifaceted disease, the symptoms of undernutrition vary from loss of appetite to sarcopenia and even cachexia. Undoubtedly, undernutrition significantly deteriorates function outcomes, survival, and increased additional costs (14). To deal with this, nutrition interventions are introduced. The prospective clinical trial EFFORT demonstrated that individualized nutritional support reduced the risk of mortality and improved functional and quality of life outcomes in cancer patients with increased nutritional risk (15). These data support universal malnutrition screening upon hospital admission followed by an individualized nutritional support strategy in these vulnerable patients to prevent adverse clinical outcomes associated with malnutrition, which was also recommended by the European Society for Clinical Nutrition and Metabolism (ESPEN) (16).

However, nutrition screening is far from ideal. Although the importance is already obvious, the practice of nutrition screening and malnutrition diagnosis is lacking (17). Li et al. (18) reported that only 0.5% of patients with cancer received nutrition screening at admission, but the prevalence of malnutrition was up to 44.9% actually in the same cohort.

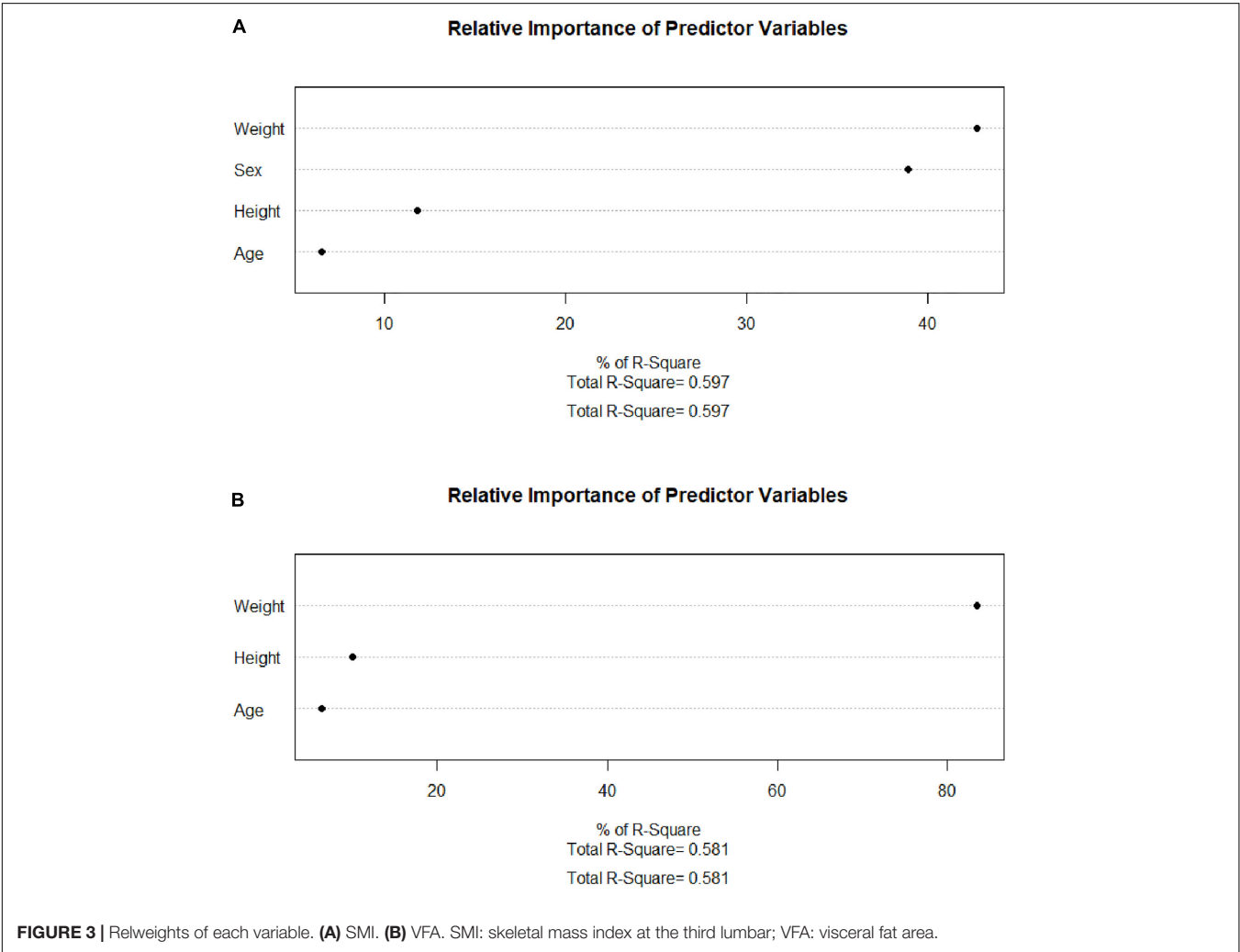
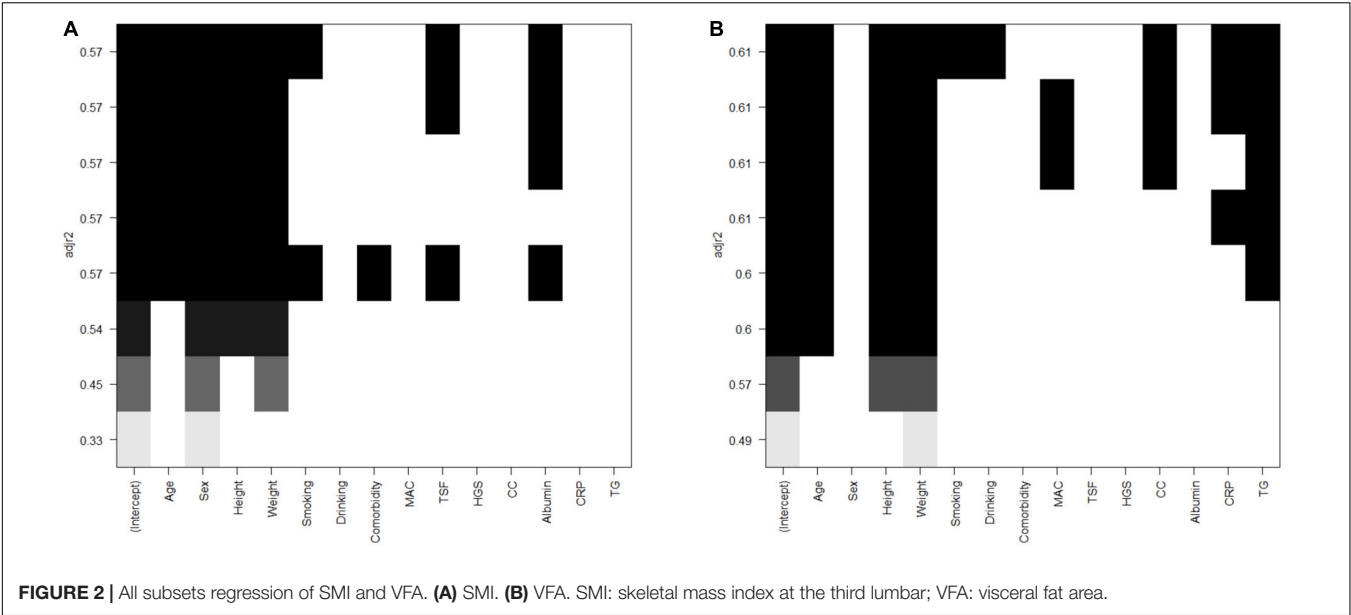


TABLE 2 | Validation of predicting models of SMI and VFA.

	Pearson correlation		Paired t-test	
	r	P	t	P
Training set				
SMI vs. SMI.pre*	0.726	<0.001	-1.391	0.165
VFA vs. VFA.pre#	0.770	<0.001	0.865	0.388
Validation set				
SMI vs. SMI.pre*	0.789	<0.001	-1.552	0.123
VFA vs. VFA.pre#	0.745	<0.001	1.042	0.299

*SMI.pre = $0.540 \times \text{weight (kg)} - 0.559 \times \text{height (cm)} - 13.877 \times \text{sex (male = 1, female = 2)} + 123.583$.

#VFA.pre = $5.146 \times \text{weight (kg)} - 2.666 \times \text{height (cm)} + 1.436 \times \text{age (year)} + 134.096$.

SMI: skeletal mass index at the third lumbar vertebra; VFA: visceral fat area.

TABLE 3 | The clinical events diagnosed by real measured and predicted parameters and their nutritional status.

Characteristics	PG-SGA		χ^2	P
	<9	≥ 9		
Sarcopenia			0.072	0.789
SMI (n = 87)	56(64.4)	31(35.6)		
SMI.pre* (n = 89)	59(66.3)	30(33.7)		
SOB#			0.035	0.851
Real (n = 13)	9(69.2)	4(30.8)		
Predicted (n = 11)	8(72.7)	3(27.3)		

SOB: sarcopenic obesity. SMI: skeletal mass index at the third lumbar; PG-SGA: patient generated subjective global assessment. VFA: visceral fat area;

*SMI.pre = $0.540 \times \text{weight (kg)} - 0.559 \times \text{height (cm)} - 13.877 \times \text{sex (male = 1, female = 2)} + 123.583$.

#SOB was diagnosed by low SMI combined with high VFA. And VFA.pre = $5.146 \times \text{weight (kg)} - 2.666 \times \text{height (cm)} + 1.436 \times \text{age (year)} + 134.096$.

The dilemma resulted from multiple reasons. Nutritional scales are commonly used like malnutrition universal screening tool (MUST), nutrition risk score-2002 (NRS-2002), and the patient-generated subjective global assessment (PG-SGA) (19). However, the implementation needs trained personnel, compliance, and time costs. Body composition directly and individually reflects nutritional status. However, body composition analysis is complex and requires investment in personnel, equipment, and technology. Therefore, simplifying the method of body composition analysis will promote the accessibility of nutritional screening.

Herein, formulas calculating SMI and VFA all by anthropometric indices were established, examined, and validated. As displayed in **Figure 2**, the SMI model with the least variables was selected with the same predictive ability. Among the VFA models, the predictive ability is slightly sacrificed given the accessibility of variables. The VFA model with the largest R^2 was not selected because the involvement of TG only increased about 1% of predictive ability. Thus, the finally selected formulas are SMI.pre = $0.540 \times \text{weight (kg)} - 0.559 \times \text{height (cm)} - 13.877 \times \text{sex (male = 1, female = 2)} + 123.583$ and VFA.pre = $5.146 \times \text{weight (kg)} - 2.666 \times \text{height (cm)} + 1.436 \times \text{age (year)} + 134.096$. Furthermore, the relweights function

reported novel information on these variables. Weight is the most important parameter, which occupies the largest share in VFA and SMI formula. Especially in the VFA formula, the relweight of weight is up to 80%. In fact, weight is considered a rough parameter of nutritional status and overall adiposity. Studies have suggested a beneficial effect of obesity, named the “obesity paradox.” The phenomenon was then clarified that it was the increased muscle that dominated the benefits, whereas the accumulated visceral fat defeated the benefits (20). In addition, it has been demonstrated that, compared with normal-weight individuals, subjects with obesity have greater thigh muscle volume, increased cross-sectional area of type I skeletal muscle fibers, increased muscle lipid content, and a lower muscle quality (21–23). These findings remind the importance of weight management. Appropriate weight means suitable muscle mass, muscle quality, and adipose tissue. In addition, sex should be emphasized in the assessment of muscle mass for the relative weight of approximately 40% in SMI. It should be noted that this result does not indicate that women are more prone to muscle depletion because of different cutoff values. Zhuang et al. (10) reported the appropriate threshold of SMI in sarcopenia, which was $34.9 \text{ cm}^2/\text{m}^2$ for women and $40.8 \text{ cm}^2/\text{m}^2$ for men. It was reported that men were more vulnerable to sarcopenia than women. Bianchi L et al. (24) reported that the prevalence of sarcopenia was 36.5% in men and 32.9% in women. Since gender is also an included variable, the gender differences should be paid attention to at least in epidemiological investigations and public health management.

Based on the principle of providing a universal and practicable method, only simple and easily available parameters were involved. From the perspective of convenient and universal nutrition screening, the formulas established should be a step forward with good consistency and no statistically significant difference between the calculated values and real values. Since the formulas were established and validated, we also recommended the same thresholds for the real measured and predicted SMI and VFA in clinical settings. However, the formulas were not proposed to substitute the ever-changing body composition tools. There are limitations. Although the training cohort and validation cohort were set to guarantee the accuracy of the formula, the formulas must be validated through large cohorts from multi-centers. As a subject still under exploration with limited sample size, it is difficult for the involved population to represent the whole. Herein, patients with digestive system cancer were applicable. The results are not necessarily generalizable to other populations. Verifying the formulas in healthy populations and populations with various diseases would further enhance the credibility.

In conclusion, SMI and VFA, the two important parameters in body composition, were calculated by basic indices, such as weight, height, sex, and age with good consistency. It appears to be a simple and valid tool for assessing the body composition of patients with cancer. The contribution of each variable involved was also reported, which explicitly reminds health providers to take care of important indices, such as weight. This project was potentially able to promote the implementation of nutrition screening.

DATA AVAILABILITY STATEMENT

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the First Hospital of Jilin

University. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

WJ and XL designed the study. YQZ and YXZ collected and analyzed the data. YH analyzed the data. JC and WL critically revised the manuscript. All authors read and approved the final manuscript.

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Acute Water Supplementation Improved the Body Composition of Young Female Adults After Water Restriction of 12 h in Baoding, China: A Randomized Controlled Trial (RCT)

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Insufficient intake of water may influence the balance of water in the human body. In this study, we explore the impacts of water supplementation on body composition indices among young adults after 12 h of water restriction, with the aim to determine the optimum volume of water for improving body water composition. A randomized controlled trial study was conducted among 64 young men and women in Baoding, China. After fasting overnight for 12 h, anthropometric measurements and urine and blood samples were collected as a baseline test at 8:00 a.m. of Day 2. Body composition was determined by measuring the ECW (extracellular water), ICW (intracellular water), and TBW (total body water) through bioelectrical impedance analysis (BIA). The participants were randomly divided into four groups, including water supplementation (WS) groups 1, 2, and 3, with 500, 200, and 100 mL of water, respectively, and a no water supplementation (NW) group. After 90 min, they were reassessed in a rehydration test (at 10:00 a.m. of Day 2). Repeated measurement ANOVA was used to assess the impact of water supplementation on body composition. Comparing the baseline and rehydration tests, interactions between time and volume were not significant among the men (all $p > 0.05$). Among women, a significant interaction was only found in ECW ($p = 0.043$), with TBW tending toward being significant ($p = 0.055$). Comparing the baseline and rehydration tests, the ECW in WS group 1, WS group 3, and the NW group all decreased ($p = 0.028$, $p = 0.001$, and $p = 0.029$), with reductions of 0.1, 0.3, and 0.2 kg, respectively; however, no significant decrease was observed in WS group 2 ($p = 0.329$). Furthermore, comparing the WS groups with the NW group in the rehydration test, the differences were not significant ($p = 1.000$, $p = 1.000$, and $p = 0.288$, respectively). Between men and women within groups, all of the body composition indices differed significantly, both at baseline and

rehydration tests (all $p < 0.05$). Water supplementation led to changes in the distribution of the water content in young female adults in this study—but not men—after 12 h of water restriction, with no decrease in ECW. Finally, we found that 200 mL was the minimum volume capable of improving the water content distribution in participants in this study.

Trial Registration: [www.chictr.org.cn], identifier [ChiCTR-IOR-17011568].

Keywords: water restriction, water supplementation, rehydration, body composition, young adults

BACKGROUND

Water is an abundant compound in the human body, representing approximately 45–75% of the body mass (1, 2). It is well-known that the total body water (TBW), consisting of intracellular water (ICW) and extracellular water (ECW)—which comprise 65 and 35% of the TBW, respectively—is constantly maintained. In humans, water is mainly secured through three routes: total drinking fluids, water from food, and metabolic water. Meanwhile, the main output of water is by urine, through excretion by the kidneys, and in sweat evaporated from the skin (3). Other routes of water loss include breathing and fecal excretion (4). Generally, there is a dynamic balance in water intake and output, which is called the hydration status of the human body. Insufficient water intake may lead to dehydration, which could degrade cognition (5, 6). Therefore, it is crucial to take in adequate water to maintain the optimal hydration status in human beings.

Studies investigating dehydration have shown that there are two types of dehydration, defined as intracellular dehydration and extracellular dehydration (7, 8). It has been revealed that cellular hydration plays a physiological role in regulating cell function. Despite the adverse effects of dehydration on human health, overhydration also needs to be avoided, which may also impede health (9–11). Therefore, analysis of the specific proportions of water in each section of the human body (e.g., TBW) is a regular diagnostic practice in some research areas. A series of studies have demonstrated that body composition information is used in various fields of medicine; for example, to supplement methods for distinguishing patients at risk of post-operative morbidity (12), to verify the effects of dietary supplements (10), and for monitoring seasonal changes in the nutritional status and body composition of athletes (13). As for patients, higher ECW/TBW (extracellular water to total body water ratio), ECW, ICW, ECW/ICW (extracellular water to intracellular water ratio), fat-free mass (FFM), and TBW/FFM have been linked with various health outcomes, including lower odds of frailty, lower survival rates, and higher level of albuminuria (14–18). Furthermore, in the COVID-19 era, the highest ECW/TBW has been observed in older adults with osteosarcopenic adiposity syndrome (OSA), indicating a heightened inflammatory state (19). As has been described previously, body composition data are useful for assessing diverse

disease conditions; furthermore, they have been associated with physiological status among healthy people (20–25).

Although some studies have evaluated the associations between body composition and health, only several studies thus far have investigated the relationship between hydration status and body composition, with inconsistent results. Studies have shown that total drinking fluids are associated with TBW, confirming the relationship between hydration status and body composition and that TBW/BW decreases with increasing dehydration among children (26–28). In addition, the impairments due to dehydration or water restriction on body composition have been evaluated clearly in studies conducted among people or animals (29–31). To our knowledge, only a few studies have analyzed the correlation between the body composition and water supplementation, in which the researchers did not obtain similar results (32, 33). Hence, more studies are needed for this issue.

Regarding the technologies for assessing the body composition of people, several methods have been used, including bioelectrical impedance analysis (BIA), dual-energy X-ray absorptiometry (DXA), quantitative magnetic resonance (QMR) EchoMRI systems, air displacement plethysmography (ADP), and magnetic resonance imaging (MRI) (34, 35). DXA is a mature technology for examining the mineral content of bone and body composition with high validity and stability (36), while the deuterium dilution technique and bromate sodium method have been recognized as reference procedures for determining the TBW and ECW, respectively (37). Nevertheless, some of these techniques and methods are expensive, invasive, and/or complicated, making them difficult to apply when considering large samples at the population level. Meanwhile, due to these revealed limitations (38), BIA has been considered as an alternative method for the quantification of water content and other components of body composition, thanks to its features of being portable, low-cost, rapid, non-invasive, safe for repeated measures, simple, and reproducible (39). A series of studies has demonstrated the validity of BIA for measuring the ICW, ECW, TBW, and FFM among healthy adults, children, and athletes with high validity (40–48). In free-living conditions, it offers a potential means to assess changes in distribution in body composition (e.g., ICW, ECW, and TBW).

Through this study, we aim to evaluate the following hypothesis: Acute water supplementation can lead to changes in some aspects of the body composition distribution, and different volumes of water may lead to different changes in the distribution

Abbreviations: TWI, Total water intake; TBW, Total body water; ICW, Intracellular water; ECW, Extracellular water; BW, Body weight.

of body composition among young Chinese men and women after 12 h of water restriction.

MATERIALS AND METHODS

Study Design

A randomized controlled trial was implemented, which included two study days.

Study Participants

Participants in a healthy state and aged 18–23 years were included in this study. The following exclusion criteria were used: participants with (a) habitual smoking, (b) high alcohol consumption (>20 g/day), (c) high-level caffeine consumption (>250 mg/day), (d) chronic diseases, and (e) other diseases (49). The participants were recruited in three ways: First, an advertisement was put up in the publicity window at a college; second, the researchers held several informative meetings, to which all college students could come if they wanted to learn about our research; third, notices of recruitment were sent through the instant messaging apps, including WeChat and QQ (Tencent Holdings Ltd., Shenzhen, China). For the selected participants, we arranged further medical examination, to exclude those with diseases. Finally, 64 participants were recruited for our study, half men and half women.

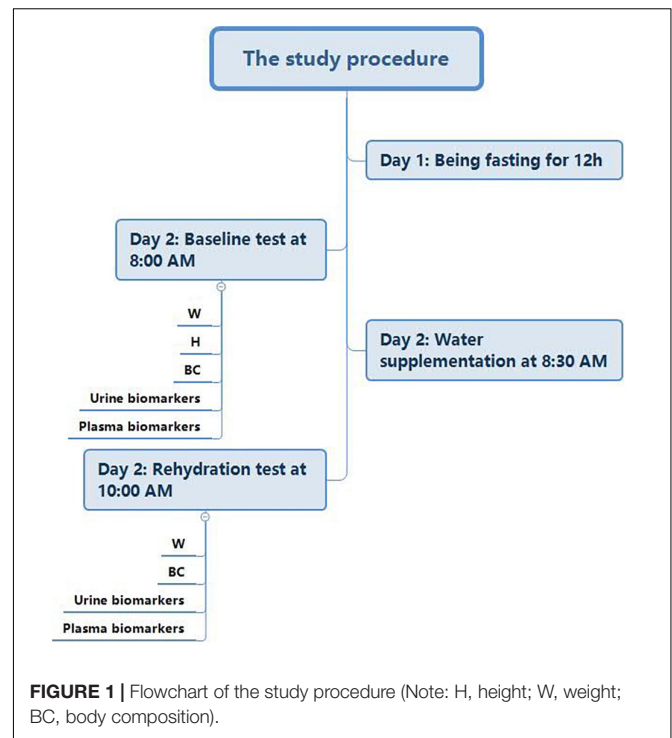
Sample Size Calculation

In one study implemented among young men, the TBW under optimal hydration and dehydration statuses were 48.8 ± 7.5 and 45.4 ± 7.3 L, respectively (50). Then, the sample size was calculated using the PASS 11.0 (NCSS, LLC, Kaysville, UT) software, with α set at 0.05 ($p < 0.05$, two-tailed) and power (1-Beta) at 0.90. In addition, a 10% drop-out rate was considered. We found that, in total, 64 participants were required.

Study Design and Procedure

A randomized controlled study design was implemented among the young men and women, which lasted 2 days.

On Day 1, all of the participants were instructed to fast for 12 h (from 8:00 p.m. of Day 1 to 8:00 a.m. of Day 2). During the fasting period, they were not allowed to have any food or drinks. On the morning of Day 2 (8:00 a.m.), the baseline test, which included the measurement of anthropometric indices (height, weight, and body composition) and urine and blood samples (to assess the osmolality of urine and plasma, as well as urine specific gravity; USG), was conducted by trained investigators. Then, the participants were randomly separated into four groups: WS groups 1, 2, and 3 (administered 500, 200, and 100 mL of purified water, respectively) and the NW group (with no water). Each group included 8 men and 8 women. At 8:30 a.m., the participants in WS groups were instructed to drink the water supplied to them within no longer than 10 min, while the participants in NW group were not allowed to drink any fluids. The interval of water supplementation was 90 min. At 10:00 a.m., participants were reassessed through a rehydration test. The study procedure is shown in **Figure 1**.



Temperature and Humidity of the Environment

The participants were college students in Hebei province, China, and were not allowed to engage in intense activities 1 week before and during the study days. The places that they were allowed access to included their dormitories and classrooms, allowing the micro-climate both outdoors and indoors to be recorded. Therefore, the temperature and humidity of the indoors and outdoors were recorded at three-time points—namely, 10:00 a.m., 2:00 p.m., and 8:00 p.m.—over the 2 days (WRB-1-H2, Exasace, Zhengzhou, China). The mean temperature and humidity indoors were 22.8°C and 72%, respectively, while those outdoors were 19.3°C and 89%, respectively.

Anthropometry

Weight and height were determined twice, to the nearest 0.1 kg and 0.1 cm, respectively, by trained investigators using standard procedures (HDM-300; Huaju, Zhejiang, China). BMI was calculated as weight (kg)/height squared (m^2).

Body composition was determined a single time by trained investigators using a bioelectrical impedance analyzer (Inbody 720; Inbody; Seoul, South Korea). Based on the four component (4C) body composition model, the instrument uses eight-point contact electrodes (two thumb electrodes, two palm electrodes, two sole electrodes, and two heel electrodes, with the surface area all bigger than 4 cm^2) to measure 30 impedance values in five segments (left and right arms and legs, trunk: RA, LA, TR, RL, and LL) at six different frequencies (1, 5, 50, 250, 500, and 1,000 kHz). The Inbody 720 uses different high- and low-frequency conditions to measure the intracellular

and extracellular water, to accurately analyze the total water content. The BIA measurements were performed in the morning of Day 2 (i.e., at 8:00 AM) after overnight fasting for 12 h, and after water supplementation for 90 min (i.e., at 10:30 AM) in the laboratory, with a room temperature of 24°C. The investigators checked the BIA before the morning of Day 2, to ensure system self-test and calibration: At least two investigators were measured by the BIA twice to ensure the normal operation of the BIA. Participants were asked to wear light clothing (e.g., shorts and short sleeves) and bare feet and to ensure that no metals were present in their clothing. Before standing on the BIA platform, participants were asked to urinate and instructed to clean their hands and feet with alcohol, and then to wait on the chair nearby with bare feet for at least 5 min. After the trained investigator had input their information, including their BMI, age, gender, and height, into the BIA, the participants were instructed to stand on the two-foot electrodes of the BIA, with the rear sole first and then the front sole completely coinciding with the electrode; to hold the two hand electrodes, with their thumb and palm in tight contact with the electrode; and to keep the angle between their trunk and arms at 15° for 2 min. They were asked to stand still with calm breathing and to stay relaxed over the 2 min. Then, the ICW, ECW, TBW, and FFM of the participants were measured separately by the BIA, and the data were stored in its database. When the body composition measurement of each participant had finished, it was printed immediately for the investigators to record. The investigators checked the impedance data (Z and Xc) for RA, LA, TR, RL, and LL on the report, which declined directly from 1 kHz to 1,000 kHz, to ensure the success of measurement. Moreover, after collection of the body composition data for all of the participants, the database was copied onto a USB drive, to ensure data integrity and security.

We collected urine samples from the participants during their water restriction between 8:00 p.m. of Day 1 to 8:00 a.m. of Day 2. Furthermore, they were asked to drink as usual before fasting overnight. The color of each urine sample was checked carefully by the researchers, to confirm the corresponding hydration status, and almost all participants showed optimal hydration status.

Thirst, Urine, and Plasma Biomarkers

Thirst was explored using the 10 cm line (51), which has been described in our previous study (5). Participants were provided with a 10 cm line and instructed to inscribe a mark anywhere on the line according to their feelings, with two extreme answers (“not at all” and “extremely”) at opposite ends of the line. Higher scores indicated that the participants were thirstier. The urine samples, including the first-morning urine and the urine after water supplementation, were collected and stored at +4°C in a special refrigerator before any measurements. After collection and centrifugation, all blood samples were retained for the following measurements. The osmolality of urine and plasma were determined using the freezing point method (SMC 30C; Tianhe, Tianjin, China). The measurement of USG (urine specific gravity) was carried out through the method of uric dry chemistry (H-800; Dirui, Changchun, China).

Statistics

Results are expressed as means and standard deviations, numbers, and percentages (the hydration status). The normality of quantitative variables was assessed using quantile plots and the Shapiro–Wilk tests. One-way ANOVA and the Bonferroni correction method were used to explore the differences in data that were normally distributed among the four groups. Chi-squared tests were used to examine the differences in the hydration status of individuals. Differences in variables between men and women within each group were analyzed using Student's *t*-test, both in the baseline test and rehydration test. The significance of differences in the variables between the baseline and rehydration tests was analyzed using Student's paired *t*-tests. Repeated measurement ANOVA was used to assess the effects of water supplementation on body composition. All statistical analyses were performed using the SPSS 21.0 software (IBM Corp., Armonk, NY, United States). A *p*-value less than 0.05 was considered to indicate statistical significance. The Bonferroni correction for multiple tests was employed, and the alpha level was set at 0.008 (0.05/6 comparisons).

RESULTS

Characteristics of Participants

In total, 64 participants were recruited and completed the study, with a completion rate of 100%. As shown in **Supplementary Table 1**, no significant differences were observed in the characteristics among the four groups (*p* > 0.05).

Water Supplementation Effects on Thirst and Hydration Status

As shown in **Supplementary Table 2**, there was a significant main effect of time (*p* = 0.037), but not volume (*p* = 0.797), and a significant interaction between time and volume was found for thirst (*p* = 0.001). When comparing the rehydration and baseline tests, for thirst, significant reductions were found in WS groups 1 and 2 (*p* = 0.003 and *p* = 0.042, respectively), with no significant decrease in WS group 3 (*p* = 0.529) and an increase in the NW group (*p* = 0.039). *Post hoc* analysis revealed that significant differences were found between the NW group and WS groups 1 (*p* < 0.001) and 2 (*p* = 0.007), but not with WS group 3 (*p* = 0.892). For urine osmolality, there was significant effect of time (*p* < 0.001), but not volume (*p* = 0.822), and a significant interaction between time and volume was found for urine osmolality (*p* < 0.001). The *t*-tests showed that WS groups 1 and 2 decreased significantly (*p* < 0.001 and *p* = 0.004, respectively), the NW group increased significantly (*p* < 0.001), and the decrease in WS group 3 was not significant (*p* = 0.596). In terms of hydration status, there were significant improvements in WS groups 1 and 2 (*p* < 0.001 and *p* = 0.004, respectively). *Post hoc* analysis demonstrated that significant differences were found between the NW group and WS groups 1, 2, and 3 (*p* < 0.001, *p* < 0.001, and *p* = 0.045, respectively); however, no significant differences were found for thirst and hydration status between WS groups 1 and 2 (*p* = 1.000 and *p* = 0.090, respectively).

Furthermore, there were no significant interactions between time and volume in any of the plasma biomarkers, including plasma osmolality (all $p > 0.05$).

Water Supplementation Effects on Body Composition

As shown in **Table 1**, in the baseline test, the body composition indices did not differ significantly among the four groups (all $p > 0.05$), except for TBW/BW ($p = 0.041$). The *post hoc* analysis demonstrated that no significant differences were found between the WS groups and the NW group ($p = 0.085$, $p = 1.000$, and $p = 0.098$, respectively). Meanwhile, during the rehydration test, no significant differences were found in any of the body composition indices considered in this study among the four groups (all $p > 0.05$). When comparing the baseline and rehydration tests, no significant interactions between time and volume were found among the four groups (all $p > 0.05$), as shown in **Table 1**. Furthermore, the main effects of time and volume were not statistically significant in ICW, ICW/TBW, ECW, ECW/TBW, ECW/ICW, TBW, TBW/BW, and TBW/FFM (all $p > 0.05$).

It can be seen, from **Table 2**, that when comparing the baseline and rehydration tests, the ICW, ICW/TBW, ECW, ECW/TBW, ECW/ICW, TBW, TBW/BW, and TBW/FFM among the men in the four groups did not differ significantly. Furthermore, comparing baseline and rehydration tests, no significant interactions were found in any of the body composition indices (all $p > 0.05$). The main effects of time and volume were not statistically significant in any of the body composition indices (all $p > 0.05$).

For women, comparing the baseline and rehydration tests, no significant differences were found in ICW, ICW/TBW, ECW, ECW/TBW, ECW/ICW, TBW, TBW/BW, and TBW/FFM among the four groups (all $p > 0.05$). Moreover, the main effects of time were statistically significant in all of the body composition indices (all $p < 0.01$), but volumes were not (all $p > 0.05$). Meanwhile, a significant interaction between time and volume was only found in ECW ($p = 0.043$), and TBW tended toward significance ($p = 0.055$). Follow-up *t*-tests indicated that the reductions of ECW in WS group 1, WS group 3, and the NS group differed significantly ($p = 0.028$, $p = 0.001$, and $p = 0.029$, respectively), while no significant change was found in WS group 2 ($p = 0.329$) when comparing baseline and rehydration tests. Compared with NW group, there were no significant differences between WS group 1 ($p = 1.000$), WS group 2 ($p = 1.000$), and WS group 3 ($p = 0.288$). When comparing the *d*-value (ECW1-ECW2), no significant differences were found among the four groups ($p = 0.909$). Furthermore, TBW decreased significantly in WS group 1, WS group 3, and the NW group ($p = 0.006$, $p = 0.001$, and $p = 0.010$, respectively), with that of WS group 2 remaining unchanged ($p = 0.169$). These *t*-tests of the TBW should be interpreted cautiously, as the interaction was not significant. Compared with the NW group, there were no significant differences between WS group 1 ($p = 1.000$), WS group 2 ($p = 1.000$), and WS group 3 ($p = 0.256$).

Referring to the differences among men and women, in the baseline test, higher ICW, ECW, TBW, and TBW/BW and lower ICW/TBW, ECW/TBW, and ECW/ICW were found in men, compared to women (all $p < 0.05$), but no significant differences were found in TBW/FFM ($p > 0.05$). Furthermore, significant differences were found in all of the body composition indices in each group, both in the baseline and rehydration tests (all $p < 0.05$).

DISCUSSION

In this study, we aimed to explore the impacts of different amounts of water supplementation on the body composition indices of healthy young male and female adults after water restriction for 12 h, for the first time in China. The osmolality of the urine and plasma was measured to determine the hydration status of the participants: on one hand, to make sure that they were all dehydrated after water restriction for 12 h; on the other hand, to determine the effects of water supplementation on body composition under the improved hydration status. Contrary to our expectations, the results revealed that, although the volumes of water supplementation differed, healthy individuals may have acute deficits of TBW. In this study, thirst decreased and the hydration status improved significantly after participants drank water, and no significant differences were found between participants who drank 500 mL compared to 200 mL. Meanwhile, the thirst increased and the hydration status got worse (with the urine osmolality increasing) among those who drank no water. Thus, we may conclude that water supplementation could prevent the adverse effects of water restriction on thirst and urine osmolality and that the minimum amount of water to attenuate the adverse effects of water restriction on thirst and urine osmolality is 200 mL.

Previous research works exploring the influence of acute water supplementation on body composition have been inconsistent. For ICW, no significant differences were revealed in this study in adults, even with different amounts of water supplementation. In results reported from an experiment involving rats, an increase in ICW was observed in rats after water and NaCl consumption; this is essentially the inverse of the findings from our study in healthy young men and women (52). The differences in the type of fluid supplementation and the type of dehydration could explain such differences. In the abovementioned study, the rats were provided supplementation of water and NaCl while, in our study, participants consumed different amounts of purified water. Moreover, the rats had acute body fluid deficits, with up to 20% reduction in ECW and up to 2% reduction in ICW; however, the participants of our study only underwent 12 h of water restriction. Interestingly, we only observed significant reductions in ECW among women who had 500 or 100 mL of water supplementation, while that of those who had 200 mL of water did not decrease; meanwhile, the *post hoc* analysis reported no significant differences among the four groups, when compared with each other. In contrast, no significant changes were found among men after water supplementation. Therefore, it could be inferred that the ECW among women after 12 h water restriction

TABLE 1 | Body composition indices of participants.

Total	Baseline test				Rehydration test				<i>P</i> interaction
	NW group (<i>n</i> = 16)	WS group 1 (<i>n</i> = 16)	WS group 2 (<i>n</i> = 16)	WS group 3 (<i>n</i> = 16)	NW group (<i>n</i> = 16)	WS group 1 (<i>n</i> = 16)	WS group 2 (<i>n</i> = 16)	WS group 3 (<i>n</i> = 16)	
ICW (kg)	19.4 ± 3.5	21.4 ± 5.3	21.2 ± 4.2	21.1 ± 2.8	19.1 ± 3.7	21.1 ± 5.4	21.0 ± 4.2	20.8 ± 3.7	0.921
ICW/TBW (%)	62.5 ± 0.6	62.4 ± 0.6	62.4 ± 0.7	62.4 ± 0.7	62.3 ± 0.6	62.3 ± 0.5	62.3 ± 0.7	62.3 ± 0.7	0.796
ECW (kg)	11.7 ± 2.0	12.8 ± 3.1	12.8 ± 2.3	12.7 ± 1.6	11.6 ± 2.1	12.7 ± 3.1	12.7 ± 2.3	12.6 ± 1.7	0.909
ECW/TBW (%)	37.6 ± 0.6	37.6 ± 0.6	37.6 ± 0.7	37.6 ± 0.7	37.7 ± 0.6	37.7 ± 0.5	37.7 ± 0.8	37.7 ± 0.7	0.796
ECW/ICW (%)	60.2 ± 1.5	60.2 ± 1.5	60.3 ± 1.8	60.3 ± 1.8	60.6 ± 1.6	60.5 ± 1.4	60.6 ± 2.0	60.5 ± 1.8	0.801
TBW (kg)	31.1 ± 5.5	34.2 ± 8.5	34.0 ± 6.4	33.8 ± 4.3	30.7 ± 5.8	33.8 ± 8.5	33.7 ± 6.4	33.4 ± 4.7	0.918
TBW/BW (%)	52.1 ± 4.3	56.4 ± 5.7	54.0 ± 4.5	56.3 ± 4.9	51.8 ± 4.5	55.7 ± 5.7	53.6 ± 4.7	55.8 ± 5.3	0.643
TBW/FFM (%)	73.9 ± 2.6	73.3 ± 0.2	73.3 ± 0.3	73.3 ± 0.2	74.0 ± 2.6	73.3 ± 0.2	73.3 ± 0.3	74.0 ± 2.6	0.819

Values are shown as the mean ± standard deviation (SD). No significant differences were found in ICW, ICW/TBW, ECW, ECW/TBW, ECW/ICW, TBW, TBW/BW, and TBW/FFM (both in the baseline and rehydration test all *p* > 0.05).

still decreased without water supplementation, with concomitant increases in thirst and urine osmolality. Too little (100 ml) or much (500 ml) water supplementation did not prevent the adverse effects of water restriction on the ECW, but the volume of 200 ml did. Thus, 200 mL of water supplementation of 200 may be the minimum that could lead to an increase in ECW among young women. The optimal volume of water supplementation that increases the ECW may be between 200 ml and 500 ml among young women after 12 h of water restriction. More studies should be conducted to explore this issue. Our results showed that the hydration status of participants who drank 500 ml and 200 ml improved and did not differ significantly between groups. Hence, 200 ml of water could prevent the adverse effects on hydration status due to 12 h of water restriction, but including the body water content (e.g., ICW and ECW), it may be that the stability of the homeostasis of water in the human body contributed to these unexpected results.

Furthermore, women were more sensitive to water supplementation than men, which was consistent with other studies, with more aspects of body composition changed in women than that of men (31). A study conducted among 56 male wrestlers has reported that, after drinking a carbohydrate–electrolyte solution, the ECW significantly decreased incrementally from the pre-dehydration test to rehydration test; even after 2 hours of rehydration, the ECW did not return to the baseline level (53). Moreover, a study evaluating the effects of different kinds of water demonstrated no changes in ECW among 88 amateur male athletes after 300 mL of water supplementation, while that of those who drank mineral water decreased, with a concomitant increase in ICW (54). This indicates that the type of fluid may lead to the differences in the changes in body composition. Notwithstanding, as there exist few related studies considering the effects of water supplementation on body composition, it was difficult to compare the results of this study with those of other studies. In this study, even after 500 mL of water supplementation, ECW still decreased. These results may be attributed to the metabolism of water in the human body and the amount of water supplied to the participants after water restriction for 12 h. In our study, participants drank the water within 10 min and the interval of the rehydration

test was 90 min after water supplementation, which was set according to the results of some studies and the pre-investigation for this study (55–57). Water may have an associated metabolic process, with many physiological reactions potentially occurring, among young adults after water supplementation. Considering the interval of this process, it may take longer than 90 min for the water to be absorbed and metabolized completely in the body. Furthermore, the results were pertinent to the debate over the dose-effect relationship of acute water supplementation on body composition, in which the amount of 500 mL of water did not have the same effect on body composition as 200 mL of water. Furthermore, although studies have shown the high validity of the BIA in measuring body composition indices, some studies arrived at an opposite conclusion, reporting the inaccuracy of the BIA (58). Maybe such a lack of validity of the BIA could have contributed to the minor differences in ECW among young women. Studies have also shown that the BIA was only valid in measuring the body composition among obese people or people with good hydration status. Furthermore, it has been shown that critical factors, such as variations in machine specifications, technical skill, the subject, and environmental factors, have an impact on the BIA results (59).

In this study, we detailed a preliminary exploration of the effects of different volumes of water on body composition indices after 12 h of water restriction. In future, more related studies should be implemented to explore this issue using the gold-standard techniques, such as isotope dilution (60), or still using BIA but among different people with different ages. The current consensus is that, in free-living conditions, no matter the amount of water that healthy people consume, the total body water remains stable. In this study, the consumption of 500, 200, and 100 mL of water may have impacted the TBW, but such results were not detected in this study due to the sensitivity of the BIA, in contrast to the results of a previous study (61). Studies have shown that the sensitivity is not only related to the volume of fluids, but also to the resistivity. The results of the study indicated that the resistivity of the ECW and ICW was different and associated with the types of the fluid, for which the resistivity of ICW was approximately twice that of ECW and increased slightly (but not significantly) following

TABLE 2 | Body composition indices of men and women.

Males	Baseline test					Rehydration test				<i>P</i> _{interaction}
	NW group (<i>n</i> = 8)	WS group 1 (<i>n</i> = 8)	WS group 2 (<i>n</i> = 8)	WS group 3 (<i>n</i> = 8)	Total (<i>n</i> = 32)	NW group (<i>n</i> = 8)	WS group 1 (<i>n</i> = 8)	WS group 2 (<i>n</i> = 8)	WS group 3 (<i>n</i> = 8)	
ICW (kg)	22.0 ± 2.6 ^b	25.3 ± 4.5 ^b	24.5 ± 2.3 ^b	23.3 ± 1.5 ^b	23.9 ± 3.1 ^b	22.1 ± 2.6 ^b	25.0 ± 4.6 ^b	24.6 ± 2.3 ^b	23.3 ± 1.3 ^b	0.354
ICW/TBW (%)	62.8 ± 0.5 ^b	62.8 ± 0.4 ^b	62.8 ± 0.7 ^b	62.8 ± 0.4 ^b	62.8 ± 0.5 ^b	62.7 ± 0.4 ^b	62.7 ± 0.3 ^b	62.7 ± 0.7 ^b	62.7 ± 0.5 ^b	0.960
ECW (kg)	13.1 ± 1.7 ^b	15.0 ± 2.7 ^b	14.7 ± 1.3 ^b	13.8 ± 1.1 ^b	14.1 ± 1.9 ^b	13.2 ± 1.7 ^b	14.9 ± 2.8 ^b	14.6 ± 1.2 ^b	13.9 ± 1.0 ^b	0.397
ECW/TBW (%)	37.2 ± 0.5 ^b	37.2 ± 0.4 ^b	37.2 ± 0.7 ^b	37.2 ± 0.4 ^b	37.2 ± 0.5 ^b	37.3 ± 0.4 ^b	37.3 ± 0.3 ^b	37.3 ± 0.7 ^b	37.3 ± 0.5 ^b	0.960
ECW/ICW (%)	59.4 ± 1.3 ^b	59.2 ± 1.0 ^b	59.2 ± 1.8 ^b	59.3 ± 1.1 ^b	59.3 ± 1.3 ^b	59.6 ± 1.0 ^b	59.5 ± 0.8 ^b	59.4 ± 1.8 ^b	59.6 ± 1.3 ^b	0.956
TBW (kg)	35.1 ± 4.3 ^b	40.3 ± 7.2 ^b	39.5 ± 3.6 ^b	37.2 ± 2.6 ^b	38.0 ± 4.9 ^b	35.2 ± 4.2 ^b	39.9 ± 7.4 ^b	39.2 ± 3.5 ^b	37.2 ± 2.3 ^b	0.355
TBW/BW (%)	55.0 ± 4.0 ^b	60.4 ± 4.5 ^b	56.5 ± 4.4 ^b	58.9 ± 4.1 ^b	57.7 ± 4.6 ^b	54.7 ± 4.0 ^b	59.7 ± 4.4 ^b	56.2 ± 4.7 ^b	59.3 ± 3.6 ^b	0.224
TBW/FFM (%)	73.3 ± 0.2	73.4 ± 0.2	73.3 ± 0.4	73.3 ± 0.1	73.3 ± 0.2	74.7 ± 3.7	73.4 ± 0.2	73.3 ± 0.4	74.6 ± 3.7	0.585
Females	NW group (<i>n</i> = 8)	WS group 1 (<i>n</i> = 8)	WS group 2 (<i>n</i> = 8)	WS group 3 (<i>n</i> = 8)	Total (<i>n</i> = 32)	NW group (<i>n</i> = 8)	WS group 1 (<i>n</i> = 8)	WS group 2 (<i>n</i> = 8)	WS group 3 (<i>n</i> = 8)	<i>P</i> _{interaction}
ICW (kg)	16.8 ± 1.9	17.4 ± 2.4	17.7 ± 1.8	18.9 ± 1.6	17.7 ± 2.0	16.2 ± 1.9	17.1 ± 2.4	17.5 ± 1.8	18.3 ± 1.7	0.088
ICW/TBW (%)	62.1 ± 0.5	62.1 ± 0.5	61.9 ± 0.4	62.0 ± 0.8	62.0 ± 0.5	61.9 ± 0.6	61.9 ± 0.4	61.8 ± 0.6	62.0 ± 0.7	0.625
ECW (kg)	10.2 ± 1.0 ^a	10.7 ± 1.6 ^a	10.9 ± 1.1	11.6 ± 1.1 ^a	10.8 ± 1.3	10.0 ± 1.0	10.6 ± 1.6	10.8 ± 1.1	11.3 ± 1.1	0.043
ECW/TBW (%)	37.9 ± 0.5	37.9 ± 0.5	38.1 ± 0.4	38.0 ± 0.8	38.0 ± 0.5	38.1 ± 0.6	38.1 ± 0.4	38.2 ± 0.6	38.0 ± 0.7	0.625
ECW/ICW (%)	61.0 ± 1.3	61.2 ± 1.3	61.5 ± 1.0	61.3 ± 2.0	61.2 ± 1.4	61.5 ± 1.5	61.5 ± 1.1	61.8 ± 1.5	61.4 ± 1.9	0.621
TBW (kg)	27.0 ± 2.8 ^a	28.1 ± 4.1 ^a	28.5 ± 2.8	30.4 ± 2.7 ^a	28.5 ± 3.3	26.2 ± 2.9	27.7 ± 4.0	28.3 ± 2.9	29.6 ± 2.8	0.055
TBW/BW (%)	49.2 ± 2.2	52.4 ± 3.6	51.5 ± 3.0	53.8 ± 4.4	51.7 ± 3.7	48.9 ± 2.8	51.6 ± 3.5	51.0 ± 3.1	52.4 ± 4.6	0.275
TBW/FFM (%)	74.5 ± 3.7	73.2 ± 0.2	73.2 ± 0.1	73.3 ± 0.2	73.6 ± 1.8	73.3 ± 0.1	73.1 ± 0.3	73.3 ± 0.2	73.3 ± 0.2	0.442

Values are shown as the mean ± standard deviation (SD). ^a*p* < 0.05 in the comparison between the baseline test and rehydration test. ^b*p* < 0.05 in the comparison between men and women within a group. For men, no significant interactions between time and volume were found in ICW, ICW/TBW, ECW, ECW/TBW, ECW/ICW, TBW, TBW/BW, and TBW/FFM (all *p* > 0.05). For women, a significant interaction between time and volume was only found in ECW (*F* = 3.096, *p* = 0.043), not in ICW, ICW/TBW, ECW/TBW, ECW/ICW, TBW, TBW/BW, and TBW/FFM (all *p* > 0.05).

infusion; meanwhile, the resistivity of ECW was significantly decreased by NaCl infusion in rats (62). Furthermore, it may be that acute water supplementation did not influence the TBW, indicating that further studies, using other methods to measure the body composition and larger volumes of water provided to the participants after water supplementation, are needed. Interestingly, the TBW decreased in women with water supplementation of 500 and 100 mL and no water supplementation, while no decrease was found among those with 200 mL of water. Perhaps amounts of water between 200 and 500 mL could improve the TBW among young women after 12 h of water restriction. Data from studies exploring the effect of water supplementation on TBW have been inconsistent. Similar to our study, some studies have not observed any beneficial effects of water supplementation on TBW. A study conducted among 13 active men has reported that the TBW was unaffected after consuming up to 591 mL of water (63). Furthermore, among 45 healthy male and female runners, the TBW remained unchanged after water replenishment of 538 mL and 533 mL, respectively (64). In a study investigating the effects of carbohydrate-electrolyte fluid on body composition among young healthy athletes, it was found that, during the 2 h of rehydration, no significant changes in TBW were observed (49). The results of a randomized study revealed that, after drinking 400 mL fluid, no significant differences in TBW were found among adults after fasting for 10 h (65). In addition, the TBW even decreased from baseline among 10 young men and women when measured 150 min after administration with 466 mL of deionized water (66). Contrary to the results of the studies, the beneficial impacts of water supplementation on TBW have been observed in some research works. One study investigating the effects of different volumes of water on TBW has revealed that, after 500 mL of water supplementation, an increase in TBW by 0.21 kg was found in men, but not in women (67). Similarly, another study has shown that participants who consumed 1,000 mL of water had higher TBW than their counterparts who drank nothing (31). The intervals used for measuring the changes in the TBW, as well as the types of fluids supplied to the participants, could explain the differences in the abovementioned studies.

Studies have demonstrated that body composition differs as a result of many factors, such as age, gender, BMI, nutrition status, and physiological stages (68, 69). Even for the same subjects, inter-daily variabilities of body composition have also been observed (70). Our data indicated differences in all of the considered indices, including ICW, ECW, TBW, ICW/TBW, ECW/TBW, and TBW/BW, between all the men and women. Consistent with a study conducted among young adults under free-living conditions, many aspects of body composition, including ICW, ECW, and TBW, differed significantly between men and women (20, 27, 71). One study conducted among children has confirmed similar differences between genders, showing that girls have lower TBW and TBW/BW than boys (26). The results of our study indicated that differences between men and women exist, even with different hydration statuses.

This research had some strengths. First, it provides the first exploration considering whether acute water supplementation has an impact on body composition among healthy young men and women after water restriction for 12 h. In addition,

a randomized controlled design was conducted among young male and female adults, to reduce the related bias induced by gender. Second, the hydration status of the participants was measured through urine osmolality and the investigators observed the lips of the participants at all times, to make sure that they followed the study procedure. Furthermore, in this study, the Inbody 720 was highly associated with DXA, with the correlation coefficient reaching 0.98. Furthermore, the result of each measurement was printed and calculated by the machine, following the 4C model. However, this study had certain limitations. For example, studies have shown a relationship between body hydration conditions, body composition, and microbiota (72), but no related biomarkers were measured in our study. Furthermore, more plasma biomarkers, including copeptin, were not explored in this study. Finally, the tiny changes in certain body composition indices, including the ECW, ICW, and TBW, may not be well-measured by the BIA. Therefore, future studies should use the BIA combined with gold-standard techniques (e.g., isotope dilution), to examine the effects of water supplementation on body composition.

CONCLUSION

Water supplementation has been shown to redistribute the water content in the body, with no decrease in ECW. Our results suggest that 200 mL of water may be the minimum volume to ensure a better distribution of the body water content among young female adults—but not men—after water restriction for 12 h. It may be that the sensitivity of the BIA did not allow for the detection of the tiny changes in certain body composition indices. More studies using other methods to measure these changes in body composition indices should be conducted, to address this issue.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The study protocol was approved by the Peking University Institutional Review Board. The ethical approval project identification code was IRB00001052-16071. The study was conducted according to the guidelines of the Declaration of Helsinki. All subjects signed the informed consent form before participating in the study.

AUTHOR CONTRIBUTIONS

JZ was responsible for the implementation of the study and drafting of the article. GM was responsible for the design of the study and provided substantive revisions to the initial

draft of the article. NZ, SD, and GM were responsible for the design of the study, quality management, and control of the implementation of the study. JZ and SL were responsible for the recruitment of participants. JZ, NZ, and SL were responsible for the implementation of the study. All authors were involved in the revision of the manuscript and approved this final version.

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Brazilian Reference Percentiles for Bioimpedance Phase Angle of Healthy Individuals

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Objectives: The present study was designed to estimate phase angle percentile curves for a broad age range of healthy individuals.

Methods: This is a cross-sectional study of healthy Brazilian individuals aged five to 80. InBodyS10 was used to assess phase angle. Reference curves were stratified by sex and estimated using Generalized additive models for location, scale, and shape as a continuous function of age. The phase angle determinants analyzed were physical activity, age, BMI, and SES variables.

Results: Data were analyzed from 2,146 individuals, 1,189 (55.2%) of whom were female. In both sexes, the phase angles showed a similar pattern (an increasing trend from childhood to the teenage phase, followed by stabilization during adult ages and a decrease in old adults). In female, the relationship between phase angle and age were associated with BMI and family income. In the male, the relationship between phase angle and age were associated with skin color and family income.

Conclusions: To the best of our knowledge, it is the first attempt to apply the GAMLSS technique to estimate phase angle percentiles in a healthy population covering most of the life cycle. We also showed that there are different phase angle determinants according to sex.

Keywords: bioimpedance (BIA), phase angle (PA), reference values, percentiles, determinants

INTRODUCTION

Phase angle (PA) from bioimpedance is measured by the potential difference of a low voltage alternating electric current introduced into the body. It is dependent on the resistive behavior and the capacitive effect on the cell membrane and other interfaces (1). PA has been proposed to indicate cellular health, where higher values reflect higher cellularity, cell membrane integrity, and better cell function (1). For this reason, it has been used as a health status tool and an important predictor of disease severity and survival in different medical conditions (2–6). However, the cut-offs used in the literature are not necessarily transferable to other populations and might thus not be applicable in the general clinical setting. This is because the cut-offs are generated primarily using the median or lowest quantile from a specific population without considering the determinants of phase angle. Phase angle reference values are still scarce, especially PA percentile curves (2).

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According to one recent meta-analysis involving more than 250,000 healthy subjects, age, sex, and BMI seem to be main independent determinants of phase angle (7). Another meta-analysis showed that physical activity also influences PA values, especially in individuals with chronic diseases, indicating that it should be considered as an associated variable (8).

Therefore, the present study was designed to estimate phase angle percentile curves for a broad age range of healthy individuals stratified by sex and understand the relationship between phase angle and physical activity, age, BMI, skin color, and family income.

MATERIALS AND METHODS

Study Design

This was a cross-sectional study that followed the STROBE statement guidelines for reporting observational studies (9).

Setting and Participants

Healthy community-dwelling individuals aged five to 80 years old, of both sexes, were invited to participate in the study. The exclusion criteria were contraindications against bioimpedance measurements, such as diseases affecting the skin's electrical resistance, pregnancy, persons with an implanted pacemaker or cardioverter-defibrillator, and amputated persons using a prosthesis/orthosis. Participants were considered healthy if they had not been diagnosed with any chronic disease or were not on continuous medication. Data were collected from December 2015 to April 2019 in public and private schools, companies, and at events in cities in southern Brazil. Recruitment occurred through word of mouth.

Data Measurements

Sociodemographic variables were obtained through structured interviews. These included age (years), sex (male or female), self-reported skin color (categorized into white, black, or others—brown, Asian, and indigenous were grouped together to homogenize the size of the categories), and location of residence (rural or urban), defined according to the IBGE Brazilian demographic census (10). Income was categorized into low and high (according to whether the families earned more or less than the median income, calculated separately for the samples of men and women).

To assess the level of physical activity, the participants answered different validated questionnaires, according to their age. The children up to 10 years of age answered the Physical Activity Checklist (11), and participants over 10 years of age answered the short version of the International Questionnaire on Physical Activity (IPAQ) (12, 13). After that, the participants were classified as active or inactive according to their physical activity level. The cut-off points to be considered active was 300 min of moderate to vigorous physical activity (MVPA) per week for children and adolescents. For the adults (18 years and older), it was 150 min of MVPA, or 75 min of vigorous physical activity (VPA) per week. These cut-off points are the same ones suggested by the World Health Organization (14).

Body mass was measured with the participants in a standing position, wearing the least possible amount of clothing and no shoes, using a calibrated digital scale (Charder MS6121). Height was measured with the participants standing barefoot with their feet parallel and heels together, arms along their body, and head in the Frankfurt plane, using a Sanny compact stadiometer and a tape measure to the nearest 0.1 cm (American Medical do Brazil Ltda, São Bernardo do Campo, Brazil). Body mass index was classified as underweight, normal weight, pre-obesity, and obesity according to the WHO BMI classification for children, adults, and the elderly (15).

Bioimpedance Multi-frequency InBodyS10 (Ottoboni, Rio de Janeiro, RJ, Brazil) was used to assess phase angle. The InBodyS10 showed excellent agreements with DEXA regarding to whole body lean mass, fat mass and percentage body fat (16). The applied current was 100 μ A (1 kHz) and 500 μ A and frequency was 50 kHz. The hand electrodes were attached to each thumb and middle finger, while the foot electrodes were positioned between the ankle bone and the heel, covering as much area as possible. The BIA was performed with the participants on a non-conductive surface in the standing position, with their legs apart and arms held away from their body and wearing the least amount of clothing possible and no metal jewelry. The standard guidelines were followed to instruct regarding fasting state of the subjects before the BIA (17). All measurements were performed by one of the four experienced researchers according to the manufacturer's instructions using a standardized technique. All the participants completed three evaluations, and the average of the three values was considered as their result.

Statistical Analysis

The data were expressed as mean (SD) or median and interquartile range (IQR, 25th–75th percentiles) for the continuous variables and absolute and relative frequencies for the categorical variables.

Generalized additive models for location, scale, and shape (GAMLSS) were used to estimate age-related phase angles and determine phase angle predictors. These models are more flexible than linear or generalized linear models. They let the data determine the relationship between the predictor and the covariables rather than enforcing a linear (or polynomial) relationship. It is also possible to use smoothing techniques and allow the covariables to model variability and shape besides the median values (18, 19).

First, the LMS R function was used to estimate the power value, possible transformation of age and select the distribution family (among BCCGo, BCPEo, and BCT families). To identify the models' optimum number of effective degrees of freedom (edf), the automated "pb" function was implemented. Models were also tested with and without age transformation, different degrees of freedom, and cubic smoothing. The models were compared using GAIC (generalized Akaike information criterion) (19, 20).

GAMLSS models were also used to explore phase angle determinants. Physical activity, BMI, skin color, and family income were analyzed one by one in a bivariable model with age to test any possible interaction between age and the

TABLE 1 | Characteristics of participants.

Characteristics	Total sample	Male	Female
<i>n</i> (%)	2,122 (100.0)	951 (44.8)	1,171 (55.2)
Age (years), <i>n</i> (%)	2,122 (100.0)	951 (44.8)	1,171 (55.2)
5–12	901 (42.4)	398 (41.8)	503 (42.9)
13–15	222 (10.4)	95 (9.9)	127 (10.8)
16–18	81 (3.8)	40 (4.2)	41 (3.5)
19–28	222 (10.4)	102 (10.7)	120 (10.2)
29–38	262 (12.3)	119 (12.5)	143 (12.2)
39–48	239 (11.2)	102 (10.7)	137 (11.6)
49–58	128 (6.0)	67 (7.0)	61 (5.2)
> 59	67 (3.1)	28 (2.9)	39 (3.3)
Body mass index, <i>n</i> (%)	2,122 (100.0)	951 (44.8)	1,171 (55.2)
Under/Normal weight	979 (46.1)	400 (42.0)	579 (49.4)
Pre-obesity	665 (31.3)	340 (35.7)	325 (27.7)
Obesity	478 (22.5)	211 (22.1)	267 (22.8)
Skin color*, <i>n</i> (%)	1,611 (100.0)	751	820
White	724 (44.9)	360 (47.9)	364 (42.3)
Black	592 (36.7)	251 (33.4)	341 (39.6)
Others (brown, Asian, and indigenous)	295 (18.3)	140 (18.6)	115 (18.0)
Missing data	511 (24.0)	200 (21.0%)	311 (26.6)
Family monthly income	1,596 (100.0)	720	876
Low income	846 (53.2)	399 (55.4)	450 (38.4)
High income	747 (46.8)	321 (44.6)	426 (48.6)
Missing data	526 (24.8)	231 (24.3)	295 (25.2)
Residence area, <i>n</i> (%)	2,122 (100.0)	951	1,171
Urban	1,304 (61.5)	634 (66.8)	670 (57.3)
Missing data	5 (0.2)	3 (0.3)	2 (0.2)
Physical activity, <i>n</i> (%)	1,599 (100.0)	729	870
Inactive	1,078 (67.4)	440 (60.3)	638 (73.3)
Missing	523 (24.9)	222 (23.3)	301 (25.7)

*Self-declared.

covariables. The covariables and interactions, significant at 5%, were maintained in the multivariable model.

All analyses were performed using the R software, version 3.2.3, with the “gamlss” package, version 5.1-5 (21).

The present study was part of an umbrella project and was conducted according to the Declaration of Helsinki (22). This project received the approval of the Research Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul (permission 2.187.802). For adult participants, the Informed Consent Term (ICT) was signed. In the case of underage participants, the consent term was obtained, and ICT signature was acquired from their parents or guardians.

RESULTS

A total of 2,122 participants aged five up to 80 years old were evaluated. The majority were females ($n = 1,171$, 55.2%); were aged between five and 12 years ($n = 901$, 42.4%); were underweight/normal weight ($n = 979$, 46.1%); self-declared their skin color as white ($n = 724$, 44.9%); were living in an urban area ($n = 1,304$, 61.5%); were inactive according to their physical activity level ($n = 1,078$, 67.4%); and were classified as low income ($n = 846$, 53.2%) (Table 1).

Phase Angle Centile Estimation

Women: The final age-related model for centile estimation was adjusted using the Box-Cox Cole and Green orig family distribution (BCCGo); there was no age transformation; $\mu = 10$, $\sigma = 4$, and $\nu = 4$, and these were all associated with age.

Men: The final age-related model for centile estimation was adjusted using the Box-Cox Power Exponential orig family distribution (BCPE); there was no age transformation; $\mu = 13$, $\sigma = 4$, $\nu = 4$, $\tau = 2$, and these were all associated with age.

The estimated phase angle percentiles showed for both sexes that the values increase through childhood, stabilize during most of adulthood, and decrease through late adulthood (Figure 1). The estimated percentiles and z-scores are shown in Table 2 for men and in Table 3 for women.

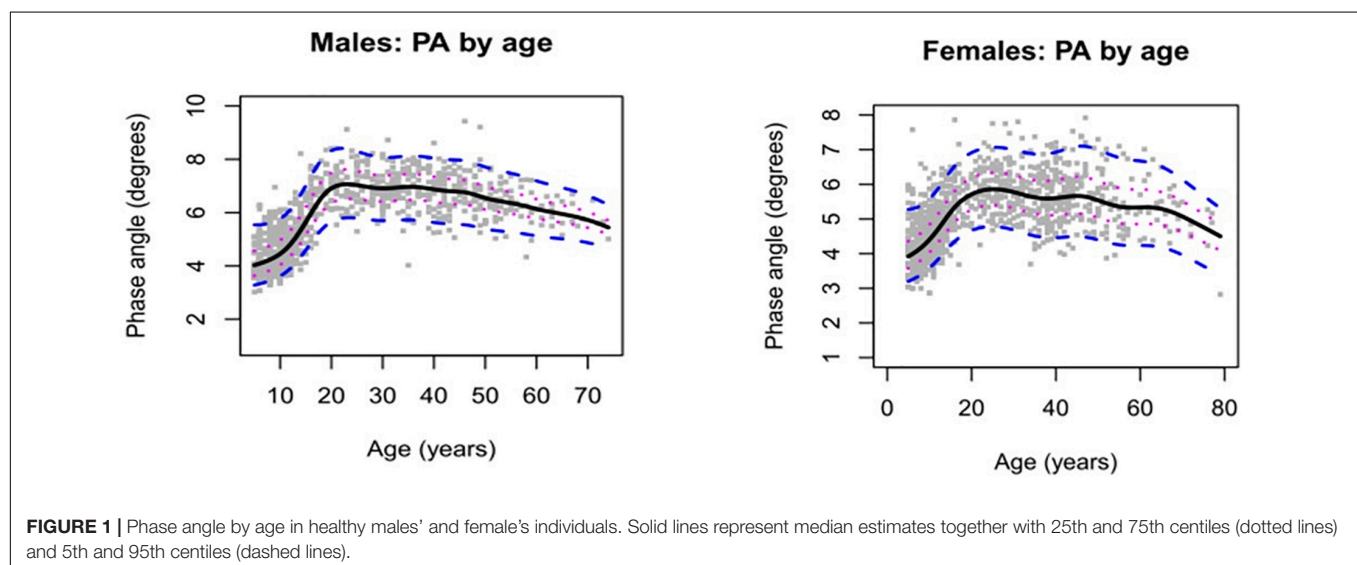


TABLE 2 | Men's phase angle percentiles and z-score.

Age	P5	P25	P50	P75	P95	−2	−1	0	+ 1	+2
5	3.28	3.63	4.03	4.56	5.54	3.18	3.49	4.03	4.86	5.99
6	3.33	3.69	4.09	4.61	5.54	3.23	3.55	4.09	4.90	5.96
7	3.38	3.75	4.15	4.67	5.56	3.28	3.61	4.15	4.95	5.95
8	3.44	3.83	4.23	4.75	5.6	3.33	3.68	4.23	5.02	5.96
9	3.52	3.92	4.33	4.84	5.67	3.41	3.77	4.33	5.10	6.00
10	3.62	4.03	4.45	4.96	5.77	3.5	3.87	4.45	5.22	6.09
11	3.74	4.18	4.61	5.12	5.91	3.62	4.01	4.61	5.38	6.22
12	3.91	4.37	4.81	5.32	6.11	3.77	4.19	4.81	5.58	6.42
13	4.11	4.60	5.05	5.58	6.37	3.96	4.41	5.05	5.84	6.67
14	4.34	4.86	5.34	5.88	6.68	4.18	4.67	5.34	6.14	6.97
15	4.59	5.15	5.65	6.2	7.01	4.42	4.94	5.65	6.47	7.31
16	4.86	5.45	5.97	6.54	7.35	4.67	5.23	5.97	6.81	7.65
17	5.11	5.74	6.28	6.86	7.69	4.92	5.51	6.28	7.14	7.99
18	5.35	6.00	6.55	7.14	7.97	5.14	5.77	6.55	7.42	8.28
19	5.53	6.21	6.77	7.36	8.19	5.31	5.97	6.77	7.64	8.49
20	5.66	6.35	6.91	7.5	8.32	5.43	6.11	6.91	7.78	8.62
21	5.74	6.45	7.00	7.59	8.39	5.51	6.20	7.00	7.86	8.68
22	5.79	6.5	7.05	7.62	8.41	5.55	6.25	7.05	7.89	8.69
23	5.81	6.52	7.06	7.62	8.39	5.56	6.27	7.06	7.88	8.67
24	5.8	6.52	7.05	7.6	8.35	5.56	6.27	7.05	7.86	8.62
25	5.79	6.50	7.03	7.56	8.29	5.54	6.26	7.03	7.81	8.56
26	5.77	6.48	7.00	7.52	8.23	5.52	6.24	7.00	7.76	8.49
27	5.74	6.46	6.96	7.47	8.17	5.49	6.21	6.96	7.71	8.42
28	5.72	6.43	6.94	7.43	8.12	5.47	6.19	6.94	7.67	8.37
29	5.71	6.42	6.92	7.41	8.09	5.45	6.18	6.92	7.64	8.33
30	5.7	6.42	6.91	7.39	8.07	5.44	6.18	6.91	7.62	8.31
31	5.7	6.42	6.91	7.39	8.07	5.43	6.18	6.91	7.62	8.31
32	5.71	6.44	6.93	7.40	8.07	5.43	6.20	6.93	7.63	8.31
33	5.72	6.45	6.94	7.42	8.09	5.44	6.21	6.94	7.64	8.33
34	5.72	6.47	6.96	7.43	8.11	5.44	6.22	6.96	7.66	8.35
35	5.73	6.48	6.97	7.44	8.12	5.44	6.23	6.97	7.67	8.37
36	5.72	6.48	6.97	7.44	8.13	5.43	6.23	6.97	7.67	8.38
37	5.71	6.47	6.96	7.43	8.12	5.41	6.22	6.96	7.66	8.37
38	5.68	6.45	6.93	7.4	8.10	5.39	6.2	6.93	7.63	8.35
39	5.66	6.42	6.90	7.37	8.07	5.36	6.17	6.90	7.60	8.33
40	5.63	6.39	6.87	7.34	8.04	5.33	6.15	6.87	7.57	8.30
41	5.61	6.37	6.85	7.31	8.02	5.30	6.12	6.85	7.54	8.28
42	5.59	6.35	6.82	7.29	8.00	5.28	6.10	6.82	7.52	8.26
43	5.57	6.33	6.8	7.27	7.98	5.27	6.09	6.8	7.5	8.26
44	5.56	6.32	6.79	7.26	7.98	5.26	6.08	6.79	7.49	8.26
45	5.56	6.32	6.78	7.24	7.97	5.25	6.07	6.78	7.48	8.25
46	5.54	6.3	6.76	7.22	7.95	5.24	6.06	6.76	7.46	8.24
47	5.52	6.27	6.72	7.18	7.91	5.22	6.03	6.72	7.41	8.20
48	5.48	6.22	6.67	7.12	7.85	5.18	5.98	6.67	7.35	8.14
49	5.43	6.16	6.61	7.05	7.78	5.14	5.93	6.61	7.28	8.07
50	5.39	6.11	6.55	6.98	7.71	5.10	5.88	6.55	7.21	8.00
51	5.35	6.07	6.5	6.93	7.65	5.06	5.84	6.5	7.16	7.95
52	5.33	6.04	6.46	6.88	7.60	5.04	5.81	6.46	7.11	7.90
53	5.31	6.01	6.42	6.84	7.56	5.02	5.79	6.42	7.07	7.86
54	5.29	5.98	6.39	6.8	7.52	5.00	5.76	6.39	7.03	7.82
55	5.27	5.96	6.36	6.76	7.48	4.98	5.74	6.36	6.99	7.78
56	5.25	5.93	6.32	6.72	7.43	4.96	5.71	6.32	6.94	7.73
57	5.22	5.9	6.28	6.67	7.38	4.94	5.68	6.28	6.89	7.68

(Continued)

TABLE 2 | (Continued)

Age	P5	P25	P50	P75	P95	−2	−1	0	+ 1	+2
58	5.19	5.86	6.24	6.62	7.32	4.91	5.65	6.24	6.83	7.62
59	5.16	5.82	6.19	6.56	7.26	4.88	5.61	6.19	6.77	7.55
60	5.13	5.77	6.14	6.51	7.19	4.85	5.57	6.14	6.72	7.49
61	5.1	5.74	6.09	6.45	7.13	4.83	5.54	6.09	6.66	7.43
62	5.07	5.71	6.05	6.41	7.08	4.81	5.51	6.05	6.61	7.38
63	5.05	5.68	6.02	6.36	7.03	4.79	5.48	6.02	6.57	7.33
64	5.03	5.65	5.98	6.32	6.98	4.77	5.46	5.98	6.52	7.28
65	5.01	5.62	5.95	6.28	6.94	4.76	5.43	5.95	6.48	7.23
66	4.99	5.59	5.91	6.24	6.88	4.74	5.41	5.91	6.43	7.18
67	4.97	5.56	5.87	6.19	6.83	4.71	5.38	5.87	6.38	7.12
68	4.94	5.52	5.82	6.13	6.77	4.69	5.34	5.82	6.32	7.06
69	4.9	5.47	5.77	6.08	6.70	4.66	5.30	5.77	6.26	6.99
70	4.86	5.43	5.72	6.01	6.63	4.62	5.26	5.72	6.19	6.92
71	4.82	5.37	5.65	5.94	6.55	4.58	5.20	5.65	6.12	6.84
72	4.77	5.31	5.58	5.86	6.46	4.53	5.15	5.58	6.04	6.74
73	4.71	5.24	5.51	5.78	6.37	4.48	5.08	5.51	5.95	6.65
74	4.65	5.17	5.43	5.69	6.27	4.43	5.01	5.43	5.86	6.54
75	4.59	5.10	5.35	5.60	6.17	4.37	4.95	5.35	5.76	6.44
76	4.53	5.03	5.27	5.52	6.07	4.31	4.88	5.27	5.67	6.34
77	4.47	4.96	5.19	5.43	5.97	4.26	4.81	5.19	5.59	6.24
78	4.42	4.89	5.11	5.35	5.88	4.21	4.75	5.11	5.50	6.14
79	4.36	4.82	5.04	5.27	5.79	4.15	4.68	5.04	5.41	6.05
80	4.30	4.75	4.97	5.18	5.70	4.10	4.62	4.97	5.33	5.95

Phase Angle Determinants

Female: In the final multivariable model, the relationship between phase angle and age was associated with BMI and family income (with a significant interaction) (**Supplementary Table 1**).

Male: In the final multivariable model, the relationship between phase angle and age was associated with BMI and skin color (with a significant interaction) (**Supplementary Table 1**).

DISCUSSION

This study used a large sample of healthy Brazilian individuals aged five to 80 years old to estimate phase angle, as measured by BIA. The models used allowed us to estimate smooth percentile curves as well as z-scores stratified by age and sex.

The estimated phase angle percentiles showed that the values increase through childhood, stabilize during most of adulthood, and decrease through late adulthood, which is consistent with the previously published meta-analysis (7). These findings reflect the physiological changes that occur throughout life. Considering that phase angle is an indicator of cell function and health, these findings reflect the different intracellular and functionality mechanisms involved in the cell membrane, which improve up to adult age but deteriorate during the late stages of life (7, 23).

We also found different phase angle determinants depending on sex. In both sexes, phase angle is associated with age and BMI. Previously study also showed that phase angle changes with sex and age and is associated with BMI (24). Its dependence on body composition is complex, being determined by BMI, %FM,

TABLE 3 | Women's phase angle means estimates and z-score.

Age	P5	P25	P50	P75	P95	-2	-1	0	+1	+2
5	3.2	3.58	3.92	4.36	5.28	3.08	3.44	3.92	4.62	5.76
6	3.26	3.65	3.99	4.43	5.29	3.14	3.5	3.99	4.68	5.72
7	3.32	3.72	4.07	4.5	5.33	3.19	3.58	4.07	4.75	5.72
8	3.39	3.81	4.17	4.59	5.39	3.26	3.66	4.17	4.83	5.75
9	3.48	3.92	4.28	4.7	5.47	3.35	3.76	4.28	4.94	5.81
10	3.58	4.03	4.4	4.83	5.58	3.44	3.87	4.4	5.06	5.9
11	3.7	4.16	4.54	4.97	5.71	3.55	4.00	4.54	5.2	6.02
12	3.82	4.31	4.7	5.13	5.86	3.67	4.14	4.7	5.36	6.16
13	3.96	4.46	4.86	5.3	6.03	3.79	4.29	4.86	5.54	6.33
14	4.1	4.62	5.03	5.48	6.21	3.93	4.44	5.03	5.71	6.5
15	4.23	4.78	5.19	5.65	6.38	4.05	4.59	5.19	5.88	6.67
16	4.36	4.91	5.34	5.80	6.53	4.17	4.72	5.34	6.04	6.82
17	4.46	5.03	5.46	5.93	6.66	4.26	4.83	5.46	6.16	6.94
18	4.54	5.12	5.56	6.03	6.76	4.34	4.92	5.56	6.27	7.05
19	4.61	5.2	5.65	6.12	6.85	4.41	5.00	5.65	6.35	7.13
20	4.67	5.27	5.71	6.19	6.91	4.46	5.06	5.71	6.42	7.19
21	4.71	5.32	5.77	6.24	6.97	4.50	5.11	5.77	6.48	7.25
22	4.75	5.36	5.81	6.28	7.01	4.53	5.15	5.81	6.52	7.29
23	4.77	5.38	5.84	6.31	7.04	4.56	5.17	5.84	6.55	7.32
24	4.78	5.4	5.85	6.33	7.06	4.57	5.19	5.85	6.57	7.34
25	4.78	5.4	5.86	6.34	7.07	4.57	5.19	5.86	6.58	7.35
26	4.78	5.4	5.86	6.34	7.07	4.56	5.19	5.86	6.58	7.35
27	4.76	5.39	5.85	6.33	7.06	4.55	5.17	5.85	6.57	7.34
28	4.74	5.37	5.83	6.31	7.04	4.52	5.15	5.83	6.55	7.32
29	4.72	5.34	5.81	6.29	7.02	4.50	5.13	5.81	6.53	7.30
30	4.68	5.31	5.78	6.26	7.00	4.46	5.10	5.78	6.5	7.28
31	4.65	5.28	5.74	6.23	6.97	4.43	5.06	5.74	6.47	7.25
32	4.61	5.24	5.71	6.20	6.94	4.39	5.03	5.71	6.44	7.22
33	4.57	5.21	5.68	6.17	6.91	4.35	4.99	5.68	6.41	7.19
34	4.54	5.18	5.65	6.14	6.89	4.32	4.96	5.65	6.39	7.17
35	4.51	5.15	5.62	6.12	6.87	4.29	4.93	5.62	6.37	7.16
36	4.49	5.13	5.61	6.10	6.87	4.26	4.91	5.61	6.35	7.16
37	4.47	5.12	5.59	6.10	6.87	4.25	4.89	5.59	6.35	7.16
38	4.46	5.11	5.59	6.10	6.88	4.24	4.89	5.59	6.35	7.18
39	4.46	5.11	5.59	6.11	6.9	4.23	4.88	5.59	6.37	7.2
40	4.46	5.11	5.6	6.12	6.93	4.24	4.89	5.6	6.39	7.24
41	4.47	5.12	5.62	6.14	6.96	4.24	4.9	5.62	6.41	7.28
42	4.48	5.13	5.63	6.17	7.00	4.25	4.91	5.63	6.44	7.32
43	4.49	5.15	5.65	6.19	7.04	4.26	4.92	5.65	6.46	7.37
44	4.49	5.16	5.66	6.21	7.07	4.27	4.92	5.66	6.49	7.41
45	4.5	5.16	5.67	6.22	7.09	4.28	4.93	5.67	6.50	7.44
46	4.49	5.15	5.66	6.22	7.10	4.27	4.92	5.66	6.50	7.46
47	4.48	5.13	5.64	6.2	7.1	4.26	4.91	5.64	6.49	7.45
48	4.46	5.11	5.62	6.17	7.07	4.24	4.88	5.62	6.46	7.44
49	4.43	5.08	5.58	6.14	7.04	4.21	4.85	5.58	6.43	7.40
50	4.4	5.04	5.54	6.10	7.00	4.19	4.81	5.54	6.38	7.36
51	4.36	5.00	5.50	6.05	6.95	4.15	4.78	5.50	6.34	7.31
52	4.33	4.96	5.46	6.00	6.90	4.12	4.74	5.46	6.29	7.26
53	4.3	4.93	5.42	5.96	6.85	4.09	4.71	5.42	6.24	7.21
54	4.28	4.90	5.38	5.92	6.81	4.07	4.68	5.38	6.21	7.16
55	4.26	4.87	5.36	5.90	6.77	4.05	4.66	5.36	6.17	7.12
56	4.24	4.86	5.34	5.87	6.74	4.04	4.64	5.34	6.15	7.09
57	4.24	4.85	5.33	5.86	6.72	4.03	4.63	5.33	6.13	7.06

(Continued)

TABLE 3 | (Continued)

Age	P5	P25	P50	P75	P95	-2	-1	0	+1	+2
58	4.23	4.85	5.33	5.85	6.70	4.03	4.63	5.33	6.13	7.04
59	4.23	4.85	5.33	5.85	6.69	4.03	4.64	5.33	6.12	7.02
60	4.24	4.86	5.33	5.85	6.67	4.03	4.64	5.33	6.12	7.00
61	4.24	4.86	5.34	5.85	6.66	4.02	4.64	5.34	6.11	6.97
62	4.23	4.86	5.34	5.84	6.64	4.02	4.64	5.34	6.10	6.94
63	4.22	4.85	5.33	5.83	6.60	4.00	4.64	5.33	6.08	6.90
64	4.21	4.84	5.31	5.81	6.56	3.99	4.62	5.31	6.06	6.85
65	4.18	4.82	5.29	5.78	6.52	3.96	4.60	5.29	6.02	6.79
66	4.15	4.8	5.26	5.74	6.45	3.92	4.58	5.26	5.98	6.72
67	4.11	4.76	5.22	5.69	6.39	3.88	4.54	5.22	5.92	6.64
68	4.07	4.72	5.18	5.64	6.31	3.83	4.50	5.18	5.86	6.55
69	4.02	4.68	5.13	5.58	6.23	3.78	4.46	5.13	5.80	6.46
70	3.96	4.62	5.07	5.51	6.14	3.72	4.41	5.07	5.72	6.36
71	3.91	4.57	5.01	5.44	6.05	3.65	4.35	5.01	5.65	6.26
72	3.84	4.51	4.95	5.37	5.95	3.58	4.29	4.95	5.57	6.16
73	3.78	4.46	4.89	5.30	5.86	3.51	4.24	4.89	5.49	6.06
74	3.71	4.4	4.83	5.23	5.77	3.44	4.18	4.83	5.41	5.96
75	3.64	4.34	4.76	5.16	5.68	3.36	4.12	4.76	5.34	5.86
76	3.57	4.28	4.70	5.08	5.58	3.28	4.06	4.70	5.26	5.76
77	3.5	4.22	4.63	5.01	5.49	3.19	3.99	4.63	5.18	5.66
78	3.43	4.15	4.57	4.93	5.4	3.11	3.93	4.57	5.10	5.56
79	3.36	4.09	4.50	4.86	5.31	3.03	3.87	4.5	5.02	5.46
80	3.29	4.03	4.44	4.78	5.22	2.95	3.81	4.44	4.94	5.36

and their interaction. However, in women there is an additional association with family income, with an age interaction; while in men there is an additional association with skin color, also with an age interaction. The comparison with the reference values already available was limited, since the statistical models, age groups and determinants were not differentiated between the studies (7, 24).

The causes of the differences observed between the sexes may include health status, cultural patterns, and biological and hormonal differences between men and women. As national economies progress, individuals with increased socioeconomic status may start taking a greater interest in their health, whereas people with a lower socioeconomic status may continue to struggle for calories. In this sense, the differences found between the determinants of phase angle in the different sexes may reflect the health inequalities still found between sexes and races (25–27).

Previous studies have shown an association between the level of physical activity and phase angle; however, we did not find this association (8). One possible explanation for our different results could be that most studies have included individuals diagnosed with a disease and they have not presented phase angle percentiles adjusted for sex and age.

This study is not free of limitations. A temporal relationship could not be defined due to the study's cross-sectional design. This article included a convenience sample of participants from only one (South) of the five regions of Brazil. However, when the study data are compared with the latest national health surveys (28, 29), we observe that these are similar in the distribution of sex, skin color, BMI, and level of physical activity.

Our study included a greater number of younger and fewer elderly individuals than the percentages in these age groups of the Brazilian population. The primary justification for including more young participants is that the data presented in this paper are part of an umbrella project with other objectives. There is a scarcity of studies that present phase angle values that include the elderly. One of the main limitations of including participants in this age group is that most have a chronic disease diagnosis. Despite the differences in the distribution of age groups, the uncertainties related to these data were expressed in confidence intervals.

To the best of our knowledge, it is the first attempt to apply the GAMLSS technique to predict future PA distributions using a healthy population and to cover most of the life cycle. In general, GAMLSS offers a flexible approach due to a large number of implemented distribution families. With GAMLSS, it is possible to assess the effect of specific parameters on the outcome variable distribution. The WHO has adopted the GAMLSS methodology for creating reference growth curves (30). The reference values in this study can be used more comprehensively in clinical practice for populations with mixed SES.

This study estimated a useful table of phase angle percentiles stratified by sex and age. To the best of our knowledge, it is the first attempt to apply the GAMLSS technique to estimate phase angle percentiles in a healthy population, covering most of the life cycle. We also showed that there are different phase angle determinants according to sex.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comitê de Ética em Pesquisas da PUCRS. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

RM: conception and design of the work, data collection, data analysis, interpretation, drafting the article, critical revision of the article, and final approval of the version to be published. PZ: conception and design of the work, data analysis, and interpretation, drafting the article, critical revision of the article, and final approval of the version to be published. EM: data collection, critical revision of the article, and final approval of the version to be published. All authors contributed to the article and approved the submitted version.

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Proposition of Cutoff Points for Anthropometric Indicators to Identify High Blood Pressure in Adolescents

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Aim: To propose cutoff points for anthropometric indicators for high blood pressure (HBP) screening in adolescents and to identify, among these indicators, those more accurately for boys and girls.

Methods: This cross-sectional study was carried out in the city of São José, SC, Brazil with 634 adolescents aged 14 to 19 years. Blood pressure levels were measured using a digital oscillometric sphygmomanometer and adolescents were classified as having HBP or not. Anthropometric indicators were calculated based on anthropometric measurements such as body mass (BM), height, waist circumference (WC), hip circumference (HC) and triceps, subscapularis, suprailiac, and midcalf skinfold thickness (SF). The Receiver Operating Characteristic Curve (ROC) was used to analyze the predictive capacity of anthropometric indicators in the identification of HBP.

Results: Higher values of Area Under the Curve (AUC) were for the anthropometric indicators BM (0.67; 95%CI: 0.62–0.72), body mass index (BMI) (0.67; 95%CI: 0.62–0.72), and WC (0.67; 95%CI: 0.62–0.71) for males. For females, no anthropometric indicator had discriminatory power for HBP screening. The cutoff points for the anthropometric indicators with discriminatory power for HBP screening in males were BM > 64.80 Kg, BMI > 21.76 Kg/m², fat percentage (FP) > 15.75, waist height to ratio (WHtR) > 0.41, WC > 73.00 cm, and HC > 92.25 cm.

Conclusion: Anthropometric indicators of body adiposity had greater discriminatory power of HBP screening in males. For females, caution is suggested because the anthropometric indicators showed AUC values (95%CI) below 0.60.

Keywords: blood pressure, anthropometry, accuracy, youth, cutoff points

INTRODUCTION

Blood pressure is an important indicator of cardiovascular and metabolic health. Children and adolescents with high blood pressure levels are highly likely of becoming hypertensive adults. Therefore, early diagnosis and treatment can prevent long-term adverse cardiovascular events (1). Recently, a study has shown that high blood pressure (HBP) at younger ages (6 to 12 years old) may be associated with damage targeting organs such as the heart, brain, kidneys, retina, and blood vessels (2).

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Epidemiological studies have shown differences between countries in the prevalence of HBP such as Africa 12.7%, China 7.7%, India 7.6%, United States 13.6%, and Brazil 14.3% (3–7). The southern region of Brazil is one of the regions with the highest prevalence of BPH in adolescents (7). Data on the prevalence of overweight in the same population is worrying, such as Mediterranean region 25.0% (2 to 13 years old), Atlantic region 19.3% (2 to 13 years old), in China, overweight is 13.2% and obesity 9.3% (adolescents aged 12–18 years), the United States, overweight prevalence is 38.7% (adolescents aged 12–15 years) and 41.5% (adolescents aged 16–19 years), and in Brazil, overweight prevalence is 17.5% and obesity is 11.7% (8–11). The southern region of Brazil has the highest prevalence of overweight adolescents in the country (18.2%) (12).

In this sense, studies have pointed out that excess weight (overweight and obesity) in the pediatric population is associated with higher blood pressure values (3, 4, 6, 13). Therefore, when body mass index (BMI) reaches the 85th percentile (overweight) in adolescents over 10 years of age, the risk of developing arterial hypertension in adulthood increases (14, 15). Furthermore, during childhood, excess weight can cause endothelial damage, consequently atherosclerosis and less arterial stiffness (16–18).

Obesity is already considered the cardiovascular risk factor with the greatest association with HBP (19). Previous studies have identified that anthropometric indicators such as BMI, waist circumference (WC), waist-to-height ratio (WHtR), waist-to-hip ratio (WHR), body adiposity index (BAI), conicity index (C), skinfolds (SF), and adiposity body shape index (ABSI) are effective in diagnosing body fat and are associated with HBP in children and adolescents (6 to 19 years old) (20–28).

However, different cutoff points have been used to classify anthropometric indicators and there is no standardization regarding HBP screening in adolescents. This is evident when verifying the different methodological procedures used such as the various protocols for assessing and measuring blood pressure, different instruments used, high range of age groups analyzed, intervals between measurements, number of incongruent measurements, and lack of consensus regarding the best cutoff point for each anthropometric indicator for HBP screening in children and adolescents (5 to 19 years old) (23, 28–36). In addition, some studies do not show diagnostic accuracy measures such as positive predictive values (PPV), negative predictive values (NPV), positive likelihood ratio (LR+), negative likelihood ratio (LR-), and area under the curve (AUC), which could provide more accurate information about cutoff points to identify the target condition or the individual's health (37).

Considering the relationship between overweight and obesity with HBP in adolescents, the use of anthropometric measures of body adiposity to identify a possible association with HBP in the pediatric population may be an effective and applicable strategy for health professionals to identify risk factors for cardiovascular diseases (13). In addition, our investigation proposes to estimate a greater number of measures of diagnostic accuracy to determine cutoff points of anthropometric indicators of body adiposity for HBP screening. This is important because the literature will have more detailed information on the cutoff points and the reader will be able to choose the anthropometric indicator that best suits

the population of interest. Therefore, this study aimed to propose cutoff points for anthropometric indicators for HBP screening in adolescents and to identify, among these indicators, those more accurately for boys and girls.

MATERIALS AND METHODS

Research Characterization

This is an observational study with a cross-sectional design using data from the school-based population research macro-project entitled Brazilian Guide for the Assessment of Physical Fitness and Health-Related Life Habits – Stage II.

Ethical Aspects

The ethics committee in research approved this study with human beings of the Federal University of Santa Catarina, under protocol No. 3.523.470 of August 21, 2019. All adolescents who participated in the research signed the consent form and, for those aged < 18 years, parents/guardians signed the Free and Informed Consent Form.

Study Location

The study was carried out in the city of São José, located in the state of Santa Catarina, southern Brazil. The Municipal Human Development Index (MHDI) of São José was 0.809 in 2010. The percentage of young people aged 15 to 17 years with the complete elementary school was 70.94%, with a life expectancy of 77.81 years, *per capita* income of R\$ 1.157,43, GINI index (income concentration of a given region) of 0.44, and low-income percentage of 1.36% (38).

Eligible Population and Sample

The target population of this research was adolescents aged 14 to 19 years enrolled in state high schools in the city of São José, SC, Brazil. The sampling process was determined in two stages: stratified by state public high schools and cluster of classes considering study shift and grade. State schools with Youth and Adult Education (EJA) that received adolescents with some type of intellectual disability were not eligible for this study.

Based on these criteria and according to the information from the State Department of Education, the municipality had 11 eligible schools, totaling 5,411 students enrolled in the first half of 2019, and for every six students in the day shift (morning, afternoon, full-time), one student was enrolled in the night shift. In the first stage, the school density was adopted as a stratification criterion (size: small, with <200 students; medium, with 200–499 students; and large, with 500 students or more). Thus, schools that predominated according to size were randomly selected, totaling seven schools. In the second stage, study shift and grade were considered.

To determine the sample size of the macro-project that resulted in different subprojects, it was decided to calculate the sample size for prevalence studies (39). A confidence level of 1.96 (95% confidence interval), tolerable error of 3.5 percentage points, prevalence of 50% (unknown outcome), and design effect of 1.5 were adopted (39). To minimize possible losses and refusals, 20% were added (40). With these parameters, the

required sample size was 1,233 students. Due to cluster sampling, all students belonging to classes were invited to participate in the research.

The sample size of day shift students was 606 and 28 students for the night shift. Regarding the grades of education, 276 students from the first year of high school, 200 students from the second year of high school, and 158 students from the third year of high school were evaluated.

Eligibility Criteria

Adolescents who refused to participate in the study, those with a physical disability that prevented them from performing physical tests, and those who did not return the Free and Informed Consent Form signed by parents or guardians (aged < 18 years) or by themselves (aged ≥ 18 years) were excluded from the research.

Data Collection

Data collection was performed using a self-administered questionnaire composed of demographic, socioeconomic, lifestyle, and sexual maturation sections. In addition, anthropometric measurements (body mass, height, perimeters, and skinfolds) and blood pressure (systolic and diastolic blood pressure) were performed.

Undergraduate and graduate Physical Education and Nutrition students with availability to carry out fieldwork were selected. Study coordinators carried out the selection and training of the team. To minimize evaluation errors in anthropometric measurements, the intra- and inter-evaluator technical measurement error (TME) of research anthropometrists was calculated during training, having as reference measurements performed by anthropometrists with level 3 certification of the International Society for the Advancement of Kinanthropometry (ISAK) (measurements used as a reference for comparisons) (Supplementary Material 1). All TME values were considered adequate, as recommended in the literature (41).

Dependent Variable

The dependent variable of the present study was blood pressure (systolic—SBP and diastolic—DBP), considering the average of two measurements performed on each adolescent (one at the beginning of data collection and another between 10 and 15 min after the first measurement) (42). However, if there was a difference <10 mmHg for SBP and/or DBP between the two measurements, a third measurement was performed, adopting the mean of the lowest blood pressure measurements and excluding the highest (43). The rest time before the first measurement was at least 10 min, also for the third measurement, if necessary. Blood pressure was measured on the right arm supported on a table at heart level and with palm facing up. To perform this measurement, the subject was seated, legs uncrossed, and feet on the floor (44). Electronic arm sphygmomanometers with a digital reading system (Omron® model HEM 742, Kyoto, Japan), previously and adequately validated for Brazilian adolescents (45), were used to measure blood pressure levels. Individuals were recommended not to

smoke, drink coffee, and ingest black tea and alcoholic beverages, not to perform physical activities of moderate to vigorous intensity 12 h before, and to empty bladder before blood pressure measurement (44).

Blood pressure was continuously and dichotomously analyzed (HBP: yes/no). Adolescents aged 13 to 17 years with SBP values ≥ 120 and/or DBP ≥ 80 mmHg and adolescents aged 18 to 19 years with SBP values ≥ 140 and/or DBP ≥ 90 mmHg were considered to have HBP (44).

Independent Variables

The body mass (BM), height, two perimeters, and skinfolds (SF) measurements were performed: WC and hip circumference (HC), triceps, subscapularis, suprailiac, and mid-calf SFs according to literature recommendations (46) (Supplementary Material 2), and the following anthropometric indicators were calculated: BMI, fat percentage (FP), WHtR, WC, C Index, BAI, WHR, and ABSI.

BMI was calculated through BM and height measurements. FP was calculated from height and WC measurement through the equation: $FP = 64 - [20 \times (\text{Height (m)} / \text{WC (m)})] + (12 \times \text{Sex})$, with zero (0) for males and one (1) for females (47).

Using WC, HC, and height measurements, WHtR and WHR indicators were calculated. The conicity index (C index) was calculated through WC, BM, and height measurements: $C \text{ index} = \text{WC (m)} / 0.109 \times [\sqrt{\text{BM (Kg)}} / \text{height (m)}]$ (48). In addition, from HC and height values, the body adiposity index (BAI) was calculated (49). ABSI was calculated through WC, BMI, and height measurements using the equation: $ABSI = \text{WC (m)} / (\sqrt[3]{\text{BMI}^2} \times \sqrt{\text{height}})$ (22). Furthermore, SF was continuously analyzed by the sum of SFs.

Sample Characterization Variables

The characterization variables of this study were: sociodemographic indicators (economic level and skin color), physical activity, eating habits, cigarette use, sleep quality, and sexual maturation, collected through a self-administered questionnaire.

The economic level was estimated using a questionnaire from the Brazilian Association of Research Companies (50). This questionnaire estimates the purchasing power of households. This questionnaire estimates the purchasing power of families based on different items that are present in adolescents' homes (bathroom, automobiles, microcomputer, dishwasher, refrigerator, freezer, washing machine, DVD, microwave, motorcycle, and clothes dryer). From the answers, the economic level can be categorized as decreasing level of purchasing power (from A to E). In the present study, the economic level was dichotomized into "high" (level A and B) and "low" (level C, D, and E). Skin color was assessed by the Brazilian census methodology that uses the words white, brown, black, yellow and indigenous to classify people's skin color or race. The following classification was adopted: "white" and "brown, yellow, indigenous and black".

Physical activity was assessed using the question "During the last 7 days, on how many days were you physically active for at least 60 minutes a day?" from the Youth Risk

Behavior Surveillance System (YRBSS) questionnaire used in the United States, translated and validated for Brazil (51). The questionnaire had a Kappa agreement index for the Brazilian population of 68.6% and the question used in the present study had a Kappa agreement index of 37.2% (51). Responses were categorized as “physically active” when active for 7 days and “not physically active” when active <7 days a week (52).

Eating habits were assessed using the question “Do you eat a balanced diet?” of the Fantastic Lifestyle questionnaire (53), translated and validated for the Brazilian population (54). The questionnaire had a Kappa agreement index for the Brazilian population of 68.6% and the question used in the present study had a Kappa agreement index of 72% (54). The instrument’s response options were categorized into “inadequate” (option 0 – almost never; option 1 – rarely; and option 2 – sometimes), and “adequate” (option 3 – relatively often; and option 4 – almost always), as explained in the questionnaire itself (55).

Cigarette use was assessed using the question “Do you smoke cigarettes?” from the Fantastic Lifestyle questionnaire (53), with the question showing 86% of the Kappa agreement index in the Brazilian population (54). The instrument’s response options were categorized into: “currently smoke” (option 0 – more than 10 per day; and option 1 – 1 to 10 a day) and “do not currently smoke” (option 2 – none in the last 6 months; option 3 – none in the past year; and option 4 – never smoked).

Sleep quality was assessed using the question “Do you sleep well and do you feel rested?” from the Fantastic Lifestyle questionnaire (53). This question had a Kappa agreement index of 55% for the Brazilian population (54). The instrument’s response options were categorized into “adequate” (option 3 – relatively often; and option 4 – almost always) and “inadequate” (option 0 – almost never; option 1 – rarely; and option 2 – sometimes), according to study that evaluated the same variable (56).

For the self-assessment of sexual maturation, we used Tanner’s scales (57), validated and reproduced for the Brazilian population with an agreement of 60.9 to 71.3% (58). Sexual maturation stages were indicated by self-assessment (figures) based on pubic hair development (males and females). Stage 1 represents the pre-pubertal stage, stages 2, 3, and 4 represent puberty, and stage 5 the post-pubertal stage. Adolescents were classified as pre-pubertal, pubertal, and post-pubertal, similarly to another study (59).

Statistical Analysis

Initially, data were entered with a double entry in the Epi Data 3.0 software. From there, descriptive statistics (mean, standard deviation, and frequencies) were performed. Differences between sexes and ages were analyzed using the Student’s *t*-test for independent samples. Data normality was verified using the Shapiro–Wilk test and, if data did not present normal distribution, the non-parametric Mann–Whitney’s test was performed.

The chi-square test was used to verify differences in prevalence between sexes and the physical, sociodemographic, and lifestyle characteristics of male and female adolescents. The ROC curve (Receiver Operating Characteristic Curve) was used to analyze the predictive capacity of anthropometric indicators to identify

TABLE 1 | General characterization of the sample (*n* = 634) adolescents from São José, SC, Brazil, 2019.

Variables	Male (<i>n</i> = 396, 62.5%)	Female (<i>n</i> = 238, 37.5%)	<i>p</i> -value
	Mean (SD)	Mean (SD)	
Age (years old)	16.63 (1.02)	16.62 (1.06)	0.92
BM (Kg)	68.11 (13.52)	58.63 (12.31)	<0.01
Height (cm)	173.62 (7.16)	160.55 (5.82)	<0.01
BMI (Kg/m ²)	22.58 (4.23)	22.72 (4.58)	0.68
FP	17.30 (4.91)	29.41 (5.86)	<0.01
WtHr	0.43 (0.05)	0.44 (0.06)	0.46
WHR	0.78 (0.04)	0.72 (0.06)	<0.01
BAI	24.10 (4.04)	29.50 (4.65)	<0.01
ABSI	0.072 (0.003)	0.069 (0.004)	<0.01
C Index	1.10 (0.04)	1.07 (0.06)	<0.01
WC (cm)	75.17 (8.60)	70.09 (9.85)	<0.01
HC (cm)	96.15 (8.73)	96.49 (8.96)	0.64
Triceps skinfold (mm)	11.18 (5.16)	17.88 (5.72)	<0.01
Subscapularis skinfold (mm)	10.72 (5.02)	14.55 (6.92)	<0.01
Supra iliac skinfold (mm)	13.91 (7.30)	17.50 (6.74)	<0.01
Calf skinfold (mm)	9.91 (4.85)	17.17 (6.86)	<0.01
Sum of skinfolds (mm)	45.73 (20.49)	67.10 (23.19)	<0.01
SBP (mmHg)	109.78 (21.71)	102.76 (15.62)	<0.01
DBP (mmHg)	65.21 (8.08)	67.35 (8.71)	<0.01

n, sample size; %, percentage; *t*-test for independent samples; Mann–Whitney’s non-parametric test; SD, standard deviation; BM, Body mass; BMI, Body mass index; FP, fat percentage; WtHr, waist-to-height ratio; WHR, waist-hip ratio; BAI, body adiposity index; ABSI, adiposity body shape index; C-index, conicity index; WC, waist circumference; HC, hip circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; the *p*-value in bold means a statistically significant difference (*p* < 0.05) between males and females.

high SBP and DBP and to find the best cutoff points that identify this association (60). For the present study, the cutoff points for anthropometric indicators of obesity were those with sensitivity above 70% to avoid the maximum number of false-negative results (61).

AUC >0.6 was considered sufficient, regardless of *p*-value, and AUC >0.7 was considered to have good diagnostic accuracy (37). In addition, sensitivity, specificity, PPV, NPV, LR+, and LR- values were calculated for all cutoff points of anthropometric indicators to identify high SBP and DBP values to interpret such cutoff points. Analyses were performed stratified by sex (male and female) and the significance level was set at 5%. Analyses were performed using the MedCalc 19.5.3 statistical software and Statistical Package for the Social Sciences software (SPSS Statistics, Chicago, USA), version 17.0.

RESULTS

A total of 634 adolescents aged 14 to 19 years completed all assessments of the present study. Most adolescents were male (62.5%). Significant differences were observed between

TABLE 2 | Physical, sociodemographic, and lifestyle characteristics of public school students in São José, SC, Brazil.

Variables	n = 634	General (n = 634)		p-value
		Male n = 396 (%)	Female n = 238 (%)	
Maturation	n = 598			0.33
Pre-pubertal		40 (10.7)	16 (7.2)	
Pubertal		250 (66.7)	158 (70.9)	
Post-pubertal		85 (22.7)	49 (22.0)	
Economic level	n = 634			0.38
High		260 (65.7)	148 (62.2)	
Low		136 (34.3)	90 (37.8)	
Skin color	n = 619			<0.05
White		200 (51.8)	143 (61.4)	
Brown/yellow/indigenous/black		186 (48.2)	90 (38.6)	
Physical activity level	n = 618			0.48
Physically active		23 (6.0)	11 (4.7)	
Not physically active		360 (94.0)	224 (95.3)	
Balanced diet	n = 634			0.42
Adequate		68 (17.2)	35 (14.7)	
Inadequate		328 (82.8)	203 (85.3)	
Smoke cigarettes	n = 616			0.45
Not currently smoker		370 (96.1)	219 (94.8)	
Currently smoker		15 (3.9)	12 (5.2)	
Sleep quality	n = 620			0.34
Adequate		147 (37.9)	79 (34.1)	
Inadequate		241 (62.1)	153 (65.9)	
HBP	n = 634			<0.01
No		278 (70.2)	211 (88.7)	
Yes		118 (29.8)	27 (11.3)	

n, sample size (number of students with information about the variable); %, percentage; Chi-square test; HBP, High blood pressure; the p-value in bold means a statistically significant difference ($p < 0.05$) in the skin color and HBP between males and females.

sexes, with higher BM, height, WHR, ABSI, C Index, WC, and SBP values for male adolescents and higher FP, BAI, triceps, subscapularis, suprailiac, calf SFs, and sum of SFs and DBP values for female adolescents (Table 1).

Yellow/brown/indigenous/black skin color students were more frequent in males (48.2%), when compared to female adolescents (38.6%). Male adolescents had higher prevalence of HBP (29.8%) when compared to female adolescents (11.3%) (p -value = 0.00) (Table 2).

For male adolescents, anthropometric indicators BM, BMI, FP, WHtR, WC, and HC showed sufficient AUC (95%CI of AUC > 0.60), ranging from 0.65 to 0.67, with 95%CI ranging from 0.60 to 0.72. Likewise, LR+ values were >1, demonstrating that individuals are more likely of having the disease with a positive result, and with LR- values lower than 50, demonstrating that an individual with a negative result is 50% less likely of having the disease, for example, an adolescent with BM >64.8 kg is 1.73 times more likely of having HBP than an adolescent with BM <64.8 kg and an

adolescent with BM <64.8 kg is 47% more likely of having HBP (Table 3).

Table 3 presents PPV and NPV, with an emphasis on NPV, demonstrating that the use of cutoff points of anthropometric indicators mentioned above presents an 80% chance of not having the disease when the test is negative, that is, if BM <64.8 kg, the adolescent is 83% more likely of not having HBP (Table 3). Anthropometric indicators such as BAI, triceps skinfold, subscapularis skinfold, supra iliac skinfold, calf skinfold, and sum of skinfolds, despite having AUC >0.60, showed 95%CI <0.60. In addition, these anthropometric indicators showed LR- ≤50%, demonstrating that an individual, even with a negative result, has a more than 50% chance of having the disease (Table 3).

Regarding cutoff points of anthropometric indicators with better accuracy for HBP screening, BM had cutoff point of >64.8 kg, BMI >21.76 kg/m², FP >15.75%, WHtR >0.41, WC >73.00 cm, and HC >92.25 cm. Furthermore, both anthropometric indicators showed sensitivity >70%, indicating that for every 100 adolescents evaluated, 70 have HBP. However, the same cannot be said about specificity, which presented values <60%, indicating that for every 100 adolescents evaluated, <60 are diagnosed as healthy, and the others are diagnosed with HBP (Table 3).

For female adolescents, BM and height presented AUC >0.60, however, with 95%CI <0.60. The cutoff points for anthropometric indicators with the best accuracy to identify HBP in female adolescents were BM >54.10 kg and height >158.50 cm (Table 4). Both anthropometric indicators showed sensitivity >70%, demonstrating that for every 100 adolescents evaluated, 70 have HBP; however, with specificity <43%, indicating that for every 100 adolescents evaluated, only 43 are diagnosed healthy, and the other 57 have HBP even though they are below the cutoff point (Table 4).

In addition, LR+ values were >1, demonstrating that individuals are more likely of having the disease with the positive result; however, 95%CI presented by BM was between 0 and 1, indicating that the chances of disease are equal between individuals with and without the disease and with LR- value ≥50, demonstrating that an individual with a negative result is 50% more likely of having the disease. Unlike height, which presented LR+ >1 and LR- values close to zero, demonstrating that individuals with the negative result are less likely of having the disease, for example, an adolescent with a height >158.50 cm is 1.54 times more likely of having HBP than an adolescent with height <158.50 cm and, in the same way, an individual with height <158.50 cm with 26% chance of having HBP. Both anthropometric indicators showed high NPV, demonstrating that the use of cutoff points showed a probability of more than 90% of not having the disease when the test is negative, that is, an adolescent with a height <158.50 cm is 90% more likely of not having HBP (Table 4).

The other anthropometric indicators (BMI, FP, WHtR, WHR, BAI, ABSI, C index, WC, HC, triceps skinfold, subscapularis skinfold, supra iliac skinfold, calf skinfold, and sum of skinfolds),

TABLE 3 | Area under the curve, cutoff point, sensitivity values, specificity values, positive likelihood ratio, negative likelihood ratio, positive predictive values, and negative predictive values of anthropometric indicators for high blood pressure screening in male adolescents ($n = 396$).

Variable	AUC (95%CI)	p-value	Cut off point	Sensitivity	Specificity	LR+	LR-	PPV	NPV
BM (kg)	0.67 (0.62–0.72)	0.00	>64.80	72.88 (63.90–80.70)	57.91 (51.90–63.80)	1.73 (1.50–2.10)	0.47 (0.30–0.60)	42.40 (38.10–46.70)	83.40 (78.60–87.30)
Height (cm)	0.54 (0.49–0.59)	0.21	>169.50	79.66 (71.30–86.50)	29.14 (23.90–34.90)	1.12 (1.00–1.30)	0.70 (0.50–1.00)	32.30 (29.80–34.90)	77.10 (69.30–83.40)
BMI (kg/m ²)	0.67 (0.62–0.72)	0.00	>21.76	70.34 (61.20–78.40)	59.71 (53.70–65.50)	1.75 (1.50–2.10)	0.50 (0.40–0.70)	42.60 (38.10–47.10)	82.60 (77.90–86.40)
FP	0.65 (0.60–0.70)	0.00	>15.75	78.81 (70.30–85.80)	48.56 (42.50–54.60)	1.53 (1.30–1.80)	0.44 (0.30–0.60)	39.40 (35.90–43.00)	84.40 (78.90–88.60)
WHtR	0.65 (0.60–0.70)	0.00	>0.41	78.81 (70.30–85.80)	48.56 (42.50–54.60)	1.53 (1.30–1.80)	0.44 (0.30–0.60)	39.40 (35.90–43.00)	84.40 (78.90–88.60)
WHR	0.57 (0.52–0.61)	0.03	>0.75	85.59 (77.90–91.40)	28.42 (23.20–34.10)	1.20 (1.10–1.30)	0.51 (0.30–0.80)	33.70 (31.40–36.00)	82.30 (74.20–88.20)
BAI	0.62 (0.57–0.67)	0.00	>22.53	70.34 (61.20–78.40)	44.60 (38.70–50.70)	1.27 (1.10–1.50)	0.66 (0.50–0.90)	35.00 (31.50–38.70)	78.00 (72.30–82.80)
ABSI	0.57 (0.52–0.62)	0.02	<0.073	70.34 (61.20–78.40)	38.49 (32.70–44.50)	1.14 (1.00–1.30)	0.77 (0.60–1.11)	32.70 (29.50–36.10)	75.40 (69.00–80.70)
C index	0.56 (0.51–0.61)	0.05	>1.09	70.34 (61.20–78.40)	38.85 (33.10–44.90)	1.15 (1.00–1.30)	0.76 (0.60–1.00)	32.80 (29.60–36.20)	75.50 (69.30–80.90)
WC (cm)	0.67 (0.62–0.71)	0.00	>73.00	72.88 (63.90–80.70)	57.91 (51.90–63.80)	1.73 (1.50–2.10)	0.47 (0.30–0.60)	42.40 (38.10–46.70)	83.40 (78.60–87.30)
HC (cm)	0.66 (0.61–0.71)	0.00	>92.25	81.36 (73.10–87.90)	47.84 (41.80–53.90)	1.56 (1.40–1.80)	0.39 (0.30–0.60)	39.80 (36.50–43.30)	85.80 (80.30–90.00)
Triceps skinfold (mm)	0.61 (0.56–0.66)	0.00	>8.90	73.73 (64.80–81.40)	45.68 (39.70–51.70)	1.36 (1.20–1.60)	0.58 (0.40–0.80)	36.60 (33.10–40.20)	80.40 (74.70–85.00)
Subescapularis skinfold (mm)	0.62 (0.57–0.67)	0.00	>8.40	72.88 (63.90–80.70)	42.45 (36.60–48.50)	1.27 (1.10–1.50)	0.64 (0.50–0.90)	35.00 (31.60–38.40)	78.70 (72.70–83.60)
Supra iliac skinfold (mm)	0.61 (0.56–0.66)	0.00	>9.80	72.88 (63.90–80.70)	42.81 (36.90–48.90)	1.27 (1.10–1.50)	0.63 (0.50–0.90)	35.10 (31.80–38.60)	78.80 (72.90–83.70)
Calf skinfold (mm)	0.60 (0.55–0.65)	0.00	>7.95	70.34 (61.20–78.40)	44.24 (38.30–50.30)	1.26 (1.10–1.50)	0.67 (0.50–0.90)	34.10 (30.70–37.70)	78.40 (72.80–83.20)
Sum of skinfolds (mm)	0.62 (0.57–0.67)	0.00	>36.00	70.34 (61.20–78.40)	45.32 (39.40–51.40)	1.29 (1.10–1.50)	0.65 (0.50–0.90)	35.30 (31.80–39.00)	78.30 (72.60–83.00)

95%CI, confidence interval at 95%; AUC, area under the curve (with lower bound CI95% > 0.60); LR+, Positive likelihood ratio; LR-, Negative likelihood ratio; PPV, Positive predictive values; NPV, Negative predictive values; BM, Body mass; BMI, Body mass index; FP, fat percentage; WHtR, waist-to-height ratio; WHR, Waist-to-hip ratio; BAI, Body adiposity index; ABSI, body shape index; C Index, conicity index; WC, waist circumference; HC, hip circumference.

which did not show sufficient AUC (95%CI AUC <0.60), also showed LR+ values close to 1 or below 1, demonstrating that individuals who presented a positive test result would have the same chances of having the disease when compared to those who presented a negative result (Table 4).

DISCUSSION

In the present study, the predictive capacity and proposition of cutoff points of anthropometric indicators to identify HBP in male and female adolescents were investigated. For males, indicators BM, BMI, FP, WHtR, WC, and HC presented sufficient AUC (i.e., 95%CI AUC > 0.60). For females, no anthropometric indicator had discriminatory power for HBP screening. This study is not intended to replace the clinical diagnosis, but to allow the identification, in a simple way, of adolescents who are more

likely of having HBP in the initial screening and who would need further care and follow-up.

High values in anthropometric indicators of body adiposity are associated with HBP in adolescents (1, 2, 13, 21–23), and this association is justified by the high concentration of fatty acids in subjects with high body fat. This condition (i.e., high concentration of fatty acids) causes insulin resistance and may induce the renal system to retain more sodium. This retention increases activation of the sympathetic nervous system, which results in increased activity of the renin-angiotensin system and increases blood pressure (62).

This study proposed to update cutoff points of anthropometric indicators for HBP screening in Brazilian adolescents and added more anthropometric indicators for the debate with literature, such as FP, ABSI, triceps, subscapular, suprailiac, calf SFs, and sum of SFs, which were not present in studies published with Brazilian adolescents, in which the use of BMI, WHtR, and WC

TABLE 4 | Area under the curve, cutoff point, sensitivity values, specificity values, positive likelihood ratio, negative likelihood ratio, positive predictive values, and negative predictive values of anthropometric indicators for high blood pressure screening in female adolescents ($n = 238$).

Variable	AUC (95%CI)	p-value	Cut off point	Sensitivity	Specificity	LR+	LR-	PPV	NPV
BM (kg)	0.62 (0.55–0.68)	0.07	>54.10	70.37 (49.80–86.20)	43.13 (36.30–50.10)	1.24 (0.90–1.60)	0.69 (0.40–1.30)	13.60 (10.70–17.10)	92.00 (86.20–95.40)
Height (cm)	0.64 (0.58–0.70)	0.01	>15.50	88.89 (70.80–97.60)	42.18 (35.40–49.20)	1.54 (1.30–1.80)	0.26 (0.09–0.80)	16.40 (14.10–18.90)	96.80 (91.00–98.90)
BMI (kg/m ²)	0.57 (0.50–0.63)	0.28	>20.13	70.37 (49.80–86.20)	34.60 (28.20–41.40)	1.08 (0.80–1.40)	0.86 (0.50–1.60)	12.10 (9.50–15.10)	90.20 (83.30–94.40)
FP	0.53 (0.46–0.59)	0.69	>24.75	70.37 (49.80–86.20)	23.70 (18.10–30.00)	0.92 (0.70–1.20)	1.25 (0.70–2.30)	10.50 (8.30–13.20)	86.30 (77.00–92.20)
WHtR	0.53 (0.46–0.59)	0.69	>0.39	70.37 (49.80–86.20)	23.70 (18.10–30.00)	0.92 (0.70–1.20)	1.25 (0.70–2.30)	10.50 (8.30–13.20)	86.30 (77.00–92.20)
WHR	0.52 (0.45–0.58)	0.79	>0.69	70.37 (49.80–86.20)	22.75 (17.30–29.00)	0.91 (0.70–1.20)	1.30 (0.70–2.50)	10.40 (8.20–13.00)	85.80 (76.20–91.90)
BAI	0.50 (0.44–0.57)	0.98	>26.43	70.37 (49.80–86.20)	27.96 (22.00–34.50)	0.98 (0.80–1.30)	1.06 (0.60–2.00)	11.10 (8.80–13.90)	88.10 (79.90–93.20)
ABSI	0.51 (0.44–0.57)	0.94	>0.067	70.37 (49.80–86.20)	27.96 (22.00–34.50)	0.98 (0.80–1.30)	1.06 (0.60–2.00)	11.10 (8.80–13.90)	88.10 (79.90–93.20)
C index	0.53 (0.46–0.59)	0.67	>1.03	70.37 (49.80–86.20)	25.12 (19.40–31.50)	0.94 (0.70–1.20)	1.18 (0.60–2.20)	10.70 (8.50–13.40)	86.90 (78.10–92.60)
WC (cm)	0.57 (0.50–0.63)	0.32	>64.20	70.37 (49.80–86.20)	29.86 (23.80–36.50)	1.00 (0.80–1.30)	0.99 (0.50–1.80)	11.30 (9.00–14.20)	88.80 (81.00–93.60)
HC (cm)	0.58 (0.51–0.64)	0.22	>93.25	70.37 (49.80–86.20)	43.13 (36.30–50.10)	1.24 (0.90–1.60)	0.69 (0.40–1.30)	13.60 (10.70–17.10)	92.00 (86.20–95.40)
Triceps skinfold (mm)	0.54 (0.48–0.61)	0.51	>15.40	70.37 (49.80–86.20)	39.34 (32.70–46.30)	1.16 (0.90–1.50)	0.75 (0.40–1.40)	12.90 (10.20–16.20)	91.20 (85.10–95.00)
Subescapularis skinfold (mm)	0.50 (0.44–0.57)	0.98	>9.80	70.37 (49.80–86.20)	27.96 (22.00–34.50)	0.98 (0.80–1.30)	1.06 (0.60–2.00)	11.10 (8.80–13.90)	88.10 (79.90–93.20)
Supra iliac skinfold (mm)	0.53 (0.46–0.59)	0.68	≤23.00	70.37 (49.80–86.20)	19.43 (14.30–25.40)	0.87 (0.70–1.10)	1.52 (0.80–2.90)	10.00 (7.90–12.50)	83.70 (73.00–90.70)
Calf skinfold (mm)	0.57 (0.50–0.63)	0.33	>13.77	70.37 (49.80–86.20)	39.34 (32.70–46.30)	1.16 (0.90–1.50)	0.75 (0.40–1.40)	12.90 (10.20–16.20)	91.20 (85.10–95.00)
Sum of skinfolds (mm)	0.52 (0.46–0.59)	0.74	>52.20	70.37 (49.80–86.20)	29.86 (23.80–36.50)	1.00 (0.80–1.30)	0.99 (0.50–1.80)	11.30 (9.00–14.20)	88.80 (81.00–93.60)

95%CI, confidence interval at 95%; AUC, area under the curve (with lower bound 95%CI ≥ 0.60); LR+, Positive likelihood ratio; LR-, Negative likelihood ratio; PPV, Positive predictive values; NPV, Negative predictive values; BM, Body mass; BMI, Body mass index; FP, fat percentage; WHtR, waist-to-height ratio; WHR, Waist-to-hip ratio; BAI, Body adiposity index; ABSI, body shape index; C-Index, conicity index; WC, waist circumference; HC, hip circumference.

prevailed (21, 23, 63, 64). Furthermore, previous Brazilian studies have shown sensitivity and specificity values (21, 23, 63, 64), and in two studies (23, 63), the 95%CI sensitivity of anthropometric indicators was below 0.60, and in one study (23), LR+ and LR- values were not presented as 95%CI. AUC allows evaluating the performance on the test, in this case, the performance of anthropometric indicators for HBP screening in adolescents. If the AUC is less than 0.60, the accuracy of the diagnostic test is considered poor, random (37), and may or may not identify HBP in adolescents.

In the present study, for males, anthropometric indicators BM, BMI, FP, WHtR, WC, and HC presented AUC (95%CI >0.60), demonstrating sufficient accuracy for HBP screening (37). The BMI cutoff point for HBP screening in males in the present study was >21.76 kg/m², which is lower than cutoff points in other studies (21, 23, 65, 66). If compared to the BMI cutoff proposed by the World Health Organization (67) to identify

obesity in adolescents [14 years (21.8 kg/m²), 15 years (22.7 kg/m²), 16 years (23.5 kg/m²), 17 years (24.3 kg/m²), 18 years (24.9 kg/m²), and 19 years (24.9 kg/m²)], the cutoff point of the present study was lower, which may have occurred due to the following factors: data from the World Health Organization are longitudinal population-based with the participation of several countries and stratified by age, while the present study has a cross-sectional design, carried out in a certain geographic region of Brazil, without stratification by age, and using the average age of adolescents. Although the cutoff point is considered low compared to other studies, this was one of the indicators that showed good accuracy for HBP screening in male adolescents in the present study, since it had an LR+ value close to two, demonstrating that an individual with a positive result is more likely of having the disease, LR- of 0.50, that is, an individual with a negative result is 50% more likely of having the disease and NPV of 82.50 (95%CI: 77.80–86.40).

Regarding the cutoff point for WHtR (>0.41) identified in this study for HBP screening in males, another study presented the same cutoff point of 0.41 (68). The cutoff point of 0.41 for WHtR is below cutoff points in other studies (21, 23, 63, 65, 66, 69–71). Since 1995 (72), the year of the creation of anthropometric indicator WHtR, a universal cutoff point (0.50) has been established to identify overweight individuals from those with normal weight and to be considered a risk factor for cardiovascular diseases, among them, hypertension, which can be used for both sexes and different age and ethnic groups (72, 73). However, this cutoff point of 0.50 was not created from AUC, therefore without diagnostic tests. Furthermore, WC measurement was performed on the umbilical line and not on the smallest portion of the trunk, which could therefore generate differences with the standardization used in the present study (72). In addition, different protocols for measuring WC were used by studies (21, 65, 66), which may interfere with the WHtR results and, consequently, with cutoff point values proposed by these studies.

The present study also identified other anthropometric measures and indicators with good accuracy for HBP screening in male adolescents, namely BM, FP, and HC. BM in the present study presented a sensitivity value of 72.88 (95%CI: 63.90–80.70), LR+ close to two, LR- <0.50 , and NPV of 83.40 (95%CI: 78.60–87.30), demonstrating good accuracy for HBP screening in male adolescents, with a cutoff point of >64.80 kg. The other studies in the literature only used BM measurement to calculate other anthropometric indicators. In this sense, this study presents this anthropometric measurement, which is easy to be performed by any professional, as a strategy to be used for the initial HBP screening.

In the present study, HC was analyzed in isolation and showed good accuracy for HBP screening in male adolescents, when high, with a cutoff point of >92.25 cm. In addition, it was used in conjunction with WC and height to calculate other anthropometric indicators, such as WHR and BAI, but without showing good AUC accuracy to identify HBP in male and female adolescents. In other studies, HC was not analyzed in isolation, only in conjunction with other anthropometric measures to calculate BAI (23) and WHR (69) indicators. The study that used BAI showed good accuracy for HBP screening in male and female adolescents and the study that used WHR, carried out only with female adolescents, showed good accuracy for HBP screening, and both studies adopted AUC (95%CI >0.50), different from the present study that adopted AUC (95%CI >0.60) (23, 69).

In the present study, FP, calculated using two anthropometric measurements (height and WC), presented an AUC of 0.65 (95%CI: 0.60–0.70) and a cutoff point of $>15.75\%$. A study carried out with Turkish adolescents aged 11 to 17 years also verified the accuracy of FP for HBP screening in adolescents; however, it was not clear how FP was calculated and, unlike the present study, AUC >0.60 was not identified for this indicator (65). FP calculated by height and HC has not yet been validated for Brazilian adolescents (74); however, this index showed better agreement with dual-energy x-ray absorptiometry (DXA) of 0.83 and 0.86 for female and male adolescents, respectively, compared to BMI and tri-ponderal mass index (TMI), which use is recommended in health care services and the school

environment (47). In addition, it presented 3.45 (95%CI: 3.26–3.63) and 3.35 (95%CI: 3.22–3.49) as mean square errors for female and male adolescents aged 15 to 19 years, respectively (47). Furthermore, FP calculated through skinfolds (triceps and subscapularis) (75) is valid for up to the age of 18 years and still depends on constants for ethnicity, which is unnecessary for the calculation of FP with the equation used in the present study that differentiates only between the sex of adolescents and presents a direct estimate of FP (47).

For female adolescents, BM and height presented AUC >0.60 ; however, with 95%CI <0.60 , demonstrating low accuracy to identify HBP in this population. In other studies (21, 23, 63, 64, 69, 70, 76), AUC >0.60 was identified for some anthropometric indicators, such as BMI, HC, WHtR, BAI, and C Index, demonstrating good accuracy for HBP screening in female adolescents. Of these, two studies presented more diagnostic accuracy measures (LR+ and LR-) (23), PPV and NPV (69). The present study did not present anthropometric indicators with good accuracy to identify HBP in female adolescents, which can be explained by the low prevalence of HBP (11.3%), which was below other studies carried out with Brazilian adolescents (7, 77).

A debate in the literature that deserves to be highlighted is the recommended AUC values for diagnostic tests and/or screening. As the present study aimed to propose cutoff points for anthropometric indicators to perform HBP screening among Brazilian adolescents, we chose to use a reference for classifying AUC values >0.60 as sufficient to identify the disease risk (37). There are other references for satisfactory AUC values, such as >0.70 (78, 79). As there are still debates in the literature with no conclusions on which recommendations to use, it was decided to use AUC values >0.60 aiming at an early screening of the risk of HBP.

This study had strengths and limitations. As a strong point, the fact that 16 anthropometric indicators were investigated and calculated through eight anthropometric measurements is highlighted, enabling verifying the accuracy of the HBP screening in adolescents aged 14 to 19 years. In addition, this study presented measures of diagnostic accuracy not present in other studies, providing additional information to the literature. Finally, this research calculated the technical error of measurement among anthropometrists and used a blood pressure monitor validated for adolescents (80), which demonstrates care during data collection and analysis.

As limitation, the size of the sample collected was smaller than the calculated one, which limits inferences of the study. In addition, despite the measurement of sexual maturation, it was only used to characterize adolescents and did not enter as adjustments in anthropometric indicators. Another limitation of this research was the cross-sectional design, making it impossible to establish a cause-and-effect relationship.

CONCLUSION

Based on the results, it could be concluded that anthropometric indicators BM, BMI, FP, WHtR, WC, and HC have good accuracy for HBP screening in male adolescents. However, for female adolescents, anthropometric indicators did not present good diagnostic characteristics for HBP screening. Thus, it is suggested

that, in a school environment, the use of anthropometric measurements is a simple, low-cost, and easy-to-apply method, which can be performed by the Physical Education teacher, being an effective strategy to contribute to obtaining information about adolescents' health.

This study is not intended to replace the clinical diagnosis of HBP through cutoff points found, but to demonstrate that male individuals with high BM, BMI, FP, WHtR, WC, and HC values should be referred for further clinical tests to verify the presence or absence of the disease.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics and Research Committee, Federal University of Santa Catarina. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

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AUTHOR CONTRIBUTIONS

DS contributed to the study construction, data interpretation, writing, manuscript supervision and review, and obtained approval from the Research Ethics Committee of the Institution to carry out the study. AG, LM, and AU contributed to data interpretation and manuscript editing. LB contributed to data analysis and interpretation, writing, manuscript editing and review, and acquired the authors' authorization for manuscript submission. All authors contributed to the article and approved the submitted version.

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Bioelectrical impedance vector analysis and body composition in cervical spinal cord injury: A pilot study

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Introduction: Body composition assessment in cervical spinal cord injury (c-SCI) individuals is important to monitor the fat free-mass (FFM) loss, due to immobilization, or gain, due to exercise practice. Single frequency bioelectrical impedance analysis (SF-BIA) is low in cost, simple and easy.

Objectives: The aims of this study are: to evaluate the concordance between the FFM values obtained using dual X-ray absorptiometry (DXA) and the three SF-BIA previous predictive equations; and to test the applicability of the bioelectrical impedance vector analysis (BIVA).

Methods: Twenty-three c-SCI males were divided into two groups: Physically active (PA; $n = 13$; at least 150 min/week) and non-active individuals (NPA) and were assessed by DXA and SF-BIA simultaneously.

Results: FFM values were similar between groups PA and NPA. Considering all participants, FFM values obtained by Kocina and Heyward ($>11\%$) and Sun ($<15.4\%$) predictive equations were different when compared to DXA ($p < 0.01$). However, Buchholz's et al. predictive equation showed FFM values similar to DXA, but presented poor concordance ($<7\%$, $p = 0.99$; concordance coefficient = 0.85). BIVA showed consistency in ellipse distribution using FFM obtained using Buchholz et al. predictive equation.

Conclusions: The use of non-specific BIA equations can lead to misinterpretation in FFM values in male c-SCI individuals. Predictive equations for this group need to be developed.

KEYWORDS

bioelectrical impedance, body composition, spinal cord injuries, tetraplegia, wheelchair sports

Introduction

Cervical spinal cord injury (c-SCI) implies serious complications, principally skeletal muscle atrophy, which are associated with adverse metabolic effects, including glucose intolerance, insulin resistance, type II diabetes and cardiovascular disease (1–3). Regardless of the age group, as time advances, SCI individuals

present a body composition profile similar to that of the elderly, with reduced fat-free mass (FFM) and increased fat mass due to reduced physical capacity.

The practice of physical exercises in c-SCI can have beneficial consequences on body composition and metabolic changes (4–6). Exercise practice has been considered an important strategy to improve FFM and reduce fat mass, decreasing risks of developing cardiometabolic diseases in different population groups (7–9). FFM is an important component of body composition, and it is related to the development of strength, power, physical performance (10, 11).

FFM can be assessed by high-accuracy methods, such as dual X-ray absorptiometry (DXA) (12, 13). However, DXA is not portable and is highly costly. On the other hand, methods such as bioelectric impedance analysis (BIA) are more affordable and portable (14). BIA is non-invasive, simple, accurate and relatively inexpensive, and it seems to be a promising method for assessing body composition in SCI individuals (13, 15–17).

BIA method estimate FFM from body electrical properties, the resistance (R) and reactance (Xc) vectors are used in predictive equations which consider sex, age, height and weight (18). Kocina and Heyward FFM predictive equation is the only BIA predictive equation validated for SCI individuals compared to FFM obtained using DXA (19). Buchholz et al. and Desport et al. validated predictive equations for SCI individuals, and the results were compared to total body water (TBW) obtained using the deuterium method. The equation developed by Buchholz et al. was aimed for chronic SCI individuals, and the one developed by Desport et al. was aimed for healthy elderly individuals. Panisset et al. and Desneves et al. observed the BIA applicability of FFM prediction in acute SCI individuals (20, 21). Both studies used a derivative of BIA known as bioimpedance spectroscopy (BIS), which measures impedance over a range of frequencies, allows the current to pass through and around the cell membrane, and provides more precise measurements of extracellular and intracellular fluid volume (22).

The spinal cord contributes to venous return through muscle contractions. In high SCI, the lack of muscle contractions can cause edema, mainly in the lower limbs (23). This characteristic makes it difficult to assess FFM using the BIA method in SCI individuals, since the BIA method assumed values for body density of 1.05 g/mL and for hydration fraction was 0.732 (24).

Bioelectrical impedance vector analysis (BIVA) is recommended for further nutritional assessment and monitoring, in particular when calculation of body composition is not feasible (25, 26), as in SCI individuals. BIVA is the graphical representation of direct measurements of the z-score of the R and Xc vectors (z-score R-Xc graph), and are plotted as bivariate vectors with tolerance intervals in the R-Xc plane (27). BIVA reflects differences in the bioelectric patterns and permits monitoring of the evolution of the nutritional status and changes associated with body composition, when comparing individual vectors and ellipses to reference populations (28, 29).

Therefore, the aims of this study were: (a) to evaluate the concordance between the FFM values obtained using dual X-ray absorptiometry (DXA) as a reference method and the three SF-BIA previous predictive equations related to SCI, and one predictive equation related to elderly people; and (b) to test the applicability of BIVA in physically active and non-physically active chronic c-SCI individuals.

Materials and methods

Participants

Twenty-three c-SCI individuals volunteers with a lesion between C5 and C7 participated in the present study. They were divided into two groups according to the frequency of physical exercise: Physically active which were wheelchair rugby players (PA; $n = 13$; age = 25.0 years (IQR 23.0–33.0); had practiced physical exercise for at least 3 months, 3 times/week or more, totaling a minimum time of 150 min/week (30); and non-physically active [NPA; $n = 10$; age = 36.5 years (32.8–40.0)]. General information was obtained through a structured questionnaire; the participants answered questions about time of injury, and how long they practiced physical sports. None of the participants were taking nutritional supplements. All participants voluntarily agreed to participate in the research, and written informed consent was obtained.

Although the sample is small, it is important to emphasize that all subjects are quadriplegic with lesions between c5 and c7, which makes the group homogeneous. Furthermore, the sample obtained here is consistent with the literature (31) showing that in investigations with subjects with SCI, the sample size is generally small. This study was approved by the National Research Ethics System (COEP 052/2009). The individuals were instructed not to eat for 4 h and to drink enough water to maintain hydration. All measurements were taken in a single day.

Anthropometric measurements and body composition

Length was measured from the top of the head to the bottom of the heel using a stadiometer (Seca®). The stadiometer was extended on the stretcher of the DXA, where the individuals were placed in the supine position.

Total body mass, total fat, and fat-free mass were determined by Lunar iDXA device with enCore 2008 software version 12.20 (GE Healthcare, Madison, Wisconsin, USA). The participants were placed in a dorsal position. Orthopedic surgical pins or other implants were identified as artifacts, and the software did not include them in the analysis. The exams were performed by a single trained and qualified professional, following the

quality control procedures recommended by the manufacturer and the official recommendations of the International Society for Clinical Densitometry (32). Measurements on the calibration block (daily) and on the calibration spine phantom (weekly) supplied by the manufacturer had coefficients of variation 0.7%. Soft tissue body composition, i.e., fat mass (kg), body fat mass (percentage), and lean tissue mass (kg), were derived from the total body scan. Total body mass (kg) was taken as the sum of fat mass, lean tissue mass and bone mineral content.

Body composition (FFM and FM, kg) was assessed using a single frequency (SF) bioimpedance analyzer (RJL, 101 Quantum, Detroit, MI), applying foot-to-hand technology. In order to avoid clinical disturbances in fluid distribution, participants were instructed to abstain from food and liquids for 4 h and to abstain from caffeine and physical activity for 24 h before SF-BIA. Bladder emptying was performed by catheter valve before arrival at the laboratory. Before each test, the analyzer was checked with the impedance calibration (resistance $R = 500$ ohms); and the components inside the bioimpedance analyzer, such as the signal generator, the sensing apparatus, the scales of weight and height, and the electrical interference were tested as suggested by Kyle et al. The average of the two repeated measurements of R and X_c was used in the subsequent analyses. SF-BIA was performed for FFM prediction, two electrodes placed on the dorsal surface of the hands and two on the surface of the feet. The device was then connected, and the voltage was detected by proximal electrodes, and the values of R and reactance (X_c) were obtained.

The predictive equations were selected based on the following criteria: (1) validated for SCI individuals or elderly, ideally using DXA; (2) BIA analyzer used to develop the equation at 50 kHz; (3) sex as a variable of the predictive equation. Three proposed predictive equations met the inclusion criteria: Kocina and Heyward (19), validated BIA predictive equation to estimate the FFM considering 91 adults with spinal cord injury and used DXA as a reference value; Buchholz et al. (33)

FFM predictive equation, developed to elderly and validated to paraplegic individuals; and Sun et al. (34) predictive equation, validated using FFM obtained using DXA, developed with representative able-bodied adults, which include elderly people, and recommended for epidemiological studies. Table 1 shows the details of the selected equations.

Bioelectrical impedance vector analysis (BIVA)

BIVA tolerance consists of plotting the experimental data in a bivariate graph considering the 95th, 75th, and 50th vector percentiles of the Z-score of the reference population. Considering the plotting position of the experimental data, it is possible to suggest an interpretation: abnormal situation, when experimental data are positioned outside of the 95th percentile ellipsis; high body cell mass, when experimental data are located above the long axis of the ellipsis; hypohydration, when experimental data are positioned to the right of the short axis of the ellipsis. Total body water is inversely related to the length of the impedance vector, and a combination of the vector length and its direction is defined as phase angle (PhA) (27). The reference population for c-SCI used in the BIVA graph was obtained from the dataset of Sun et al. and Buchholz et al. predictive equations, with similar sex, age, and BMI range as the present study. R and X_c values from the Kocina et al. predictive equation were not available for BIVA use; for this reason, they were not applied in this study.

Statistical analysis

Statistical analyses were performed using SPSS software version 19 (IBM Corporation, Armonk, NY, USA) and MedCalc Statistical Software version 14.8.1 (MedCalc Software, Ostend,

TABLE 1 BIA equations found for the prediction of FFM in SCI individuals and able-bodied people*.

Author	n (sex)	Age (years)	Characteristics	Predictive equations	SEE (kg)	R ²
Sun et al. (34)*	1,829 (734 males)	12–94	Able-bodied	FFM = $-10.68 + 0.65 \text{ Ht}^2/\text{R} + 0.26 \text{ W} + 0.02 \text{ R}$	0.4	0.90
Kocina and Heyward (19)	91	18–73	SCI lesion level not reported	FFM = $18.874 + \text{Ht}^2/\text{R} (0.367) + \text{W} (0.253) - \text{age} (0.081) - \text{sex} (5.384)$	3.2	0.87
Buchholz et al. (33)	93 (19 males)	34.2 ± 8.8	Paraplegia	FFM = $\text{TBW}/0.732$ TBW = $2.11 - (0.1 \times \text{age}) + (3.45 \times \text{sex}) + (0.34 \times \text{W}) + (28) \text{ Ht}^2/\text{R} - (0.086) \text{ sex} \cdot \text{W}$	1.86	0.95

FFM, Fat Free Mass in kilograms; TBW, Total Body Water in liters; sex is 0 for men and 1 for women; W, weight in kilograms; Ht, height (cm); R, resistance (ohms); SEE, standard error of the estimate; R², determination coefficient.

TABLE 2 c-SCI individuals' general characteristics according to physical exercise.

Variable	Median (IQR), PA (<i>n</i> = 13)	Median (IQR), NPA (<i>n</i> = 10)	<i>P</i> -value
Age (years)	25.0 (23.0–33.0)	36.5 (32.8–40.0)	0.029
Time since injury (years)	3.3 (2.8–7.4)	14.0 (9.7–17.9)	0.023
Total body mass (kg)	64.9 (59.7–73.2)	70.9 (61.4–74.8)	0.619
Height (cm)	172.0 (165.0–179.0)	172.2 (169.3–176.1)	0.803
BMI (kg/m ²)	21.9 (20.9–23.2)	23.4 (20.8–24.5)	0.784
FFM (kg)			
DXA	47.5 (45.2–50.1)	45.3 (41.4–48.4)	0.313
Sun et al. (34)	40.3 (38.8–47.7)	43.0 (37.5–46.7)	0.927
Kocina and Heyward (19)	52.9 (51.1–55.9)	52.5 (49.0–56.5)	0.642
Buchholz et al. (33)	46.7 (45.0–49.9)	46.0 (42.0–51.4)	0.522

Values are presented as median and interquartile range [IQR (25% and 75% percentiles)]. BMI, body mass index; FFM, fat free mass; DXA, dual energy X-ray absorptiometry. Mann–Whitney test $P < 0.05$ was considered significant.

Belgium; <http://www.medcalc.org>; 2014). Statistical tests were considered significant at the significance level of 5% ($P \leq 0.05$).

Anthropometric measurements and body composition compartments were expressed as median and interquartile range. Mann–Whitney test was used to determine differences between groups, according to physical exercise (physically active and non-active). Wilcoxon test was used to determine the difference between the FFM values obtained by the three BIA predictive equations and values obtained using DXA. Lin's concordance correlation coefficient (35) was used considering the strength-of-agreement criteria described by (36) (almost perfect: >0.99 ; substantial: 0.95 – 0.99 ; moderate: 0.90 – 0.95 ; and poor: <0.90). The root means square error (RSME) between FFM observed (DXA) and predicted equation values were calculated. The mean absolute percentage error (MAPE) was calculated in percentage (37) for the FFM predictions performed with the two methods. BIVA was performed using specific software, which was kindly provided by Dr. Antonio Piccoli (*in memoriam* - Institute of Internal Medicine, Division of Nephrology, and Clinical Nutrition Unit, University of Padova, Padova, Italy).

Results

General characteristics of the participants are shown in Table 2. The PA group showed lower chronological age ($p = 0.029$) and shorter time since injury ($p = 0.023$) than NPA. Since there was no significant difference between the groups in other variables, the statistical analysis considered all participants.

Comparison between FFM values obtained using DXA and BIA showed that Kocina and Heyward ($p \leq 0.001$) and Sun et al. ($p = 0.012$) predictive equations were significantly different from DXA. However, Buchholz et al. predictive equation showed

no difference in FFM when compared to the value obtained using DXA (Table 3).

Bland–Altman plots showed that Kocina and Heyward predictive equation presented higher bias (-5.9 kg) than Sun et al. (4.7 kg) and Buchholz et al. (0.1 kg) predictive equations. However, other concordance parameters showed that Sun et al. predictive equation presented higher SEE (5.21), RMSE (7.07) and MAPE (15.4%), and lower R^2 (0.70) and CCC (0.65) than Kocina and Heyward, and Buchholz et al. predictive equations (Figure 1).

When Z-score Xc and R values obtained by Sun et al. were used as reference population in the BIVA graph, the vectors were displaced below the high axis of the tolerance ellipses, showing little body cell mass. However, when plotted with Z-score Xc and R values obtained by Buchholz et al. predictive equation, most individuals were located within the tolerance ellipses (Figure 2).

Discussion

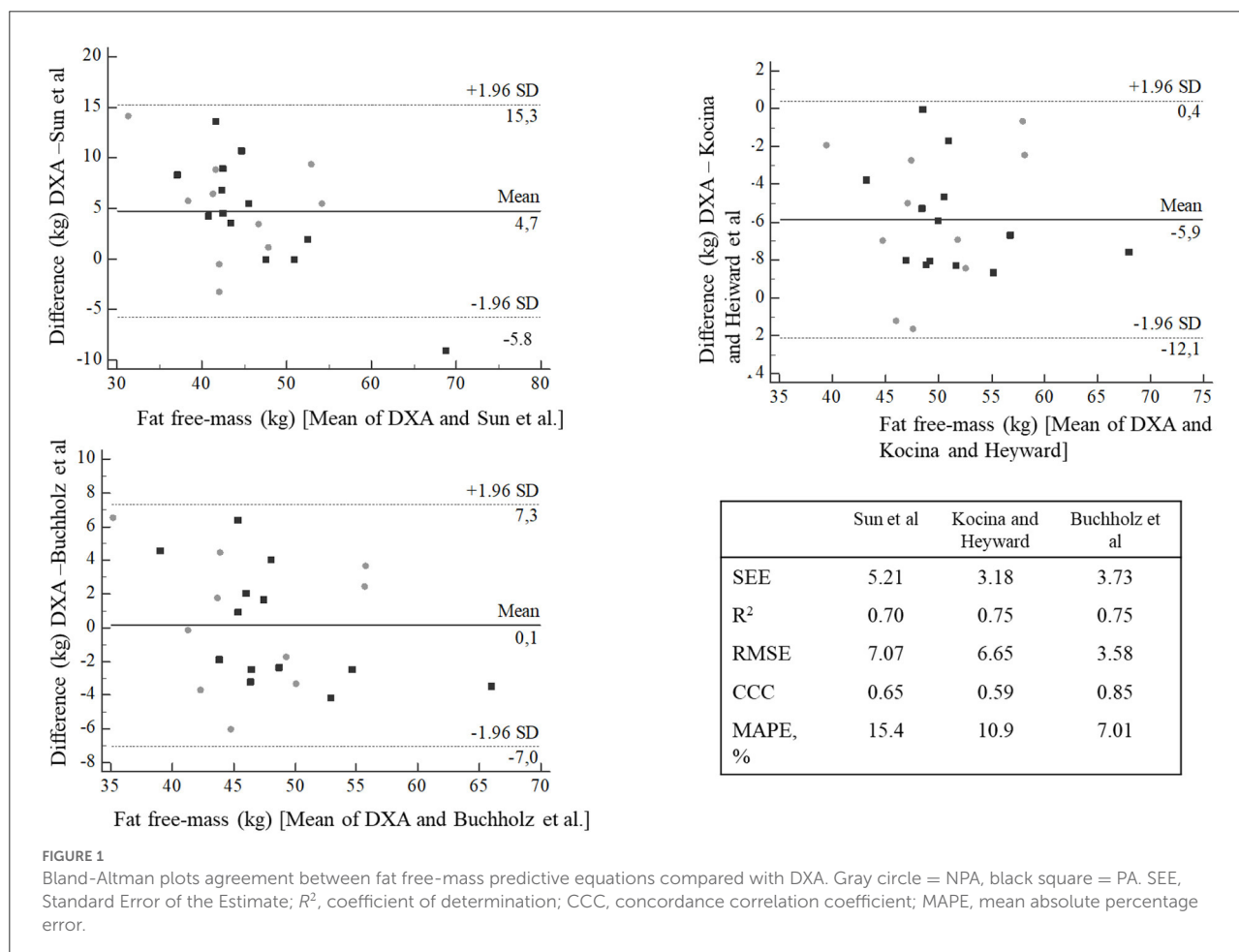
This pilot study showed that Buchholz et al. FFM–BIA predictive equation presented the highest concordance coefficient with FFM obtained using DXA; however, this is not sufficient to recommend it to c-SCI individuals. Therefore, BIVA could be used with caution, because according to the population reference used, different conclusions can be observed, such as demonstrated in this study. To our knowledge this is the first study to test BIVA in SCI individuals.

Regular physical activity practice for at least 150 min/week may be responsible for the improvement of body composition, hormonal profile and bone health in SCI individuals (38–41). Despite the benefits of exercise, this study failed to observe differences in the FFM values between the PA and NPA groups, possibly due to significant difference in age and time of injury between the groups.

TABLE 3 FFM obtained using DXA and BIA predictive equation (kg).

	DXA	Sun et al. (34)	Kocina and Heyward (19)	Buchholz et al. (33)
Median	47.0	41.7	52.9	46.7
IQR (25–75%)	43.8–49.3	38.1–47.5	50.3–56.3	43.5–51.0
p-value		0.012	<0.001	0.999

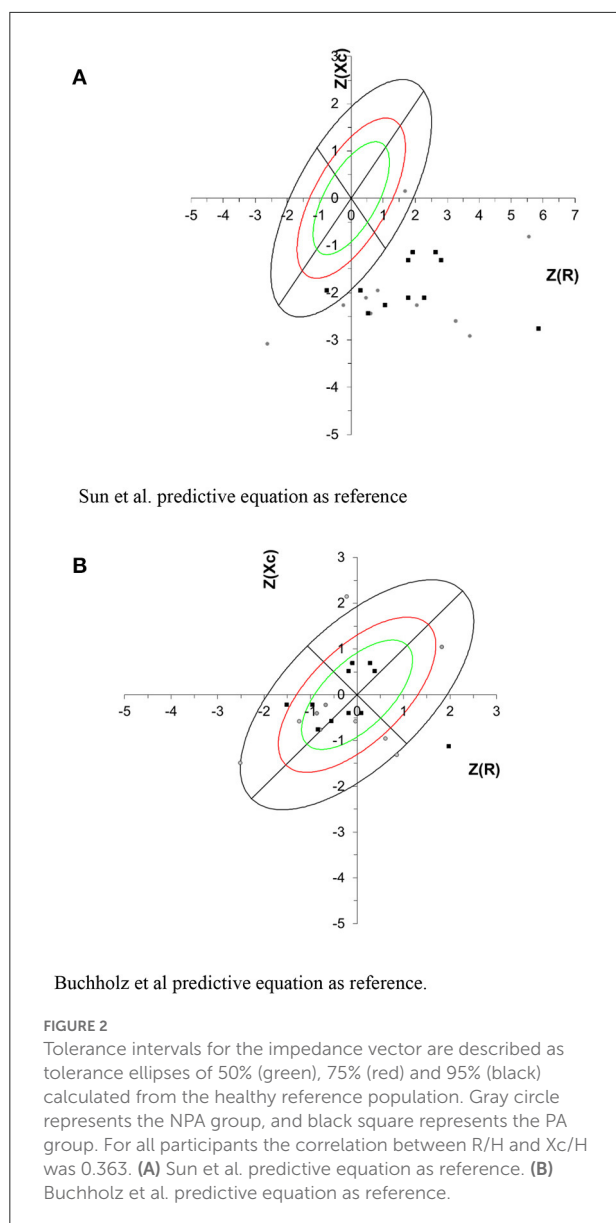
Interquartile range (IQR). p-values by Wilcoxon test. $P \leq 0.05$ was considered significant. MAPE, mean absolute percentage error.



Sun et al. predictive equation was developed with 734 able-bodied physically active males, including elderly individuals. c-SCI individuals have a body composition similar to that of the elderly population, presenting, for example, FFM loss and fat mass increase (42). In the present study, FFM obtained by Sun et al. predictive equation presented higher MAPE value (15.4%) than Kocina and Heyward and Buchholz et al. FFM predictive equations, and lower concordance coefficient than Buchholz et al. predictive equation. Possibly this happens because Sun's predictive equation was developed and validated for elderly individuals.

Plotting the R and Xc z-score in BIVA tolerance graph, using Sun's predictive equation as reference, it was observed that all c-SCI individuals were located under the highest axis, which can be attributed to the lowest body cell mass (BCM). BCM is the metabolically active cell mass involved in energy production and energy expenditure and is an important component of FFM (43). Our results using Sun's et al. predictive equation in BIVA were consistent with the c-SCI individuals body composition profile when compared to able-bodied individuals.

Kocina and Heyward predictive equation was developed for SCI adults; however, the abstract does not present information about sample size, height and time of injury of the participants,



resistance and reactance values. In the present study, FFM values obtained using the Kocina and Heyward predictive equation presented a high mean error percentage represented by MAPE (10.9%) and the lowest value of concordance coefficient, suggesting that despite having been developed with SCI individuals, it was not suitable for the group of the present study, possibly due to the wide range age group (18–73 years). The Kocina and Heyward predictive equation was not used in BIVA as a reference group, because there is no information on the bioelectrical data.

Buchholz et al. FFM BIA predictive equation was developed for healthy elderly subjects and tested and validated for paraplegic individuals, using the TBW method as reference,

which reflects the lean soft tissue as muscle mass, considering the hydration constant (0.732) (44). In the present study, FFM values obtained using Buchholz et al. predictive equation presented higher concordance coefficient and lower MAPE than Kocina and Heyward and Sun et al. predictive equations. FFM values obtained by Panisset et al. using Buchholz et al. predictive equation observed a good concordance and low underestimation bias, in acute SCI obese individuals, whereas this study aimed for chronic c-SCI individuals.

In the present study, BIVA tolerance ellipses of SCI individuals shifted to the right, when Buchholz et al. predictive equation was used as reference, indicating high cell mass and fluid content, which can be attributed to better cell functioning (45). This profile is consistent when a specific equation for SCI individuals was used as reference; differently observed when the Sun et al. equation was used as a reference.

In conclusion, the present study was able to show that BIVA is sensitive to adaptations in body composition and to hydration status, responding consistently to the equations used as a reference, despite some limitations, such as: lack of hydration status control, and difference between age and time of physical exercise. In addition, future studies on new FFM predictive equations, specific for SCI individuals, should be developed according to spinal lesion characteristics and with a more representative sample size. The inaccuracies in assessing body composition and fluids at the c-SCI group may compromise an adequate assessment and monitoring of body fluids and may interfere with the clinical care of this specific and vulnerable group. Therefore, caution should be applied when interpreting data extracted from generalized equations.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by National Research Ethics System (COEP 052/2009). The patients/participants provided their written informed consent to participate in this study.

Author contributions

FF performed data collection. AB, AC, FF, and JK analyzed the data and participated in the conceptualization of the work. AB wrote the original manuscript. AC and JK were involved in proofreading and editing. All authors read and agreed with the published version of the manuscript.

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Conflict of interest

Author FF was employed by Brazilian Paralympic Committee.

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Age-, sex-, and maturity-associated variation in the phase angle after adjusting for size in adolescents

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Background: Applied research using the phase angle (PhA) in children and adolescents has increased notably. Using multilevel modeling in a fully Bayesian framework, we examined the relationships between PhA, age, sex, biological maturity status, and body size in 10–16-year-old adolescents.

Methods: The sample comprised 519 adolescents (women, $n = 241$; men, $n = 278$) from Campinas, São Paulo, Brazil. Biological maturity status was assessed with self-examination of pubertal development for sexual maturity and maturity offset protocol to estimate age at peak height velocity (PHV) for somatic maturity status. Stature and body mass were measured by anthropometry. Phase angle was calculated based on raw resistance and reactance values (50 kHz frequency) obtained by bioelectrical impedance with the foot-to-hand technology.

Results: The multilevel regression analysis revealed that boys had significantly higher values of phase angle than girls, adjusting for age group and sexual maturity status. Overall, older and more mature adolescents had higher values of phase angle. When considering aligning variation in the phase angle by distance to estimated PHV (maturity offset), there was a higher association between the phase angle and time before and after predicted age at PHV for boys ($r = 0.31$, 90% CI: 0.23 to 0.39) than girls ($r = 0.2$, 90% CI: 0.11 to 0.28). When including body mass in the multilevel models, corresponding changes in the overall body mass mediate most of the influence of the maturity status and age group on the phase angle.

Conclusion: The present study demonstrated that the variability in phase angle is related to inter-individual variation in sex, age, and maturity status, as well as differences in body size. Research with adolescents considering phase angle should use multilevel modeling with standardized parameters as default to adjust for the concurrent influence of sex, age, maturity status, and body size.

KEYWORDS

pediatric populations, body mass, body composition, youth, Bayesian methods, multilevel modeling

Introduction

Bioelectrical bioimpedance analysis (BIA) has been an attractive method to assess body composition (1). The BIA provides an easy-to-handle, non-invasive, portable method with good reproducibility, which is viable for clinical practice and epidemiological studies (2–5). BIA measures the values of resistance (R) and reactance (X_c) of a current as it passes through tissues of the body measured (6, 7). R represents the opposition offered by the body to the flow of an alternating electrical current and is inversely related to the water and electrolyte content of tissues (5). X_c represents the resistive effect produced by the tissue interferences and cell membranes (5, 6). Body composition has indirectly been estimated using prediction equations from R and X_c measurements derived with BIA (7). However, age-, ethnic-, sex- or clinical-conditions-associated variations in body shape, relations between the trunk and leg lengths, and hydration levels limit the validity of the equations (6, 7), allowing for the validity of the modeling assumptions of the prediction equations.

Information about body fluids distribution among intracellular and extracellular compartments and tissue integrity can be obtained from raw BIA measurements, R and X_c (2, 6, 8). The values of R and X_c of a current as it passes through tissues of the body by BIA can be used to calculate the phase angle, PhA (2, 9). Since bioelectric impedance (Z) results from the two vectors representing R and X_c , PhA is the angle between Z and R (10, 11). PhA is influenced by the body quantity of cells, with respective cell membranes, cell membrane integrity, related permeability, and the amounts of intracellular and extracellular fluids (1, 7). Consequently, factors such as age, sex, body dimensions and compositions, level of physical activity, or fluid status should be considered when interpreting PhA (1, 2, 12, 13). PhA is a relevant health parameter in clinical use as it reflects the body fluid distribution among intracellular and extracellular compartments (2, 8). Furthermore, PhA can indicate the nutritional status of different populations and malnourished children, healthy children, and adolescents (14–16).

Recently, the interest in applied research interpreting PhA in children and adolescents, particularly in youth sports, has increased notably (14–19). The PhA values should increase with adolescence and should be more pronounced in boys than girls (10, 13). During the pubertal growth spurt, gains in fat-free mass are higher in boys, while relative fatness, i.e., fat mass as a percentage of body mass, tends to decline in boys but increase in girls (20). Phase angle increases with age in adolescence, particularly in boys, due to a increase in reactance which parallels the gains of fat-free mass (particularly muscle mass) and a decrease in resistance due to the increasing proportion of body water at the expense of reduction of relative fatness.

Substantial body dimensions and composition changes mark pubertal growth, and sexual dimorphism becomes apparent (21). However, biological maturity-associated variation between

individuals in body size and composition is considerable during pubertal years (22). Moreover, within a small chronological age range during adolescence, differences in maturity status may be significant within and between boys and girls. Hence, pubertal growth changes and maturity-associated variation in body dimensions and composition likely influence the interpretation of PhA, particularly among adolescents around peak height velocity (PHV) age (23). However, data considering the influence of maturity status on PhA is limited and mostly based on samples of young athletes (24–28). Overall, the interpretation of maturity-related variation aligned with adolescents' chronological age, sex, and body size merits further study.

Interpretations of physiological outcomes, particularly considering children and adolescents, need to consider cross-classified nesting within and between groups (e.g., sex, maturity status, age group), which often requires coping with an imbalance in sample size and heterogeneity among individuals. Therefore, multilevel models should be used as default, as they allow and explicitly model the data structure by allowing for residual components at each level in the hierarchy or cluster (29, 30). Nevertheless, Traditional single-level regressions continue to be used to deal with data in pediatric physiology, which is a concern, particularly, in settings with a low group-level variation where multiple comparisons exist (31).

In the present study, using Bayesian multilevel modeling, we examined the age-, sex- and maturity-associated variation in PhA adjusting for the influence of body size in adolescents aged 10 to 16.

Materials and methods

Study design and sample

This study used a cross-sectional design on 519 adolescents (girls, $n = 241$; boys, $n = 278$) from Campinas, São Paulo, Brazil. Participants in this study were adolescents enrolled adequately at the local school, detaining a regular frequency of physical education. In addition, the study did not consider children with physical disabilities (permanent or temporary), children impeding participation in any of the procedures, or those using prescribed medicine. For each participant, all the measurements were obtained in the morning, after an overnight regular fast (8 h), refraining from vigorous exercise for at least 15 h, avoiding caffeine and alcohol during the preceding 24 h, and consuming a normal evening meal the night before.

The Ethics Committee of the Pontifical Catholic University of Campinas (CAAE: 79625817.6.0000.5481) approved the research. All procedures followed Resolution No.466 of 2012 of the National Health Council of the Ministry of Health of Brazil and were conducted following the Declaration of Helsinki.

Participants and their parents or legal guardians provided informed written consent.

Age and anthropometry

Chronologic age was calculated and recorded to the nearest 0.1 years by subtracting the birth date from testing. The participants were categorized by age group as follows: 10–11 years old (10 to 11.9 years); 12–13 years old (12 to 13.9 years); and 14–15 years old (14 to 15.9 years). Stature was measured with a vertical portable stadiometer (Sanny, SBC, SP, Brazil) to the nearest 0.1 cm. Body mass was measured with a calibrated portable balance (Sanny Digital Glass 200 Control, SBC, SP, Brazil) to the nearest 0.05 kg. Technical errors of measurement were 0.29 cm for stature and 0.51 kg for body mass, based on replicated measurements of 20 participants.

Maturity status

We estimated the maturity status using two approaches: (i) sexual maturation, using self-examination of pubertal development, and (ii) somatic maturation, using estimations with the maturity offset and age at PHV using sex-specific equations (32).

Sexual maturation

Before the self-physical examination, the participants were provided with a standardized series of realistic color images with an explanatory text to individually assess their pubertal development (32), following the sexual maturity stage criteria described by Tanner (33). For example, in girls, breast development was classified from 1 (pre-puberty) to 5 (mature), and stage 2 (appearance of the buttoned breast) marks the beginning of pubertal development. In boys, genital development was classified from 1 (pre-puberty) to 5 (mature); stage 2 marks puberty onset. Participants were asked to read brief descriptions of each stage and check the box on the image that best represents the development component. All assessments were carried out in a private room. Participants were grouped as pre-puberty (classified as 1), early puberty (classified as 2), mid-puberty (classified as 3), late puberty (classified as 4), and mature (classified as 5).

Somatic maturation

We used the simplified versions of the gender-specific maturity offset protocol (32) to determine participants' maturity status. The offset equations estimate time before or after PHV based on their chronological age and stature. Thus, negative

values indicate the time before PHV, and positive values indicate the time after PHV.

Phase angle

PhA was assessed using a single frequency (50 kHz) BIA device, model Quantum II (RJL Systems, Detroit, MI, USA). All adolescents were instructed to remove all objects containing metal before taking the BIA measurement. Next, participants were laid barefoot, in a supine position, with the legs abducted at a 45° angle, arms far from the trunk, and hands pronated on a table isolated from electrical conductors. After 5 min of resting, the participants' skin was cleaned with alcohol, and two electrodes were placed on the surface of the right hand and two others on the surface of the right foot, according to the recommended protocol (9). The evaluation lasted approximately 1 min.

Bioelectrical bioimpedance analysis provided the value of R and X_c in ohms (Ω), and, from these variables, PhA was calculated using the following published equation (9):

$$PhA = \arctan(X_c/R) \times (180/\pi) \quad (1)$$

Based on replicated measurements of 23 participants, the technical errors of measurement were 3.54 and 0.49 Ω for R and X_c , respectively. Corresponding coefficients of variation were 0.35 and 0.33% for R and X_c , respectively.

Statistical analysis

We used a fully Bayesian approach in our analysis. By fitting the multilevel models within a Bayesian framework (34), the parameters are treated as random variables combining both prior distribution information and sample data to estimate a (posterior) probability distribution that reflects the uncertainty associated with how well they are known based on the data (35, 36). Thus, Bayesian methods allow a direct probabilistic interpretation of CIs (also referred to as confidence or compatibility intervals) and posterior probabilities, relevant in applied human biology research, where the interest frequently lies in estimating small effects.

Our estimations were based on the Bayesian multilevel models considering the variation in PhA, adjusting for cross-classified nesting by age group, sex, and maturity status among young Brazilian adolescents. We standardized (z-score) all the outcomes for interpretative convenience and computational efficiency. Given the limitations in the agreement between maturity indicators, we explored the influence of maturity status by considering the sexual maturity status as a discrete variable with five levels (pre-puberty, early puberty, mid-puberty, late puberty, and mature) and the somatic maturity status using the maturity offset as a continuous variable.

To model the influence of age group, sex, and sexual maturity status on PhA, we used varying-intercept models where each participant's outcome (intercept) was estimated as a function of his/her age group, sex, and estimated sexual maturity status (model 1). Hence, for individual i , we used indexes a , s , and m for age group, sex, and sexual maturity status, respectively. The group-level effect terms (also referred to as random effects) and the data-level terms (also referred to as level-1 residuals) were drawn from normal distributions with variances to be estimated from

the data:

$$y_i = \beta_0 + \alpha_{a[i]}^{age\ group} + \alpha_{s[i]}^{sex} + \alpha_{m[i]}^{maturity\ status} + \epsilon_i \quad (2)$$

$$\alpha_{a[i]}^{age\ group} \sim N(0, \sigma_{age\ group}^2), \text{ for } a = 1, 2, 3. \quad (3)$$

$$\alpha_{s[i]}^{sex} \sim N(0, \sigma_{sex}^2), \text{ for } s = 1, 2. \quad (4)$$

$$\alpha_{m[i]}^{maturity\ status} \sim N(0, \sigma_{maturity\ status}^2), \text{ for } m = 1, 2, 3. \quad (5)$$

$$\epsilon_i \sim N(0, \sigma_{y_i}^2) \quad (6)$$

We replicated the model 1 structure, adding body mass as a populations level effect (model 2):

$$y_i = \beta_0 + \beta_1^{body\ mass} + \alpha_{a[i]}^{age\ group} + \alpha_{s[i]}^{sex} + \alpha_{m[i]}^{maturity\ status} + \epsilon_i \quad (7)$$

$$\alpha_{a[i]}^{age\ group} \sim N(0, \sigma_{age\ group}^2), \text{ for } a = 1, 2, 3. \quad (8)$$

$$\alpha_{s[i]}^{sex} \sim N(0, \sigma_{sex}^2), \text{ for } s = 1, 2. \quad (9)$$

$$\alpha_{m[i]}^{maturity\ status} \sim N(0, \sigma_{maturity\ status}^2), \text{ for } m = 1, 2, 3. \quad (10)$$

$$\epsilon_i \sim N(0, \sigma_{y_i}^2) \quad (11)$$

TABLE 1 Distribution of stages of pubic hair (PH) in the sample of adolescents by sex and age group.

	PH1	PH2	PH3	PH4	PH5
<i>Female</i>					
10–11 years	2	20	33	26	1
12–13 years	0	2	21	71	20
14–15 years	0	1	2	31	11
<i>Male</i>					
10–11 years	3	11	56	9	1
12–13 years	0	4	42	80	12
14–15 years	0	1	19	28	12

TABLE 2 Mean, standard deviation and range of maturity offset in the sample of adolescents by sex and age group.

	Mean	Standard deviation	Range
<i>Female</i>			
10–11 years	−0.77	0.55	−2.04 to 0.51
12–13 years	0.77	0.70	−0.60 to 2.36
14–15 years	2.09	0.49	1.13 to 3.02
<i>Male</i>			
10–11 years	−2.06	0.48	−3.21 to −0.97
12–13 years	−0.62	0.63	−1.86 to 0.83
14–15 years	0.81	0.56	−0.39 to 2.60

TABLE 3 Descriptive statistics (mean and standard deviation) for adolescents by sex.

	Female ($n = 22$)	Male ($n = 35$)
Chronological age (yrs)	12.7 (1.3)	12.8 (1.3)
Maturity offset (yrs)	0.49 (1.20)	−0.72 (1.17)
Stature (cm)	152.9 (9.0)	156.2 (11.0)
Body mass (kg)	48.2 (11.5)	51.4 (14.6)
Phase angle (degree)	5.50 (0.70)	5.88 (0.77)

Considering the somatic maturity status, we used a varying-intercept and a varying-slope model to model PhA as a function of his/her estimated maturity offset, age group, and sex (model 3). We allowed for individuals' maturity offset to vary by sex

TABLE 4 Multilevel regression models posterior estimations and 90% credible intervals of variation in phase angle by sex, age group, stages of pubic hair (PH) (model 1), and adjusting for body mass (model 2) among adolescents.

	Model 1 (variation by sex, PH, and age group)	Model 2 (variation by sex, PH, and age group, adjusted for body mass)
Population-level effects (90% credible interval)		
Intercept	−0.03 (−1.20 to 1.11)	0.01 (−1.01 to 1.04)
Body mass	-	0.34 (0.26 to 0.41)
Group level estimates (90% credible interval)		
Level 2, standard deviation		
Sex	0.73 (0.22 to 1.65)	0.67 (0.18 to 1.58)
PH	0.33 (0.10 to 0.75)	0.19 (0.02 to 0.49)
Age group	0.58 (0.19 to 1.31)	0.35 (0.06 to 0.98)
Level 1 standard deviation	0.92 (0.87 to 0.97)	0.88 (0.83 to 0.98)

All outcomes were standardized in the models.

(varying slope):

$$y_i = \beta_0 + \beta_{s[i]}^{maturity\ offset} + \alpha_{s[i]}^{sex} + \alpha_{a[i]}^{age\ group} + \epsilon_i \quad (12)$$

$$\begin{bmatrix} \beta_0 \\ \beta_{s[i]}^{maturity\ offset} \end{bmatrix} \sim MVNormal \left(\begin{bmatrix} \beta_0 \\ \beta_{maturity\ offset} \end{bmatrix}, \Sigma \right) \quad (13)$$

$$\Sigma \sim \begin{bmatrix} \sigma_{\beta_0} & 0 \\ 0 & \sigma_{\beta_{maturity\ offset}} \end{bmatrix} R \begin{bmatrix} \sigma_{\beta_0} & 0 \\ 0 & \sigma_{\beta_{maturity\ offset}} \end{bmatrix} \quad (14)$$

$$\alpha_{s[i]}^{sex} \sim N(0, \sigma_{sex}^2), \text{ for } s = 1, 2. \quad (15)$$

$$\alpha_{a[i]}^{age\ group} \sim N(0, \sigma_{age\ group}^2), \text{ for } a = 1, 2, 3. \quad (16)$$

$$\epsilon_i \sim N(0, \sigma_{y_i}^2) \quad (17)$$

Again, we added body mass as a population-level effect to the model, but in this case, a varying-intercept model was

used (model 4):

$$y_i = \beta_0 + \beta_1^{maturity\ offset} + \beta_2^{body\ mass} + \alpha_{s[i]}^{sex} + \alpha_{a[i]}^{age\ group} + \epsilon_i \quad (18)$$

$$\alpha_{s[i]}^{sex} \sim N(0, \sigma_{sex}^2), \text{ for } s = 1, 2. \quad (19)$$

$$\alpha_{a[i]}^{age\ group} \sim N(0, \sigma_{age\ group}^2), \text{ for } a = 1, 2, 3. \quad (20)$$

$$\epsilon_i \sim N(0, \sigma_{y_i}^2) \quad (21)$$

We used weakly informative priors to regularize our estimates, a normal prior (0,5) for the intercept (population-level parameter, also referred to as fixed effect) and a normal prior (0,1) for group-level parameters. For the data-level residuals (ϵ_i), we used the “brms” default prior, Student- t (3, 0, 2.5) (37). Considering the outcome standardization, by using a normal (0,1) prior for the parameters, we state that the group-level estimates are unlikely to be greater than one standard deviation.

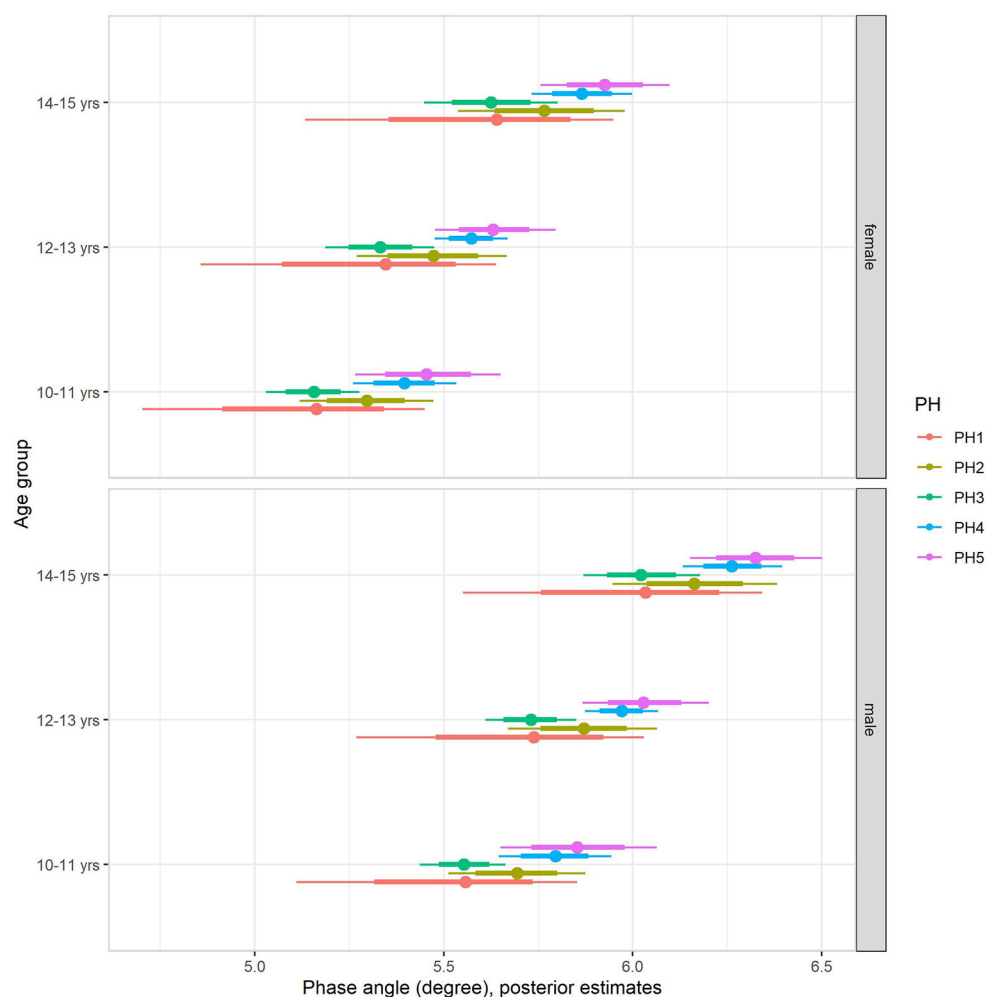


FIGURE 1
Posterior estimations (67 and 90% CIs) for the phase angle by age group and sexual maturity among Brazilian girls and boys.

To check whether the models successfully partitioned the influence of body mass (when included in the model), we inspected the residual plots against the body mass to check the homoscedasticity of the residuals. We ran four chains for 2,000 iterations with a warm-up length of 1,000 iterations in each model. The convergence of Markov chains was inspected with trace plots. We used posterior predictive checks to be confident in our models and estimations (34). We fitted the models in R (38) using the “brms” package (37), which calls Stan (39).

Results

The distribution of stages of pubic hair within age groups by sex is given in Table 1. The girls aged 10–11 were mainly distributed in early puberty, mid-puberty, and late puberty. Boys aged 10–11 years old were mainly classified as mid-puberty. Both

girls and boys aged 12–13 were mainly classified as mid-puberty and late puberty, and 20 out of 104 girls in the age group were classified as mature. Girls aged 14–15 were mainly in late puberty (PH4) or mature. Boys aged 14–15 years were mainly in late puberty (~47%), but about 32% were in mid-puberty, and 20% were mature.

Estimated maturity offset in the sample of adolescents by sex and age group is summarized in Table 2. On average, offset values were higher for girls across all age groups. In addition, values of offset increased as participants were older. However, the range of values in each age group was extensive (about 2 years), indicating significant variations between participants in the somatic maturity status within age groups.

The characteristics of the sample by sex are summarized in Table 3.

The multilevel regression models examining variation in PhA associated with gender, age group, and sexual maturity

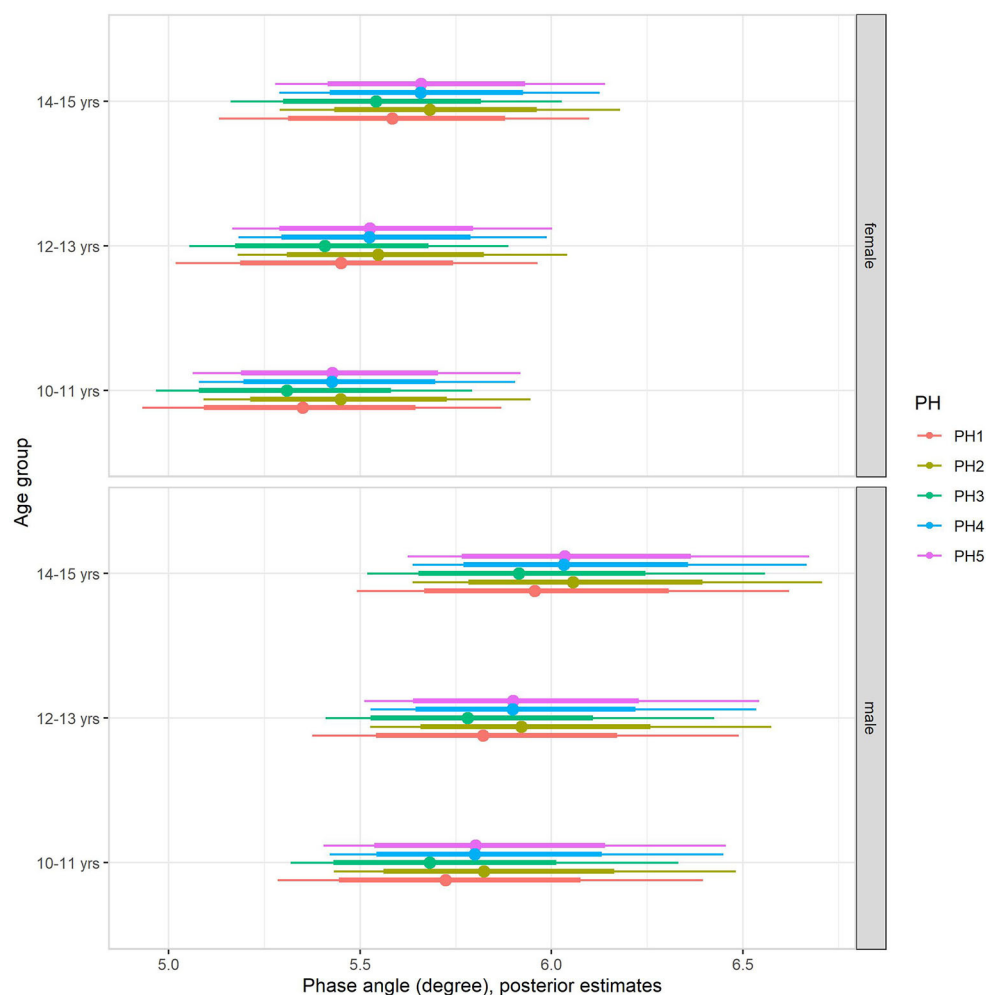


FIGURE 2
Posterior estimations after adjusting for body mass (67 and 90% CIs) for the phase angle by age group and sexual maturity among Brazilian girls and boys.

stage are summarized in model 1 of Table 4. Note that outcomes were standardized in the models, and the table summaries are presented on a z-score scale. Back transformed posterior estimates of adolescents' PhA are given in Figure 1, contrasting boys and girls by sexual maturity stage within each age group. Overall, older and more mature adolescents had higher values of PhA. The results of model 1 show substantial variation by sex. Boys had higher values than girls, adjusting for age group and sexual maturity stage (Figure 1). Also, the results showed an influence of age group and sexual maturity stage on PhA.

Back transformed posterior estimates of PhA adjusted for body mass, sex, age, and maturation are given in Figure 2, contrasting boys and girls by sexual maturity stage within each age group. When including body mass in the multilevel regression model (model 2, Table 4), it became clear that body mass substantially influenced PhA, independent of sex, age group, or sexual maturity stage. The sex-associated variation was attenuated but remained substantial (Figure 2). Adjusting for body mass substantially decreased the influence of age group and sexual maturity stage on PhA, and particularly the latest remained small at best. Residual analysis showed no spurious correlation between the residuals and body mass, indicating that our model successfully partitioned the influence of body mass on PhA (see Supplementary material).

The multilevel regression models examining variation in PhA associated with gender, age group, and somatic maturity (model 3) and adjusting for body mass (model 4) are summarized in Table 5. Figure 3 illustrates the relationships between PhA and maturity offset (time before and after predicted age at PHV), contrasting by sex. There was a substantially higher association between PhA and time before and after predicted age at PHV for boys ($r = 0.31$, 90% CI: 0.23 to 0.39) than girls ($r = 0.2$, 90% CI: 0.11 to 0.28). When adjusting for body mass in the model, the association of PhA with the estimated maturity offset became small ($r = 0.08$, 90% CI: 0.02 to 0.15) and similar for girls and boys (Figure 4). The association of body mass with PhA, adjusted for sex and maturity offset, was 0.34 (CI: 0.26 to 0.42). Nevertheless, boys presented higher PhA, adjusted for body mass, than girls when aligned by the time before and after the predicted age at PHV. Residual analysis showed no spurious correlation between the residuals and body mass, indicating that the model successfully partitioned the influence of body mass on PhA (see Supplementary material).

Discussion

PhA has been considered an important tool for the diagnosis of malnutrition and clinical prognosis, which can be associated with changes in cell membrane integrity, changes in fluid balance, and information on cell health and integrity (i.e., high

TABLE 5 Multilevel regression models posterior estimations and 90% credible intervals of variation in phase angle by sex, age, group, maturity offset (model 3), and adjusting for body mass (model 4) among adolescents.

	Model 3 (variation by sex, age group and maturity offset)	Model 4 (variation by sex, age group and maturity offset, adjusted for body mass)
Population-level effects		
(90% credible interval)		
Intercept	0.06 (−1.08 to 1.21)	0.01 (−1.04 to 1.05)
Maturity offset	0.22 (−0.46 to 0.82)	−0.06 (−0.20 to 0.08)
Body mass	-	0.37 (0.28 to 0.45)
Group level estimates		
(90% credible interval)		
Level 2, standard deviation		
Age group	0.21 (0.01 to 0.72)	0.49 (0.09 to 0.1.25)
Sex		
Intercept	0.90 (0.33 to 1.83)	0.59 (0.09 to 1.51)
Maturity offset (varying slope)	0.42 (0.03 to 1.33)	-
Level 1 standard deviation	0.92 (0.87 to 0.97)	0.88 (0.84 to 0.93)

All outcomes were standardized in the models.

phase angle values are associated with better permeability of cell membrane and cell function) (7, 40). The present study examined the concurrent influence of sex, age, maturity status, and body size on PhA among Brazilian adolescents. Variation in maturity status significantly influenced the PhA of female and male adolescents aged 10–15 years, adjusting for sex- and age-associated variation. Within each age group, adolescents in more advanced stages of pubic hair, particularly those in late puberty and maturity, had higher values of PhA. Nevertheless, the maturity- and age-associated variation on PhA was significantly accounted for when partitioning for body size. Hence, the influence of body size appears to mediate maturity- and age-associated variation on PhA in adolescents, independent of sex.

The growth characteristics of this sample of Brazilian adolescents were consistent with other reports of healthy young populations (41–43). In addition, the patterns of pubertal growth between individuals and sex-associated variations in the maturity status in the present sample were consistent in longitudinal growth studies in female and male adolescents (20, 21). Also, there is a need to adjust for sex difference in somatic and sexual maturation rates when interpreting maturity-associated variation between girls and boys (44). Hence, age and maturity status must be modeled jointly to analyze sex effects on physiological outcomes. The limitations

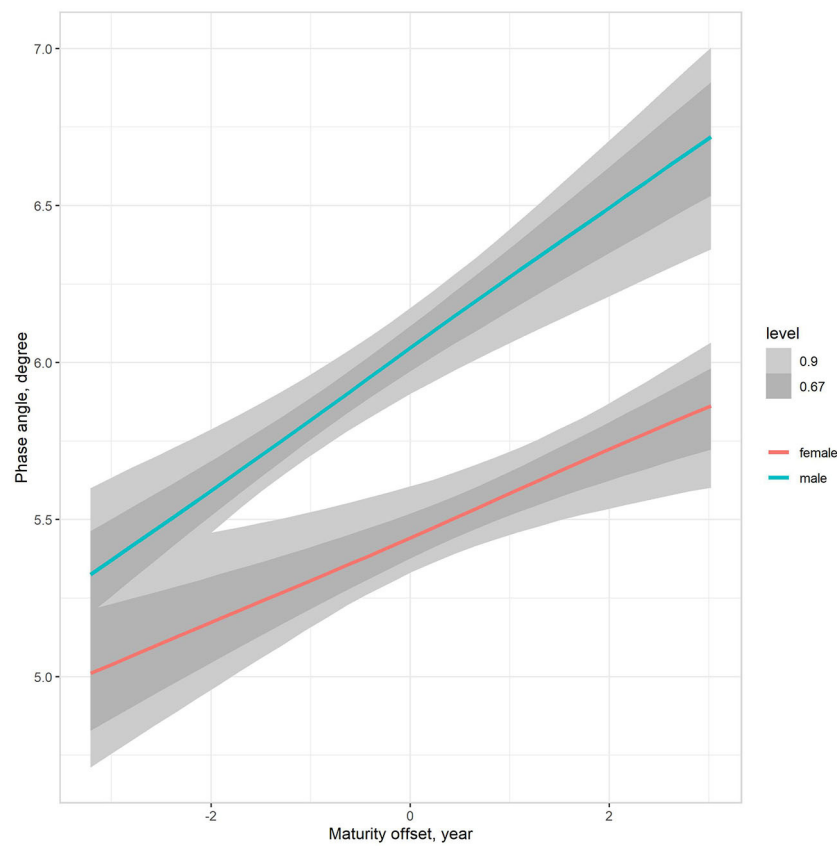


FIGURE 3

Posterior estimations (67 and 90% CIs) of the association of the phase angle with estimated time before and after the age at PHV (maturity offset) by sex.

of the maturity indicators used in this study imply the need for conservative interpretations (45, 46). Nevertheless, the offset equations and the self-examination of pubertal development provide possible options for having a reference of maturity status when only cross-sectional observations are available, assuming their limitations.

Available data consider mainly age-related variation in PhA in adolescents but do not control interindividual differences in the biological maturity status (13, 26, 47–49). Therefore, it appears reasonable to interpret that PhA is likely associated with increased cell mass with age (13). The observations of the present study on Brazilian adolescents indicated substantial variation associated with the stage of pubertal development, adjusting for age. Furthermore, we observed a linear increase in PhA when aligning for the age before and after PHV. Within an age group during adolescence, late-maturing girls and boys appear to have higher values of PhA. Overall, the results suggested an influence of age *per se* on PhA when individual variability is aligned to biological maturity indicators; in the present study, on self-examination of pubertal development and time before or after PHV, it has been noted that PhA values

were higher, albeit with small magnitude, in early maturing adolescent football players than in late maturing players, considering the skeletal maturity status using the Tanner-Whithouse-3 method (50). A similar trend has been noted in young football players using somatic maturity status (25, 27), albeit the variation was small. Often, samples of young athletes are relatively homogeneous in maturity status, body size and composition, and sport-specific performance, with a consistent trend of overrepresentation of early maturers (51). Hence, our observations highlight that maturity-associated variation in PhA among non-athletic adolescents is likely substantial and must be accounted for when interpreting PhA.

Biological maturation likely influences PhA through associated variation in somatic features, including size *per se* and lean and fat mass. Pubertal growth is marked by many neuroendocrine changes that mediate changes in size, physique and body composition, and various body systems (45). The process of pubertal growth and maturation, i.e., progress toward the mature state, is related and appears to influence PhA. In particular, our observations suggest that maturity-associated variations in body size and composition, considering body

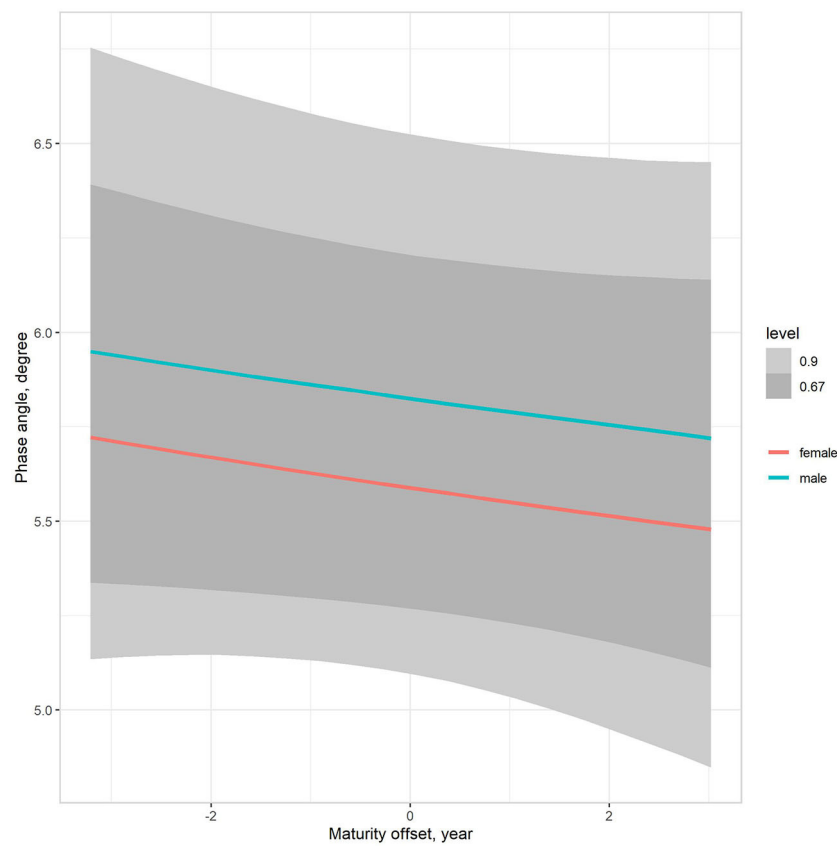


FIGURE 4

Posterior estimations (67 and 90% CIs) of the association of the phase angle with estimated time before and after the age at PHV (maturity offset) by sex, adjusting for body mass.

mass as a surrogate of body size and composition, likely explain a substantial portion of age- and maturity-related variation in PhA. Note that the interpretations are similar when considering stature as a surrogate of body size (results available as [Supplementary material](#)). In particular, this trend may be related to adolescent growth spurts in the body, and free-fat mass since maximal growth in body and muscle mass occurs after PHV (22).

Our observations showed a clear trend of sex variation, adjusting for age and maturity status. When aligning PhA by age before and after PHV, we observed a distinct increase in trends in PhA, with girls having a lower increase than boys. Nevertheless, the observed sex-related variation in PhA during adolescence became similar between girls and boys when body mass was adjusted in the models. Lean mass is largely constituted of body water (52) and is an excellent conductor of electricity, offering low resistance to the passage of electric current (3, 53). Hence, the low resistance values contribute directly to higher phase angle values (2). Sex dimorphic growth and development are most pronounced during adolescence (54). Girls usually begin adolescent growth before boys and progress at a faster

rate than boys (44). Moreover, body fat levels rise substantially in girls during adolescence. Body fat distribution is mainly determined by sex steroids, with increased body fat in girls' subcutaneous, gluteal, and femoral regions (54). The relative contribution of lean mass to total body mass usually declines once consideration is given to the relative contribution of fat mass (22, 54). Furthermore, lean tissue hydration values tend to decline with age in girls, specifically with decreases in water content and increases in density with increasing age. Hence, sex-associated variation in PhA between girls and boys should be expected.

The adequacy and appropriateness of the adopted multilevel regression models were demonstrated by the near-zero relationships between the predicted PhA outputs and body mass and the examination of residuals, which showed a normal distribution (see [Supplementary material](#)). Hence, this study illustrates an approach to dealing with concurrent influences of sex, age, maturity status, and body size on physiological outcomes in adolescents. Data and codes for replication of our models are available at <https://osf.io/j2yez/>.

Our analysis did not account for body composition differences between the adolescents (e.g., fat mass and lean mass). Furthermore, although we took advantage of Bayesian inference in our models, the sample was from the same region within a cross-sectional design, limiting generalizations. Therefore, future studies should adjust PhA considering biological, contextual, and fitness characteristics based on longitudinal observations.

In summary, our study demonstrated significant interindividual variation in PhA among female and male adolescents. Furthermore, the variability in PhA is related to interindividual variation in sex, age, maturity status, and body size differences. Overall, the present study results highlight the need to account for the transient influence of pubertal growth on body size, shape, and composition and its effect on the interpretation. PhA has been proposed to assess body composition using whole-body BIA that can represent the intracellular/extracellular water ratio (5), providing a meaningful clinical interpretation of cell damage, inflammation, or dehydration during pubertal development. Furthermore, the multilevel regression models incorporating body mass indicate that corresponding changes in overall body mass mainly mediate the influence of the sexual and somatic maturity status on PhA. Therefore, for investigating changes in PhA during adolescence, multilevel modeling with standardized parameters is recommended to normalize data, allowing any disproportionate increase in PhA associated with maturity status, sex and body size to be identified.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found at: <https://osf.io/j2yzez/>.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the Pontifical Catholic University of Campinas (CAAE: 79625817.6.0000.5481) approved the research. All procedures followed Resolution No.466 of 2012 of the National Health Council of the Ministry of Health of Brazil. Written informed consent to participate in

this study was provided by the participants' legal guardian/next of kin.

Author contributions

AM, HC, and GG-J: conceptualization. RQ, AL, and HC: data curation. AM and HC: formal analysis and writing-original draft. GG-J: project administration. AM and GF: investigation. AM, RQ, AL, GF, HC, and GG-J: writing-review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnut.2022.939714/full#supplementary-material>

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Bioimpedance analysis combined with sagittal abdominal diameter for abdominal subcutaneous fat measurement

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Abdominal subcutaneous fat tissue (ASFT) is an independent predictor of mortality. This prospective observational study aimed to establish a rapid, safe, and convenient estimation equation for abdominal subcutaneous fat area (SFA) using bioimpedance analysis (BIA) combined with sagittal abdominal diameter (SAD). A total of 520 adult subjects were recruited and were randomly divided into 2/3 ($n = 346$) and 1/3 ($n = 174$) to form a modeling group (MG) and a validation group (VG), respectively. Each subject's abdomen was scanned using computed tomography to obtain target variables (SFA_{CT}). Predictor variables for all subjects included bioimpedance index (h^2/Z), anthropometric parameters height (h), weight (W), waist circumference (WC), hip circumference (HC), and SAD, along with age and sex (male = 1, female = 0). SFA estimation equation $SFA_{BIA+SAD}$ was established for the MG using stepwise multiple regression analysis. Cross-validation was performed using VG to evaluate the performance of the $SFA_{BIA+SAD}$ estimation equation. Stepwise multiple regression analysis was applied from the MG, including $SFA_{BIA+SAD} = 49.89 + 1.09 SAD - 29.90 Sex + 4.71 W - 3.63 h^2/Z - 1.50 h$ ($r = 0.92$, $SEE = 28.10 \text{ cm}^2$, $n = 346$, $p < 0.001$). Mean differences in $SFA_{BIA+SAD}$ relative to SFA_{CT} were -1.21 ± 21.53 , 2.85 ± 27.16 , and $-0.98 \pm 36.6 \text{ cm}^2$ at different levels of obesity (eutrophic, overweight, obese), respectively. This study did not have a large number of samples in different fields, so it did not have completely external validity. Application of BIA combined with SAD in anthropometric parameters achieves fast, accurate and convenient SAF measurement. Results of this study provide a simple, reliable, and practical measurement that can be widely used in epidemiological studies and in measuring individual SFA.

KEYWORDS

abdominal obesity, bioelectrical impedance, cross-validation, sagittal abdominal diameter (SAD), anthropometric

Introduction

Obesity has become a global medical problem over the past few decades, and is projected to only worsen in the foreseeable future (1). Overweight and obesity are closely associated with chronic disease and increased morbidity and mortality. Related issues often lead to cardiovascular disease or metabolic syndrome. Direct and indirect medical expenses also place a huge economic burden on society (2).

Abdominal subcutaneous fat tissue (ASFT) and abdominal visceral adipose tissue (AVAT) have different effects on metabolic homeostasis, but their quantity and distribution are both risk factors for cardiometabolic diseases (3). Abdominal obesity is a major risk factor for diabetes and cardiovascular disease. Excess visceral and subcutaneous fat are key contributors to abdominal obesity. Visceral and subcutaneous fat differ in structure, metabolic activity, and functional significance. The current study suggests that a positive caloric balance in individuals with impaired adipogenesis may lead to adipocyte hypertrophy of the ASFT. This in turn leads to impaired energy storage and ASFT dysfunction (4). Insufficient ASFT reservoirs can lead to redistribution of free fatty acids to ectopic tissues such as liver, and skeletal muscle, thereby increasing metabolic risk (5). Metabolic syndrome may develop when ASFT stores fat in ectopic locations, which may lead to developing insulin resistance or lipotoxicity (6, 7). When AVAT is higher than ASFT, the risk of atherosclerotic cardiovascular disease and hemodynamic abnormalities increases (8, 9).

Several methods for indirect assessment of abdominal fat include body mass index (BMI), waist-to-hip ratio (WHR), and skin-fold thickness measurement (10–12) or dual-energy X-ray absorptiometry (DXA) (13). However, to obtain more accurate measurements, non-invasive methods such as computed tomography (CT) or magnetic resonance imaging (MRI) are required, which can accurately identify specific fat areas. Several studies have compared CT and MRI techniques (14, 15), and the use of MRI or CT to measure abdominal fat has been validated since the 1980s (16, 17). Recent fully automated CT and MRI can accurately measure visceral and subcutaneous fat in obese people (18, 19). However, the high cost and time requirements of CT and MRI are the main limitations of their widespread use even though they yield precise measurements when performing medical examinations or academic research.

Bioelectrical impedance analysis (BIA) is a simple, safe, rapid, and non-invasive method for assessing body composition. Many studies have compared the measurement results of the calibration method and BIA, and they are widely used in clinical and epidemiological studies (20, 21). BIA can also be used to measure AVAT or visceral fat area (VFA) (22, 23) but the application of BIA to the measurement of ASFT or abdominal subcutaneous fat area (SFA) is very limited. Therefore, this study applied bioimpedance measurement combined with

anthropometry, and used computed tomography as a reference method to establish and verify the estimation equation of BIA in SFA.

Subjects and methods

Study design and subjects

In this prospective observational and cross-sectional study. Subjects were recruited through hospital advertisements and word of mouth at Puzi Hospital in southern Taiwan. The subjects were tested by the non-random purposive sampling method. Potential participants were healthy adults who came to the hospital for their free NHS continuing healthcare checklist (24). Answering questionnaires and signing experimental consent forms was under the introduction of a trained research assistant. Long-term bedridden persons, those who had a change in weight in the previous year or recently, and those who had undergone abdominal surgery in the past were excluded from this experiment. Patients with malignant tumors and chronic liver disease were also excluded from this experiment. A total of 520 subjects were ultimately included in the study. This study complied with the ethical guidelines of the 1975 Declaration of Helsinki. Participants filled out personal data, including medical history and health status. Included subjects were adults over age 20 years who were free from endocrine, nutritional or growth disorders, or any major chronic diseases. Subjects previously diagnosed with diabetes, cancer, liver disease with renal insufficiency, or chronic asthma or pregnancy were excluded. The 520 included subjects were randomly divided into groups of 2/3 ($n = 346$) and 1/3 ($n = 174$) to form a MG and a VG, respectively.

Ethical considerations

The study protocol was approved by the Human Trials Committee of Taso-Tun Psychiatric Center, Ministry of Health and Welfare, Nan-Tou, Taiwan (IRB 109043). After volunteers met the inclusion criteria, and received explanation of the study from the researchers, all included subjects provided signed informed consent to participate.

Anthropometry

Participants' body weights were measured to the nearest 0.1 kg using a body composition analyzer BC418MA (Tanita Co, Tokyo, Japan). Each subject's barefoot height was measured to the nearest 0.5 cm using a height ruler (Holtain, Cosswell, Wales, UK). Body mass index (BMI) was defined as weight (kg) divided by height (meters) squared. Waist Circumference

(WC) is measured with the feet together, the abdominal muscles relaxed, the arms naturally on the sides, normal breathing, and the narrowest position of the body below the ribs and above the navel. Hip Circumference (HC) is measured at the widest point of the buttocks (25); both were measured using a standard tape measure to the nearest 0.1 cm. Each anthropometric measurement was performed by trained observers. All subjects wore hospital cotton/polyester blend gowns and minimal underwear. The measuring tool was calibrated weekly by the same observer.

Computerized tomography

Participants' abdominal region was scanned using a 64-slice computed tomography scanner (Somatron Sensation 64 CT System, Siemens Corp., Germany) together with operating software (software version, Syngo CT2005A). Each participant was placed supine on the CT scanning platform, the lumbar region was scanned, with scanning voltage 120 Kv, tube current 120 mAs, X-ray width 1.5 mm, scanning time 0.5 s, slice thickness 5 mm, and images were captured at 2 mm intervals. The image reconstruction kernel index was B20.

Two image analysts (observers) were trained by radiologists in localizing human anatomy in relation to the L3–L4 lumbar spine. A fixed image analysis program was used to calculate the abdominal cross-sectional area (ACSA) at the L3–L4 lumbar height. Image processing Slice-O-Matic version 4.3 software (Tomovision, Magog, QC, Canada), and Slice-O-Matic image analysis program was used for quantitative analysis of the image area. The image format was DICOM (Digital Imaging and Communications in Medicine) and Slice-O-Matic was used for image opening. Image analysts used Slice-O-Matic to circle the VFA and SFA, expressed in VFA_{CT} and SFA_{CT} . Sagittal abdominal diameter (SAD) was measured at the largest supine anteroposterior diameter at lumbar vertebra levels of L3–L4. Transverse abdominal diameter (TAD) is defined as the largest spanned width of the body in the sliced image. As shown in Figure 1, its diameter was measured by manually fitting the smallest possible rectangle, including the entire abdominal area in the image slice.

Bioelectrical impedance

Subjects were not allowed to drink alcoholic beverages 48 h before the test. No diuretics were used for the seven days prior to the test. Subjects had to fast for 4 h and avoid vigorous activity and alcohol for 24 h before the test. On arrival, subjects were asked to remove all metal objects and empty their bladder. After standing up for at least 10 min, subjects were placed on the BIA with barefoot and arms separated from the trunk. Thumbs, hands, feet and heels were positioned in contact with the

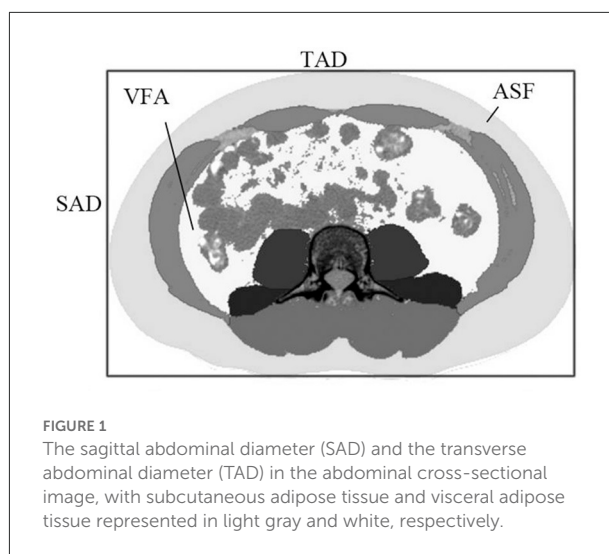


FIGURE 1
The sagittal abdominal diameter (SAD) and the transverse abdominal diameter (TAD) in the abdominal cross-sectional image, with subcutaneous adipose tissue and visceral adipose tissue represented in light gray and white, respectively.

corresponding electrodes. Two measurements were taken from each individual. For accurate measurements, wet hands and feet with an electrolyte paper towel before measurement. Female subjects were excluded from testing during menstruation. All subjects had no history of nutritional, endocrine or growth disorders. A standing 8-contact electrode impedance analyzer BC418MA (Tanita Co., Tokyo, Japan) was used for impedance measurements. During the test, the subject stands on the base platform, holds the handles embedded with the transmitting and sensing electrode plates with both hands, and the soles of the feet naturally touch the sensing and transmitting and sensing electrode pairs with body weight pressure. The BC418MA applies a single alternate current of 0.55 mA with the frequency of 50 kHz to measure the impedance of the left and right upper limbs, lower limbs and whole body, respectively.

The coefficient of variation of the impedance measurements of the current flow path throughout the whole body was evaluated within-day and between-days. Five males and five females were tested. The subjects repeated the impedance measurement 10 times within 1 h of the day, and the impedance measurement was carried out at the same time period over 5 days.

Statistical analysis

Values in this study are presented as mean \pm SD. Values shown in parentheses are the minimum and maximum values. Continuous variables in this study include weight, height, age and BMI and all of them were normally distributed according to the Shapiro-Wilk tests. Levene's test was performed to test its homogeneity. The Akaike Information Criterion (AIC) were presented to evaluate the precision of the estimation equations. Given that a sample size of 218 subjects was calculated

considering a power of 95% and a type 1 error of 5% to achieve a medium effect size for the coefficient of determination (r^2) increases in the estimation equation with the inclusion of 5 predictors (G*power 3.1) (26), or sample size of 346 athletes was sufficient for assuring an adequate power analysis in model development. Multiple linear regression analysis with stepwise variable selection, and SFA_{CT} was used as the response variable in the MG. The bioimpedance index “ h^2/Z ” was combined with the anthropometric parameters of height (h), weight (W), age (Age), gender (sex, female = 0, male = 1), WC, HC, BMI, WHR, SAD and TAD were predictor variables. The parameters Forward ($F_{in} = 4.00$) and Backward ($F_{out} = 3.99$) were used to obtain the selected predictor variables. When the correlation between predictor variables was too high, variance inflation factor (VIF) ≥ 5 was applied to remove the predictor variables from the estimation equation. The estimation equation SFA ($SFA_{BIA+SAD}$) was constructed, and the corresponding regression coefficient, standard error of the estimate (SEE), and r^2 were obtained to evaluate the performance of the estimation equation. The $SFA_{BIA-SAD}$ obtained by applying VG data was analyzed by correlation and Bland-Altman plot with SFA_{CT} as the reference value. BMI was the obesity criterion. All subjects were divided into three groups, eutrophic (BMI $< 25 \text{ kg/m}^2$), overweight ($25 \text{ kg/m}^2 \leq \text{BMI} < 30 \text{ kg/m}^2$), and obese (BMI $> 30 \text{ kg/m}^2$). One-way ANOVA was used to compare the differences of $SFA_{BIA+SAD}$ and SFA_{CT} between the different obesity groups. All statistical analyses were performed using the statistical analysis software SPSS Version 20 (IBM SPSS, Armonk, NY, USA). The level of statistically significant difference was set at $p < 0.05$.

Results

Subjects were randomly divided into an MG of 346 subjects and a VG of 174 subjects. The MG included 206 males (age: 37.8 ± 15.9 years old, BMI: $26.1 \pm 3.5 \text{ kg/m}^2$), 140 females (age: 41.3 ± 16.6 years, BMI: $24.9 \pm 3.8 \text{ kg/m}^2$). The VG included 107 males (age: 37.1 ± 14.9 years, BMI: $26.0 \pm 3.6 \text{ kg/m}^2$) and 67 females (age: 42.4 ± 16.8 years, BMI: $25.1 \pm 3.6 \text{ kg/m}^2$). The abdominal subcutaneous fat area (SFA_{CT}) was $100.1 \pm 68.3 \text{ cm}^2$ in males and $142.0 \pm 73.4 \text{ cm}^2$ in females. The continuous variables were normally distributed (weight, height, age, and BMI). The measurement results for SFA_{CT} and other variables are shown in Table 1. For whole-body impedance measurements within 1 day, the coefficient of variation of the subjects was 0.3–0.8%. The coefficient of variation for the same subjects on the between-days was 0.9–1.8%.

In multiple linear regression analysis with stepwise variable selection, TAD, WC, HC, age, and BMI were excluded, and SAD, Sex, W, h^2/Z , and h were predictor variables of SFA_{CT} in sequence. When each estimated variable was added to the SFA_{CT} estimation equation one by one, its r^2 , SEE and the

regression coefficient of each estimated variable changed, as shown in Table 2. The $SFA_{BIA+SAD}$ estimation equation is shown in equation (1):

$$\begin{aligned} SFA_{BIA+SAD} = & 2.08 \text{ SAD} - 57.26 \text{ Sex} + 1.39 \text{ W} \\ & - 3.67 h^2/Z - 1.50 h \\ (r^2 = & 0.842, SEE = 28.10 \text{ cm}^2, p < 0.001, n = 346) \end{aligned} \quad (1)$$

Figure 2 depicts the regression line obtained by equation (1), the distribution and its mean difference, the limit of agreement (LOA) in the distribution diagram and the Bland-Altman Plots in the MG. In Figure 2A, the regression line equation is $SFA_{CT} = 1.014 SFA_{BIA+SAD} - 2.341$; in Figure 2B, Bias $\pm 1.96 \text{ SD}$ was -51.29 and 51.32 cm^2 .

Figure 3 shows the regression line obtained by applying equation (1), the distribution and its mean difference, the LOA on the distribution plot and the Bland-Altman Plots on the VG. In Figure 3A, the regression line equation is $SFA_{CT} = 0.972 SFA_{BIA+SAD} - 5.72$, $r = 0.930$. In Figure 3B, bias $\pm 1.96 \text{ SD}$ was -50.23 , 50.67 cm^2 .

Figure 4, Bar charts of Equation (1) was fitted from the three groups of eutrophic nutritional status (BMI $< 25 \text{ kg/m}^2$), overweight ($25 \text{ kg/m}^2 \leq \text{BMI} < 30 \text{ kg/m}^2$), and obese (BMI $> 30 \text{ kg/m}^2$). As shown in Figure 4A, the mean differences of $SFA_{BIA+SAD}$ and SFA_{CT} in the three groups were -1.21 , 2.85 , -0.98 cm^2 and SD were 21.53 , 27.16 , 36.60 cm^2 , respectively. As shown in Figure 4B, the mean differences in the male group (Males, $n = 309$) were -8.62 , 4.54 , and 3.15 cm^2 , respectively, and the SDs were 19.01 , 27.32 , and 37.37 cm^2 , respectively. As shown in Figure 4C, the mean differences in the female group (Females, $n = 204$) were 4.70 , -0.36 , -12.61 cm^2 , and the SDs were 21.8 , 36.6 , 33.36 cm^2 , respectively.

The correlation coefficients between the response variable SFA_{CT} and each estimated variable were SAD ($r = 0.782$), WC ($r = 0.750$), BMI ($r = 0.738$), HC ($r = 0.690$), W ($r = 0.516$), h^2/Z ($r = -0.321$), WHR ($r = 0.287$), sex ($r = -0.265$), h ($r = -0.262$), TAD ($r = 0.220$), and age ($r = 0.216$).

Discussion

The present study is the first to use BIA combined with accurate abdominal computed tomography anthropometric parameters to verify the established SFA estimation equation with homogeneity. The current estimates of abdominal fat or abdominal obesity are based primarily on AVAT. Compared with AVAT, the current methods for estimating SFA are limited (27). Therefore, it is particularly important to establish a simple, safe and valuable SFA estimation equation for abdominal obesity or SFA measurement.

Many studies have shown that SFA correlated highly with anthropometric indicators such as WC, HC and BMI. However,

TABLE 1 Demographic and physical characteristics of the study participants.

Variable	Modeling group (<i>n</i> = 346)				<i>P</i>
	Males (<i>n</i> = 206)		Females (<i>n</i> = 140)		
Age (year)	37.8 ± 15.9	(18.2, 81.5)	41.3 ± 16.6	(18.5, 73.5)	**
Height (cm)	171.7 ± 7.2	(151.2, 197.3)	160.7 ± 6.0	(149.0, 176.0)	**
Weight (kg)	77.1 ± 12.1	(50.5, 124.5)	63.7 ± 1.1	(45.5, 110.2)	**
BMI (kg/m²)	26.1 ± 3.5	(19.7, 41.4)	24.9 ± 3.8	(18.8, 39.5)	*
ACSA _{CT} (cm)	488.0 ± 123.7	(286.3, 854.3)	432.7 ± 98.2	(273.4, 702.5)	**
VFA _{CT} (cm)	63.4 ± 48.5	(15.2, 201.4)	49.6 ± 33.4	(16.3, 192.5)	**
SFA _{CT} (cm)	99.0 ± 69.6	(12.2, 443.3)	145.2 ± 76.5	(45.7, 488.3)	**
WC (cm)	82.9 ± 10.1	(65.0, 122.0)	79.7 ± 1.6	(58.0, 122.0)	*
HC (cm)	98.4 ± 7.5	(65.0, 123.0)	97.3 ± 8.8	(61.5, 129.0)	
WHR	0.84 ± 0.07	(0.72, 1.24)	0.82 ± 0.07	(0.65, 1.09)	
Z (ohm)	523.1 ± 61.0	(372.0, 729.0)	663.7 ± 79.7	(506.0, 856.5)	**
H²/Z (cm²/ohm)	57.3 ± 8.2	(38.7, 81.8)	39.5 ± 5.2	(30.1, 51.8)	**
SAD (cm)	19.2 ± 2.6	(14.8, 29.8)	18.5 ± 2.8	(14.1, 30.1)	*
TAD (cm)	29.8 ± 3.7	(25.6, 37.7)	29.6 ± 3.9	(22.5, 40.6)	
Validation group (<i>n</i> = 174)					
	Male (<i>n</i> = 107)		Female (<i>n</i> = 67)		<i>P</i>
Age (years)	37.1 ± 14.9	(20.0, 61.1)	42.4 ± 16.8	(20.3, 84.8)	**
Height (cm)	172.6 ± 8.0	(148.0, 195.0)	159.9 ± 6.7	(143.3, 176.0)	**
Weight (kg)	77.8 ± 13.6	(43.2, 106.7)	63.8 ± 11.0	(43.2, 109.5)	**
BMI (kg/m²)	26.0 ± 3.6	(19.2, 37.9)	25.1 ± 3.6	(18.2, 37.9)	*
ACSA _{CT} (cm²)	483.0 ± 109.7	(294.3, 820.5)	432.1 ± 95.3	(280.4, 6,952)	**
VFA _{CT} (cm²)	65.2 ± 46.6	(17.2, 194.4)	49.6 ± 31.7	(17.3, 182.1)	**
SFA _{CT} (cm²)	102.1 ± 66.1	(12.0, 349.3)	135.4 ± 66.4	(30.8, 342.9)	**
WC (cm)	82.7 ± 10.3	(64.0, 124.0)	79.1 ± 1.8	(56.0, 120.0)	*
HC (cm)	98.8 ± 7.9	(62.0, 125.0)	96.3 ± 8.3	(62.1, 128.8)	
WHR	0.83 ± 0.06	(0.71, 1.22)	0.81 ± 0.06	(0.64, 1.10)	
Z (ohm)	528.2 ± 59.6	(411.3, 554.7)	668.1 ± 83.7	(496.6, 892.3)	**
h²/Z (cm²/ohm)	57.3 ± 8.3	(32.5, 66.0)	39.0 ± 6.3	(27.2, 58.6)	**
SAD (cm)	19.2 ± 2.7	(13.8, 26.5)	18.3 ± 2.8	(13.9, 24.9)	*
TAD (cm)	29.7 ± 3.1	(25.3, 36.7)	29.7 ± 3.7	(23.7, 39.3)	

WHR, Waist-hip ratio; SAD, Sagittal abdominal diameter; ACSA, abdominal cross-sectional area; BMI, Body mass index; SFA, subcutaneous fat area; VFA, visceral fat area; h, height; TAD, Transvers abdominal diameter; **p* < 0.05; ***p* < 0.001.

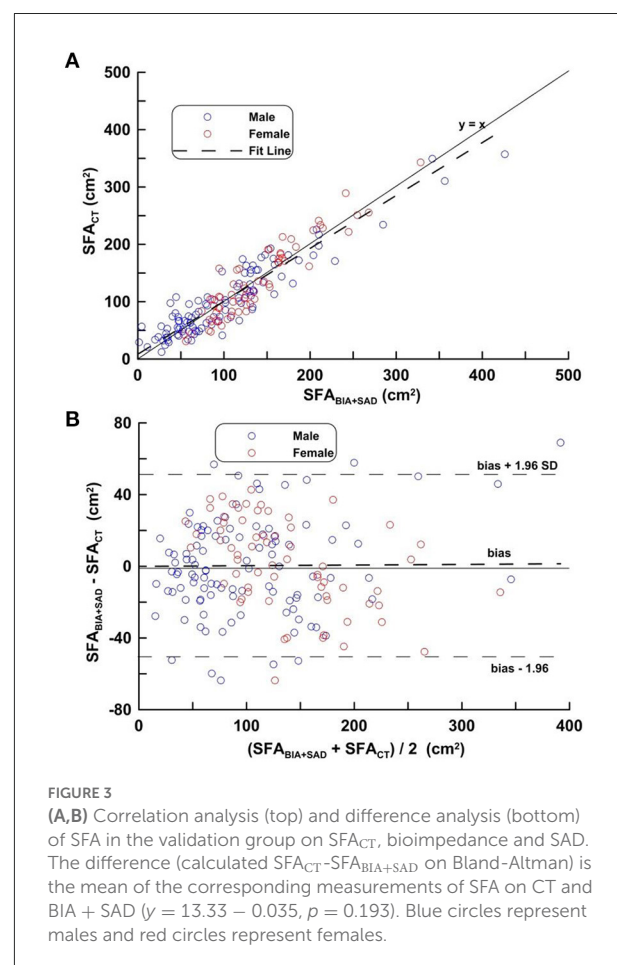
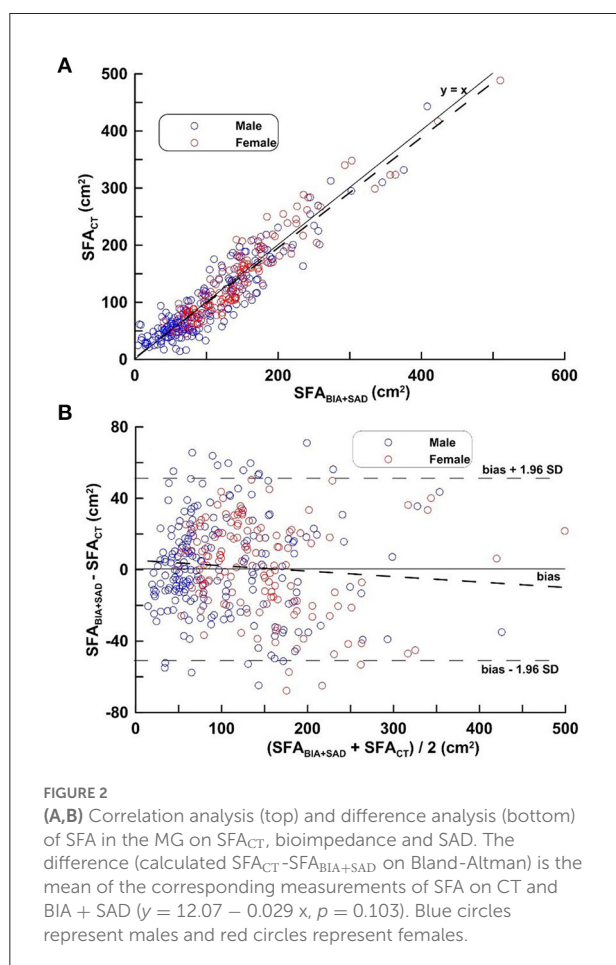
results have been inconsistent across studies, ranging from 0.23 to 0.92 for the correlation of SFA with anthropometric measures (28, 29). This discrepancy may be due to the different characteristics of subjects in different age groups, ethnic groups or in different studies. In the stepwise regression analysis used in this study, SAD, gender, weight, bioimpedance index, and height were obtained sequentially as the predictor variables of SFA_{CT}. However, age, WC, HC, BMI, and TDA were not selected as predictor variables in the SFA estimation equation in the stepwise regression analysis. SAD was the estimated variable that correlated most with the response variable “SFA” in this

study, followed by WC, BMI, and HC. In fact, SAD was the first variable to be selected in stepwise regression analysis, which has the highest correlation with SFA_{CT} and is also the most explanatory of SFA among all predictor variables. WC, BMI, and HC had high collinearity with SAD, and the correlation coefficients were 0.825, 0.821, 0.668, respectively, but were not selected in the estimation model. This study reported a correlation coefficient of 0.78 between SAD and SFA, regardless of gender, which is similar to previous studies showing that the correlation coefficients between SAD and SFA were 0.66–0.78 and 0.72–0.76 in male and female, respectively (30–32). SAD acts

TABLE 2 Multiple regression analysis of sagittal abdominal diameter (SAD) measured with bioelectrical impedance measures as predictor variable and SFA_{CT} as response variable (Modeling group).

Cumulative dependent variable used in model ($n = 346$)								
SAD	+ Sex	+ W	+ h^2/Z	+ h	Intercept	SEE	r^2	ACI
2.08 ± 0.09 (1.00)**	–	–	–	–	$-277.17 \pm 17.10^{**}$	44.12	0.612	854
2.26 ± 0.07 (1.03)**	-57.26 ± 3.86 (1.03)**	–	–	–	$-276.83 \pm 12.64^{**}$	34.40	0.764	832
1.82 ± 0.09 (2.17)**	-72.31 ± 4.38 (1.47)**	1.39 ± 0.22 (2.82)**	–	–	$-277.82 \pm 12.65^{**}$	32.62	0.788	812
1.39 ± 0.9 (2.75)**	-36.69 ± 5.44 (2.86)**	3.82 ± 0.32 (4.60)**	-3.67 ± 0.39 (4.70)**	–	$-199.48 \pm 14.02^{**}$	29.08	0.831	801
1.09 ± 0.11 (3.88)**	-29.90 ± 5.43 (3.06)**	4.71 ± 0.36 (4.81)**	-3.63 ± 0.37 (4.71)**	-1.50 ± 0.30 (3.11)**	43.89 ± 50.69	28.10	0.842	798

Regression coefficient estimate \pm SEE (VIF, variance inflation factor); r^2 , determination coefficient; SAD, sagittal abdominal diameter; SEE, standard estimate error; h^2/Z , bioimpedance index; h, height; *, $P < 0.05$; **, $P < 0.001$; r^2 , coefficient of determinations; Sex (female = 0, male = 1); W, weight; AIC, Akaike's information criteria.



as an indicator for estimating abdominal obesity and is highly correlated with cardio metabolic risk factors, anthropometric parameters and body fat estimates (33).

The bioimpedance index has good power and correlation with the body's fat-free mass or lean mass or body fluids (34). In the present study, the power or correlation of the bioimpedance index to SFA_{CT} was lower than that of many

anthropometric variables. The power of the bioimpedance index for total body fat mass was also not high. The impedance measurement used in this study was a standing whole-body measurement mode. The dual-impedance method (35), which directly measures abdominal impedance, should theoretically increase the correlation with SFA, and may improve the measurement accuracy of BIA in SFA. VFA estimate has

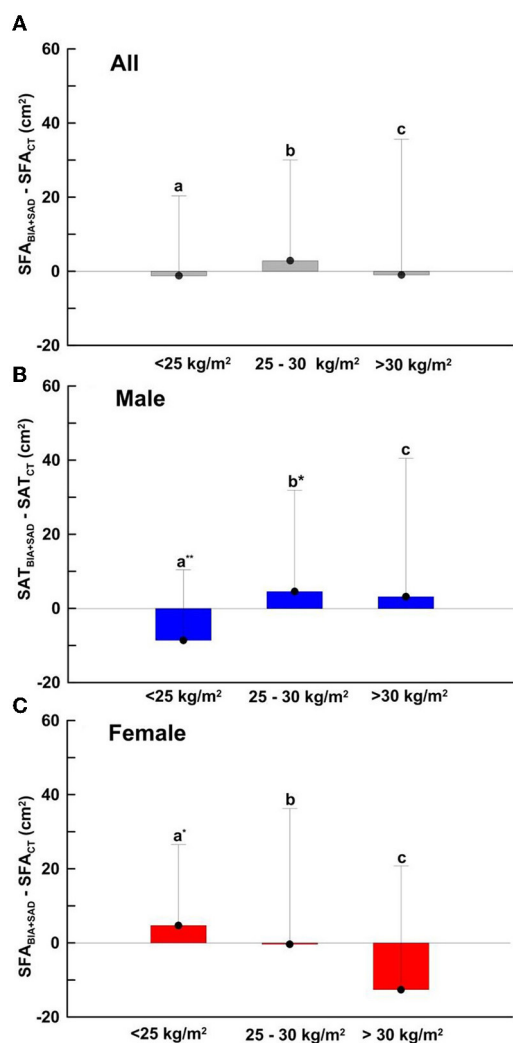


FIGURE 4
SFA-dependent bias of $SFA_{BIA+SAD}$ compared with SFA_{CT} in (A) total ($n = 520$), BMI < 25 kg/m², $n = 154$; 25 kg/m² ≤ BMI < 30 kg/m², $n = 275$; BMI > 30 kg/m², $n = 104$ (B) male ($n = 310$), BMI < 25 kg/m², $n = 67$; 25 kg/m² ≤ BMI < 30 kg/m², $n = 180$; BMI > 30 kg/m², $n = 62$ and (C) female ($n = 210$), BMI < 25 kg/m², $n = 86$; 25 kg/m² ≤ BMI < 30 kg/m², $n = 96$; BMI > 30 kg/m², $n = 22$. Data are presented as the mean difference ± SD. Means with symbol are significantly different, $p < 0.05$ (*), $p < 0.01$ (**).

been provided by some of the BIA models using abdominal dual BIA or segmental BIA methods. The Inbody 720/770 model measures impedance of five segments of the body and VFA estimate is derived from a regression analysis using the segmental impedance. In contrast, a DUALSCAN HDS-2000 (Omron Healthcare Co., Kyoto, Japan), an abdominal dual BIA, provides a direct measurement of VFA. For dual abdominal BIA, electric current is applied to the limb electrodes for calculating fat-free mass and to the eight abdominal surface electrodes for calculating subcutaneous fat thickness with subject in a supine position (36). Compared to the dual abdominal BIA method,

this study provided a more convenient way to measure SFA with subjects in a standing position. Furthermore, this study showed that SAD was highly correlated with SFA and can be used to predict VFA.

Potential variables for model fitting were age, sex, W, BMI, WC, HC, TAD, SAD and h^2/Z in this study. TAD, WC, HC, age and BMI were excluded from the model during variable selection process. Finally, variables selected for the best regression model for estimating SFA_{BIA} included SAD, Sex, W, h^2/Z . Age, sex, W, BMI, WC, HC, TAD and SAD have been shown to be correlated with SFA by previous studies. This study further identified h^2/Z as a negatively correlated variable of the SFA_{BIA} estimation equation. The existing research literature rarely explored the relationship between SFA and h^2/Z , except for the DUALSCAN HDS- 2000 study (35). The above findings are also another contribution of this study.

In the SFA estimation model of the present study, the estimated variable that best reflected SFA was SAD, while the traditional measurement of SAD was measured with calipers (32). However, with the substantial increase in computing power, artificial intelligence and image processing power, 3-dimensional optical body scanner (3DO) could be used in this study to replace CT in future applications to obtain accurate anthropometric parameters. 3DO provides a fast, widely integrated method for automated body composition estimation (37, 38). Therefore, in the future, the standing BIA measurement model combined with the standing 3D image body scanner could be used to integrate the predictor variables such as bioimpedance index and anthropometric measurements. We would expect this quick, automatic method to be widely used in SFA estimation.

In the present study, the standing position was used to measure the impedance of the whole body. Compared with the traditional supine impedance measurement, the impedance value obtained for the same measurement path or part was significantly different. The gravity factor of the standing impedance measurement mode affects the distribution of water in the human body, which in turn affects the impedance value of the measurement site. An average difference of about 10 ohms is found in the standing body or right hand to foot impedance values relative to the supine impedance. Standing impedance measurements decrease with time as the standing measurement time increases (39). Compared with traditional supine impedance measurement, standing bioimpedance measurement still has its limitations.

Among the existing anthropometric methods for SFA, the established measurement mode may have a good correlation coefficient, but the number of people who established the model was either too small or cross-validation was not performed (11). Therefore, the application value of related measurement methods is limited. In this study, 520 subjects were used in the MG (346) and the VG (174), and cross-validation was performed. Statistical indicators such as correlation, SEE,

and LOA in MG or VG all showed that the SFA estimation model established in this study had a certain reference value. In addition; this study specifically explored the estimation error in different genders and different obesity levels, possibly making the results of this study more valuable than those from similar studies.

In this study, we used random sampling instead of stratified sampling to divided subjects into two groups. Since our study sample was homogeneous, our random sampling yielded homogeneous samples. In addition, studies used the same tools and methods and were controlled. We also run a Levene's test to test whether two groups have equal variances for BMI, showing a p -value of < 0.01 , suggesting equal variances for two groups. Therefore, homogeneity of the data can be ensured in both cross-validation or BMI groupings. BMI was categorized according to the WHO BMI Classification: BMI ≤ 18.5 kg/m² as underweight, between 18.5 and 24.9 kg/m² as normal, between 25 and 29.9 kg/m² as overweight and ≥ 30 kg/m² as obesity (40). AIC is a penalized likelihood, balanced between model fit and the number of estimated variables (41). AIC has been applied to evaluate the performance of the series of estimation equations in this study. NHS continuing healthcare checklist is a screening tool that can be used to help researchers or medical personnel understand the needs of subjects or patients for medical care. After completing the checklist, the health status of the subjects can be clearly understood. It can be used to determine whether the volunteer can be accepted in this study. In this study, some subjects who did not meet the acceptance criteria need to be excluded, or those who met the research purpose and health conditions. The recruitment of the subjects belongs to the non-random purposive sampling method.

The cross-validation of this study was to use an independent sample separated from the constructed sample of estimation equations to verify the prediction equation. In theory, cross-validation is performed by independent samples consistent with the applicable conditions of the estimation equation. Therefore, in order to meet this condition, all the subjects were randomly divided into 2/3 and 1/3 as the model establishment group and the validation group for cross-validation (42, 43). In addition, we could also apply the k-fold or leave-one-out method to BIA for cross-validation of body composition estimation equations (44, 45). The Least absolute shrinkage and selection operator (LASSO) developed by Tibshirani in 1996 can increase the preset accuracy and interpretability of statistical models (46). Compared with the stepwise regression analysis method used in this study, each has its own advantages in different application conditions (47). Therefore, in order to improve the performance of the estimation model established in the future, LASSO is one of the statistical suitable methods that can be selected.

This study has a few limitations, first that it included only Asian ethnic groups in Taiwan, which may limit generalization to other populations or ethnic groups; whether the results of this study are applicable to other ethnic groups needs to be further

explored. In addition, only adults were selected as subjects, and the hydration status of children and adolescents differs from that of adults. Therefore, the SFA estimation model established in this study is not applicable to subjects under the age of 20. Because of the limitations of funding and manpower, this study cannot conduct research with a large, and multiple sampling method in different medical fields. Therefore, it cannot fully solve the problem of external validity, which is the limitation of this study. To increase the adequacy of cross-validation, similar studies may consider applying LASSO for model selection, or using k-fold or leave-one-out method in future studies.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Human Trials Committee of Taso-Tun Psychiatric Center, Ministry of Health and Welfare, Nan-Tou, Taiwan (IRB 109043). The patients/participants provided their written informed consent to participate in this study.

Author contributions

C-LL, H-KL, and K-CH interpreted the results, critically reviewed the manuscript, and supervised the study. K-CH designed the study and conducted the research. C-LL, L-PC, and K-CH contributed to subject recruitment and data collection. H-KL, C-LL, A-CH, H-YC, and K-CH performed the laboratory analysis and statistical analysis, interpreted the results, and wrote the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

Author K-CH was employed by Department of Research and Development, Starbia Meditek Co., Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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External validation of BIA equations to estimate appendicular skeletal muscle mass in older adults: Importance of the bias analysis and derivation of correction factors to achieve agreement

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There are several equations based on bioelectrical impedance analysis (BIA) to estimate with high precision appendicular skeletal muscle mass (ASM). However, most of the external validation studies have reported that these equations are inaccurate or biased when applied to different populations. Furthermore, none of the published studies has derived correction factors (CFs) in samples of community-dwelling older adults, and none of the published studies have assessed the influence of the dual-energy X-ray absorptiometry (DXA) model on the validation process. This study assessed the agreement between six BIA equations and DXA to estimate ASM in non-Caucasian older adults considering the DXA model and proposed a CF for three of them. This analysis included 547 non-institutionalized subjects over 60 years old from the northwest of Mexico who were physically independent and without cognitive impairment: 192 subjects were measured using DXA Hologic, while 355 were measured by DXA Lunar. The agreement between each of the equations and DXA was tested considering the DXA model used as a reference method for the design of each equation, using the Bland and Altman procedure, a paired *t* test, and simple linear regression as objective tests. This process was supported by the differences reported in the literature and confirmed in a subsample of 70 subjects measured with both models. Only six published BIA equations were included. The results showed that four equations overestimated ASM_{DXA}, and two underestimated it ($p < 0.001$, 95% CI for Kim's equation: -5.86 – -5.45 , Toselli's: -0.51 – -0.15 , Kyle's: 1.43 – 1.84 , Rangel-Peniche's: 0.32 – 0.74 ,

Sergi's: 0.83–1.23, and Yoshida's: 4.16–4.63 kg). However, Toselli's, Kyle's and Rangel-Peniche's equations were the only ones that complied with having a homogeneous bias. This finding allowed the derivation of CFs, which consisted of subtracting or adding the mean of the differences from the original equation. After estimating ASM applying the respective CF, the new ASM estimations showed no significant bias and its distribution remained homogeneously distributed. Therefore, agreement with DXA in the sample of non-Caucasian was achieved. Adding valid CFs to some BIA equations allowed to reduce the bias of some equations, making them valid to estimate the mean values of ASM at group level.

KEYWORDS

appendicular skeletal muscle mass, non-Caucasian older adults, predictive equations, bioimpedance analysis, dual-energy X-ray absorptiometry, external validation

Introduction

Skeletal muscle performs a broad range of mechanical, structural and metabolic functions (1). It increases from childhood and remains constant between 18 and 40 years. From the age of 45, the skeletal muscle mass is progressively lost (2). The loss of skeletal muscle is associated with malnutrition, sarcopenia, loss of functionality and other adverse effects in older adults (3–7). Therefore, it should be a priority to assess this body composition component in this age group. Currently, there are several precise and accurate methodologies to measure skeletal muscle such as magnetic resonance imaging (MRI), computed tomography, dual-energy X-ray absorptiometry (DXA), and deuterated creatine (D^3 -creatine) dilution. However, they are expensive and not feasible or available for regular epidemiological or clinical practice. A less expensive, non-invasive, and reliable alternative method is bioelectrical impedance analysis (BIA). From this methodology, variables such as resistance and reactance can be obtained. These variables, together with other anthropometric or sociodemographic variables, can be included in BIA predictive models or equations to estimate total or appendicular skeletal muscle mass (ASM) considering MRI or DXA as reference methods.

Various predictive models based on BIA have been developed worldwide (8–17). Some have been validated through internal (8, 10, 11, 15, 17) or external validation procedures (18–20) to estimate ASM in older adults. However, high values of the coefficient of determination (R^2), low standard error of the estimate (SEE) of the predictive model, or the results of internal or external validation in a particular group, do not guarantee the validity of the predictive models to estimate ASM in other populations with specific or different characteristics (11, 12, 14, 16, 20–22). Very few studies have considered valid some BIA equations to estimate ASM within the same ethnic group

(18), or with different health conditions (19, 23). In general, it is recognized that BIA equations that estimate ASM or any other body composition compartment are only precise, accurate and unbiased in populations with similar characteristics to the sample or ethnic group where it was generated (24, 25). These findings and others support that the published BIA equations should not be applied interchangeably. They also highlight the need for external validation in the population of interest. This validation procedure will determine whether or not the predictive models could be generalized (26).

Currently, it is noticeable that most studies aim to generate new precise models to estimate body composition components or compartments based on the assumption (8, 10) and their own results (9, 11, 12, 14, 16) that the existing models are not valid in certain populations. Meanwhile, in other studies, it is possible to notice the efforts to use the existing equations, and in this way, avoid the generation of new ones unjustifiably (18, 19, 23, 27, 28). However, based on the results of these studies, some of them have resulted inaccurate or have not achieved agreement. In this case, an effective strategy may be the analysis of the bias during the validation process. This analysis consists on evaluating the trend of bias: verifying that it remains constant regardless of the amount of ASM presented. This allows discarding the use of published predictive equations, provide the bases for the development of new age, gender, or ethnic specific BIA equations, or determine the possibility of generating CFs for existing equations. These CFs are derived from the mean differences between the equations' estimates and the measurements of the reference method. The condition to derive one, is that the bias must be distributed homogeneously throughout the average of both methods. If it meets this criterion, it will be possible to add or subtract the mean difference to the estimated value of the original equation. In this way, it is possible to achieve agreement. However, it is important to clarify that a simple correction factor might

not eliminate the prediction error in individuals, since these data come from the values of R^2 and the standard error in the estimation of the original equation.

In the case of Mexico, there is only one study (11) where two published BIA equations were applied to estimate ASM in healthy non-institutionalized older adults. The results of this external validation study showed that Kyle's and Sergi's equations were inaccurate in the validation sample. Likewise, the researchers generated, and internally validated a new specific BIA equation for older Mexican adults from the center of the country. In the aforementioned study, the bias of the Kyle and Sergi's equations was not explored, and it has not currently been explored whether the equation generated in older adults from the center of Mexico and other published BIA equations could be valid for older adults from the northwest of Mexico. This, taking into account that it was previously reported that older adults from the northwest of Mexico had less ASM compared to those from the center of the country (29). This may probably be due to differences in total and central fat. Women and men from northwestern Mexico were fatter than those from central Mexico. A positive association has been shown between fat mass and some markers of inflammation, such as C-reactive protein (CRP), and a negative association between CRP and ASM (30).

In general, there are no studies where the equations' bias has been critically analyzed, nor where correction factors have been proposed for the existing equations based on BIA to estimate ASM. This could stop the generation of models that may never be used. The external validation and bias analysis can provide alternatives and close the gap between equation development and implementation of equations, in this case, for estimation at the group level (26).

Moreover, the influence of the DXA model used as reference method, is a factor which has not been explored in external validation studies. Currently, the most widely used models are DXA Lunar and DXA Hologic, of which significant differences in body composition measurements have been reported between both models (31, 32). Considering this evidence, it is possible that the ASM estimated by an equation may not be entirely comparable or equivalent when compared to ASM measurements with a different DXA model than the one used for the generation of the equation. This could lead to bias in external validation studies. For all of the above, the objective of this study was to assess the agreement between six equations based on BIA and dual-energy X-ray absorptiometry to estimate ASM in non-Caucasian older adults, considering the DXA model. The bias was also analyzed in order to propose correction factors.

Materials and methods

This is a secondary analysis generated from various studies with a cross-sectional design (33–35) and the baseline data

of one randomized clinical trial (36) carried out in the Body Composition Laboratory of the Food and Development Research Center, (CIAD, A.C.). This analysis included a large sample of older men and women from Hermosillo, Sonora, México. The methodology has already been described previously in the mentioned studies, but a brief description is provided.

Subjects

Independently of the cited studies, all participating subjects were adults over 60 years of age or older, who were invited to participate through flyers, telephone calls and home visits. The corresponding study protocol was explained to them, as well as the procedures to which they would undergo. All volunteers underwent body composition measurement by different methodologies including DXA and BIA. The subjects were categorized according to their body mass index (BMI, kg/m^2) using to the WHO classification (37). Likewise, various questionnaires and scales were applied to determine the health status, including functionality and cognition. All the subjects were free of physical disability according to the Lawton and Brody scale (38) or the Barthel Index (39), and the majority were free of cognitive impairment according to the Pfeiffer Scale (40) or the Mini Mental State Examination (41). Also, information on demographic and socioeconomic conditions was collected. All these procedures were conducted at CIAD, A.C. From the cited studies, a primary database was built.

All the volunteers selected for this study, had to have a physical file, which had to contain complete information on age, sex, waist circumference, resistance and reactance variables, and DXA scans. They had to be free of diseases, conditions or medications that could affect body composition or hydration status. Regardless of their BMI, men and women older than 60 years were included. All those subjects who did not have complete data on the variables necessary for this external validation protocol, and those who had atypical data or outliers detected by the exploratory analysis were excluded. The identification of outlier variables was carried out through the visual identification of variables that were separated from the set of points of the scatter plot.

Anthropometry

Body weight was measured without shoes and minimum of clothes or disposable gown and recorded by the HV-200KGL scale (A&D Weighing, CA, US), which was previously calibrated with a known weight. Height was measured in the same condition, placing the subject's head according to the Frankfurt plane and using a digital stadiometer (SECA stadiometer 274, Hamburg, Germany). Afterwards, the BMI was calculated. Waist circumference was measured just above the superior border of the iliac crest. The measurement was made with the

subject standing and using a fiberglass measuring tape (Lafayette Instruments Company Inc., Lafayette, IN, USA).

Appendicular skeletal muscle mass measurements

Body composition in some of the cited studies was assessed using DXA Lunar Radiation Corp; Madison, WI, USA or DXA Hologic Discovery WI QDR Series; Waltham, USA. It is important to point out that it has been reported that, quantitatively, these two models do not measure the exact same amount of body composition components such as ASM, or compartments such as fat mass. Regarding the appendicular lean mass (ALM), Shepherd et al. (31) showed significant differences in the measurement of ALM by both models (16.176 kg using Hologic Discovery vs. 15.715 kg with GE Lunar, $p < 0.01$). For this study, we analyzed the ASM measurements in a subsample of 70 older adults, who had been measured with both DXA models. DXA measurements were performed in the same day, following the same protocol for the whole scan and scan editing for ASM determination. A paired t -test was used to determine if the mean difference of the measurements between both methods was different from zero.

Protocol of DXA measurement, DXA scan edition for ASM and calibration were performed according to a published study (42). Participants were measured wearing a disposable gown and free of plastic or metal objects. The ASM determined by DXA (ASM_{DXA}) was considered as reference. For those that did not fit in the DXA scan area, half-body scans were performed, and the remaining side was duplicated as described by Rothney et al. (43). In the case of two subjects who wore non-removable metal accessories, the opposite half of the body to where they had the accessory, was duplicated. In addition to ASM, fat mass measured by DXA was considered to estimate the fat mass index (kg of fat/height in m^2).

Bioimpedance analysis

For the purposes of this secondary analysis, resistance (R) and reactance (Xc) were measured by a RJL Systems single frequency bioimpedance (50 kHz), Detroit, Mich, USA, which complied with a daily calibration protocol with a resistance of 500 ohms. BIA measurements were according to the methodology published previously (24, 44). Both the DXA and BIA measurements were performed after an 8 h fast, with an empty bladder and without having consumed food or liquid prior to the measurement.

Selected BIA predictive models

English-language articles on topics of BIA equations or predictive models to estimate ASM published between 2000 and

2022 were identified in the PubMed database. The keywords for the search were “appendicular skeletal muscle mass,” “muscle mass,” “BIA equation” and “older adults.” The search yielded a total of 34 related articles. The selection of BIA equations was based on the reported precision, and it was decided to include those equations that had an R^2 value ≥ 0.85 and a SEE ≤ 1.8 kg (45). These cut-off points were considered since R^2 is expected to be as close to 1 and SEE as close to zero. An equation with these values can assure considerable precision.

Only BIA equations generated including older adults aged 60 to 90 years, non-institutionalized of any nationality or ethnic group, and with DXA as the reference method were included. Also, the BIA equations had to include any of the following variables: age in completed years, sex, R, Xc, resistance index (height in cm^2/R), or body weight. Age, sex, and body weight are both clinically and statistically associated with ASM, and together with BIA variables, the predictive model can yield more precise and accurate results of the ASM. Finally, the selected equations must have been generated with a single or multi-frequency bioimpedance model. Also, there was no discrimination regarding the method of generation and validation of the equations, nor the nutritional status of the subjects that integrated the generation or validation sample.

Statistical analysis

The data was analyzed using STATA version 16 (StataCorp LP, TX, USA). An exploratory analysis of the primary data was carried out to observe the behavior of the data and detect atypical data or outliers. The significance of the differences between men and women was determined using an independent sample t -test and the results are presented as mean \pm standard deviation. To test if the differences between the ASM measured by DXA Lunar and Hologic were different from zero, a paired t -test was used in the sample of 70 adults.

Regarding the validation procedure, the agreement between methods was evaluated using the Bland and Altman procedure, which considers that the average of the two methods is the best estimator. Objectively agreement was tested by a paired t test and by simple linear regression analysis. The paired t -test assessed if the mean differences between the estimation of each equation and the ASM measurement by DXA were statistically different from zero, and the simple linear regression analysis, which assessed the homogeneity of the dependent variable. To visually analyze the mean of the differences and the distribution of the differences between methods, Bland and Altman (46) plots were incorporated.

Taking the above into account, the following criteria was established to test agreement between methods: The paired t test must prove that the mean of the difference between each BIA equation and DXA as reference method is equal to zero (a p -value > 0.05 is expected). Additionally, the simple regression

analysis must test that the differences are randomly distributed. For this, a p -value of the beta coefficient >0.05 was expected. This would prove the homogeneity of the bias, that is, the homogeneous distribution of the differences along the spectrum of the mean of ASM between methods. If these two conditions were met, agreement was accomplished, meaning that the BIA equation can be considered as an interchangeable method to DXA to assess ASM in this large sample of non-Caucasian older adults. This methodology to establish agreement has been described and applied in other validation studies (33, 47).

This bias analysis supports or rejects the possibility of deriving a CF. In order to propose one, the bias distribution must be homogeneous, and the mean of the differences must be different from zero. That is, the p -value of β of the simple linear regression must be >0.05 , and the p -value of the paired t -test must be <0.05 . If so, the equation can be corrected by subtracting or adding the mean difference to the respective equation. This CF does not change the behavior of the variables included in the equation, but it makes it possible to reduce the average of the differences (bias) in the estimates at group level. This correction has been proposed in other studies (33, 48), and has provided the opportunity to improve the estimates according to the equations where applicable.

Results

The initial sample made up of all the subjects participating in the previously mentioned studies was of 649 participants. Ninety-five volunteers were excluded due to lack of BIA data. After removing subjects with incomplete or duplicate records, or with outliers ($n = 7$), a sample of 547 subjects who had complete data on the variables required for this external validation study was formed. The sample consists of 338 women (61.8%) and 209 men (38.2%), with a mean age of 70 years (age range: 60–94 years). Some of them reported a previous diagnosis of hypertension, controlled type 2 diabetes, and dyslipidemia, with their respective pharmacological control. Other diseases reported were colitis, gastritis, bronchitis, rheumatoid arthritis, bronchial asthma, or controlled hypothyroidism, with stable weight according to self-report.

The mean value of BMI was 27.9 kg/m^2 (range: 16–44.4 kg/m^2). According to their BMI classification, 6 subjects were underweight (1.1%), 129 had a normal weight (23.6%), 259 were overweight (47.4%), and 153 had obesity (27.9%). The mean value of ASM in the whole sample was of 17.4 kg. According to the DXA model, the mean ASM measured by DXA Hologic was 15.9 kg, while that measured by Lunar DXA was 18.2 kg. The general characteristics of both samples are found in Table 1.

TABLE 1 General characteristics of the sample.

Variables	Total sample of subjects measured with both DXA models		Sample of subjects measured with DXA Hologic		Sample of subjects measured with DXA Lunar	
	Men ($n = 209$)	Women ($n = 338$)	Men ($n = 40$)	Women ($n = 152$)	Men ($n = 169$)	Women ($n = 186$)
Age (years)	70.1 ± 6.8	69.3 ± 6.7	71.7 ± 7.8	$70.3 \pm 6.9^*$	69.6 ± 6.4	68.5 ± 6.5
Weight (kg)	76.1 ± 11.7	$69.2 \pm 11.6^*$	80.1 ± 12.9	$71.5 \pm 12.5^*$	75.2 ± 11.3	$67.3 \pm 10.6^*$
Height (m)	1.6 ± 0.1	$1.5 \pm 0.1^*$	1.6 ± 0.1	$1.5 \pm 0.1^*$	1.6 ± 0.1	$1.5 \pm 0.1^*$
BMI (kg/m^2)	26.7 ± 3.5	$28.6 \pm 4.4^*$	27.7 ± 3.7	$29.6 \pm 4.6^*$	26.5 ± 3.4	$27.8 \pm 4.1^*$
FMI (kg/m^2)	8.1 ± 2.6	$12.5 \pm 3.3^*$	9.1 ± 2.6	$13.1 \pm 3.2^*$	7.8 ± 2.5	$11.9 \pm 3.2^*$
WC (cm)	97.8 ± 10.4	98.5 ± 12.1	100.8 ± 11.6	99.4 ± 12.1	97.1 ± 10	97.7 ± 11.9
Resistance (Ω)	505.4 ± 62.7	$585.6 \pm 73.9^*$	489.3 ± 51.1	$560.7 \pm 67.2^*$	509.2 ± 64.7	$605.9 \pm 73.2^*$
Reactance (Ω)	49.4 ± 9.2	50.9 ± 9.8	46.9 ± 10.1	48.2 ± 9.5	50 ± 8.9	$53.2 \pm 9.4^*$
RI (cm^2/R)	57.2 ± 8.2	$41.9 \pm 6.2^*$	59.6 ± 8.1	$43.5 \pm 6.3^*$	56.6 ± 8.1	$40.5 \pm 5.8^*$
ASM _{DXA} (kg)	21.4 ± 3.1	$14.9 \pm 2.4^*$	20.8 ± 3.1	$14.6 \pm 2.6^*$	21.6 ± 3.1	$15.1 \pm 2.1^*$
ASM _{Kim} (kg)	15.2 ± 1.4	$10.3 \pm 1.2^*$	15.6 ± 1.6	$10.6 \pm 1.3^*$	15.1 ± 1.4	$10.1 \pm 1.1^*$
ASM _{Kyle} (kg)	22.2 ± 2.9	$15.6 \pm 2.4^*$	23.1 ± 3.1	$16.1 \pm 2.5^*$	22.1 ± 2.8	$15.2 \pm 2.1^*$
ASM _{Rangel} (kg)	21.2 ± 2.5	$14.4 \pm 2.1^*$	22.1 ± 2.5	$15.1 \pm 2.1^*$	21.1 ± 2.4	$14.1 \pm 1.9^*$
ASM _{Sergi} (kg)	20.8 ± 2.6	$15.3 \pm 2.1^*$	21.5 ± 2.7	$15.8 \pm 2.3^*$	20.6 ± 2.5	$15.1 \pm 1.9^*$
ASM _{Toselli} (kg)	22.2 ± 2.1	$15.1 \pm 1.8^*$	22.8 ± 2.3	$15.5 \pm 1.9^*$	21.6 ± 2.1	$14.4 \pm 1.6^*$
ASM _{Yoshida} (kg)	24.8 ± 3.3	$18.2 \pm 2.5^*$	26.1 ± 3.5	$18.8 \pm 2.6^*$	24.5 ± 3.2	$17.7 \pm 2.2^*$

* $p < 0.05$ when performing the t -test for independent samples between sex. Means \pm standard deviation. BMI, body mass index; FMI, fat mass index; WC, waist circumference; RI, resistance index; ASM, appendicular skeletal muscle mass.

Selected BIA predictive models

Regarding the BIA equations to estimate the ASM, a total of 25 equations were found, of which 10 were generated in older adults. Of these, only 5 had reported an internal validation process, and 6 have been externally validated in other studies. Only 6 equations which met the selection criteria were selected: Kim's, Kyle's, Rangel-Peniche's, Sergi's, Toselli's and Yoshida's equations. The characteristics of these equations are shown in Table 2. These equations were applied to the complete sample, and with this, the variables ASM_{Kim} , ASM_{Kyle} , ASM_{Rangel} , ASM_{Sergi} , $ASM_{Toselli}$ and $ASM_{Yoshida}$ were obtained. Importantly, Kim's and Toselli's equations generated with DXA Lunar, were tested on subjects measured with DXA Lunar, while BIA equations generated using DXA Hologic as the reference method, were tested on those measured with that model. This, in order to eliminate the effect or possible bias due to DXA model in this validation procedure.

Differences in ASM measurements between DXA lunar and DXA hologic

The results of the paired *t*-test between the measurements by both DXA models in the subsample of 70 subjects, showed a mean difference different from zero (16.7 kg using DXA Hologic vs. 17.1 kg using DXA Lunar, mean difference of -0.4 kg; $p < 0.001$). These differences between DXA models support the decision to validate the equations according to the DXA model taken as reference, since the measurements between both models are not interchangeable.

Validation of the BIA equations to estimate ASM

The mean value of ASM estimated by the Kim's and Toselli's equations in the sample of subjects measured by DXA Lunar was 12.5 and 17.8 kg, respectively. Regarding the Kyle, Rangel-Peniche, Sergi and Yoshida equations, the mean value of ASM was 17.6, 16.5, 17.0 and 20.4 kg, respectively. When each of the six BIA equations were compared with their respective reference method, the mean of the differences between the Kim, Toselli, Kyle, Rangel, Sergi and Yoshida equations and the ASM_{DXA} was -5.6 , -0.3 , 1.6 , 0.5 , 1.0 and 4.4 kg, respectively (Figures 1, 2). Clearly, these results indicate that 2 equations underestimated ASM_{DXA} , while 4 overestimated it (Table 3).

The statistical analysis showed that the mean of the differences between each of the equations and the ASM_{DXA} was statistically different from zero ($p < 0.001$) (Table 4). However, Toselli's, Kyle's and Rangel-Peniche's equations showed a homogeneous distribution of the bias over the entire range of

ASM values between methods ($\beta = -0.038$, $p = 0.091$; $\beta = 0.048$, $p = 0.087$; and $\beta = -0.014$, $p = 0.641$, respectively) (Table 3; Figure 1). This indicates that these equations do not significantly underestimate or overestimate as ASM increases. Having a homogeneous bias allows us to suggest a correction factor, which could correct the significant differences found in the paired *t* tests in these three equations.

This wasn't possible for Kim's, Sergi's and Yoshida's equations. In addition to the paired *t*-tests results, the simple linear regression showed a non-homogeneous bias in these equations ($\beta = 0.409$, $p < 0.001$; $\beta = -0.105$, $p < 0.001$; and $\beta = 0.098$, $p = 0.001$, respectively) (Tables 3, 4). In these cases, the overestimation or underestimation of these equations as the ASM increases is significant, so they cannot be corrected.

Derivation of the correction factors and validation of the corrected equations

Considering the finding of homogeneous bias, correction factors were proposed by considering the mean difference between DXA and both equations. The bias of each one of the equations was subtracted or added as following:

$$\begin{aligned} ASM_{ToselliCF} &= 5.982 + (0.188 \times RI) + (0.014 \\ &\times WC) + (0.046 \times weight) + (3.881 \times sex) \\ &- (0.053 \times age) + 0.332 \\ ASM_{KyleCF} &= -4.211 + (0.267 \times RI) + (0.095 \times weight) \\ &+ (1.909 \times sex) + (-0.012 \times age) + (0.058 \\ &\times reactance) - 1.639 \\ ASM_{RangelCF} &= -0.05376 + (0.2394 \times RI) + (2.708 \times sex) \\ &+ (0.065 \times weight) - 0.533 \end{aligned}$$

$ASM_{ToselliCF}$, corrected Toselli's equation. ASM_{KyleCF} , corrected Kyle's equation. $ASM_{RangelCF}$, corrected Rangel-Peniche's equation. RI, resistance index (height in cm^2 /resistance). WC, waist circumference in cm. Weight in kilograms. Sex: 0 for women and 1 for men. Age in years.

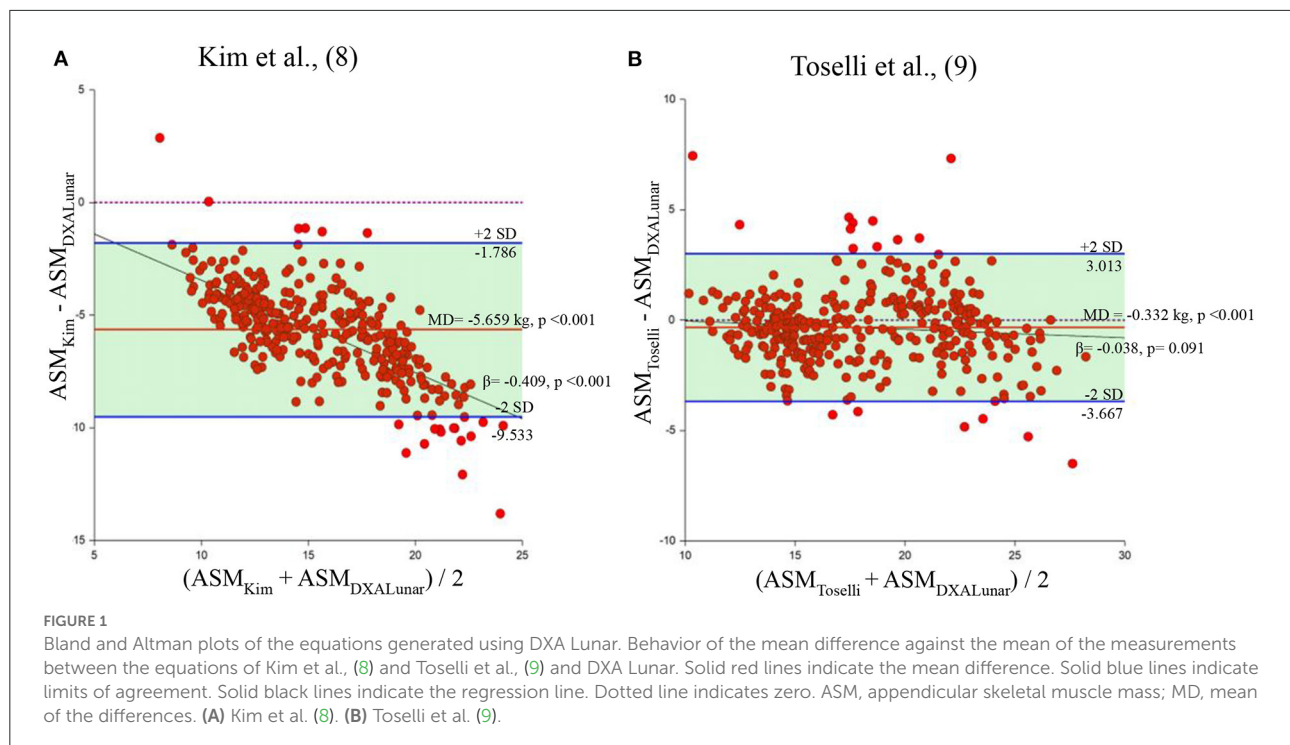
The mean value of ASM estimated by the corrected Toselli's equation ($Toselli_{CF}$) in the sample of subjects measured by DXA Lunar was 18.2 kg. On the other hand, the mean value of ASM estimated by the corrected Kyle's equation ($Kyle_{CF}$) and the corrected Rangel-Peniche's equation ($Rangel_{CF}$) in the sample of subjects measured by DXA Hologic was 15.9 kg for both equations. When these three corrected BIA equations were compared with their respective reference method, the mean differences were less than 0.01 kg.

By carrying out the same tests applied previously (paired *t* test and simple linear regression), and considering the criteria to determine agreement, it was possible to achieve agreement between the three corrected BIA equations and the ASM_{DXA} .

TABLE 2 Selected equations and their characteristics.

Reference	Equations	n/sex	Age (years)	BMI (kg/m ²)	ASM DXA (kg)	R ²	SEE (kg)	DXA model used
Kim et al. (8)	$ASM_{(kg)} = (0.104 \times RI) + (0.050 \times age) + (2.954 \times sex) + (0.055 \times weight) + 5.663$	483/M 642/W	73.5 ± 5.6	24.4 ± 3.2	M: 20.1 ± 2.6 W: 13.6 ± 1.8	0.88	1.35	DXA Lunar Corporation, Madison, WI
Toselli et al. (9)	$ASM_{(kg)} = 5.982 + (0.188 \times RI) + (0.014 \times WC) + (0.046 \times weight) + (3.881 \times sex) - (0.053 \times age)$	26/M 92/W	71.2 ± 7.2	27.9 ± 5.1	16.2 ± 3.5	0.86	1.35	Lunar DPX-MD
Kyle et al. (10)	$ASM_{(kg)} = -4.211 + (0.267 \times RI) + (0.095 \times weight) + (1.909 \times sex) + (-0.012 \times age) + (0.058 \times Xc)$	459/M 311/W	20-94	V: 25 ± 3.2 P: 24.6 ± 4.4	M-V: 25.8 ± 3.6 M-P: 22.1 ± 2.8 W-V: 17.3 ± 2.5 W-P: 15.2 ± 2.8	V: 0.95 P: 0.91	V: 1.12 P: 1.5	DXA Hologic QDR4500A
Rangel-Peniche et al. (11)	$ASM_{(kg)} = -0.05376 + (0.2394 \times RI) + (2.708 \times sex) + (0.065 \times weight)$	55/M 158/W	68 ± 5.9	-	15 ± 3.4	0.91	1.01	DXA Hologic Explorer QDR-4500W
Sergi et al. (12)	$ASM_{(kg)} = 3.964 + (0.227 \times RI) + (0.095 \times weight) + (1.384 \times sex) + (0.064 \times Xc)$	117/M 179/W	71.4 ± 5.4	27.0 ± 3.4	18.6 ± 4.1	0.92	1.14	DXA Hologic QDR Discovery A
Yoshida et al. (13)	$MME_{men (kg)} = (0.197 \times RI) + (0.179 \times weight) - 0.019$ $MME_{women (kg)} = (0.211 \times RI) + (0.170 \times weight) + 0.881$	141/M, 109/W.	73.5 ± 5.6	23.4 ± 3.4	17.8 ± 3.8	M:0.87 W:0.89	M: 0.98 W: 0.81	DXA Hologic QDR 4500 A

Means ± standard deviation. BMI, body mass index; R², coefficient of determination; SEE, standard error of the estimate; ASM, total appendicular skeletal muscle; RI, resistance index; Xc, reactance; WC, waist circumference; M, men; W, women; V, volunteers; P, patients; Sex variable: 0 for female, 1 for male. Weight in kilograms. ASM, appendicular skeletal muscle mass; n, number of subjects.



The paired t -tests (Table 5) showed that the mean differences between these BIA equations and the ASM_{DXA} were not statistically different from zero ($p = 0.997$ for Toselli's corrected equation, $p = 0.993$ for Kim's corrected equation and 0.992 for Rangel-Peniche's corrected equation). The homogeneous bias distribution of these BIA estimations remained the same graphically and objectively, tested by simple linear regression ($\beta = -0.038$, $p = 0.091$; $\beta = 0.048$, $p = 0.087$; and $\beta = -0.014$, $p = 0.641$, respectively) and in the Bland and Altman plot, it was possible to observe a mean difference almost above the zero line in these three corrected equations (Figure 3, Table 6). This analysis gave us three corrected equations with a bias very close to zero, which is not statistically significant, and which maintained a homogeneous bias in the estimation.

Discussion

The purpose of this study was to validate some published BIA equations for estimating ASM. None of these BIA equations met the criteria for agreement in this sample. However, the analysis of bias permitted to derive CFs, which, when applied to some equations, showed agreement with DXA. A valid corrected equation for this group of older adults can be a useful tool for epidemiological studies. To the best of our knowledge, in Mexico, low muscle mass has only been assessed at the national level using calf circumference (49). From our perspective, estimating it with accurate and practical tools, such as BIA

equations could guarantee a better estimate of skeletal muscle, particularly ASM.

All the BIA equations selected for this study have already been tested in other populations previously, where they were discarded for its inaccuracy in certain populations due to the difference in age ranges (11, 12, 21), nutritional status (20, 50), differences in body composition and anthropometry measurements related to ethnicity (18), health status (19), differences in functional status (14), or BIA device employed (18).

For example, in other external validation studies (18, 20), Kim's equation was found to have the highest mean difference compared to DXA Lunar ASM estimations. In these studies, authors discuss that it is most likely due to the fact that it was developed for an Asian population, but also because the authors used a multifrequency bioimpedance device, operating at a single frequency of 250 Hz. It is already well recognized, that low frequencies predominantly measure extracellular water. At higher frequencies, in contrast, cell membranes are permeable to current, so both intracellular and extracellular water are measured (51). In this way, it is understood that multifrequency devices measure body composition in a slightly different way. In our study, the Kim equation yielded the highest mean difference of all (-5.659 kg), followed by the Yoshida equation (4.401 kg). Both equations were generated in older Asian adults and using multi-frequency BIA devices, thus, we hypothesize that these two characteristics may have been an important factor contributing to bias in this sample as well.

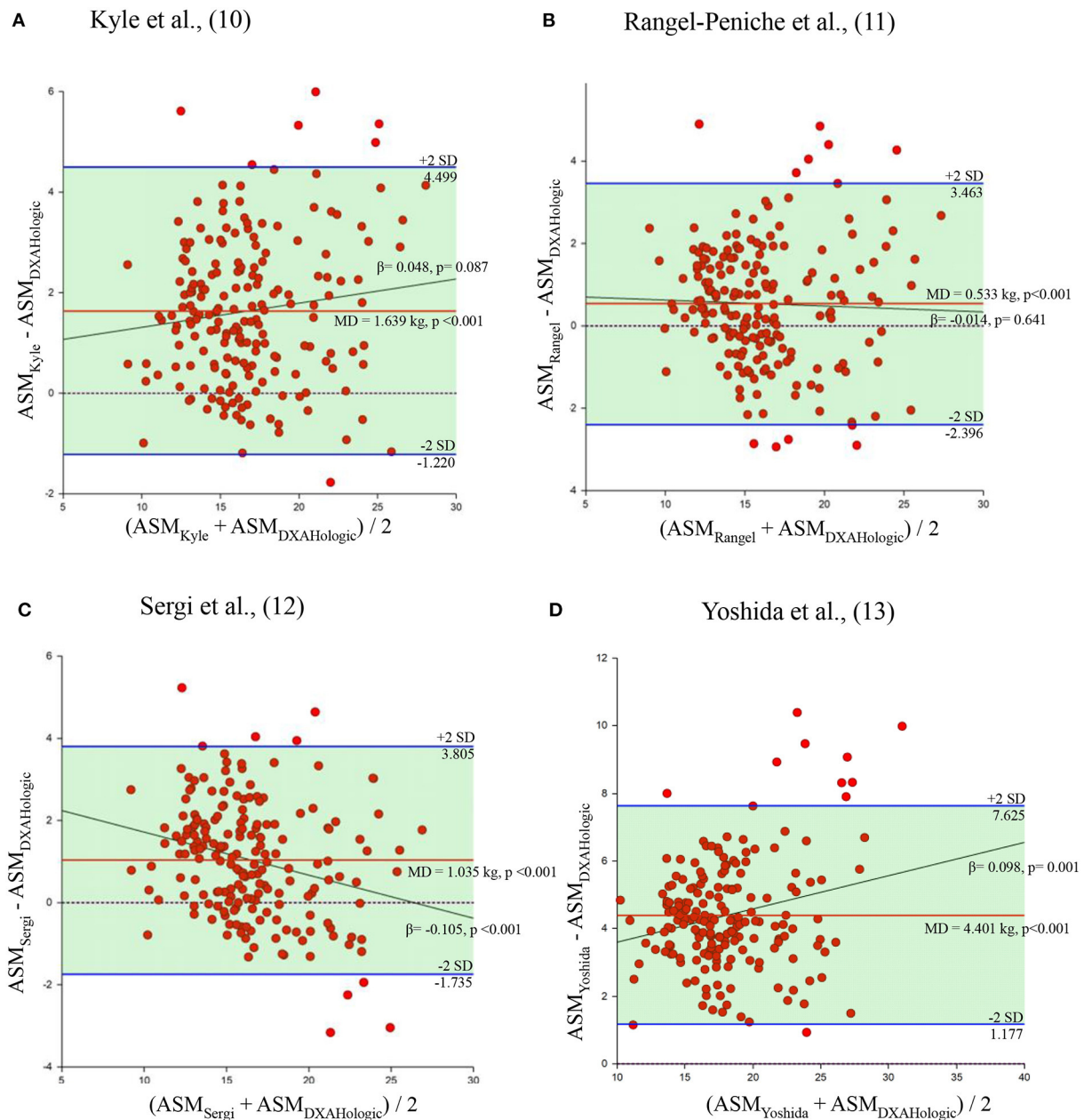


FIGURE 2

Bland and Altman plots of the equations generated using DXA Hologic. Behavior of the mean difference against the mean of the measurements between the equations of Kyle et al., (10), Rangel-Peniche et al., (11), Sergi et al., (12) and Yoshida et al., (13), and DXA Hologic. Solid red lines indicate the mean difference. Solid blue lines indicate limits of agreement. Solid black lines indicate the regression line. Dotted line indicates zero. ASM, appendicular skeletal muscle mass; MD, mean of the differences. (A) Kyle et al. (10). (B) Rangel-Peniche et al. (11). (C) Sergi et al. (12). (D) Yoshida et al. (13).

Sergi's equation was generated in Caucasian subjects, and it only included older adults for its generation process. Even though their generation sample has very similar characteristics to ours, the equation had a very high bias, and like the others, the mean of the differences was significant. It is important to remember that several studies have described

the differences in body composition between different ethnic groups (42, 52, 53), which could also have contributed to the bias of this equation as well. In addition to the high and significant bias found in these aforementioned equations, the Kim, Yoshida, and Sergi equations did not have a homogeneous bias distribution (β coefficient p -value > 0.05).

TABLE 3 Validation data of the six BIA equations.

Equations	Estimated ASM (kg)	Mean difference (kg)	Limits of agreement	95% Confidence interval	β of SLR	p -value of SLR
Kim	12.5	-5.6	-9.5, -1.7	-5.8, -5.4	-0.409	<0.001
Toselli	17.8	-0.3	-3.6, 3.0	-0.5, -0.1	-0.038	0.091
Kyle	17.6	1.6	-1.2, 4.4	1.4, 1.8	0.048	0.087
Rangel-Peniche	16.5	0.5	-2.3, 3.4	0.3, 0.7	-0.014	0.641
Sergi	17.0	1.0	-1.7, 3.8	0.8, 1.2	-0.105	<0.001
Yoshida	20.3	4.4	1.1, 7.6	4.1, 4.6	0.098	0.001

SLR, simple linear regression between the mean difference and the mean of ASM by both methods.

TABLE 4 Comparison of the mean values of the estimated ASM and the ASM_{DXA}.

DXA model	Equations	Estimated ASM (kg)	ASM DXA (kg)	p -value
Lunar	Kim et al. (8)	12.5	18.2	<0.001
	Toselli et al. (9)	17.8		<0.001
Hologic	Kyle et al. (10)	17.6	15.9	<0.001
	Rangel-Peniche et al. (11)	16.5		<0.001
	Sergi et al. (12)	17.0		<0.001
	Yoshida et al. (13)	20.3		<0.001

TABLE 5 Comparison of the mean values of the estimated ASM and the ASM_{DXA}.

DXA model	Equations	Estimated ASM (kg)	ASM DXA (kg)	p -value
Lunar	Toselli _{CF}	18.2	18.2	0.997
Hologic	Kyle _{CF}	15.9	15.9	0.993
	Rangel-Peniche _{CF}	15.9		0.992

Toselli_{CF}, corrected Toselli's equation; Kyle_{CF}, Kyle's corrected equation; Rangel-Peniche_{CF}, corrected Rangel-Peniche's equation; Eq, equation.

This did not allow a correction factor to be proposed for these models.

Kyle's equation was developed for Swiss adults in the age range of 22 to 94 years. Many studies have tried to validate it in external validation protocols. In almost all validation studies (11, 12, 14, 16, 18, 19, 21–23, 50, 54), the equation has overestimated the ASM in different conditions, which the authors consider is due to the fact that it is not specific for a particular age group. Therefore, this equation is usually discarded for use in certain populations. In our study, this equation overestimated 1.639 kg, and we agree that this was probably because it was not generated including subjects similar to those in this sample, and that it was not specific for older adults.

Toselli and Rangel-Peniche equations were the ones with the mean of the differences closest to zero (-0.332 and 0.533 kg, respectively). In the case of the Rangel-Peniche equation, this must be since it was developed in a group of individuals of

the same nationality as our sample. Despite this, this equation does not meet the established criteria for agreement in this sample of older adults from the northwest of the same country. This confirms the nature of the equations to be specific for the population where it was generated and very similar populations. In fact, another study by Rangel-Peniche et al. (29) evaluated the differences in body composition of older adults from central Mexico and older adults from the northwest of the country. After adjusting for age, body weight, height, health status, estimated energy expenditure, and some demographic variables, ASM and the appendicular muscle mass index in older adults from central Mexico were significantly higher compared to the older adults from the northwest of Mexico. This could be one reason why Rangel-Peniche's equation was not valid for our sample. In other studies, such as the one by Yu et al. (18), the Rangel-Peniche's equation also underestimated the ASM when applied to Australian adults, with a mean error of 1.82 kg. In another study (19), it overestimated approximately 0.51 kg when applied to subjects with anorexia. In the study by Coëffier et al. (20) the equation had a mean difference even further from zero, of -2.68 kg. Due to these values, these studies have decided to rule out the use of this equation.

On the other hand, this is the first study to externally validate Toselli's equation. This model, which includes waist circumference among the predictor variables, turned out to have a very low bias in our sample (-0.332 kg). In their study, the authors discuss the relationship between waist circumference and ASM. We believe that having taken this variable into

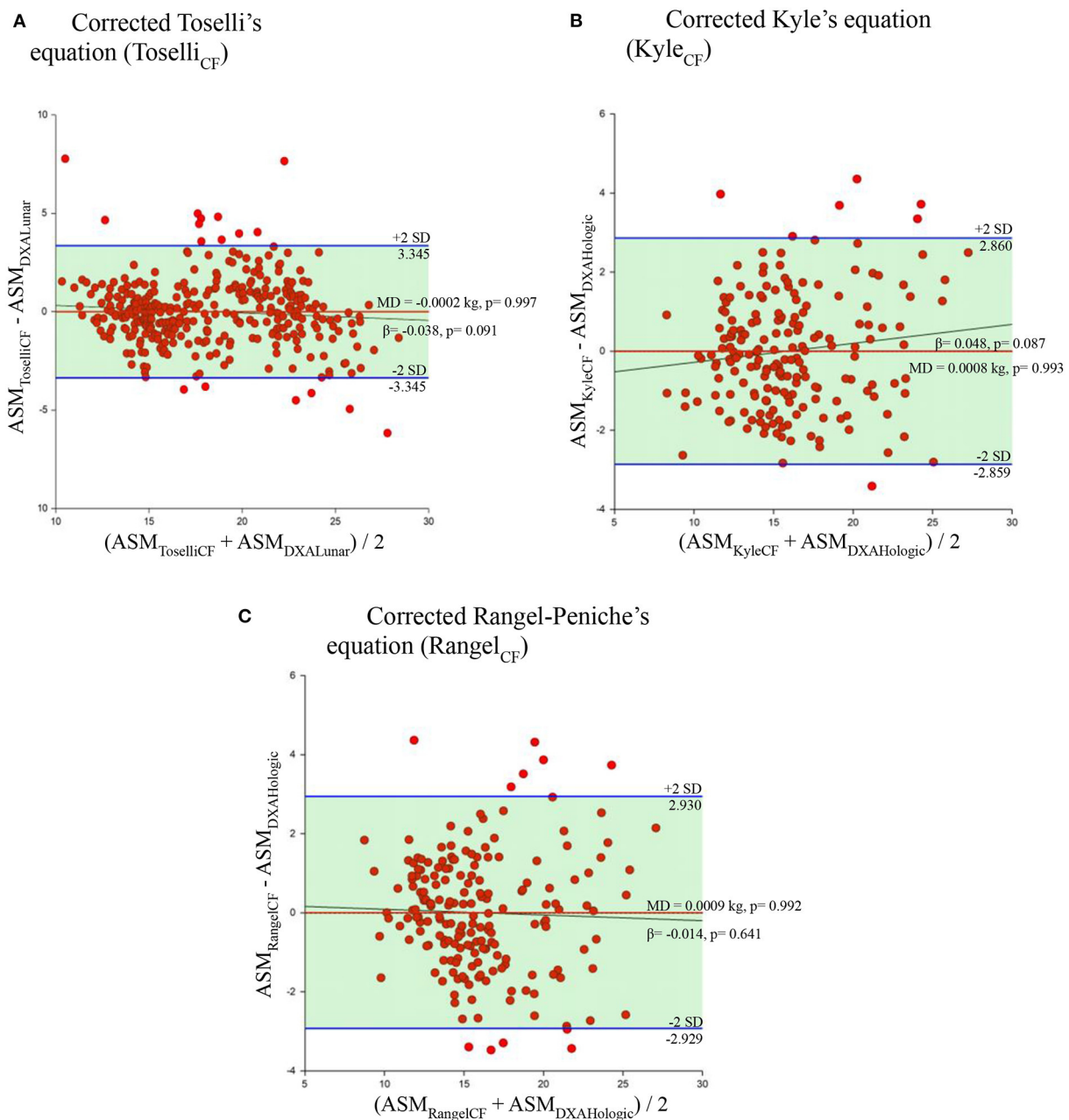


FIGURE 3

Bland and Altman plots and simple linear regression of the selected equations applying the correction factors. Behavior of the mean difference against the mean of the measurements between the corrected equations and their respective reference method. Solid red line indicates the mean difference. Solid blue line indicates the limits of agreement. Solid black line indicates the regression line. Dotted line indicates zero. ASM, appendicular skeletal muscle mass. MD, mean of the differences. (A) Corrected Toselli's equation ($Toselli_{CF}$). (B) Corrected Kyle's equation ($Kyle_{CF}$). (C) Corrected Rangel-Peniche's equation ($Rangel_{CF}$).

account in this model and applying it to a sample with a high mean waist circumference, could be the reason why it had the smallest mean difference.

According to our results, none of the selected equations was valid for older adults from the northwest of Mexico. However, an important finding achieved when analyzing the

bias of the equations, is that we realized that the Toselli, Rangel-Peniche and Kyle equations had a homogeneous bias. This allowed them to be further improved to yield accurate data in this sample of older Mexican adults. By deriving a correction factor for Toselli's, Kyle's and Rangel-Peniche's equations, precise, accurate, and bias-free ASM estimates were

TABLE 6 Validation data of the three corrected BIA equations.

Corrected equation	Estimated ASM (kg)	Mean difference (kg)	Limits of agreement	95% Confidence interval	β of SLR	p -value of SLR
Toselli _{CF}	18.2	−0.0002	−3.3, 3.3	−0.1, 0.1	−0.038	0.091
Kyle _{CF}	15.9	0.0008	−2.8, 2.8	−0.2, 0.2	0.048	0.087
Rangel-Peniche _{CF}	15.9	0.0009	−2.9, 2.9	−0.2, 0.2	−0.014	0.641

SLR, simple linear regression between the mean difference and the mean of ASM by both methods; Toselli_{CF}, Toselli's corrected equation; Kyle_{CF}, Kyle's corrected equation; Rangel_{CF}, Rangel-Peniche's corrected equation.

obtained. Importantly, this was possible after the analysis of the bias in this external validation study. This turned out to be a very useful strategy to use the existing equations in the literature, and thus not contribute to the development of more equations, which would have been generated unjustifiably and that, as mentioned in the systematic review by Beaudart et al. (25) would have been redundant.

This study has several advantages: to our knowledge, it is the first study to propose correction factors for BIA equations to estimate ASM, derived from a validation study with a large sample that included subjects of a wide nutritional range, age range, physically independent and without uncontrolled diseases that affected body composition. Likewise, it is the first study that considers the DXA model in the validation process. Many external validation studies have treated the DXA model indistinctly, despite the differences that are already recognized in the literature (31, 32, 55–57). In this study, in addition to considering these differences, we tested if the measurements taken by both DXA models were different in a subsample of subjects. Once confirmed, we chose to separate the validation according to the DXA model: the equations generated with a model, were applied only in subjects measured with that same model. This reduces the influence of the DXA model in the validation process, which could have been an important contributing bias factor.

Another advantage is that this validation confirms that single frequency bioimpedance devices are a valid tool for ASM estimation compared to DXA. These models are cheaper and more practical compared to others, and they can be a portable alternative for epidemiological studies.

A final advantage that we find are the criteria established in this article to determine agreement between methods. When assessing other validation studies, we noticed that some of them only carry out paired *t*-tests between methods, some use the pure error, or the Pearson or Lin coefficient. Some others are satisfied with only determining which was the lowest mean error of the selected equations. We also notice that most studies do not analyze the bias distribution. We opted for the criteria mentioned in the Materials and Methods section, because, by adding paired *t*-tests and simple linear regression to the statistical methods, we address more than what is included in the

Bland and Altman plot, testing agreement not only subjectively, but also objectively. These steps should be fundamental in validating equations.

One disadvantage of this study is that, due to its nature, the CF may not be generalizable to other populations. Likewise, this CF could be more viable for overweight and obese subjects, since approximately 75.2% of our sample in this validation study is made up of these subjects. A very little percentage of our subjects is made up of low-weight subjects, so it could be less valid for this group of individuals. Another disadvantage is that, despite that this study has a larger sample compared to others published, our sample is not representative or randomized, so our results are only valid in this sample, and we hypothesize that it may be valid in subjects with similar characteristics.

Moreover, it is important to mention that in this study, agreement was proven statistically, and this is not synonymous with clinical significance. It is notorious that, when applying these CFs, there are no changes or improvements in the amplitude of the limits of agreement of the estimations, and it only allows the reduction of the mean difference. Given this, the corrected equations by this CFs are only useful for estimating mean values of ASM on populations and are not valid if applied at the individual level, since the estimates exceed clinically significant physiological values. Because of this, first, we recommend exploring through regression models, which are the variables associated with bias in each of the equations, to obtain a broader picture of the main contributing bias factors. Subsequently, knowing the variables associated with the bias in these equations, we recommend generating more complex correction equations, to obtain values closer to the real ones at the individual level.

We also recommend validating these CFs on an independent sample, as long as the DXA model used as the reference method is considered. Furthermore, we consider that clinically acceptable limits of accuracy need to be defined when estimating the ASM.

Conclusion

None of the published BIA equations met the criteria to achieve agreement with DXA. However, the bias analysis

done after stratifying by DXA model, was determinant to derive and apply correction factors to Toselli's (generated with DXA Lunar), Kyle's and Rangel-Peniche's equations (generated with DXA Hologic). Incorporating the correction factors to the corresponding BIA equations showed an extremely low bias. Therefore, these three corrected BIA equations could be used to estimate the mean values of ASM at group level in older adults from the northwest of Mexico.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: The database from which this article was derived, is available only by request. Requests to access these datasets should be directed to HA-M, helio@ciad.mx.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

Author contributions

HA-M: design. MC-R and HA-M: drafting of manuscript. MC-R, HA-M, JE-R, and RG-A: data analysis. HA-M, RG-A, MR-T, RU-R, DR-P, and GF-P: recruitment and data collection. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The Body Adiposity Index is not applicable to the Brazilian adult population

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Background: Obesity is a serious disease that burdens public health systems around the world. It is a risk factor for the development of several non-communicable chronic diseases that are related to the amount and distribution of body fat. Body composition assessment using simple and low-cost techniques can help in the early detection of excess fat, allowing for the prevention and treatment of both obesity and associated diseases. Thus, identifying and proposing valid anthropometric indices for this purpose can be a great ally of health programs.

Objective: To verify the validity of the Body Adiposity Index (BAI) in relation to Dual Energy X-Ray Absorptiometry (DXA) for estimating body fat percentage in Brazilian adults, as well as to propose a new mathematical model to estimate the fat-free mass of this population.

Methods: In a cross-sectional study, 424 subjects (of which 220 were women), aged between 20 and 59 years, were evaluated by BAI and DXA, then randomly divided into two groups stratified by sex: the development group ($n = 283$) and the cross-validation group ($n = 141$). Statistical analyses to test the validity of BAI as a predictor of fat mass, in addition to proposing a new mathematical model for estimating fat-free mass, using DXA as a reference method. The analysis included paired t -test, stepwise multiple regression, coefficient of concordance correlation, and Bland-Altman plots.

Results: The BAI validity analysis showed a low correlation coefficient of agreement [CCC = 0.626; ρ (precision) = 0.795; C_b (accuracy) = 0.787]; in addition, the mean difference in the Bland-Altman plot was different from zero in the cross-validation group ($p < 0.01$) and limits of agreement (LOA) ranged between -8.0 and 14.4 kg, indicating a poor agreement between the BAI and the reference method. The new mathematical model for estimating FFM showed a high correlation coefficient of agreement (CCC = 0.952; $\rho = 0.953$; $C_b = 0.999$), in addition to acceptable LOA in the Bland-Altman plot (-6.7 and 6.7).

Conclusion: In the studied sample, the BAI showed low validity for estimating body fat, while the new proposed model was found to be a good option to assess the body composition of Brazilian adults.

KEYWORDS

obesity, physical evaluation, body composition, Body Adiposity Index, fat percentual, body fat percentage, fat-free mass

Introduction

Obesity is a severe disease overloading public health systems worldwide. It is an important risk factor for developing chronic diseases such as hypertension and diabetes, as well as cardiovascular and cerebrovascular events (1). Such a risk impact on secondary conditions is related to body fat distribution, i.e., an abnormal amount of body fat exerts an independent risk according to morbidity and represents a different impact in relation to other forms of excessive weight (2, 3).

Body composition assessments, i.e., determining the proportions of fat mass and fat-free mass, are an essential factor for prescribing and monitoring dietary and exercise programs, as well as verifying the response to such programs and health treatments (4). The most accurate method for the body composition assessment is the four-compartment model (4C) which is the criterion method for assessing fat mass (FM) and fat-free mass (FFM) at the molecular level, given that the variability of the main FFM components (water, protein, and minerals) is assessed (5). Furthermore, techniques such as magnetic resonance imaging (MRI) and dual-energy X-ray absorptiometry (DXA) rely on high-cost equipment and qualified personnel, so relatively simple and low operating cost techniques such as anthropometry and bioimpedance have been preferred in clinical and field settings (5–7).

Several mathematical models and anthropometric indices have been proposed to estimate different body components and health risk factors, in order to simplify the role of health professionals in clinical practice or field assessments (8–10).

Previously, in a study by Bergman et al. (11), a new anthropometric index for the estimation of body fat mass was presented, called the Body Adiposity Index (BAI). In this study, the BAI was successfully validated using DXA as a reference method, for the determination of body fat percentage (BF%) in a sample of American and Mexican men and women. Subsequently, Barreira et al. (12) tested the BAI in a large sample of 3,851 subjects in Baton Rouge (USA) and concluded that the BAI is not a valid predictor of BF%. Likewise, a study conducted in Brazil (13) also pointed out that the BAI is not an efficient predictor of BF% in young Brazilian athletes of both sexes.

Considering the inconsistent findings regarding the validity of the BAI for determining BF% in different population groups, we hypothesized that the BAI may not be suitable for all populations. We verified the need to test the validity of the BAI to estimate the results obtained by DXA for BF% in different Brazilian subpopulations. Thus, the objective of this study was to verify the validity of the BAI in relation to DXA for estimating BF% in Brazilian adults, as well as to propose a new mathematical model to estimate the fat-free mass of this population.

Methods

Sample

Four hundred and twenty-four participants (51.9% women, 20–59 years old), from the northeast region of Brazil, who were recruited through dissemination among the participants of university extension projects from the Physical Education Department of the Federal University of Rio Grande do Norte (UFRN) were included in this cross-sectional study.

After their inclusion in the study, the sample was randomly divided into two groups, i.e., the group used for the development of a mathematical model for FFM ($n = 283$) and the cross-validation group ($n = 141$). For the sample size calculation, using FFM as a primary outcome, we considered a medium to small effect size (0.10) with six predictors (independent variables), with a type I error of 5% and a power of 95%. Using these parameters, a total of 132 participants was required.

Procedures

All data collection was conducted in a single visit by each participant to the laboratory to perform anthropometric measurements and DXA assessments, after consenting to the ethical terms approved by the Ethical Committee of the University Hospital Onofre Lopes–HUOL/UFRN–ID CODE: 34804414.7.0000.5292. Individuals with any physical deficiency, prosthesis, under specific diets, or reporting diuretic problems or edema were excluded from the sample.

Anthropometric evaluation

Weight (W) was measured using a Sanny[®] digital scale (BL200PP, American Medical, São Bernardo do Campo, Brazil), with 0.1 kg precision. Participants were barefoot and wearing light clothes. The height (Ht) was measured using the stadiometer Caprice Sanny[®] (American Medical do Brasil, São Bernardo do Campo, Brasil) with 0.1 cm precision, and participants were barefoot, in an orthostatic position. Hip circumference (HC) was measured using a Sanny[®] measuring tape with 0.1 cm precision, at the level of the maximum extension of the buttocks posteriorly in a horizontal plane, as described in the original BAI article (11). Waist circumference (WC) was measured at the midpoint between the iliac crest and the lower border of the last rib, using a Sanny[®] anthropometric metal tape measure with 0.1 cm precision, with the participants standing and the tape measure over bare skin at the measurement site. The body mass index (BMI) was determined as the body mass (kg)/height² (m).

Body adiposity index

The BAI was calculated using the height (Ht) and the hip circumference (HC) in Bergman et al. (11) equation as:

$$BAI = \frac{HC (cm)}{Ht (m) \sqrt{Ht (m)}} - 18$$

Dual-energy X-ray absorptiometry

The DXA scan was performed using the Lunar Prodigy, model NRL 41990 (GE Lunar[®], Madison, WI, USA) with participants in the supine position, feet attached and stabilized to the stretcher, and hands in pronation. Measurements were performed following the recommendations proposed by Nana et al. (14). The body composition was determined by enCORE software (GE Healthcare[®], version 15.0, Madison, WI, USA).

Statistics

The Kolmogorov-Smirnov test was applied to verify the normal distribution of the data. The descriptive analysis consisted of the mean and standard deviation of all study variables, and the comparisons between groups were performed using Student's *t*-test for independent samples.

To test the validity of the BAI to estimate BF%, the means of the results obtained by BAI and measured by DXA were compared using the paired *t*-test. In addition, Pearson's correlation coefficient (*r*), coefficient of determination (*r*²), and standard error of the estimate (SEE) were calculated. The approach proposed by Lin (15) was used for the concordance

correlation coefficient (CCC) analysis to verify the validity (*ρ*) and accuracy (*C_b*) between the estimated and measured BF% values.

The stepwise multiple regression analysis was used to propose the new mathematical model for FFM. The stepwise regression analysis was conducted using FFM obtained by DXA as a dependent variable and age, sex, weight, height, hip circumference, and waist circumference as possible independent variables. During model development, the normality of the residuals and homogeneity of variance were tested. Significance at *p* < 0.05 was established as the criterion for inclusion of a predictor, whereas removal criteria were set at *p* > 0.1. If more than one variable remained in the model, and to assess multicollinearity, a variance inflation factor (VIF) and the tolerance (reciprocal of VIF) were calculated for each independent variable, and a VIF < 10 or tolerance higher than 0.1 was considered appropriate (16, 17). To verify the validity of the proposed model, the same approach as described for the BAI was used. For the cross-validation of the new model proposed in this study, a multiple regression analysis was performed. In turn, the new model accuracy was evaluated using pure error (PE), which was calculated as the square root of the mean of the sum of squared differences between the measurement and estimate of FFM (18). The Bland-Altman (19) plots were used to verify bias and concordance between FFM measurement and estimate, in which the limits of agreement (LOA) were defined as the mean of differences ± 1.96 standard deviations, including the analysis of the correlation between the mean and the difference of the methods. Analyses were carried out with the statistical package SPSS v.20.0 (SPSS Inc., IBM Corp., Armonk, New York, NY, United States) and MedCalc version 12.5.0. Statistical significance of *p* < 0.05 was considered for all tests.

Results

Table 1 describes the physical characteristics and body composition variables for the developmental and cross-validation groups, as well as for the whole sample with no differences observed between the two groups (i.e., developmental and cross-validation) (*p* > 0.05).

When testing the validity of the BAI to estimate the body fat percentage, although the correlation with DXA was high (*r* = 0.795; *p* < 0.01), it was found that there was a significant difference when the mean results were compared with the measurements obtained by DXA (DXA = 30.7 ± 9.8; BAI = 28.2 ± 5.2; *p* < 0.01). The other validity criteria used are presented in Table 2, together with the performance of the cross-validation of the model proposed in this study for the estimation of fat-free mass.

Table 3 shows the regression model for predicting FFM (kg). The possible independent variables used were: age (years), sex, weight (kg), height (cm), hip circumference (cm), and waist circumference (cm). Only the variables that contributed to the

TABLE 1 Descriptive characteristics and body composition of development and cross-validation groups (mean \pm sd).

	Development group (DG)			Cross-validation group (CVG)		
	Male (<i>n</i> = 136)	Female (<i>n</i> = 147)	Whole sample (<i>n</i> = 283)	Male (<i>n</i> = 68)	Female (<i>n</i> = 73)	Whole sample (<i>n</i> = 141)
Age (yrs)	36.6 \pm 12.5	38.7 \pm 13.0	37.7 \pm 12.8	38.8 \pm 12.8	40.0 \pm 13.0	39.4 \pm 12.9
Weight (kg)	78.4 \pm 14.1	65.7 \pm 13.3	71.8 \pm 15.1	80.4 \pm 13.0	66.8 \pm 10.9	73.4 \pm 13.7
Height (cm)	174.9 \pm 7.4	161.3 \pm 6.3	167.9 \pm 9.6	174.5 \pm 7.2	161.6 \pm 6.9	167.8 \pm 9.6
BMI (kg/m ²)	25.6 \pm 4.0	25.2 \pm 4.9	25.4 \pm 4.4	26.4 \pm 3.9	25.7 \pm 4.4	26.0 \pm 4.2
FM (kg)	19.2 \pm 8.6	25.1 \pm 9.2	22.2 \pm 9.4	21.0 \pm 8.0	25.8 \pm 8.6	23.5 \pm 8.6
FM (%)	23.8 \pm 7.2	37.2 \pm 7.0	30.7 \pm 9.8	25.5 \pm 7.0	37.9 \pm 7.7	31.9 \pm 9.6
FFM (kg)	59.2 \pm 9.1	40.6 \pm 5.9	49.6 \pm 12.0	59.4 \pm 8.1	41.0 \pm 5.9	49.9 \pm 11.6
Hip circumference (cm)	99.6 \pm 7.6	100.4 \pm 9.5	100.0 \pm 8.6	99.3 \pm 8.6	102.4 \pm 8.6	100.9 \pm 8.7
Waist circumference (cm)	88.2 \pm 11.7	82.3 \pm 12.9	85.1 \pm 12.6	90.6 \pm 10.8	83.6 \pm 14.6	87.0 \pm 13.3
BAI (BF%)	25.1 \pm 3.4	31.1 \pm 5.0	28.2 \pm 5.2	25.2 \pm 4.2	32.0 \pm 5.3	28.2 \pm 5.2

BMI, Body Mass Index; FM, fat mass; FFM, fat-free mass; BAI, Body Adiposity Index; BF%, body fat percentage.

TABLE 2 Cross-validation of FFM predictive new model, and validation of BAI for BF%.

	FFM (kg)	<i>p</i> -value*	CCC Analysis				PE (kg)
			CCC	ρ	C _b	r ²	
DXA	49.9 \pm 11.6						
New model	49.5 \pm 11.5	0.844	0.952	0.953	0.999	0.91	3.40

	BF%	<i>p</i> -value*	CCC Analysis				PE (%)
			CCC	ρ	C _b	r ²	
DXA	30.7 \pm 9.8						
BAI	28.2 \pm 5.2	<0.001	0.625	0.795	0.787	0.63	6.54

BAI, Body Adiposity Index; FFM, fat-free mass; BF%, body fat percentage; CCC, Concordance Correlation Coefficient; ρ , accuracy; C_b, validity; PE, pure error. *Differences between predictive models and reference method by paired t-test.

estimates using a backward stepwise approach were used in the model. The performance of the developed model can be observed by the high coefficient of determination ($r^2 = 0.91$) and low standard error of the estimate ($SEE = 3.67$ kg).

The resulting prediction model included is shown below, including FFM (fat-free mass) in kg, height (Ht) in cm, weight (W) in kg, sex (male = 0; female = 1), age in years, hip circumference (HC) in centimeters and waist circumference (WC) in cm:

$$\text{FFM} = 26.771 + 0.143\text{Ht} + 0.725\text{W} - 7.942\text{Sex} - 0.087\text{Age} - 0.328\text{HC} - 0.154\text{WC}$$

From the results of FFM, it is possible to calculate FM in kilograms by subtracting FFM from body mass ($\text{FM} = \text{BM} - \text{FFM}$). Then, it is also possible to calculate body fat percentage by the mathematical expression: $\text{BF\%} = (\text{FM} \times 100)/\text{BM}$.

Estimated FFM by the new model developed in this study did not present significant differences in comparison with the value determined by DXA for both the development and cross-validation groups. All parameters used for proposing and validating the model confirmed their validity. Additionally, no association was found between the mean and the difference in the methods ($r = 0.08$; $p = 0.356$).

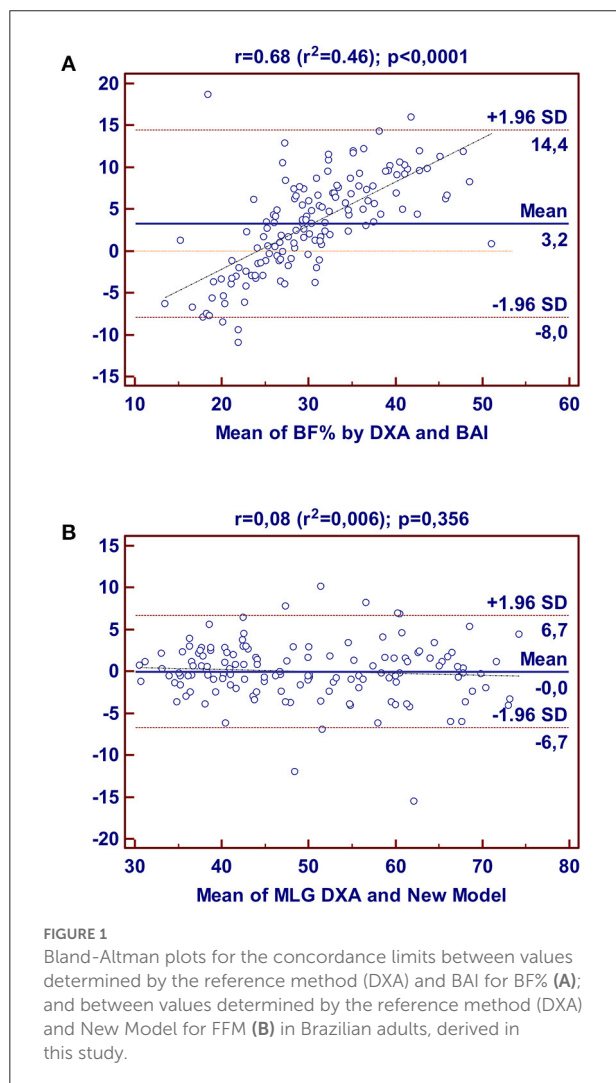
Figure 1 presents the LOA in the cross-validation group for BF% between the standard method (DXA) and the BAI, and the LOA for FFM between DXA and the new model developed in this study.

The mean difference in the Bland-Altman plot was different from zero in the cross-validation group for BAI ($p = 0.006$) but was not different from zero for FFM by the new model ($p = 0.728$). For BAI, the LOA ranged between -8.0 and 14.4 for the body fat percentage, while the LOA ranged between -6.7 and 6.7 kg for fat-free mass, indicating poor agreement between BAI

TABLE 3 Regression model for the prediction of FFM (kg).

Variables included in the model	Regression coefficient	r^2	SEE	p -value	Collinearity statistics	
					Tolerance	VIF
Constant	+26,771			<0.001		
Height	+0.143	0.648 ^a	7.138	<0.001	0.293	3.409
Weight	+0.725	0.806 ^b	5.307	<0.001	0.130	7.695
Sex	−7.942	0.884 ^c	4.118	<0.001	0.355	2.816
Age	−0.087	0.893 ^d	3.963	<0.001	0.799	1.252
Hip Circumference	−0.328	0.902 ^e	3.791	<0.001	0.182	5.489
Waist Circumference	−0.154	0.909 ^f	3.669	<0.001	0.251	3.989

SEE, standard error of the estimate; VIF, variance inflation factor. Predictors: ^a(Constant), Height; ^b(Constant), Height, Weight; ^c(Constant), Height, Weight, Sex; ^d(Constant), Height, Weight, Sex, Age; ^e(Constant), Height, Weight, Sex, Age, Hip Circumference; ^f(Constant), Height, Weight, Sex, Age, Hip Circumference, Waist Circumference. The r^2 change was significant for a, b, c, d, e, and f.



and DXA, but an acceptable agreement between the new model and the reference method.

Discussion

The objective of the present study was to evaluate the validity of the BAI, using DXA as a reference, to estimate body fat percentage in subjects from the Brazilian population. The results did not confirm the validity of the BAI for estimating body fat in Brazilian adults, which justifies the need to create new mathematical models to estimate the body composition of this population. Thus, we developed and cross-validated a model for estimating fat-free mass through anthropometric measurements, using DXA as a reference method.

The BAI is an alternative DXA-based low-cost method for determining body adiposity with a simplified protocol and acceptable accuracy verified in different populations. However, except for the applicability of BAI for the diagnosis of excessive body fat, there is no consensus in the literature regarding its validity in different populations (7, 20).

The equations for the development of BAI are based on body height and hip circumference since these variables correlate with the BF% verified by DXA. The original study—named BetaGene—used men and women from the adult Mexican-American population of the USA. Different populations show distinct anthropometric characteristics, including body fat distribution (21, 22). Thus, an index based on a certain population may not attend to the specific characteristics of another population. Such conflict in the applicability of BAI has been evidenced by several studies (13, 20, 23–25). These reports consistently show that the BAI does not offer a valid estimation of BF% in subpopulations such as Caucasians, Europeans, adult Americans, female athletes, Brazilian women, and children. In line with the above, the main finding in our study was the poor validity of BAI, in relation to DXA, to estimate BF% in Brazilian adults.

An issue that deserves to be highlighted is that the use of BAI in men and women may have different associations with

the body fat values obtained by DXA, which may be related to the distribution of body fat, as it is more concentrated in the gluteal-femoral region in women, in contrast to the abdominal region in men (26), thus interfering with the results of the BAI, which has hip circumference as one of its variables. In this way, perhaps a model that also uses waist circumference would provide better results.

Several studies have shown unsatisfactory results with the use of the BAI. Miazgowski et al. (27) analyzed 234 Caucasian women aged between 20 and 40 years and found a moderate correlation between the BF% values provided by the BAI and DXA. The validity of the BAI was also tested in 106 Asian adults by Lam et al. (24), and their results showed that the values obtained by BAI underestimated the BF% of individuals by an average of 5.77% in relation to DXA. Furthermore, Freedman et al. (28) found that BAI values reported a 75% overestimation in BF% of men and a 70% underestimation in women in a large sample of 1,151 adults of different ethnicities (37% Caucasian, 27% Black, 25% Hispanic, 8% Asian, and 3% others).

Differences in fat distribution in different ethnicities have been previously reported as being associated with environmental and cultural factors, such as nutrition habits and physical activity (21, 22). In this sense, despite the original study's validation of the BAI for the evaluation of BF% in North Americans, it seems that this validation was biased by the ethnicity of the sample, which was mostly Black.

The present study also found that there is a large difference in the limits of agreement between the BAI and DXA, according to the Bland-Altman plot. It was verified that the BAI might underestimate BF% as much as 8.0% or overestimate it up to 14.4% in adult Brazilians, which indicates that the BAI is not an adequate tool for the determination of BF% in healthy adult Brazilians. Convergenly, Cerqueira et al. (20) previously detected a tendency for an overestimation when applying the BAI for the determination of BF% in Brazilians with low fat mass, as well as an underestimation in Brazilians with obesity.

Another study carried out in Brazil analyzed the validity of the BAI to estimate the BF% of 144 adults with severe obesity (candidates for bariatric surgery), using air displacement plethysmography (BOD POD®) as the standard technique (29). The authors showed poor validity of the BAI and LOA very close to those of the present study for the percentage of body fat (-7.48 to 14.84). Thus, the authors chose to develop a new mathematical model for the study population.

In this sense, the creation of a mathematical model for estimating body composition in the population of the present study, using simple anthropometric measurements, seems to be a viable way to obtain better results than the BAI. Thus, we developed and tested the validity of several models, and the one that presented the best results of validity and cross-validation was the one that proposed the estimation of fat-free mass and included as dependent

variables height, weight, sex, age, hip circumference, and waist circumference, with excellent prediction performance observed by the high coefficient of determination ($r^2 = 0.91$) and low standard error of the estimate (SEE = 3.67 kg).

The low cost and ease of use may explain the number of studies that have been carried out to propose anthropometric mathematical models for estimating body composition in different groups or to validate existing ones (30–35). However, most of these equations were proposed to meet the specificities of the group under study, such as breast-feeding children (30), children (34), adolescent athletes (33), healthy adults (31), or those with chronic diseases (32) and elite athletes (35), among many others, which cannot be generalized to groups other than the population of origin.

Considering that there is no consensus on generalizable anthropometric prediction equations to validly estimate body composition, a comprehensive study was carried out to develop and validate practical anthropometric predictions for lean body mass, fat mass, and percent fat in adults (men, $n = 7,531$; women, $n = 6,534$) participating in the National Health and Nutrition Examination Survey 1999–2006 (36). The authors derived several regression models, with different anthropometric measurements, including circumferences and skinfolds, and concluded that a practical equation including age, race, height, weight, and waist circumference had a high predictive ability for lean body mass and fat mass and that the inclusion of other circumferences and skinfold thickness slightly improved the prediction model.

There have been few studies carried out in Brazil with the aim of proposing mathematical models based on circumference measurements to estimate body composition. In the same geographic region of the country where we carried out our study, another study was previously carried out that proposed predictive equations for fat mass and fat-free mass of adolescents aged 10 to 16 years, based on anthropometric measurements, and concluded that the equations developed to estimate fat mass in females and fat-free body mass in all genders had high adjusted coefficients of determination (37).

More recently, in southern Brazil, a study was carried out to propose mathematical models for estimating the percentage of body fat in women aged 18 to 35 years, based on body circumferences (38). However, the authors only used Pearson's correlation coefficient and the paired *t*-test, in relation to DXA as a reference method, and lacked more robust statistical analyses to validate and carry out the cross-validation of the developed models.

In the same year, a mathematical model was developed to estimate the BF% of Brazilian subjects with severe obesity, and

candidates for bariatric surgery, demonstrating high validity and limits of agreement similar to the present study, but this model does not apply to our sample since the anthropometric characteristics of the source population are very different from our sample (29).

This study has several strengths. To our knowledge, this is the first study to develop and cross-validate a predictive equation for FFM by body circumference, using DXA as a reference method, in Brazilian adults in the northeast region. The mathematical model developed in our study showed a high coefficient of determination and good limits of agreement in relation to the reference method, and all the parameters used for the proposition and cross-validation of the model confirmed its validity for the population studied (15, 18, 19, 39), which can be used to monitor changes in FFM resulting from dietary and exercise programs (40).

However, there are limitations to our study that must be addressed. The sample included adults from only one region of the country, and ethnicity was not assessed. Other studies carried out in Brazil for the development of predictive equations by anthropometry (38, 41) also used ethnically mixed samples, miscegenation, and ethnic differences, which suggests the need to validate the equation proposed in the study in other regions of the country and with subjects from different ethnic origins. Another important issue concerns the standard technique used. The 4C model is the most appropriate reference method to assess FM and FFM at the molecular level (5). However, due to the complexity of the technique (42), the use of DXA to derive anthropometric equations has been widely accepted (7, 34, 36, 37). It is noteworthy that the new equations are only useful for Brazilian adults with similar characteristics. In addition, more research should be carried out to test the accuracy of the new model in tracking FFM.

Conclusion

In the studied sample, the BAI showed low validity for estimating body fat, while the new proposed model proved to be a good option to assess the body composition of Brazilian adults. However, we are aware that the validity of the proposed model must be tested in other regions of the country and in other population groups in order to verify its applicability.

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Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Ethics statement

The studies involving human participants were reviewed and approved by Ethical Committee of the University Hospital Onofre Lopes–HUOL/UFRN ID CODE: 34804414.7.0000.5292. The patients/participants provided their written informed consent to participate in this study.

Author contributions

JR: responsible for the concept/design, the data collection, the data analysis/interpretation, and drafting the article. RC: responsible for the interpretation and drafting the article. CG and MO: responsible for data collection, drafting the article, and critical revision of the article. PA-N and GD: responsible for translate to English, drafting the article, and critical revision of the article. BC: responsible for project supervision, data analysis/interpretation, and drafting the article. PD: responsible for the concept/design, project supervision, the data collection, drafting the article, and critical revision of the article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Prediction of fat-free mass in a multi-ethnic cohort of infants using bioelectrical impedance: Validation against the PEA POD

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Background: Bioelectrical impedance analysis (BIA) is widely used to measure body composition but has not been adequately evaluated in infancy. Prior studies have largely been of poor quality, and few included healthy term-born offspring, so it is unclear if BIA can accurately predict body composition at this age.

Aim: This study evaluated impedance technology to predict fat-free mass (FFM) among a large multi-ethnic cohort of infants from the United Kingdom, Singapore, and New Zealand at ages 6 weeks and 6 months ($n = 292$ and 212 , respectively).

Materials and methods: Using air displacement plethysmography (PEA POD) as the reference, two impedance approaches were evaluated: (1) empirical prediction equations; (2) Cole modeling and mixture theory prediction. Sex-specific equations were developed among ~70% of the cohort. Equations were validated in the remaining ~30% and in an independent University of Queensland cohort. Mixture theory estimates of FFM were validated using the entire cohort at both ages.

Results: Sex-specific equations based on weight and length explained 75–81% of FFM variance at 6 weeks but only 48–57% at 6 months. At both ages, the margin of error for these equations was 5–6% of mean FFM, as assessed by the root mean squared errors (RMSE). The stepwise addition of clinically-relevant covariates (i.e., gestational age, birthweight SDS, subscapular skinfold thickness, abdominal circumference) improved model accuracy (i.e., lowered RMSE). However, improvements in model accuracy were not consistently observed when impedance parameters (as the impedance index) were incorporated instead of length. The bioimpedance equations had mean absolute percentage errors (MAPE) < 5% when validated. Limits of agreement analyses showed that biases were low (< 100 g) and limits of agreement were narrower for bioimpedance-based than anthropometry-based equations, with no clear benefit following the addition of clinically-relevant variables. Estimates of FFM from BIS mixture theory prediction were inaccurate (MAPE 11–12%).

Conclusion: The addition of the impedance index improved the accuracy of empirical FFM predictions. However, improvements were modest, so the benefits of using bioimpedance in the field remain unclear and require further investigation. Mixture theory prediction of FFM from BIS is inaccurate in infancy and cannot be recommended.

KEYWORDS

air displacement plethysmography (ADP), bias, bioelectrical impedance analysis (BIA), bioelectrical impedance spectroscopy (BIS), body composition, fat-free mass (FFM), validation

Introduction

Measurements of body composition are increasingly being used among pediatric cohorts. Knowledge of the composition and distribution of tissue compartments can inform health risk and uncover associations between longitudinal body composition changes and health outcomes. However, measurement of body composition in infancy is complicated by rapid growth, changes in adiposity, and shifts in the makeup of the fat-free mass compartment (FFM)—consisting of essential lipids, intra- and extracellular water (ICW and ECW, respectively), protein, glycogen, and minerals (1). A multi-compartment model involving the measurement of fat mass (FM) and three or more components of the FFM compartment is an appropriate reference for measuring body composition in infancy (2). However, the technique is seldom used due to its level of complexity (3–5). Other tools vary in their accuracy, feasibility, and cost. For example, although air displacement plethysmography (ADP, i.e., the PEA POD) and dual-energy X-ray absorptiometry (DXA) are reproducible and widely used in pediatric research (1, 6–8), these tools are expensive, not readily portable, require technical expertise in their use and, in the case of DXA, present an, albeit small, radiation hazard.

Bioimpedance techniques, on the other hand, are comparatively inexpensive compared to ADP, DXA, or MRI, simple to use, and portable, making them appropriate field tools; however, bioimpedance is not considered a reference technique and may be inaccurate in infancy (9–11).

Bioimpedance techniques involve measuring the opposition to the flow of a small, harmless alternating electrical current through the body. Impedance (Z) is the combined effect of resistance (R) and reactance (X_c), with resistance being related to total body water (TBW) and reactance to cell membrane capacitance (12). Single-frequency bioimpedance analysis (SFBIA), which measures impedance at one frequency only, is the most commonly used technique (13). However, devices are also available that measure at a few frequencies, generally <8 (multifrequency BIA, MFBIA), and over a large range of frequencies, typically >50 (Bioimpedance spectroscopy, BIS). BIS devices have the added benefit of being able to distinguish between ICW and ECW through the extrapolation of measured impedances to zero and infinite frequencies *via* Cole simple circuit impedance modeling to represent the impedance of biological tissues (14). BIS modeled data can then be used to predict TBW, and hence FFM, according to mixture theory (15). A purported benefit of using

BIS is that population-specific empirical prediction equations are not required. However, the coefficients used in mixture theory prediction may need to be population-specific (10).

A recent systematic literature review identified 15 studies that reported a total of 46 bioimpedance (both SFBIA and MFBIA) equations suitable for use in infancy (<24 months) (11). None could be recommended due to methodological issues or not offering improved performance beyond anthropometric prediction equations (11). Furthermore, BIS has not been adequately evaluated in infancy (16, 17). Indeed, mixture theory prediction has seldom been used in any pediatric cohort (16, 18). Therefore, we aimed to evaluate different BIA approaches in infancy among a multi-ethnic cohort of infants at 6 weeks and 6 months of age by developing and validating empirical prediction equations for FFM, considering PEA POD as the reference standard, as well as evaluating mixture theory prediction. We also aimed to determine whether the prediction of FFM with bioimpedance empirical equations could be improved by the inclusion of additional clinically relevant covariates.

Materials and methods

Participants were born between April 2016 and January 2019 to mothers participating in the Nutritional Intervention Preconception and During Pregnancy to Maintain Healthy Glucose Metabolism and Offspring Health (NiPPeR) study (19). The NiPPeR study is a multinational randomized controlled trial that recruited women prior to conception across three centers in the United Kingdom, Singapore, and New Zealand. The trial's primary aim was to determine if a twice-daily nutritional drink would regulate maternal glucose tolerance at 28 weeks of gestation; no differences in gestational glycaemia were observed (20). Secondary offspring outcomes included neonatal and infant body composition. The trial was registered on 16 July 2015 (ClinicalTrials.gov NCT02509988; Universal Trial Number U1111-1171-8056) and was conducted according to the guidelines laid down in the Declaration of Helsinki (21). Ethics approval was granted by the appropriate committees: Southampton–Health Research Authority National Research Ethics Service Committee South Central Research Ethics Committee (15/SC/0142); Singapore–the National Healthcare Group Domain Specific Review Board (2015/00205); and New Zealand–the Northern A Health and Disability Ethics Committee (15/NTA/21/AM20). Written informed consent was obtained from the mothers of the included offspring.

Comprehensive inclusion and exclusion criteria for the NiPPeR study are reported elsewhere (19). Briefly, women were eligible to participate if they were: aged 18 to 38 years; lived in Southampton, Singapore, or Auckland; planned to conceive within 6 months; and had future maternity care planned at one of the study centers. Their infants were assessed at multiple time points during infancy, with BIS data collection commencing at 6 weeks of age (Supplementary Figure 1). NiPPeR offspring were

included in the current study if they were born to mothers who conceived within a year of starting the NiPPeR intervention; were born at term ($37^{0/7}$ – $41^{6/7}$ weeks of gestation); and at 6 weeks of age (37–54 days) or 6 months of age (169–204 days) had each of the following anthropometric measurements collected: weight, recumbent crown-heel length, subscapular and triceps skinfold thicknesses, and abdominal, upper arm and chest circumferences; and had valid body composition data from BIS and PEA POD collected on the same day.

Of the 584 offspring born to mothers participating in the NiPPeR study (excluding one stillbirth and one neonatal death), 519 were assessed at 6 weeks and 533 at 6 months. The following exclusions applied to the 6-week cohort: 1 infant with trisomy 21, 96 without BIS measurements, 37 without a PEA POD measurement, 19 with BIS and PEA POD measurements completed on different days, 16 who were < 37 or > 54 days old at the time of measurement, 21 who were born pre- or post-term, and 37 who had one or more missing anthropometric measurement (Supplementary Figure 2). At 6 months, the following exclusions applied to the cohort: 1 infant with trisomy 21, 48 without BIS measurements, 229 without a PEA POD measurement, 10 with BIS and PEA POD measurements completed on different days, 11 who were < 169 or > 204 days old at the time of measurement, 17 who were born pre- or post-term, and 5 who had one or more missing anthropometric measurements (Supplementary Figure 2). A total of 292 offspring had complete data available for analysis at 6 weeks and 212 at 6 months (Supplementary Figure 2).

Birth outcomes

Gestational age at birth was determined using a pre-specified algorithm based on menstrual data (22). First-trimester fetal crown-rump length measurement was used if there was a >7-day discrepancy between the last menstrual period (LMP) and scan dates, an uncertain LMP date, irregular cycles, or hormonal contraception use within the prior 3 months. Sex- and gestational age-specific birthweight standard deviation scores (SDS) were calculated using the INTERGROWTH-21st international standards for newborn weight (23), with birthweight obtained from hospital records.

Anthropometry

Anthropometric measurements were done accordingly to standardized protocol by trained staff. Recumbent length (L) was measured in duplicate to the nearest 0.1 cm using a Harpenden neonatometer or infantometer (Holtain Ltd., Crymych, UK). Abdominal circumference (AC) was measured immediately above the umbilicus and chest circumference (CC) at the point where the ribs meet the sternum. Both measurements were made at the end of expiration and

in duplicates. Mid-upper arm circumference (MUAC) was measured at the mid-point of the left upper arm. Measurements were made in triplicate using an unmarked non-stretchable tape which was then measured against a fixed metal rule to 1 mm resolution. Triceps skinfold thickness was measured at the horizontal level of the MUAC measurement, and subscapular skinfold thickness was measured immediately below the inferior angle of the scapula. Skinfold thickness were measured in triplicate using a calibrated Holtain metal caliper (Holtain Ltd.) after a count of 2 s, with mean values used in analyses.

PEA POD

PEA POD machines were calibrated daily prior to use and measurements were carried out according to the manufacturer's recommendations. Following measurement of recumbent length, the infant was placed nude inside the PEA POD measurement chamber with hair flattened with oil or fully covered by a tightly fitting cap. The integrated scales of the PEA POD measured weight, while the displacement of air in the chamber measured volume, thus allowing calculation of body density. The default age- and sex-specific reference data of FFM density from Fomon et al. (24) was used as studies have shown better alignment with isotope dilution when using Fomon's reference compared to Butte's (25–27). This procedure was repeated in all infants at 6 weeks and, where possible, at 6 months of age, as the device has a weight restriction of approximately 10 kg.

Bioelectrical impedance spectroscopy

Bioelectrical impedance spectroscopy (BIS) was measured at each study site using the ImpediMed SFB7 (ImpediMed, Queensland, Australia), which measures bioimpedance parameters over a frequency range of 3 to 1,000 kHz, resulting in 256 measurements per assessment. The device's calibration was checked daily prior to use with a test cell provided by the manufacturer. ImpediMed single-tab Ag-AgCl gel electrodes (25 × 23 mm) were used to attach sense leads to the dorsum wrist and ankle, and source leads to the palm at the metacarpal heads and the sole at the metatarsal heads on the same side of the body (28). Electrode sites were cleaned with 70% isopropyl alcohol wipes prior to the attachment of electrodes. Infants were measured on non-conductive examination tables with their legs separated and arms by their sides. Insulating materials (e.g., thin blankets) were used to separate body parts, where necessary, to prevent short-circuiting of the current. Any clothing with metal (e.g., clips or buckles) was removed prior to measurement to avoid electrical interference. Otherwise, clothing was only removed to access electrode sites. The infant was measured in a relaxed state and, where possible, while asleep, as movement

during measurement has been found to affect impedance values in infancy (29).

Data were fitted to the Cole model as a plot of reactance against resistance (14) using software provided by the manufacturer (BioImp version 5.4.0, ImpediMed). All measurements were analyzed using the following software settings: frequency range 5–1,000 kHz; time delay (Td) correction off; 1% data rejection limit. Data were screened for poor quality files (as determined visually by a poorly fitted Cole plot) which were removed, and the mean impedance values of multiple measurements used in further analyses. Multiple measurements were obtained for each infant. For the entire NiPPeR cohort, 16 and 25% of files at 6 weeks and 6 months, respectively, were identified as being poorly fitted and were subsequently removed; however, only 21 and 25 participants had no BIS data remaining following the removal of poor quality files (15 and 25 for the current analysis).

The following impedance parameters were used in developing the empirically-derived equations:

- Resistance at infinite frequency (R_{∞})
This extrapolated value reflects TBW as at high frequencies, the electrical current can pass across the cell membrane, and both ICW and ECW can be measured (30).
- Resistance at zero frequency (R_0)
At low frequencies, the cell membrane acts as an imperfect capacitor, and current cannot pass through it; therefore, the resistance at zero frequency reflects ECW only (30).
- Magnitude of the body impedance at the characteristic frequency (Z_c)
The characteristic frequency (f_c) is the frequency where reactance is maximal in an individual. At this frequency, the ratio of current flow through extra- and intracellular paths is independent of the membrane capacitance (17). Z_c has therefore been suggested as an alternative predictor of TBW (30).
- Resistance at 50 kHz (R_{50})
Most SFBIA devices use resistance at this frequency to predict TBW and FFM. At this frequency, both ICW and ECW are represented, although ECW predominates.

Whole-body (wrist-ankle) BIA models the human body as a series of inter-connected cylinders (legs, arms, and trunk). A cylinder's electrical resistance is directly proportional to its length (L) and inversely proportional to its cross-sectional area (A):

$$R = \rho L/A$$

where ρ is the specific resistivity (ohm.cm) of the material of the cylinder. Since conductive volume (V , body water) equals $L \times A$, this equation can be rearranged to

$$V = \rho L^2/R$$

Thus, L^2/R , termed the “impedance index,” was used in the development of the predictive equations (31), where R is the values measured at the different frequencies listed above.

In addition, body composition was estimated using mixture modeling. TBW was estimated using mixture theory (32) with coefficients appropriate for this age group by sex taken from the literature: body proportions, K_b (16, 33); intra- and extracellular resistivities, ρ_{ICF} and ρ_{ECF} , respectively (16, 32); and body density, D_b (16, 32, 34). FFM was then derived from TBW by dividing TBW by age- and sex-specific hydration factors (24).

Data analysis

The accuracy of BIA in estimating body composition was assessed using a similar approach to that used by Tint et al. (28). Our population of infants with valid data were split into equation derivation (~70%) and validation (~30%) cohorts using a random number generator. Predictive regression equations were developed using bi-directional stepwise multivariable linear regression analysis combined with data minimization techniques, with separate equations being developed for each time point (6 weeks and 6 months). Differences between derivation and validation groups were assessed using two-sample t -tests for continuous variables and Fisher's exact test for categorical variables.

The equations were developed considering FFM (from PEA POD) as the outcome, with weight (W , kg) and either length (L , cm) alone or in combination with impedance values (L^2/R , cm^2/Ω). The contribution of birth characteristics (gestational age and birthweight SDS) and additional anthropometric measurements (triceps and subscapular skinfold thickness, their sum, as well as abdominal, chest, and upper arm circumferences) were also assessed. Gestational age, birthweight SDS, subscapular skinfold thickness, and abdominal circumference were included in the final equations as these covariates were statistically significant predictors for most age- and sex-specific groups. We also evaluated other combinations of these clinically-relevant variables, but no notable improvements to FFM prediction were observed (data not shown). Standardized regression coefficients and the adjusted coefficient of determination (aR^2) were used to assess the contribution of each variable to the prediction of FFM. Model performance was assessed using aR^2 and root mean squared error (RMSE).

As there are sex-specific differences in the association between these anthropometric variables and body composition (35–37), sex-specific equations were derived rather than including sex as a factor in the equations. Likewise, there

may be ethnicity-specific differences (38, 39); therefore, we also explored ethnic differences by developing ethnicity-specific equations among the two largest ethnic sub-groups (White Caucasian and Chinese), which were subsequently compared to the main equations.

The final predictive equations were applied to their respective validation cohorts. Agreement between measured and predicted FFM was assessed using Passing and Bablok regression scatterplots (40), Pearson's correlation coefficient (r), and Lin's concordance coefficient (CCC) (41). Bland and Altman's limits of agreement (LOA) method (42) were used to assess method agreement. The bias (mean difference) between the methods indicates whether the equations under- or overestimate (negative and positive bias, respectively) the mean FFM and by how much. The 95% LOA (± 1.96 SD) indicate the possible extent of variation between the reference and predicted body composition values for any individual. Finally, the slope of the regression line indicates if there is a proportional bias between the two methods, i.e., whether the bias was largely equal across the range of measurements (42). Finally, the performance of each equation was ranked using mean absolute percentage error (MAPE). The equations were then cross-validated in infants of similar ages (6 weeks and 4.5 months) from the study by Lingwood et al. (17), with agreement and intra-individual differences evaluated using the aforementioned methods. The FFM estimates obtained from the mixture theory analyses were also validated against PEA POD FFM measurements using the aforementioned methodology.

Statistical analyses were conducted in R (version 4.0.3, R Foundation for Statistical Computing, Vienna, Austria). All statistical tests were two-tailed with significance set at $p < 0.05$. Descriptive statistics are presented as means \pm SD or medians \pm IQR for continuous variables and n (%) for categorical variables.

Results

Characteristics of the study population are described in Table 1. There were no differences between the development and validation cohorts at either time point for either sex (Supplementary Tables 1, 2).

Relationship between impedance and fat-free mass

Impedance was inversely correlated with FFM from the PEA POD at both ages. At 6 weeks, the strength of the inverse correlation was greater among boys, whereas at 6 months, the inverse correlation of impedance with FFM was

TABLE 1 Characteristics of the included cohort.

	6 weeks		6 months	
	Males (<i>n</i> = 123)	Females (<i>n</i> = 169)	Males (<i>n</i> = 85)	Females (<i>n</i> = 127)
Gestational age at birth (weeks)	39.5 ± 1.2	39.6 ± 1.1	39.4 ± 1.1	39.6 ± 1.1
Birthweight SDS ⁺	0.20 ± 0.96	0.24 ± 0.99	0.08 ± 0.94	0.24 ± 0.98
Age at visit (days)	44 ± 4	43 ± 4	184 ± 8	183 ± 8
Weight (kg)	4.98 ± 0.51	4.59 ± 0.51	7.81 ± 0.67	7.16 ± 0.66
Recumbent length (cm)	56.8 ± 2.0	55.7 ± 2.0	67.4 ± 2.3	65.7 ± 2.2
PEA POD fat-free mass (kg)	3.9 ± 0.4	3.6 ± 0.4	5.7 ± 0.5	5.2 ± 0.5
PEA POD fat mass (kg)	1.1 ± 0.2	1.0 ± 0.3	2.1 ± 0.5	2.0 ± 0.5
PEA POD fat mass (%)	21.1 ± 4.0	21.7 ± 4.8	26.7 ± 4.8	27.7 ± 5.4
Resistance at 0 kHz (Ω)	743 ± 83	811 ± 95	783 ± 91	856 ± 102
Resistance at ∞ kHz (Ω)	483 ± 89	522 ± 106	541 ± 102	588 ± 118
Characteristic frequency (kHz) [§]	434 ± 265	395 ± 232	237 ± 142	235 ± 120
Impedance at Fc (Ω) [§]	616 ± 78	670 ± 93	666 ± 87	726 ± 102
Resistance at 50 kHz (Ω)	690 ± 79	748 ± 96	729 ± 90	790 ± 106
Upper arm circumference (cm)	12.5 ± 0.9	12.0 ± 1.0	14.9 ± 1.0	14.3 ± 1.1
Chest circumference (cm)	39.0 ± 1.7	37.8 ± 1.7	44.0 ± 2.1	42.8 ± 2.2
Abdominal circumference (cm)	38.6 ± 2.4	37.4 ± 2.5	43.3 ± 3.4	41.9 ± 3.6
Triceps skinfold (mm)	8.6 ± 1.8	8.2 ± 1.8	10.2 ± 2.0	10.2 ± 2.3
Subscapular skinfold (mm)	8.0 ± 1.7	7.8 ± 1.6	8.2 ± 1.9	7.8 ± 1.7
Sum of skinfolds (mm) ^{&}	16.6 ± 3.1	16.0 ± 3.0	18.4 ± 3.2	18.0 ± 3.1
Ethnicity				
White Caucasian	60 (48.8%)	93 (55.0%)	47 (55.3%)	71 (55.9%)
Chinese	44 (35.8%)	54 (32.0%)	27 (31.8%)	37 (29.1%)
South Asian	4 (3.3%)	7 (4.1%)	4 (4.7%)	2 (1.6%)
Malay	6 (4.9%)	7 (4.1%)	3 (3.5%)	9 (7.1%)
Other	9 (7.3%)	8 (4.7%)	4 (4.7%)	8 (6.3%)
Study site				
UK	30 (24.4%)	39 (23.1%)	22 (25.9%)	32 (25.2%)
SG	49 (39.8%)	60 (35.5%)	29 (34.1%)	43 (33.9%)
NZ	44 (35.8%)	70 (41.4%)	34 (40.0%)	52 (40.9%)
Randomisation group				
Intervention	55 (44.7%)	82 (48.5%)	40 (47.1%)	61 (48.0%)
Control	68 (55.3%)	87 (51.5%)	45 (52.9%)	66 (52.0%)

Data are means ± SD or medians ± IQR for continuous variables and n (%) for categorical variables.

⁺INTERGROWTH-21st birthweight standard deviation scores (SDS).

[§]Frequency at which reactance is maximal in an individual (Fc).

[&]Sum of triceps and subscapular skinfold thicknesses.

stronger among girls (**Supplementary Figure 3**). Nonetheless, correlations were weak at $r \leq 0.4$ (**Supplementary Figure 3**). The inverse correlations between FFM and impedance indices (L^2/R) were typically improved in comparison to those between FFM and impedance alone ($r = 0.32$ – 0.65); however, the strength of correlations were greater still for length and FFM ($r = 0.53$ – 0.71), except among girls at 6 months (**Supplementary Figure 4**).

Prediction of PEA POD fat-free mass

The various impedance parameters (R_{50} , R_0 , R_{∞} , and Z_c as their respective indices) had similar performances;

therefore, results are only reported for R_{50} here. Equations incorporating the other impedance parameters can be found in **Supplementary Table 3**.

At 6 weeks, the standardized regression coefficient was greatest for weight, indicating that it was the strongest contributor to the prediction of FFM (**Table 2**). The simple anthropometric equations based on weight and length (**Table 2**, equation 1A) explained 81.2 and 75.0% of the variance in FFM for males and females, respectively, with RMSE ± 0.185 kg (4.7% of mean FFM) and ± 0.191 kg (5.3% of mean FFM) (**Table 2**). Substituting length with the impedance index (equation 1B) marginally improved the prediction of FFM in males, reducing RMSE by 5 g, down to 0.180 kg (4.6% of mean FFM); in females, the reverse was observed, with

TABLE 2 Multivariable linear regression analysis of weight (W) and (A) length (L) or (B) impedance index (L^2/R_{50}), (1) alone or in combination with (2) gestational age (GA) and birthweight standard deviation score (BW_{SDS}), or (3) GA, BW_{SDS} , subscapular skinfold thickness (SS), and abdominal circumference (AC) for predicting PEA POD fat-free mass (FFM) among the 6-week and 6-month-old derivation cohorts.

		aR ²	RMSE	Standardized coefficients					Prediction equation for FFM	
				W	L or L ² /R ₅₀	GA	BW _{SDS}	SS	AC	
6 weeks										
Males (n = 86)										
1A	W + L	0.812	0.185 (4.74%)	0.766***	0.171*					−1.07 + 0.62W + 0.03L
2A	W + L + GA + BW _{SDS}	0.856	0.159 (4.08%)	0.629***	0.058	0.231***	0.165**			−2.48 + 0.51W + 0.01L + 0.08GA + 0.07BW _{SDS}
3A	W + L + GA + BW _{SDS} + SS + AC	0.881	0.144 (3.69%)	0.594***	0.089	0.200***	0.066	−0.140**	0.169**	−3.14 + 0.48W + 0.02L + 0.07GA + 0.03BW _{SDS} − 0.04SS + 0.03AC
1B	W + L ² /R ₅₀	0.821	0.180 (4.62%)	0.795***	0.176**					0.23 + 0.65W + 0.10L ² /R ₅₀
2B	W + L ² /R ₅₀ + GA + BW _{SDS}	0.867	0.157 (4.03%)	0.637***	0.090	0.222***	0.148*			−2.00 + 0.52W + 0.05L ² /R ₅₀ + 0.08GA + 0.06BW _{SDS}
3B	W + L ² /R ₅₀ + GA + BW _{SDS} + SS + AC	0.878	0.145 (3.72%)	0.640***	0.024	0.207***	0.089	−0.134**	0.153**	−2.42 + 0.52W + 0.01L ² /R ₅₀ + 0.07GA + 0.04BW _{SDS} − 0.03SS + 0.03AC
Females (n = 118)										
1A	W + L	0.750	0.191 (5.31%)	0.692***	0.223**					−1.06 + 0.51W + 0.04L
2A	W + L + GA + BW _{SDS}	0.794	0.172 (4.78%)	0.512***	0.183**	0.168***	0.215***			−2.39 + 0.38W + 0.03L + 0.06GA + 0.08BW _{SDS}
3A	W + L + GA + BW _{SDS} + SS + AC	0.811	0.164 (4.56%)	0.584***	0.142*	0.150***	0.164**	−0.152**	0.107	−2.27 + 0.43W + 0.03L + 0.05GA + 0.06BW _{SDS} − 0.04SS + 0.02AC
1B	W + L ² /R ₅₀	0.738	0.196 (5.44%)	0.788***	0.127*					0.60 + 0.58W + 0.08L ² /R ₅₀
2B	W + L ² /R ₅₀ + GA + BW _{SDS}	0.782	0.177 (4.92%)	0.607***	0.073	0.174***	0.213***			−1.08 + 0.45W + 0.04L ² /R ₅₀ + 0.06GA + 0.08BW _{SDS}
3B	W + L ² /R ₅₀ + GA + BW _{SDS} + SS + AC	0.804	0.166 (4.61%)	0.698***	0.052	0.152***	0.164**	−0.183***	0.073	−1.11 + 0.52W + 0.03L ² /R ₅₀ + 0.05GA + 0.06BW _{SDS} − 0.04SS + 0.01AC
6 months										
Males (n = 59)										
1A	W + L	0.565	0.337 (5.91%)	0.491***	0.353**					−2.94 + 0.37W + 0.09L
2A	W + L + GA + BW _{SDS}	0.614	0.312 (5.47%)	0.386***	0.289**	0.224*	0.117			−3.49 + 0.29W + 0.07L + 0.06GA + 0.12BW _{SDS}
3A	W + L + GA + BW _{SDS} + SS + AC	0.642	0.295 (5.18%)	0.496**	0.198	0.169	0.081	−0.220*	0.025	−1.63 + 0.37W + 0.05L + 0.04GA + 0.09BW _{SDS} − 0.06SS + 0.004AC
1B	W + L ² /R ₅₀	0.537	0.348 (6.11%)	0.603***	0.247*					1.26 + 0.46W + 0.14L ² /R ₅₀
2B	W + L ² /R ₅₀ + GA + BW _{SDS}	0.581	0.325 (5.70%)	0.496***	0.161	0.123*	0.220			−0.11 + 0.37W + 0.09L ² /R ₅₀ + 0.06GA + 0.12BW _{SDS}
3B	W + L ² /R ₅₀ + GA + BW _{SDS} + SS + AC	0.632	0.299 (5.25%)	0.625***	0.117	0.149	0.097	−0.270**	−0.051	0.74 + 0.47W + 0.07L ² /R ₅₀ + 0.05GA + 0.08BW _{SDS} − 0.08SS − 0.01AC
Females (n = 88)										
1A	W + L	0.475	0.343 (6.73%)	0.503***	0.323***					−2.32 + 0.37W + 0.07L
2A	W + L + GA + BW _{SDS}	0.491	0.334 (6.55%)	0.482***	0.266**	0.130	0.113			−3.68 + 0.35W + 0.06L + 0.06GA + 0.06BW _{SDS}
3A	W + L + GA + BW _{SDS} + SS + AC	0.525	0.319 (6.25%)	0.774***	0.144	0.084	0.119	−0.235*	−0.221	−0.88 + 0.56W + 0.03L + 0.04GA + 0.06BW _{SDS} − 0.06SS − 0.03AC
1B	W + L ² /R ₅₀	0.481	0.341 (6.69%)	0.491***	0.337***					1.40 + 0.36W + 0.21L ² /R ₅₀
2B	W + L ² /R ₅₀ + GA + BW _{SDS}	0.522	0.324 (6.35%)	0.444***	0.321***	0.178*	0.136			−1.47 + 0.32W + 0.20L ² /R ₅₀ + 0.08GA + 0.07BW _{SDS}
3B	W + L ² /R ₅₀ + GA + BW _{SDS} + SS + AC	0.625	0.283 (5.55%)	0.787***	0.367***	0.086	0.126	−0.286***	−0.364***	0.76 + 0.57W + 0.23L ² /R ₅₀ + 0.04GA + 0.06BW _{SDS} − 0.08SS − 0.05AC

aR², adjusted coefficient of determination; RMSE, root mean squared error; W, weight (kg); L, recumbent crown-heel length (cm); L^2/R_{50} , impedance index (cm^2/Ω); GA, gestational age (weeks); BW_{SDS} , INTERGROWTH-21st gestational age and sex specific birthweight standard deviation score; SS, subscapular skinfold thickness (mm); AC, abdominal circumference (cm); FFM, fat-free mass (kg).

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ for statistically significant standardized regression coefficient from multivariable linear regression.

RMSE increasing by 5 g, up to 0.196 kg (5.4% of mean FFM) (Table 2).

At 6 months, weight remained the strongest contributor to the prediction of FFM. The simple anthropometric equations explained less than 60% of the variance in FFM, but the equations incorporating the impedance index and other important variables increased explained variance to 63% (Table 2). The simple anthropometric equations (equation 1A) predicted FFM with RMSE of ± 0.337 kg (5.9% of mean FFM) and ± 0.343 kg (6.7% of mean FFM) for males and females, respectively. In contrast to the 6-week equations, substituting length with the impedance index (equation 1B) increased RMSE for males while marginally reducing RMSE in females (+11 g and -2 g, respectively).

The addition of birth characteristics (i.e., gestational age at birth and birthweight SDS) improved the prediction of FFM at both ages, with aR^2 increasing and RMSE decreasing; however, RMSE was only reduced by an average of 0.5% of mean FFM (Table 2). RMSE was further reduced by the addition of subscapular skinfold thickness and abdominal circumference (Table 2). Overall, the inclusion of additional covariates (gestational age at birth, birthweight SDS, subscapular skinfold thickness, and abdominal circumference) increased the aR^2 by 0.06 to 0.13 and decreased RMSE on average by 1% of mean FFM, which is an approximately 15% reduction in RMSE in comparison to the simple equations containing only weight and length or the impedance index.

At 6 weeks, the final anthropometric equations (equation 3A) incorporating length, weight, gestational age, birthweight SDS, subscapular skinfold thickness, and abdominal circumference predicted FFM with RMSE of less than 5% of mean FFM. Substituting length with the impedance index (equation 3B) increased the RMSE marginally (+1 g for males and +2 g for females). At 6 months, the final anthropometric equations (equation 3A) predicted FFM with RMSE of less than 6.5% of mean FFM. Substituting length with the impedance index (equation 3B) resulted in increased RMSE in males (+4 g) but decreased RMSE in females (-36 g).

Validation of PEA POD fat-free mass equations

Results from the internal validation are reported in Figures 1–4. As there was a slight reduction in RMSE for the prediction equations using L^2/Z_c at 6 months compared to those using L^2/R_{50} (-0.1% of mean FFM), these equations were also validated, with results reported in Supplementary Figures 5, 6.

When the equations were internally validated, the impedance equations had improved concordance with measured FFM compared to their respective anthropometric equations, except for the simple equations (equations 1A, 2A) among females at 6 weeks (Lin's concordance correlation

coefficient = 0.779 vs. 0.788) (Figures 1–4). The mean absolute percentage errors for each equation were largely comparable (< 5.5%), with slight improvements seen following the addition of gestational age and birthweight SDS (< 5%). Among 6-week-old males, mean absolute percentage error was reduced from 4.0% to 3.5% with the addition of abdominal circumference and subscapular skinfold thickness. Further reductions were seen following the addition of subscapular skinfold thickness and abdominal circumference among 6-week-old males only (MAPE: 3.5% vs. 4.0%).

The LOA analyses revealed that bias for each impedance equation were smaller than their respective anthropometric equations (Figures 5–8), except for the 6-month female equations incorporating birth characteristics (-0.115 vs. -0.141 kg for equations 2A,B, respectively). Limits of agreement were narrower, except for the female 6-week equations, which were marginally increased (ranging from ± 3 to ± 14 g), and the final equations (equations 3A,B) among 6-week-old males and 6-month-old females (increased by ± 6 g and ± 16 g, respectively). The greatest improvements in prediction at the individual level (i.e., reduction in the limits of agreement) were observed when comparing the simple anthropometry and simple impedance equations (equations 1A,B). Bias decreased by approximately 150 g at 6 weeks and 240 g at 6 months, resulting in biases of less than 100 g (equivalent to < 2% of mean FFM), except among 6-week-old girls, where bias was initially low at -53 g and reduced to 35 g ($\pm 0.6\%$ of mean FFM) following the addition of impedance. Limits of agreement narrowed modestly by ± 23 g among 6-week-old boys, though they increased by ± 28 g among girls. At 6 months, limits of agreement narrowed by ± 88 g among males and ± 47 g among females.

Cross-validation with an independent cohort

Characteristics of the University of Queensland cohort are detailed in Supplementary Table 4. When the equations were cross-validated in the University of Queensland cohort, mean absolute percentage errors were largely comparable to the internal validation results (Table 3). The addition of birth covariates (GA and BW_{SDS} , equations 2A,B) resulted in the narrowing of the limits of agreement and the removal of a proportional bias among 6-week-old males. No improvements were seen among the other groups. Likewise, the final models containing additional anthropometric variables (equations 3A,B) did not improve prediction compared to the simpler equations. Therefore, further discussion refers only to the simple equations (equations 1A,B).

At 6 weeks, although concordance was greater and bias reduced with the impedance equations in comparison to the anthropometry equations, the LOA were wider. At 4.5 months, among boys, the 6-month impedance equations had greater

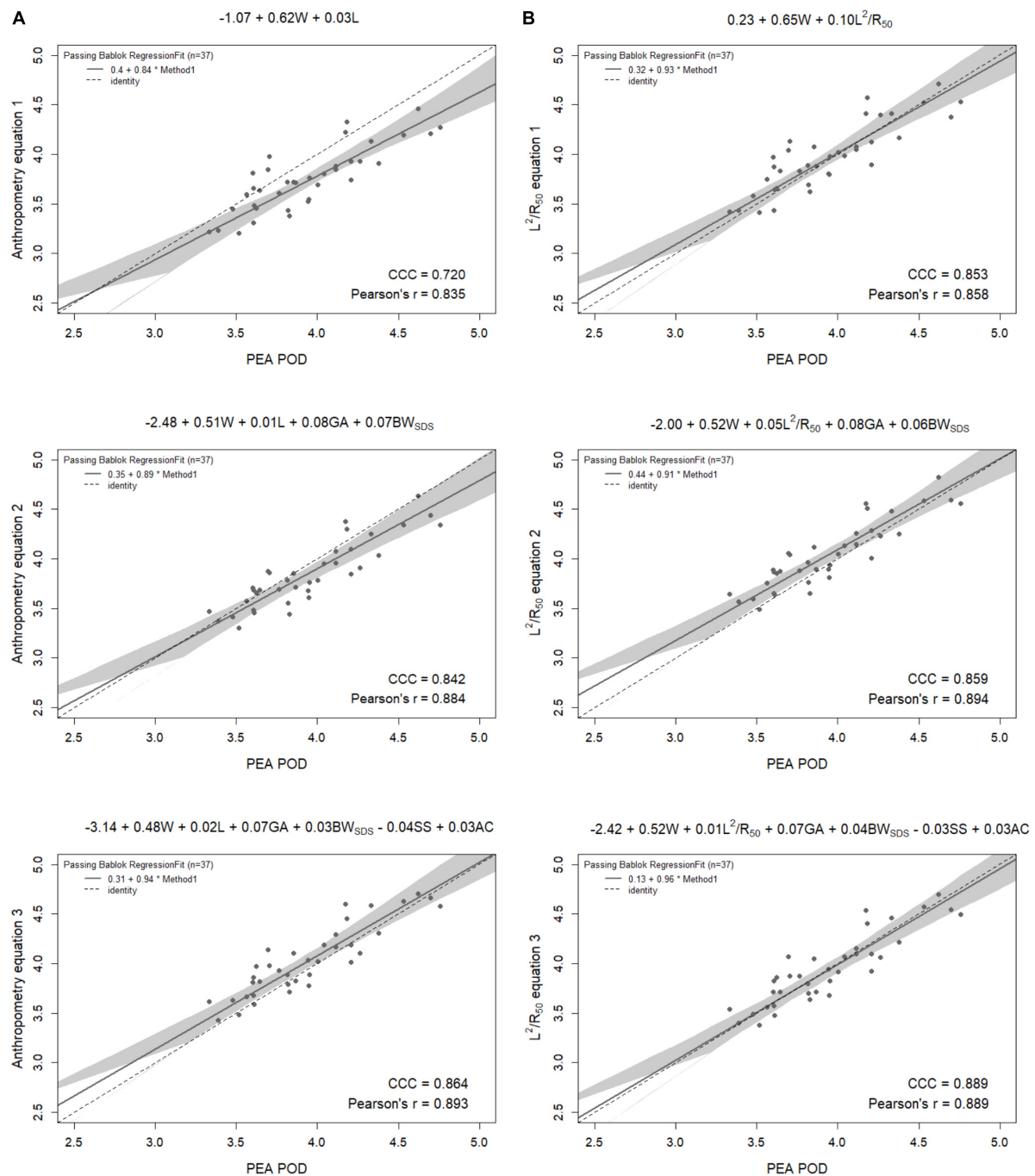


FIGURE 1

Scatterplots of fat-free mass (kg) of 6-week-old males measured by PEA POD and from prediction equations based on weight (W) and (A) recumbent crown-heel length (L) or (B) impedance index (L^2/R_{50}) with stepwise addition of gestational age (GA), birthweight SDS (BW_{SDS}), subscapular skinfold thickness (SS), and abdominal circumference (AC). Dashed lines are the lines of identity. Individual points below the line of identity indicate an underestimation, while those above are an overestimation. CCC is Lin's concordance correlation coefficient and r is Pearson's correlation coefficient.

concordance, reduced bias, and narrower LOA compared to their anthropometric counterparts (Table 3). Among girls, although concordance was lower and bias greater, LOA were narrower with the impedance equations (Table 3).

When the University of Queensland equations were validated in NiPPeR, although bias was often reduced, the LOA were wider than when the simple NiPPeR equations were validated in the University of Queensland (Table 3).

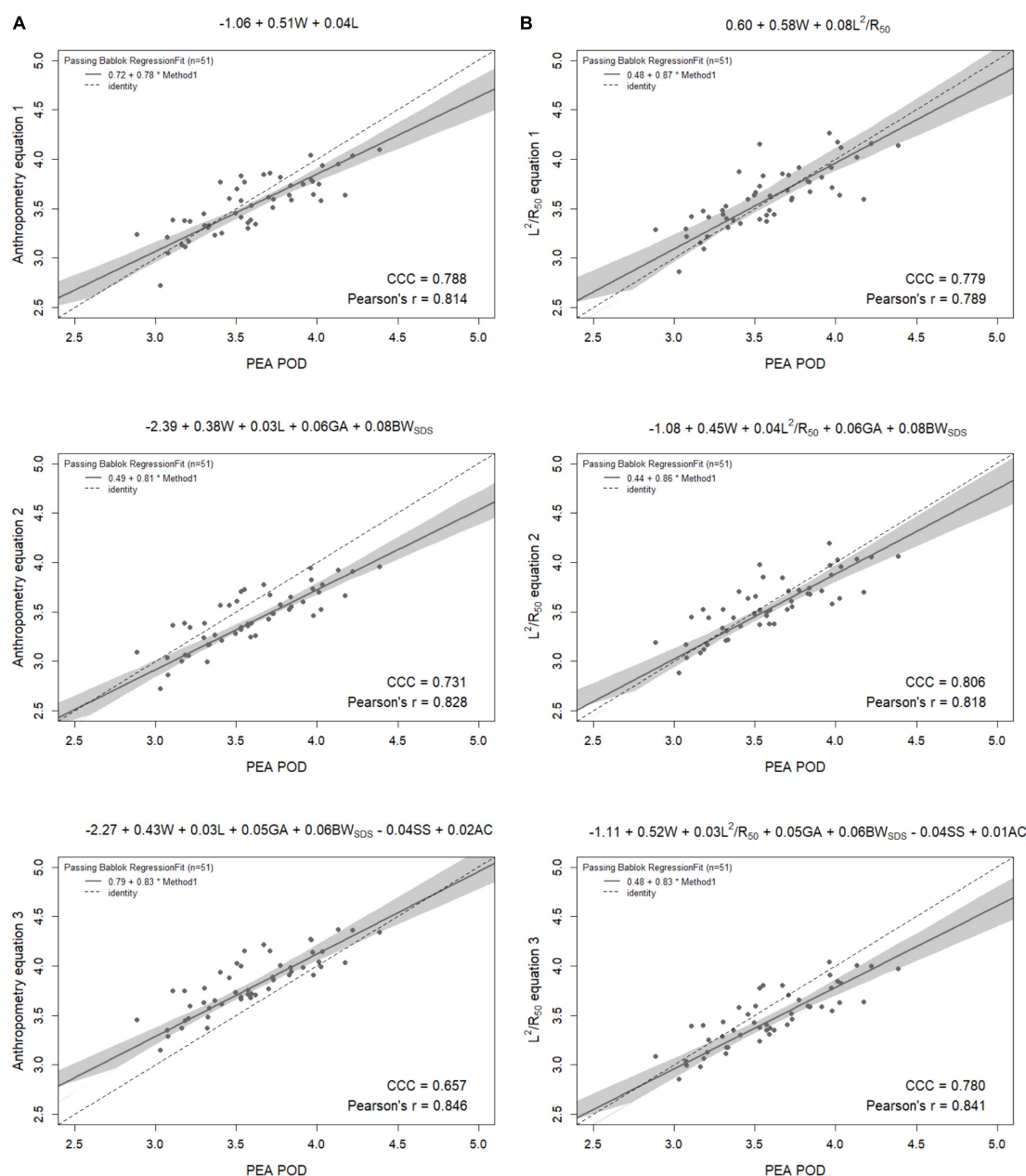


FIGURE 2

Scatterplots of fat-free mass (kg) of 6-week-old females measured by PEA POD and from prediction equations based on weight (W) and (A) recumbent crown-heel length (L) or (B) impedance index (L^2/R_{50}) with stepwise addition of gestational age (GA), birthweight SDS (BW_{SDS}), subscapular skinfold thickness (SS), and abdominal circumference (AC). Dashed lines are the lines of identity. Individual points below the line of identity indicate an underestimation, while those above are an overestimation. CCC is Lin's concordance correlation coefficient and r is Pearson's correlation coefficient.

Impact of ethnicity on prediction equations

Characteristics of the included White Caucasian and Chinese cohorts are detailed in [Supplementary Tables 5, 6](#). There were no differences in any characteristics between the development and validation groups.

The ethnicity-specific equations are detailed in [Supplementary Table 7](#). The contribution of each variable to the prediction of FFM varied between the ethnicities; however, weight still predominated. Impedance was generally a greater contributor to the prediction of FFM among Chinese than White Caucasian infants. Among Chinese infants, the equations based on impedance had lower

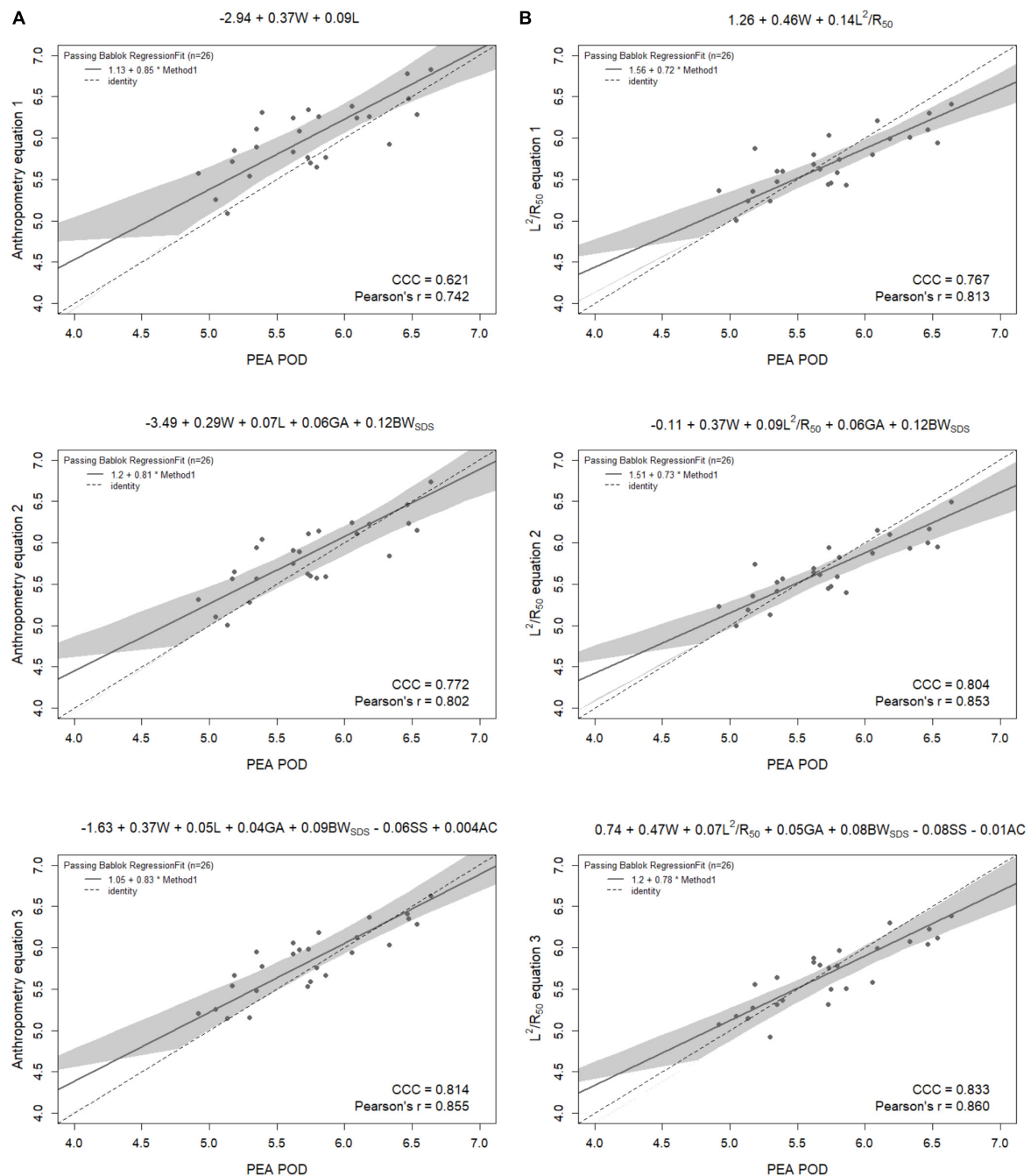


FIGURE 3

Scatterplots of fat-free mass (kg) of 6-month-old males measured by PEA POD and from prediction equations based on weight (W) and (A) recumbent crown-heel length (L) or (B) impedance index (L^2/R_{50}) with stepwise addition of gestational age (GA), birthweight SDS (BW_{SDS}), subscapular skinfold thickness (SS), and abdominal circumference (AC). Dashed lines are the lines of identity. Individual points below the line of identity indicate an underestimation, while those above are an overestimation. CCC is Lin's concordance correlation coefficient and r is Pearson's correlation coefficient.

RMSE than their anthropometry counterparts, except for the 6-month male equations. The reverse was true among White Caucasian infants, with the anthropometry equations predicting FFM with lower RMSE, except among 6-month females (Supplementary Table 7). RMSE was

consistently lower for the ethnicity-specific equations than the main equations; however, when applied to the ethnicity-specific validation cohorts, there was no clear benefit in using the ethnicity-specific equations (Supplementary Tables 8, 9).

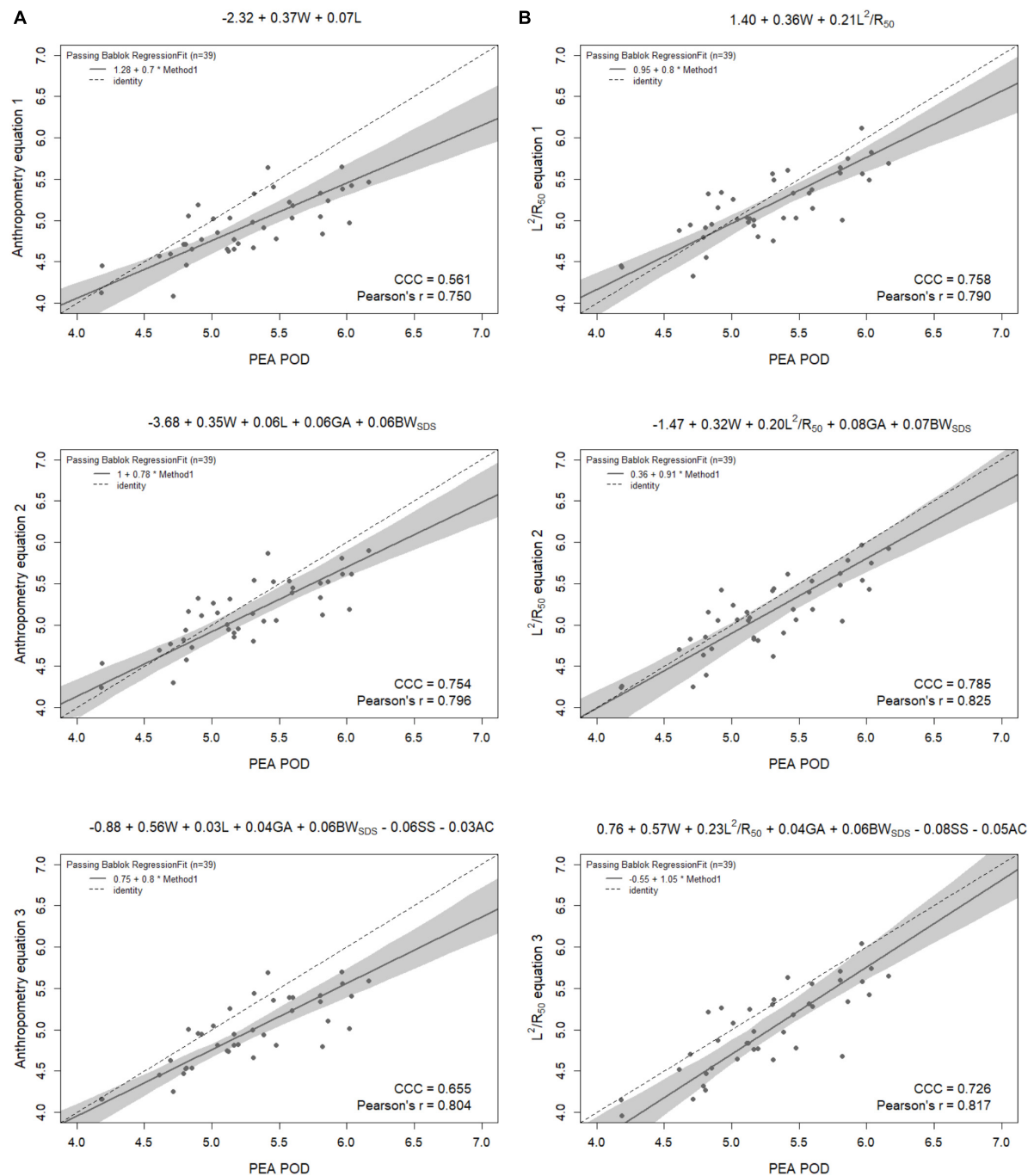


FIGURE 4

Scatterplots of fat-free mass (kg) of 6-month-old females measured by PEA POD and from prediction equations based on weight (W) and (A) recumbent crown-heel length (L) or (B) impedance index (L^2/R_{50}) with stepwise addition of gestational age (GA), birthweight SDS (BW_{SDS}), subscapular skinfold thickness (SS), and abdominal circumference (AC). Dashed lines are the lines of identity. Individual points below the line of identity indicate an underestimation, while those above are an overestimation. CCC is Lin's concordance correlation coefficient and r is Pearson's correlation coefficient.

Mixture theory modeling

Several combinations of mixture theory coefficients (ρ_{ECF} , ρ_{ICF} , Kb, and Db) were assessed. These were mostly equivalent,

except for the equation by Moissl et al. (43), which consistently had the worst performance. The defaults built into the SFB7 device (i.e., BioImp defaults— ρ_{ECW} and ρ_{ICW} 235.5 and 894.2 Ω/cm for females and 273.9 and 937.2 Ω/cm for males; Db

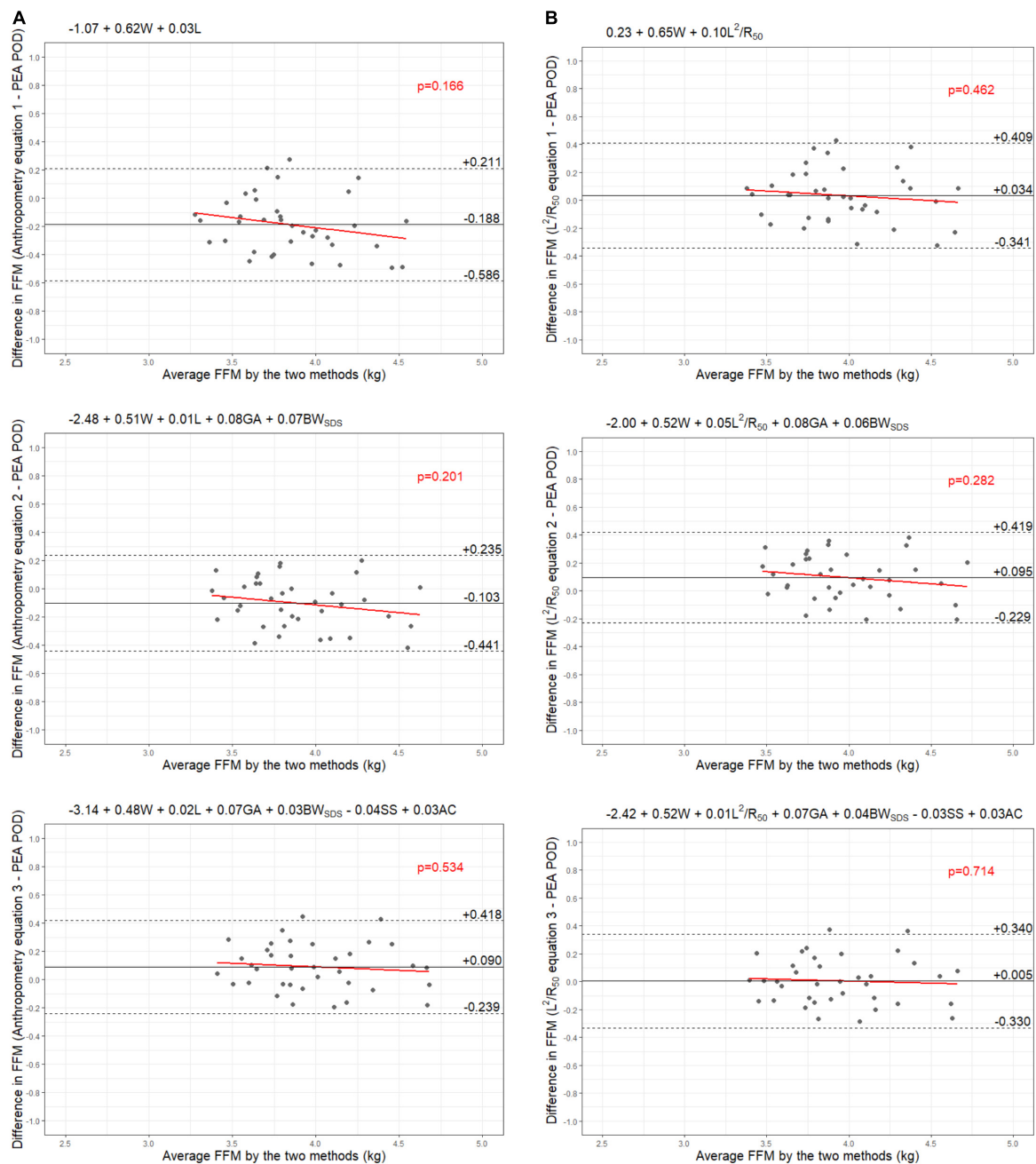


FIGURE 5

Bland-Altman plots comparing fat-free mass (FFM) (kg) of 6-week-old males measured by PEA POD and from prediction equations based on weight (W) and (A) recumbent length (L) or (B) impedance index (L^2/R_{50}) with stepwise addition of gestational age (GA), birthweight SDS (BW_{SDS}), subscapular skinfold thickness (SS), and abdominal circumference (AC).

1.05 g/L), when combined with the Kb from Collins et al. (16) (i.e., 3.78), performed best in our cohort (Supplementary Figures 7–10 and Supplementary Tables 10, 11). Nonetheless, performance was poor compared to the empirical equations, with an overall mean absolute percentage error of approximately 11% at 6 weeks and 12% at 6 months. At 6 weeks, bias was low at 38 g overall (70 g for males and 12 g for females), but

LOA were very wide at ± 1 kg. At 6 months, bias was larger at 0.333 kg overall (0.364 kg for males and 0.313 kg for females), with LOA being larger still (± 1.4 kg, $\pm 27.9\%$ of mean FFM). At 6 months, the use of the Bioimp default Kb value (i.e., 4.3) resulted in reduced bias (~ 0.120 kg) but marginally increased LOA (± 1.5 kg, $\pm 25.7\%$ of mean FFM) (Supplementary Figures 7–10 and Supplementary Tables 10, 11).

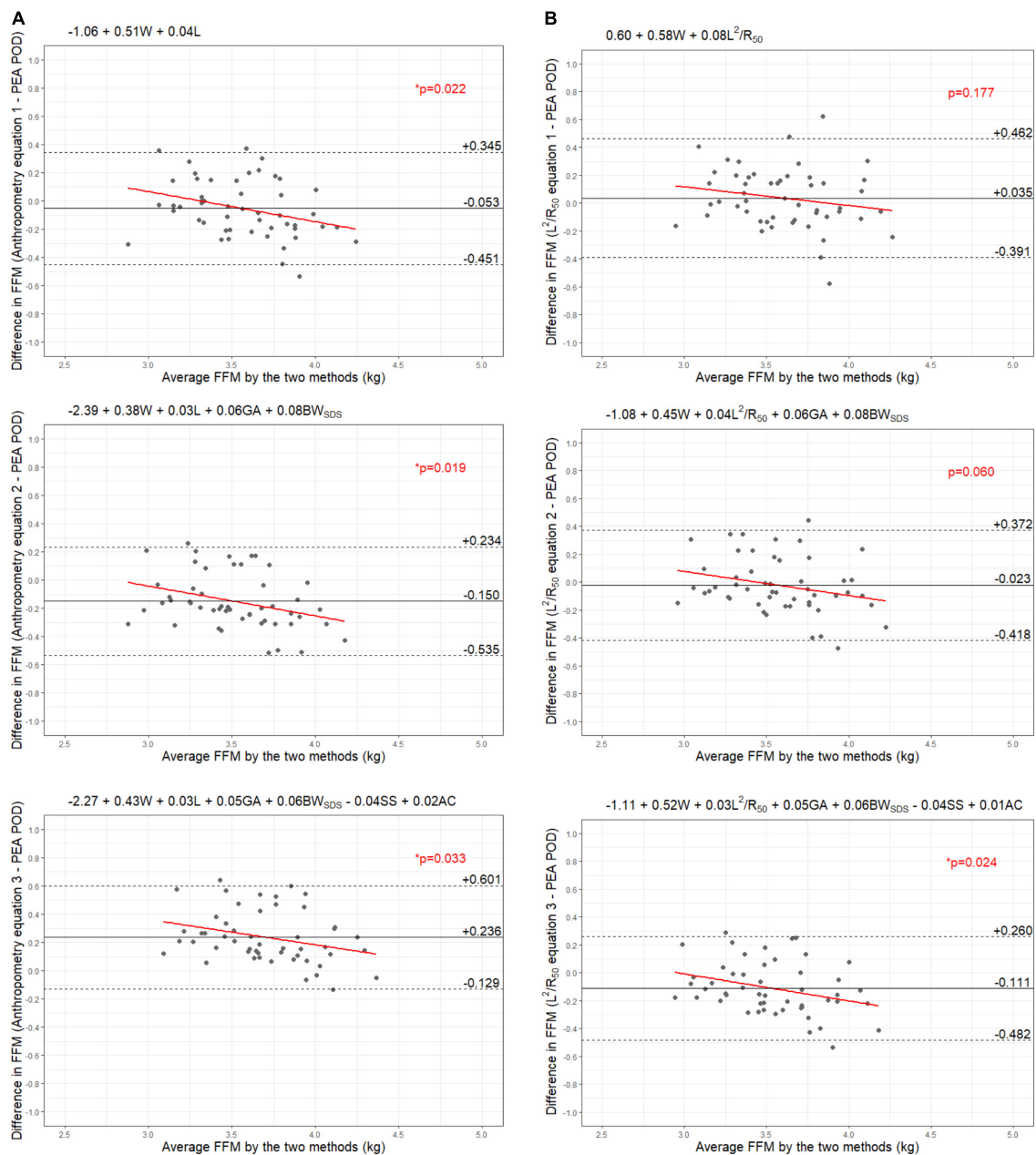


FIGURE 6

Bland-Altman plots comparing fat-free mass (FFM) (kg) of 6-week-old females measured by PEA POD and from prediction equations based on weight (W) and (A) recumbent length (L) or (B) impedance index (L^2/R_{50}) with stepwise addition of gestational age (GA), birthweight SDS (BW_{SDS}), subscapular skinfold thickness (SS), and abdominal circumference (AC).

Discussion

Prediction equations for FFM were developed considering weight, length or impedance, gestational age, birthweight SDS, subscapular skinfold thickness, and abdominal circumference as predictors. Substitution of length with the impedance index marginally increased the accuracy of the equations among boys at 6 weeks and girls at 6 months but decreased accuracy among

boys at 6 months and girls at 6 weeks. When internally validated, the impedance equations improved group and individual-level accuracy (smaller biases and narrower LOA, respectively). However, improvements were modest and not consistently observed (e.g., LOA marginally increased among 6-week-old girls). Adding clinical predictors (i.e., gestational age, birthweight SDS, subscapular skinfold thickness, and abdominal circumference) only marginally improved model accuracy,

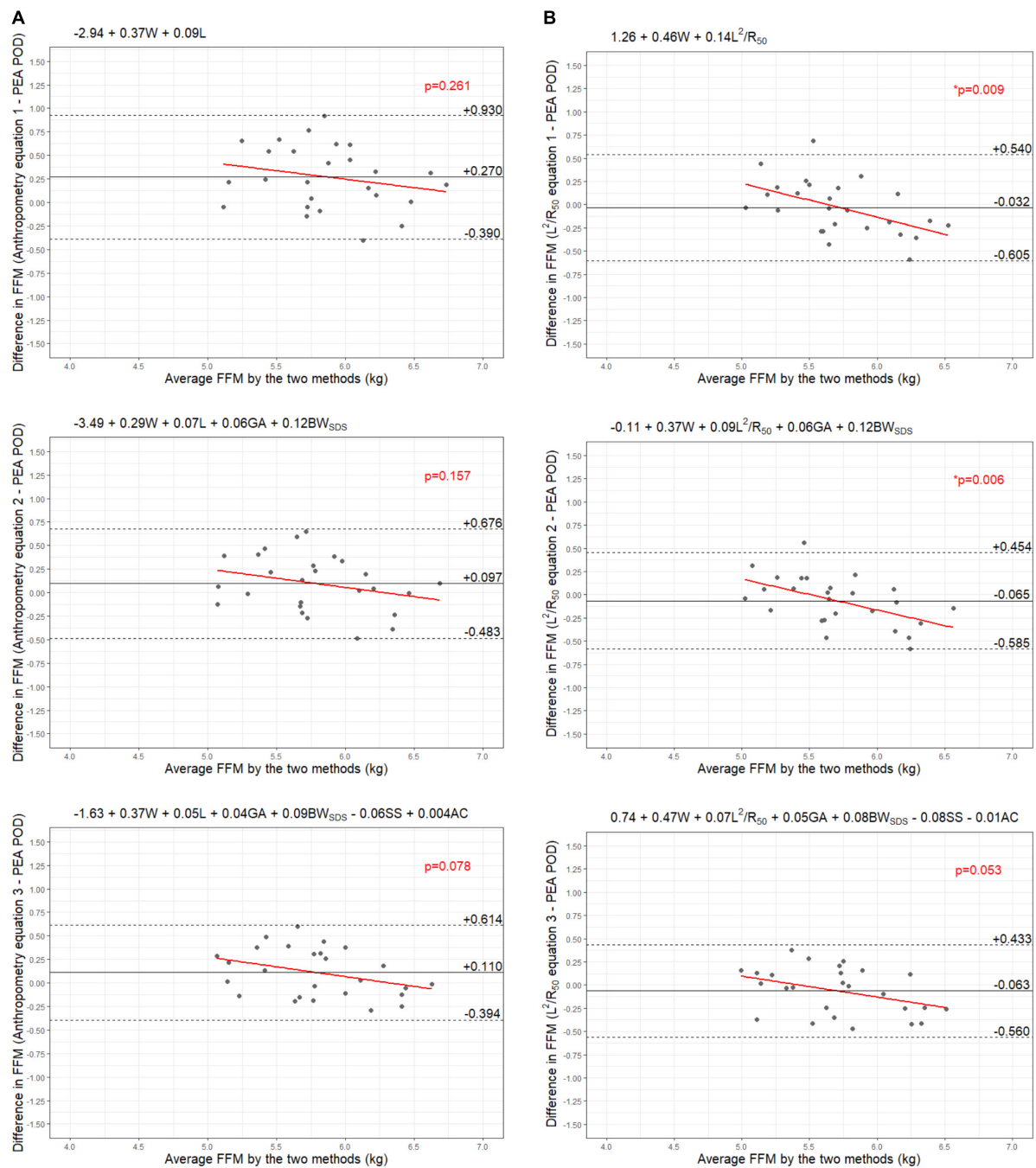


FIGURE 7

Bland-Altman plots comparing fat-free mass (FFM) (kg) of 6-month-old males measured by PEA POD and from prediction equations based on weight (W) and (A) recumbent length (L) or (B) impedance index (L^2/R_{50}) with stepwise addition of gestational age (GA), birthweight SDS (BW_{SDS}), subscapular skinfold thickness (SS), and abdominal circumference (AC).

and improved performance was not consistently observed when the equations were validated. While empirical equations could accurately predict FFM, mixture theory estimates were dramatically different to the reference FFM derived from air displacement plethysmography. Our findings add to the limited literature evaluating the validity of bioimpedance in infancy.

Previous impedance studies have reported biases ranging from 0 to 15% and LOA from $\pm 5\%$ to $\pm 36\%$ of mean FFM or TBW (11). Similar to our evaluation of mixture theory modeling, Collins et al. (16) found estimates of TBW derived from mixture theory to be inaccurate compared to stable isotope dilution. In contrast, Tint et al. (28) developed empirical

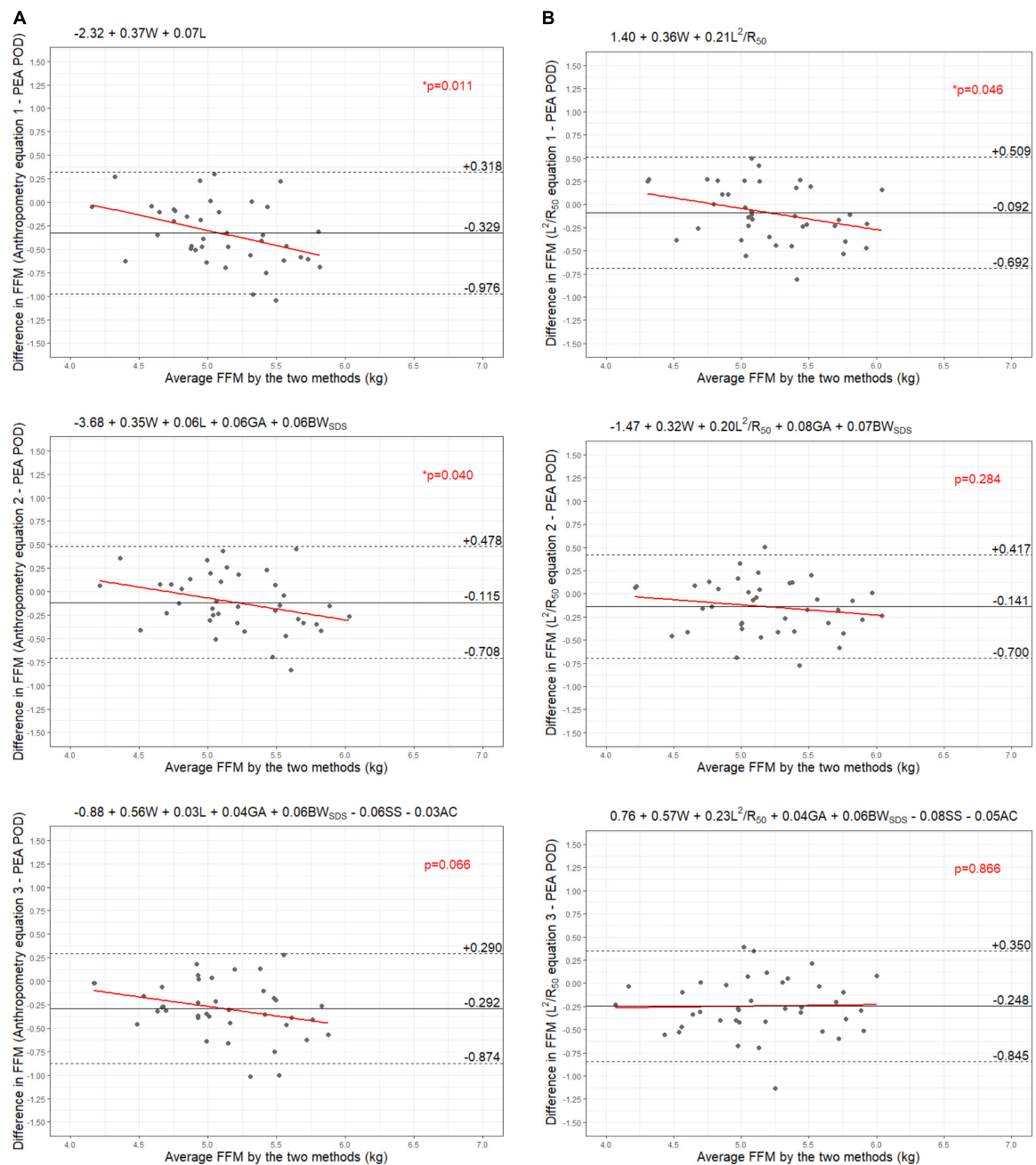


FIGURE 8

Bland-Altman plots comparing fat-free mass (FFM) (kg) of 6-month-old females measured by PEA POD and from prediction equations based on weight (W) and (A) recumbent length (L) or (B) impedance index (L^2/R_{50}) with stepwise addition of gestational age (GA), birthweight SDS (BW_{SDS}), subscapular skinfold thickness (SS), and abdominal circumference (AC).

equations for FFM among neonates in reference to PEA POD. When validated, these equations produced small biases and narrow LOA, with comparable results only when externally validated among infants of a similar age (28). When our equations were cross-validated among infants from the study by Lingwood et al. (17), bias and LOA were largely comparable, although bias was increased when the 6-month equations were

validated among the cohort of 4.5-month-olds (males: 0.56% vs. 2.04%; and females 1.74% vs. -5.00%); corroborating the need to have a suitable age-match when applying empirical impedance equations.

At both 6 weeks and 6 months, we observed the greatest benefit of using bioimpedance when substituting length with the impedance index when comparing the simple equations

TABLE 3 Cross-validation of the NiPPeR and the University of Queensland (UQ) BIA prediction equations.

Origin		Parameters		Equation	Destination		MAPE	CCC	Bias	SD	LOA	p	
NiPPeR	6 week	M	W + L	$-1.07 + 0.62W + 0.03L$	UQ	6 week	M	6.82%	0.729	-0.279 (-6.64%)	0.154 (3.67%)	-0.581, 0.023	0.053
		F	$-1.06 + 0.51W + 0.04L$	F		4.35%	0.753	-0.129 (-3.39%)	0.183 (4.82%)	-0.488, 0.230	0.093		
		M	W + L + GA + BW _{SDS}	$-2.48 + 0.51W + 0.01L + 0.08GA + 0.07BW_{SDS}$		M	4.28%	0.831	-0.165 (-3.93%)	0.144 (3.43%)	-0.448, 0.118	0.581	
		F	$-2.39 + 0.38W + 0.03L + 0.06GA + 0.08BW_{SDS}$	F		6.43%	0.633	-0.226 (-5.95%)	0.186 (4.89%)	-0.591, 0.139	0.056		
		M	W + L + GA + BW _{SDS} + SS + AC	$-3.14 + 0.48W + 0.02L + 0.07GA + 0.03BW_{SDS} - 0.04SS + 0.03AC$		M	2.80%	0.908	0.001 (0.02%)	0.154 (3.67%)	-0.300, 0.302	0.410	
		F	$-2.27 + 0.43W + 0.03L + 0.05GA + 0.06BW_{SDS} - 0.04SS + 0.02AC$	F		5.63%	0.694	0.172 (4.53%)	0.187 (4.92%)	-0.195, 0.540	0.046		
		M	W + L ² /R ₅₀	$0.23 + 0.65W + 0.10L^2/R_{50}$		M	3.23%	0.884	-0.021 (-0.50%)	0.194 (4.62%)	-0.402, 0.360	0.048	
		F	$0.60 + 0.58W + 0.08L^2/R_{50}$	F		3.29%	0.783	-0.020 (-0.53%)	0.197 (5.18%)	-0.406, 0.366	0.047		
		M	W + L ² /R ₅₀ + GA + BW _{SDS}	$-2.00 + 0.52W + 0.05L^2/R_{50} + 0.08GA + 0.06BW_{SDS}$		M	3.04%	0.903	0.051 (1.21%)	0.154 (3.67%)	-0.252, 0.353	0.926	
		F	$-1.08 + 0.45W + 0.04L^2/R_{50} + 0.06GA + 0.08BW_{SDS}$	F		3.96%	0.750	-0.090 (-2.37%)	0.197 (5.18%)	-0.477, 0.297	0.038		
		M	W + L ² /R ₅₀ + GA + BW _{SDS} + SS + AC	$-2.42 + 0.52W + 0.01L^2/R_{50} + 0.07GA + 0.04BW_{SDS} - 0.03SS + 0.03AC$		M	3.13%	0.882	-0.086 (-2.05%)	0.158 (3.76%)	-0.395, 0.223	0.852	
		F	$-1.11 + 0.52W + 0.03L^2/R_{50} + 0.05GA + 0.06BW_{SDS} - 0.04SS + 0.01AC$	F		5.70%	0.636	-0.191 (-5.03%)	0.197 (5.18%)	-0.577, 0.195	0.011		
	6 month	M	W + L	$-2.94 + 0.37W + 0.09L$	4.5 month	M	6.42%	0.784	0.308 (5.70%)	0.213 (3.94%)	-0.110, 0.726	0.353	
		F	$-2.32 + 0.37W + 0.07L$	F		4.84%	0.564	0.102 (2.08%)	0.303 (6.18%)	-0.490, 0.695	0.270		
		M	W + L + GA + BW _{SDS}	$-3.49 + 0.29W + 0.07L + 0.06GA + 0.12BW_{SDS}$		M	7.30%	0.725	0.350 (6.48%)	0.231 (4.28%)	-0.102, 0.802	0.075	
		F	$-3.68 + 0.35W + 0.06L + 0.06GA + 0.06BW_{SDS}$	F		6.02%	0.486	-0.170 (-3.47%)	0.324 (6.61%)	-0.806, 0.466	0.301		
		M	W + L + GA + BW _{SDS} + SS + AC	$-1.63 + 0.37W + 0.05L + 0.04GA + 0.09BW_{SDS} - 0.06SS + 0.004AC$		M	5.91%	0.735	0.266 (4.93%)	0.262 (4.85%)	-0.248, 0.780	< 0.001	
		F	$-0.88 + 0.56W + 0.03L + 0.04GA + 0.06BW_{SDS} - 0.06SS - 0.03AC$	F		6.13%	0.404	0.156 (3.18%)	0.354 (7.22%)	-0.537, 0.849	0.076		

(Continued)

TABLE 3 (Continued)

Origin		Parameters	Equation	Destination		MAPE	CCC	Bias	SD	LOA	<i>p</i>		
UQ	6 week	M	$W + L^2/R_{50}$	$1.26 + 0.46W + 0.14L^2/R_{50}$	M	3.46%	0.920	0.110 (2.04%)	0.191 (3.54%)	−0.263, 0.484	0.723		
		F		$1.40 + 0.36W + 0.21L^2/R_{50}$	F	5.73%	0.526	−0.245 (−5.00%)	0.276 (5.63%)	−0.786, 0.295	0.330		
		M	$W + L^2/R_{50} + GA + BW_{SDS}$	$−0.11 + 0.37W + 0.09L^2/R_{50} + 0.06GA + 0.12BW_{SDS}$	M	5.54%	0.822	0.260 (4.81%)	0.196 (3.63%)	−0.124, 0.645	0.082		
		F		$−1.47 + 0.32W + 0.20L^2/R_{50} + 0.08GA + 0.07BW_{SDS}$	F	6.43%	0.480	−0.258 (−5.27%)	0.303 (6.18%)	−0.851, 0.335	0.328		
		M	$W + L^2/R_{50} + GA + BW_{SDS} + SS + AC$	$0.74 + 0.47W + 0.07L^2/R_{50} + 0.05GA + 0.08BW_{SDS} − 0.08SS − 0.01AC$	M	5.13%	0.780	0.104 (1.93%)	0.296 (5.48%)	−0.477, 0.685	< 0.001		
		F		$0.76 + 0.57W + 0.23L^2/R_{50} + 0.04GA + 0.06BW_{SDS} − 0.08SS − 0.05AC$	F	7.02%	0.443	0.099 (2.02%)	0.392 (8.00%)	−0.671, 0.868	0.295		
	4.5 month	6 week	W + S + L	$0.260 + 0.528W − 0.125S + 0.022L$	NiPPeR	6 week	M	4.63%	0.822	0.092 (2.36%)	0.200 (5.13%)	−0.300, 0.485	< 0.001
						F	4.48%	0.810	0.073 (2.03%)	0.199 (5.53%)	−0.317, 0.462	< 0.001	
			W + S + L ² /R ₀	$1.169 + 0.568W − 0.128S + 0.032L^2/R_0$		M	7.08%	0.695	0.243 (6.23%)	0.199 (5.10%)	−0.148, 0.633	< 0.001	
						F	6.76%	0.702	0.195 (5.42%)	0.206 (5.72%)	−0.209, 0.598	< 0.001	
		W + S + L ² /R _∞	$1.322 + 0.588W − 0.148S + 0.009L^2/R_{\infty}$		M	4.74%	0.808	0.101 (2.59%)	0.205 (5.26%)	−0.300, 0.502	< 0.001		
					F	4.93%	0.798	0.067 (1.86%)	0.207 (5.75%)	−0.339, 0.472	< 0.001		
		W + S + L ² /Z _c	$1.253 + 0.585W − 0.143S + 0.001L^2/Z_c$		M	4.81%	0.804	0.107 (2.74%)	0.205 (5.26%)	−0.294, 0.508	< 0.001		
					F	4.98%	0.795	0.072 (2.00%)	0.207 (5.75%)	−0.333, 0.478	< 0.001		
		W + S + L ² /R ₅₀	$1.248 + 0.584W − 0.142S + 0.002L^2/R_{50}$		M	4.48%	0.825	0.088 (2.26%)	0.199 (5.10%)	−0.302, 0.479	< 0.001		
					F	4.79%	0.806	0.058 (1.61%)	0.204 (5.67%)	−0.342, 0.458	< 0.001		
	6 month	W + S + L	$−0.044 + 0.397W − 0.427S + 0.045L$		M	4.66%	0.693	−0.051 (−0.89%)	0.339 (5.95%)	−0.715, 0.613	< 0.001		
					F	6.64%	0.541	−0.264 (−5.08%)	0.346 (6.65%)	−0.942, 0.414	< 0.001		
		W + S + L ² /R ₀	$1.909 + 0.280W − 0.279S + 0.305L^2/R_0$		M	4.63%	0.666	−0.063 (−1.11%)	0.343 (6.02%)	−0.735, 0.610	< 0.001		
					F	6.35%	0.539	−0.256 (−4.92%)	0.345 (6.63%)	−0.932, 0.420	< 0.001		
		W + S + L ² /R _∞	$2.484 + 0.416W − 0.430S + 0.040L^2/R_{\infty}$		M	4.56%	0.669	−0.091 (−1.60%)	0.343 (6.02%)	−0.764, 0.582	< 0.001		
					F	6.07%	0.562	−0.263 (−5.06%)	0.336 (6.46%)	−0.921, 0.396	< 0.001		
		W + S + L ² /Z _c	$2.059 + 0.313W − 0.320S + 0.201L^2/Z_c$		M	4.61%	0.674	−0.134 (−2.35%)	0.337 (5.91%)	−0.795, 0.528	< 0.001		
					F	6.33%	0.566	−0.284 (−5.46%)	0.330 (6.35%)	−0.931, 0.363	< 0.001		
		W + S + L ² /R ₅₀	$2.203 + 0.334W − 0.361S + 0.185L^2/R_{50}$		M	4.87%	0.673	−0.100 (−1.75%)	0.351 (6.16%)	−0.788, 0.587	< 0.001		
					F	6.04%	0.585	−0.247 (−4.75%)	0.336 (6.46%)	−0.906, 0.412	< 0.001		

AC, abdominal circumference (cm); BW_{SDS} , INTERGROWTH-21st birthweight standard deviation score; CCC, Lin's concordance correlation coefficient; GA, gestational age (weeks); L, length (cm); LOA, 95% limits of agreement; MAPE, mean absolute percentage error; R_0 , resistance at very low, i.e. 0 kHz (Ω); R_{50} , resistance at 50 kHz (Ω); R_{∞} , resistance at very high, i.e. ∞ kHz (Ω); SD, standard deviation; SS, subscapular skinfold thickness (mm); W, weight (kg); Z_c , impedance at the characteristic frequency (Ω).

based only on weight combined with length or impedance. Tint et al. (28) reported that substitution of length with the impedance index resulted in reduced bias at birth (−80 g) and marginally reduced bias (−20 g) but increased LOA at 2 weeks (± 6.8 vs. $\pm 6.4\%$ mean FFM). In contrast, Lingwood et al. (17) saw increased bias and LOA, except among their 4.5-month-old cohort, concluding that bioimpedance may improve prediction in older infants. In our study, bias was reduced, and LOA decreased (or largely unchanged, i.e., 6-week-old females) when comparing impedance-based equations to anthropometry-based equations. Prediction of FFM may be improved by use of an impedance-based prediction equation, though improvements are modest and may not be sufficient to justify routine use of BIA in infancy.

Although the overall percentage of FFM variance explained by the prediction equations decreased with increasing age, the contribution of length and impedance increased while the contribution of weight decreased. Among 6-week-old boys, length and the impedance index had similar standardized beta coefficients, whereas, for girls, length was a stronger predictor. At 6 months, the reverse was observed. These findings are consistent with the observation that the correlations between length or impedance and FFM varied according to sex. Studies have previously reported that correlations between FFM and impedance increase with increasing age in infancy (17, 28); however, correlations have not been reported separately according to sex. The divergent trends observed in our cohort have not previously been reported. These data suggest that in late infancy and early childhood, the inclusion of bioimpedance parameters may improve the prediction of FFM; however, sex differences may be apparent.

In addition to impedance and weight, prior studies have also considered sex (17, 28, 44–46) and gestational age (43) as potentially important covariates in bioimpedance-based prediction equations. Raghavan et al. (47) evaluated whether the addition of gestational age improved the estimation of TBW among their cohort of very low birthweight (< 1,200 g), preterm neonates, with the inclusion of gestational age improving the aR^2 from 90 to 97%. In the study by Aris et al. (48), inclusion of gestational age marginally improved anthropometry-based prediction of neonatal FM; however, in contrast to the previous study, neonates were all term-born ($37^{0/7}$ – $41^{6/7}$). In our study, pre- and post-term infants were excluded from the analyses ($n = 17$ and 21 , at 6 weeks and 6 months, respectively), and infants were measured beyond the neonatal period, during which gestational age is likely to have a larger impact on body composition (49). Nonetheless, the addition of gestational age and birthweight SDS increased the absolute aR^2 by approximately 4 to 5%, with standardized regression coefficients being greater for birthweight SDS than gestational age among girls. In contrast, the reverse was observed among boys. To our knowledge, no previous study has evaluated the

contribution of birthweight SDS to the prediction of body composition in infancy.

We also evaluated whether the inclusion of additional anthropometric measurements could improve the prediction of FFM. Although skinfold thicknesses have previously been used in anthropometry-based prediction equations (35, 48, 50–54), we are not aware of any study that has evaluated bioimpedance in combination with skinfold thicknesses in infancy. The addition of skinfold thickness increased the percentage of explained variance by 2% among 3-month-olds in the Baby-bod study (54), whereas in another study, R^2 was increased by 9% at 3 days and 15 weeks and by 23% at 54 weeks (35). Among our cohort at 6 weeks, the addition of subscapular skinfold thickness and abdominal circumference increased the absolute aR^2 by 1 to 2%, whereas aR^2 increased by 5 to 10% at 6 months, suggesting greater importance of these variables in late infancy. Nonetheless, the inclusion of these covariates did not consistently improve the prediction of FFM when the equations were validated internally and externally, which may be related to the high degree of measurement error associated with these anthropometric parameters (55, 56).

Strengths of the current study include using a device capable of BIS, evaluating multiple bioimpedance parameters, evaluating both empirical equations and mixture theory prediction, including additional clinically-relevant covariates, and the availability of an external cohort for validation. By using a device capable of BIS, in addition to being able to evaluate both empirical equations and mixture theory prediction, poor quality files could easily be identified and deleted. Standardization of measurements can be challenging in infancy, as evidenced by the many poor quality files removed prior to analysis; if SFBIA had been used, these might not have been identified. Though we evaluated multiple bioimpedance parameters (R_{50} , R_0 , R_{∞} , and Z_c), performance was similar; therefore, we reported equations based on resistance at 50 kHz. This will enable those with SFBIA devices to use our equations. We also evaluated several equations (including those based on weight, sex, and impedance only); therefore, our equations can be used among cohorts who did not collect additional data (i.e., gestational age, birthweight, subscapular skinfold thickness, and abdominal circumference). When externally validated, the prediction of FFM was not improved by the inclusion of these additional covariates. However, the University of Queensland cohort was measured using different skinfold calipers (Harpender skinfold caliper, Baty International, Burgess Hill, West Sussex, UK). Further validation of our equations in external cohorts may help determine whether there is any added benefit to the prediction of FFM with bioimpedance from the inclusion of these covariates.

A limitation of our study was the use of the PEA POD as a reference standard. Although reproducible and widely used in pediatric studies (6), when the PEA POD was validated against a multi-compartment model, estimates of body fat percentage were very wide, at $\pm 44\%$ of mean body fat

percentage (3). Notably, the PEA POD's weight restriction limited the number of infants available to be studied at 6 months. Thus, the cohort was not reflective of the overall NiPPeR cohort, as larger offspring could not be measured. We were also unable to standardize several factors that may influence bioimpedance measurements: feeding, voiding, and movement. Though fed versus fasted status may influence bioimpedance measurements, it would not be ethical to fast infants prior to measurement. Nonetheless, Gridneva et al. (57) found that differences between pre- and post-feed measurements in infants were not statistically significant. We observed no differences in impedance parameters according to the category of time of last meal (<30 min, 30 min–1 h, 1–2 h, >2 h) nor time of last void (<30 min or \geq 30 min) among our cohort at 3.5 years (58). Likewise, Randhawa et al. (59) observed no differences in mean body fat percentage from bioimpedance measurements according to feeding, voiding, or exercise among adults.

In summary, we developed empirical prediction equations to estimate FFM in infancy. While the inclusion of impedance in the equations instead of solely anthropometric parameters improved performance in most cases, the difference was small. BIA appears to be a useful modality to improve the estimation of FFM in infancy, when available. The addition of clinically relevant covariates (gestational age, birth weight SDS, subscapular skinfold thickness, and abdominal circumference) did not improve the prediction of FFM when the empirical equations were externally validated, though differences existed between the cohorts. Mixture theory estimates of FFM from BIS were inaccurate. Further investigation is required before routine use of BIA in infancy can be recommended.

Data availability statement

The datasets presented in this article are not readily available because the participants did not consent to open access data sharing, and this is an ongoing longitudinal study in which there will be further future analyses conducted. Requests to access the datasets should be directed to WC, w.cutfield@auckland.ac.nz.

Ethics statement

The studies involving human participants were reviewed and approved by the Health Research Authority National Research Ethics Service Committee South Central Research Ethics Committee (Southampton–15/SC/0142), the National Healthcare Group Domain Specific Review Board Singapore (2015/00205), and in New Zealand, the Northern A Health and Disability Ethics Committee (15/NTA/21/AM20). Written

informed consent to participate in this study was provided by the participant's legal guardian/next of kin.

Author contributions

WC, TK, and BA supervised all aspects of the research study. KG, S-YC, and WC led the NiPPeR trial conception and design. JL-R, LW, M-TT, TK, KG, and WC planned the analyses. JL-R and LW prepared the data for analysis. LW analyzed the data using mixture theory modeling. JL-R and JD carried out all other statistical analyses. JL-R wrote the manuscript with critical input from all other authors. All authors have approved the final version of this manuscript and had agreed to be accountable for all aspects of this work.

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Conflict of interest

CM and JR are employees of Société des Produits Nestlé S.A. LW provides consultancy services to ImpediMed Ltd., (a manufacturer of devices for bioelectrical impedance analysis). ImpediMed Ltd. was not involved in the inception and conduct of this research, or in the writing of this manuscript. KG had received reimbursement for speaking at conferences sponsored by companies selling nutritional products, and KG, S-YC, and WC are part of an academic consortium that has received research funding from Abbott Nutrition, Nestec, BenevolentAI Bio Ltd., and Danone.

The remaining authors declare that the research was conducted in the absence of any commercial or financial

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnut.2022.980790/full#supplementary-material>

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The impact of an early intervention home-based program on body composition in preterm-born preschoolers with very low birth weight

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Background and aims: Early child interventions focused on the family prevented neurodevelopmental and behavioral delays and can provide more knowledge regarding responsive feeding, thus creating learning opportunities to promote better quality nutrition and preventing failure to thrive. The aim is to verify the impact of a continuous program of early home-based intervention on the body composition of preschool infants who were born preterm with very low birth weight (VLBW).

Methods: This is a longitudinal analysis from a randomized controlled trial, including VLBW preterm children, born in a tertiary hospital in Southern Brazil and followed up at the high-risk institutional ambulatory clinic. Participants were divided into the intervention group (IG): skin-to-skin care with the mother (kangaroo care), breastfeeding policy, and tactile-kinesthetic stimulation by mothers until hospital discharge. Subsequently, they received a program of early intervention with orientation and a total of 10 home visits, independently from the standard evaluation and care that was performed following the 18 months after birth; conventional group (CG): standard care according to the routine of the newborn intensive care unit (NICU), which includes kangaroo care, and attending to their needs in the follow-up program. Body composition estimation was performed using bioelectrical impedance analyses (BIA), and physical activity and feeding practices questionnaires were evaluated at preschool age, as well as anthropometric measurements and biochemical analysis.

Results: Data of 41 children at 4.6 ± 0.5 years old were evaluated (CG $n = 21$ and IG $n = 20$). Body weight, height, body mass index, waist and arm circumferences, and triceps and subscapular skinfold did not differ between groups. The IG presented higher segmented fat-free mass (FFM) when

compared to the CG (right arm FFM: 0.74 vs. 0.65 kg, $p = 0.040$; trunk FFM: 6.86 vs. 6.09 kg, $p = 0.04$; right leg FFM: 1.91 vs. 1.73 kg, $p = 0.063$). Interaction analyses showed that segmented FFM and FFM Index were associated with higher iron content in the IG. In the CG, interaction analyses showed that increased visceral fat area was associated with higher insulin resistance index.

Conclusion: An early intervention protocol from NICU to a home-based program performed by the mothers of VLBW preterm children of low-income families presents a small effect on FFM.

KEYWORDS

premature birth, very low birth weight (VLBW), early intervention, body composition, blood chemical analysis, preschool child

Introduction

Preterm birth is considered a health problem worldwide, with a higher incidence in low-income countries, with an average of 12%, compared to 9% in high-income countries (1). Despite preterm birth being the leading cause of death of children aged under 5 years, the improvement of neonatal care and follow-up programs to support this population is increasing survival rates, allowing more children to enter adulthood (2). Furthermore, preterm infants, mainly those born extremely preterm, present a higher incidence of developmental deficits (cognitive, motor, behavioral, communicative, learning, and sensory disorders) and a higher risk for delayed neurodevelopment, a condition that demands early implementation of multidisciplinary actions that may prevent negative outcomes (3, 4). Moreover, preterm infants present a higher risk for chronic and metabolic diseases with aging (5, 6).

Among the factors that increase their vulnerability to developing growth and developmental issues (5) is body composition, which has been associated with neurodevelopment in very low birth weight (VLBW) infants (7, 8). A higher rate of fat-free mass (FFM) was associated with improved cognitive and motor scores at 12 months of corrected age (9), and, on the other hand, a deficit of FFM was associated with neurological impairment in VLBW infants at 24 months of corrected age (10). Body composition studies have shown that preterm infants reach full-term equivalent age with less FFM and a higher percentage of total body fat (%TBF) when compared to their full-term equivalent counterparts (11, 12), as well as lower bone mineral density (13, 14) and increased abdominal adipose tissue (15). Since body growth and mineral accretion occur mainly in the last trimester of gestation, reduced muscle mass and skeletal mineralization could be related to preterm infants (12). Moreover, body fat and FFM gains in preterm infants are associated with several areas of cognitive function (16). Thus,

early intervention, follow-up care for preventive action, and timely detection of possible adverse health outcomes are critical for preterm infants' growth health.

The "first 1,000 days," from conception to 24 months (17), are characterized as a window of opportunity to stimulate a child's developmental domains, such as physical, language, cognitive, and social-emotional (18, 19). A systematic review showed that early intervention significantly affects child development, but it does not affect linear growth, which is more associated with nutritional intervention (20). The literature describes that nutritional intervention promotes short- and long-term health effects after preterm birth (21). Moreover, early tactile and kinesthetic stimulation in VLBW preterm children promoted a borderline higher psychomotor development and increased cognitive development assessed at 2 years corrected age, which did not affect weight, length, and head circumference (22). Early physical therapy intervention also presented a positive impact on VLBW preterm, hence reducing the incidence of motor delay (23). However, it is still not clear if an early intervention program could affect the body composition of the preterm population with advanced age. A single-blind cluster randomized controlled trial showed that early physical activity in the first months of life in term-born children promoted a reduced sum of skinfold, when compared to the non-stimulated group, without any differences in motor development (24).

There are few studies evaluating body composition in preschool VLBW preterm children subjected to early intervention and continuous clinical follow-up. Thus, our main goal is to investigate if a protocol of early home-based intervention program during the first 18 months of corrected age in VLBW preterm affected the body composition once they reach preschool age, comparing them with a group subjected to conventional care protocol. Also, this study investigated if the body composition results in response to the intervention

protocol were related to neonatal, growth, and biochemical characteristics evaluated during these follow-ups.

Materials and methods

Design and study population

This was a longitudinal analysis from a randomized controlled trial that investigated preschool VLBW preterm children that were subjected to an early, continuous, and global intervention with a parent's orientation program in the first 12–18 months of corrected age. This program was a randomized clinical trial (RCT), performed from 2016 to 2019, previously described in the study protocol (4), with children born in the Hospital de Clínicas de Porto Alegre (HCPA), a level-3 referral center for high-risk neonates in South Brazil, with regular follow-up until 5 years of age. The study population was preterm children born at less than 32 weeks of gestational age (GA) or children of birth weight less than 1,500 g during the 48 h after childbirth. Newborns with a major congenital malformation or inborn errors of metabolism, STORCH complex infections, HIV, or autoimmune conditions were excluded.

For the follow-up study, the parents were invited to accompany their children, aged 3 and 5 years, to perform a body composition analysis (Figure 1). Exclusion criteria for this investigation were children that died before reaching preschool age, children with severe motor, cognitive, or organic sequelae that prevented the use of bioimpedance scale (cerebral palsy, autism spectrum disorder, use of orthoses, tracheostomized children, and gastrostomy), and children who did not complete 3 years of age during the study period. The recruitment was performed by phone calls and use of social media to locate the parents with no updated phone numbers in the medical records.

The preschool children evaluated in this study were those who were randomized to one of the two groups from the previous clinical trial (4). The intervention group (IG) was characterized by skin-to-skin care (kangaroo care), breastfeeding policy, and massage therapy made by the mothers until hospital discharge. After discharge, mothers received orientation for continuous global stimulation at home plus 10 home visits by the research team, regardless of the standard evaluation and care provided in the follow-up clinic to all preterm children. The systematic early intervention program was based on developmental milestones, anticipating by a month the evolutionary step acquisition of motor and/or cognitive expected for corrected age. The conventional group (CG) was characterized as receiving standard care according to the routine care of the newborn intensive care unit (NICU) and according to the subject's needs in the follow-up program. The follow-up program used in this study is an extension of neonatal and perinatal care, in which the research team provides conditions to monitor growth, development,

and common morbidities with a multidisciplinary team who can fully assess the child and the caregivers, parents, family members, and the school.

Outcome measures

Physical exam during newborn intensive care unit and follow-up at an institutional ambulatory clinic

Physical exams for measurements of weight (kg), height (cm), head circumference (cm), and body mass index (BMI, kg/m^2) were performed at birth, at NICU discharge, and at monthly and annual assessments as a routine appointment at the ambulatory clinic until the appointment for bioelectrical impedance analysis (BIA). All measurements in the ambulatory clinic were performed by a Neonatologist, using standard techniques and calibrated instruments (electronic scale and stadiometer). Z-scores of weight-for-age (W/A), length-for-age (L/A), and BMI-for-age (BMI/A) were evaluated using the *Anthro*® software (25), considering overweight: BMI/A Z-score $> +1$, obesity: BMI/A Z-score $> +2$, and underweight: BMI/A Z-score < -2 .

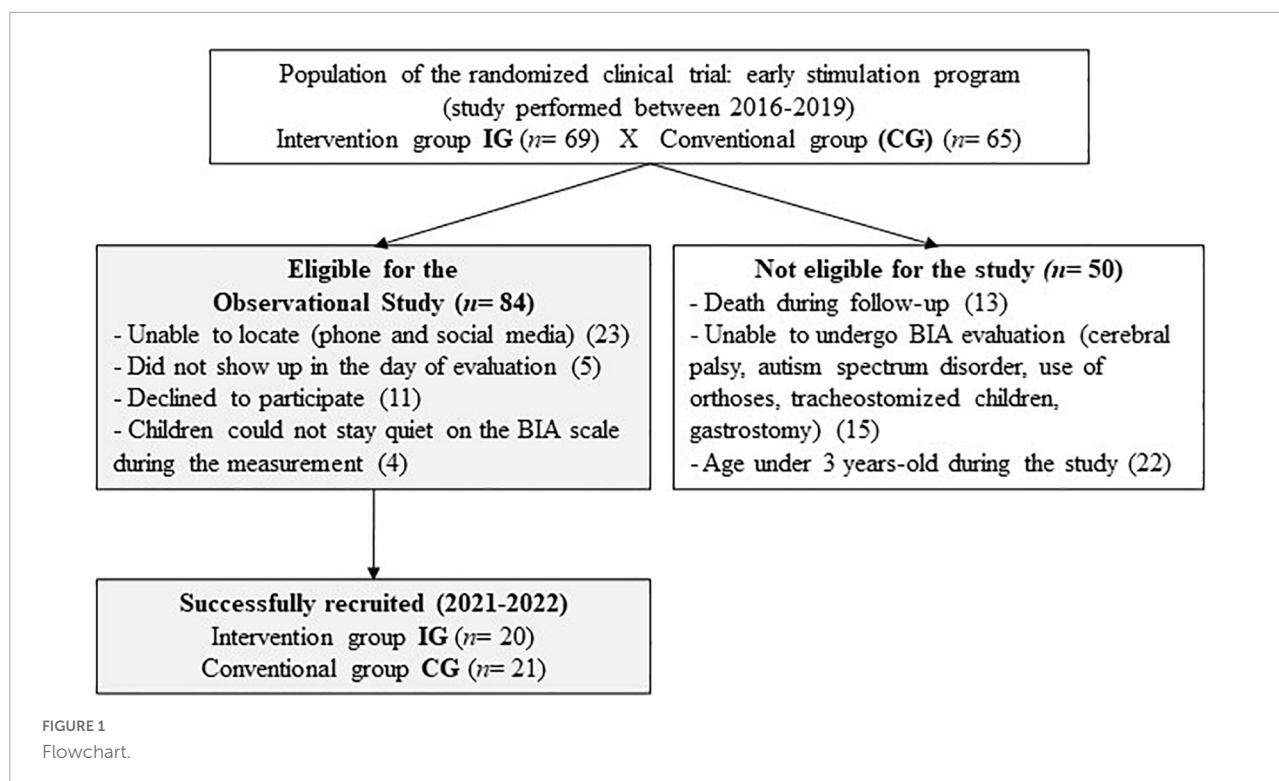
At the evaluation appointment for BIA in the preschool-age children, all anthropometric measurements were performed in duplicate for which the average results were considered: waist circumference (cm) measured with an inelastic measuring tape (in cm) placed at the umbilical scar level at the end of the child's exhale in orthostatic position; arm circumference (cm), measured in the mid-point of the upper arm; triceps skinfold thickness (mm), measured by the midpoint between the acromion and the olecranon; and, subscapular skinfold thickness (mm), measured diagonally below the inferior angle of the scapula. Skinfold measurements (triceps and subscapular) were assessed using a skinfold caliper (*Lange*®, Ann Arbor, Michigan, USA).

Socioeconomic status

Data related to the mother's schooling level (in years) and household income (in BRL per month) were assessed on the day of the follow-up appointment when participants reached preschool age, on the same day of BIA.

Neonatal and follow-up data

Neonatal information from the NICU stay, follow-ups, and clinical appointments was collected from in-hospital patient records. During the NICU period, maternal variables were collected (maternal age, preeclampsia, gestational diabetes, and use of corticosteroid). The neonatal variables were gestational age (GA—evaluated by last menstrual period and confirmed by early obstetrical ultrasound and neonatal clinical examination), birth weight, gender, the status of small for gestational age (SGA; defined as birth weight < 10 th percentile) (26), 5-min Apgar



score, length of hospital stay, necrotizing enterocolitis, central nervous system injury (periventricular hemorrhage and/or leukomalacia), bronchopulmonary dysplasia as oxygenotherapy at 36 weeks corrected age, retinopathy of prematurity, and packed red blood cell transfusion. Anthropometric measures were collected at NICU discharge.

In addition to physical exams in the follow-up clinic, information regarding breastfeeding practices was examined and classified as exclusive breastfeeding, exclusive infant formula, or combination feeding. Exclusive breastfeeding is the action of feeding solely with human milk coming from the mother's breast, with no other liquids or solids (27).

A biochemical exam was also assessed in the records in the follow-up consultation at the 24th month of corrected age. Venous blood sampling (12 h fasting) was collected for routine blood tests according to the follow-up program for measuring hemogram; serum iron ($\mu\text{g/dL}$); total iron binding capacity ($\mu\text{g/dL}$); ferritin and transferrin (mg/dL); total cholesterol; LDL-cholesterol; HDL-cholesterol; non-HDL cholesterol and triglycerides (mg/dL); glucose (mg/dL); insulin ($\mu\text{U/mL}$); and cortisol ($\mu\text{g/dL}$). Dyslipidemia was determined when one or more lipid markers presented values, such as total cholesterol $> 200 \text{ mg/dL}$; LDL-cholesterol $> 130 \text{ mg/dL}$; HDL-cholesterol $< 40 \text{ mg/dL}$; non-HDL cholesterol $> 145 \text{ mg/dL}$; and triglycerides $> 100 \text{ mg/dL}$ (28). The insulin resistance index was calculated as a homeostatic model assessment for insulin resistance (HOMA-IR) $[(\text{glucose (mg/dL)} \times \text{insulin (}\mu\text{U/mL)})/405]$.

Body composition, feeding practices, and physical activity at preschool age

Body composition was evaluated using a multi-frequency bioelectrical impedance analysis (BIA) using InBody 770® (Biospace, South Korea), a tetrapolar electrode configuration system. Parents were instructed to fast their children for at least 3 h before the measurement, and diapers were changed before children got up on the scale. Each participant was positioned in an orthostatic position on a platform with lower electrodes for the feet and the hands holding onto upper electrodes, in which children were to hold this position for 1 min until completion of the measurement. This evaluation accurately measures body weight (BW in kg), the body water content in liters (total body water (TBW), water inside and outside cells, and water in the segments), fat mass (FM; in kg), total body fat (%TBF), fat-free mass ($\text{FFM} = \text{BW} - \text{fat mass}$, in kg; segmented FFM of arms, legs, and trunk—the head is not considered in the segmented measurements), FFM Index (FFMI , in kg/m^2), lean mass ($\text{LM} = \text{water} + \text{proteins} + \text{non-osseous mineral}$; in kg), skeletal muscle mass (in kg), proteins and minerals (in kg), bone mineral content ($\text{BMC} = \text{osseous mineral}$; in kg), visceral fat area (in cm^2), cellular body mass (in kg), arm circumference (in cm), and basal metabolic rate (BMR, in kCal).

Physical activity levels were measured using a structured questionnaire that estimates the sedentary and active time of children, considering activities on weekdays or weekends and day shifts (morning, afternoon, and night), presented as total hours of activity per week. The measure of physical activity

is expressed by the daily time of participation in games and outdoor play while the measure of sedentary behavior is based on the time spent watching television.

Data analysis

Descriptive statistics are shown as mean with standard deviation (\pm SD) or median with interquartile range for continuous variables, according to the Shapiro-Wilk normality test, and counts with proportions for categorical variables. Between-group comparisons were performed with Student's *t*-test, Mann-Whitney *U*-test, Pearson's Chi-squared test, or Fisher's exact test. Cohen's *d* was used to measure effect size for quantitative variables. The power to compare the FFM segment averages between conventional and intervention groups was calculated considering a 5% significance level and the sample size of this study (IG = 20 and CG = 21). First, the relationship between neonatal data (GA, birth weight, and head circumference), physical exam results at 12 months (body weight, height, and BMI/A), and biochemical variables with body composition were explored (TBW, FM, FFM, and BMC) by Pearson's or Spearman's correlation (a hypothesis-generating analyses). Analysis of covariance (ANCOVA) was performed to compare body composition outcomes between groups and to analyze the relationship between them and the biochemical variables. They were adjusted for gender due to literature differences between outcomes by gender (29–31). *P*-values under 0.05 were considered statistically significant. All analyses were performed using the SPSS (Statistical Package for the Social Science) Program Version 18.0 (IBM SPSS Statistical for Windows, Armonk, New York, USA).

Results

Our investigation evaluated 41 VLBW preterm children at preschool age who participated previously in a clinical trial of an early intervention program developed from February 2016 to December 2019. Briefly, of the 134 children from the previous clinical trial, 84 were eligible to participate in this new investigation (Figure 1). For the current study, BIA and physical exam results were collected from December 2020 to June 2022 from the children whose guardians have accepted to participate.

Table 1 shows the characteristics of infants of the general sample population, as well as their respective groups, CG (*n* = 21) and IG (*n* = 20). The mean age of children when BIA was performed was 4.7 ± 0.5 years, 17 (41%) girls and 24 (59%) boys. There were no differences in anthropometric variables, such as body weight, height, waist and arm circumferences, and skinfold measurements from the children of both groups. Overall, 22% of the sample were classified as overweight or obese

and 7% as underweight, without any significant differences between groups. Maternal schooling level and family income did not differ between groups. Regarding neonatal data of the preterm children randomly assigned in each group, the mean gestational age was 28 ± 2 weeks, birth weight $1,073 \pm 318$ g, head circumference 25.5 ± 2.4 cm, and 4 (11%) were SGA without statistical differences between groups. Also, neonatal comorbidities were not statistically different between groups. In both groups, birth weight correlated positively with TBW (IG: $r = 0.462$, $p = 0.041$; CG: $r = 0.501$, $p = 0.021$) and with FFM (IG: $r = 0.461$, $p = 0.041$; CG: $r = 0.487$, $p < 0.025$). Birth weight also correlated positively with FM only in CG: $r = 0.472$, $p = 0.031$ (IG $r = 0.289$, $p = 0.22$), and no significant association with BMC was observed. GA did not present a correlation with TBW, FFM, FM, and BMC when analyzed in each group separately ($p > 0.05$).

Physical examination performed during the first follow-up appointment after NICU discharge and 12 months corrected age did not show a statistical difference between groups. The nutrition offered to the preterm children in the first weeks of life at home did not vary between groups (Table 2). Biochemical analyses performed at 24 months of corrected age did not show statistical differences among groups (Table 3).

Table 4 shows the results of the BIA in preschool age. FM and %TBF did not differ significantly between CG and IG groups. Although the IG presented a non-significant increase in total FFM, FFMI, and skeletal muscle mass when compared to the GC group, they demonstrated a significant increase in segmented FFM in the children subjected to early intervention (right arm ($p = 0.040$), left arm ($p = 0.053$), trunk ($p = 0.040$), and an overall tendency in right leg ($p = 0.063$) and left leg ($p = 0.054$). The statistical power to test if there were a minimal difference of 0.09 kg in the mean of right arm FFM between groups was 57%. For the means of trunk FFM, a power of 53% was calculated, considering a minimal difference of 0.77 kg between groups. Moreover, the means of left leg FFM yielded a 51% power, considering a minimal difference of 0.19 kg among groups. These power values were obtained considering a 5% statistical level. TBW content also showed an increased pattern, as observed in the FFM from IG, although not significant. BMC, BMR, and visceral fat area were similar between groups. Finally, we observed that TBW/FFM ratio in preterm children was 74.3% (95% CI 74.0–74.5).

Regarding physical activity, total active hours during the week were significantly higher in CG (median 16 h (14–19.5) when compared to IG [10 h (6–18); $p = 0.015$]) (data not shown).

Analyses of interaction were performed to investigate if the association between body composition and biochemical analyses differed among groups. Overall, Figure 2 shows that FFM with iron content differed between IG and CG, even after adjusting by gender. IG group showed that FFMI increased by 0.26 kg/m^2 (95%CI: 0.002–0.049; $p = 0.037$) per unit of increased serum iron, but no increase was observed in CG.

TABLE 1 Current and neonatal characteristics of preterm children subjected to the early intervention program compared to conventional care.

Characteristic	General (<i>n</i> = 41)	Conventional (<i>n</i> = 21)	Intervention (<i>n</i> = 20)	<i>P</i> -value	Cohen- <i>d</i>
Current period (preschoolers)					
Age at examination, years	4.6 ± 0.5	4.5 ± 0.5	4.7 ± 0.5	0.758	0.40
Female/male, <i>n</i> (%)	17 (41)/24 (58)	9 (43)/12 (57)	8 (40)/12 (60)	0.602	–
Weight, Kg	18.1 ± 4.0	17.4 ± 3.3	18.8 ± 4.6	0.481	0.35
Weight-for-age, Z-score	0.03 ± 1.54	−0.10 ± 1.46	0.17 ± 1.65	0.762	0.17
Height, m	1.05 ± 0.06	1.04 ± 0.06	1.06 ± 0.06	0.522	0.33
Length-for-age, Z-score	−0.39 ± 1.25	−0.49 ± 1.35	−0.29 ± 1.16	0.666	0.16
Body mass index (BMI), Kg/m ²	15.6 (14.6–17.7)	15.6 (14.5–17.2)	15.2 (14.7–18.9)	0.824	–
BMI-for-age, Z-score	0.43 ± 1.59	0.33 ± 1.18	0.52 ± 1.96	0.946	0.12
Overweight/obesity, <i>n</i> (%)	9 (22)	3 (14)	6 (30)		–
Underweight, <i>n</i> (%)	3 (7)	0 (0)	3 (2)		–
Waist circumference, cm	51.5 (47–55)	52 (48–53.9)	51.5 (47.5–58.5)	0.686	–
Arm circumference, cm	16.9 ± 2.6	16.3 ± 2.3	17.5 ± 2.7	0.096	0.48
Triceps fold, mm	8.5 (6.8–11.0)	8.0 (6.0–11.3)	9.5 (7.0–10.5)	0.370	–
Subscapular fold, mm	5.6 (4.0–7.2)	5.0 (4.0–6.0)	6.5 (4.3–8.0)	0.212	–
Current socioeconomic status					
Maternal education, years	11.1 ± 3.12	10.9 ± 3.1	11.3 ± 2.8	0.719	0.13
Family income, Brazilian R\$ (×1000)	2.9 (1.5–3.8)	3.0 (1.2–3.8)	2.5 (1.5–4.6)	0.694	–
Neonatal period					
Gestational age (GA), weeks	28.1 ± 2.0	28.0 ± 1.8	28.2 ± 2.2	0.701	0.10
Birth weight, g	1,073 ± 318	1,175 ± 308	1,172 ± 335	1.000	0.00
Birth length, cm	36.0 (33.5–39.7)	35.5 (33–39.7)	37.0 (34–39.7)	0.551	–
Birth head circumference, cm	25.5 ± 2.4	25.7 ± 2.2	25.4 ± 2.6	0.739	0.12
Small for GA (SGA), <i>n</i> (%)	4 (10.8)	2 (10)	2 (11)	0.298	–
Apgar score 5 min	8 (7–9)	8 (6–8)	8 (7–9)	0.312	–
Maternal age, years	31 (23–35)	33 (29–36)	28.5 (19–31)	0.009	–
Pre-eclampsia, <i>n</i> (%)	16 (39)	8 (38)	8 (40)	0.901	–
Gestational DM (GDM), <i>n</i> (%)	2 (5)	1 (5)	1 (5)	0.972	–
Antenatal corticosteroids, <i>n</i> (%)	37 (90)	21 (100)	16 (80)	0.031	–
Use of surfactant, <i>n</i> (%)	25 (61)	14 (67)	11 (55)	0.444	–
BPD, <i>n</i> (%)	13 (32)	5 (24)	8 (42)	0.217	–
Parenteral nutrition (PTN), days	15 (11–23)	14 (11–25)	15 (10–18)	0.539	–
Full enteral feeding, days	23 (12–30)	16 (12–28)	18 (15–30)	0.428	–
Necrotizing enterocolitis, <i>n</i> (%)	5 (12)	2 (9)	3 (15)	0.881	–
Lesion CNS, <i>n</i> (%)	18 (44)	9 (42)	9 (45)	0.890	–
PIVH, <i>n</i> (%)	16 (39)	8 (38)	8 (40)	0.901	–
Leukomalacia, <i>n</i> (%)	3 (5.6)	2 (9)	1 (5)	0.935	–
Ductus arteriosus, <i>n</i> (%)	19 (46)	10 (47)	9 (39)	0.867	–
Ibuprofen, <i>n</i> (%)	16 (39)	10 (42)	6 (30)	0.248	–
ROP, <i>n</i> (%)	11 (27)	5 (24)	6 (30)	0.655	–
Packed RBC transfusion	2 (0–4)	1 (0–4)	2 (0–4)	0.894	–
NICU stay, days	64 (52–99)	64 (55–100)	59 (49–100)	0.382	–
Body weight at discharge, g	2,691 ± 544	2,764 ± 660	2,615 ± 390	0.387	0.27
Length at discharge, cm	45.5 ± 2.8	46.0 ± 3.0	45.0 ± 2.4	0.402	0.36
Head circumference at discharge, cm	33.4 ± 1.6	33.4 ± 2.0	33.3 ± 1.1	0.810	0.06

Data presented as mean ± SD, Median (P25–75), or absolute number (*n*) and proportion (%).

Parametric variables: independent sample *t*-test.

Non-parametric variables: Mann-Whitney *U*-test. Categorical variable: Chi-square test.

BMI, Body Mass Index; GDM, Gestational diabetes Mellitus; BPD, bronchopulmonary dysplasia; CNS, Central Nervous System; PIVH, peri intraventricular hemorrhage; ROP, Retinopathy of prematurity; RBC, red blood cells; NICU, neonatal intensive care unit.

TABLE 2 Physical exam in the first ambulatory follow-up visit after NICU discharge and 12 months corrected age of preterm children subjected to early intervention program compared to conventional care.

Characteristic	General	Conventional	Intervention	P-value	Cohen-d
First ambulatory appointment after discharge	n = 39	n = 20	n = 19		
Chronological age, weeks	13 ± 5	15 ± 5	12 ± 4	0.133	–
Body weight, kg	3.06 (2.74–3.86)	3.55 (2.65–4.53)	2.96 (2.74–3.45)	0.175	–
Length, cm	48 (46–51)	50 (47–54)	47 (46–51.49)	0.063	–
Cephalic perimeter, cm	35.9 ± 2.4	36.7 ± 2.8	35.2 ± 1.7	0.058	0.65
Body Mass Index, kg/m ²	13.7 ± 2.0	14.0 ± 2.2	13.5 ± 1.8	0.429	0.25
Breast milk, n (%)	4 (10)	2 (10)	2 (10)		–
Combination feeding, n (%)	19 (46)	12 (60)	7 (37)		
Infant formula, n (%)	16 (39)	6 (30)	10 (53)	0.319*	
Ambulatory appointment at 12 months of corrected age	n = 37	n = 20	n = 17		
Chronological age, months	15 ± 1	15 ± 1	15 ± 1	0.361	–
Body weight, kg	9.03 ± 1.68	9.29 ± 1.59	8.73 ± 1.64	0.304	0.34
Length, cm	74 ± 4	75 ± 4	73 ± 4	0.357	0.50
Cephalic perimeter, cm	45.7 ± 1.9	46.1 ± 1.7	45.4 ± 2.1	0.266	0.37
Body mass index, kg/m ²	16.1 ± 1.5	16.3 ± 1.5	15.9 ± 1.5	0.374	0.27

Data presented as mean ± SD, Median (P25–75), or absolute number (n) and proportion (%).

Parametric variables: independent sample *t*-test. Non-parametric variables: Mann-Whitney *U*-test. Categorical variable: Chi-square or Fisher's exact test*.

TABLE 3 Biochemical analysis in the follow-up ambulatory care at 24 months corrected the age of preterm infants subjected to an early intervention program compared to conventional care.

Biochemical parameter	General (n = 31)	Conventional (n = 15)	Intervention (n = 16)	P-value	Cohen-d
Hemoglobin, g/dL	12.6 ± 1.1	12.4 ± 1.0	12.7 ± 1.2	0.615	0.27
Hematocrit, %	36.8 ± 3.2	36.6 ± 3.1	37.0 ± 3.5	0.792	0.12
Serum iron, µg/dL	83.4 ± 25.1	77.3 ± 24.8	89.2 ± 24.8	0.192	0.48
Ferritin, mg/dL	33.2 (23.4–55.8)	25 (21.4–50.7)	37.5 (24.0–54.4)	0.257	–
Transferrin, mg/dL	282 ± 45	287 ± 52	278 ± 40	0.644	0.19
Transferrin saturation, %	24.1 ± 8.1	23.0 ± 8.3	25.3 ± 8.17	0.459	0.28
Total iron binding capacity, µg/dL	252 (226–305)	258 (222–309)	251 (226–312)	0.949	–
Glucose, mg/dL	86.5 ± 8.6	86.9 ± 9.6	86.0 ± 7.8	0.789	0.10
Insulin, µU/mL	2.9 (2.3–4.1)	2.6 (1.8–3.6)	3.9 (2.5–5.0)	0.101	–
HOMA-IR	0.74 ± 0.45	0.59 ± 0.24	0.89 ± 0.56	0.107	0.69
Cortisol, µg/dL	9.8 ± 4.6	9.5 ± 5.8	10.0 ± 3.2	0.775	0.11
Total cholesterol, mg/dL	143 ± 26	141 ± 23	145 ± 30	0.709	0.15
LDL-cholesterol, mg/dL	85 ± 30	85 ± 22	85 ± 37	0.995	0.00
HDL-cholesterol, mg/dL	43 ± 11	39 ± 8	46 ± 12	0.084	0.68
Triglycerides, mg/dL	89 (66–133)	71 (61–99)	114 (86–170)	0.074	–
Dyslipidemia, n (%)	19 (65)	10 (71)	9 (60)	0.782	–

Data presented as mean ± S.D., Median (P25–75), or absolute number (n) and proportion (%).

Parametric variables: independent sample *t*-test. Non-parametric variables: Mann-Whitney *U*-test.

HOMA-IR, homeostatic model assessment for insulin resistance.

Moreover, IG showed an increase of segmented FFM per unit of iron content (adjusted by gender): right arm: 0.004 kg (95%CI: 0.000–0.008; *p* = 0.036), left arm 0.004 kg (95%CI: 0.000–0.008; *p* = 0.051), trunk 0.025 kg (95%CI: 0.008–0.058; *p* = 0.132), right leg 0.009 kg (95%CI: 0.001–0.017; *p* = 0.037), and left leg 0.009 kg (95%CI: 0.00–0.017; *p* = 0.043) per unit of iron content. Visceral fat area interaction significantly decreased to 37 cm² (95%CI: –50 to –25; *p* < 0.001) per unit

of HOMA-IR in IG, an opposite response when compared to CG (**Figure 2E**) (removing the two outliers, interaction is still significant, *p* = 0.005**).

There was no significant interaction between the FFMI and segmented FFM with physical activity when adjusted by gender. Also, no association was established between feeding practices and FFM (data not shown).

TABLE 4 Body composition in preterm infants subjected to early intervention program compared to conventional care.

Body composition analysis	General (<i>n</i> = 41)	Conventional (<i>n</i> = 21)	Intervention (<i>n</i> = 20)	<i>P</i> -value	Cohen- <i>d</i>
Total body water (TBW), liters	10.7 ± 1.6	10.4 ± 1.3	11.1 ± 1.7	0.125	0.47
Intracellular water, liters	6.6 ± 1.01	6.4 ± 0.8	6.9 ± 1.1	0.110	0.52
Extracellular water, liters	4.1 ± 0.6	3.9 ± 0.5	4.2 ± 0.6	0.166	0.54
Fat mass (FM), Kg	2.7 (1.9–5.4)	2.7 (2.0–4.7)	3.0 (1.5–5.7)	0.938	–
Total body fat (TBF%)	16.3 (12.4–26.7)	16.3 (13.1–25.1)	17.6 (9.2–17.5)	0.657	–
Fat mass Index (FMI), kg/m ²	2.6 (1.8–4.8)	2.4 (1.9–4.2)	2.6 (1.3–5.2)	0.804	–
Fat-free mass (FFM), Kg	14.5 ± 2.1	14.0 ± 1.9	15.0 ± 2.3	0.146	0.48
FFM Index (FFMI), Kg/m ²	12.9 ± 0.8	12.8 ± 0.6	13.1 ± 0.9	0.156	0.39
Bone mineral content, Kg	0.68 ± 0.15	0.67 ± 0.15	0.69 ± 0.14	0.741	0.14
Lean mass, Kg	13.8 ± 2.0	13.3 ± 1.7	14.3 ± 2.2	0.126	0.51
Skeletal muscle mass, Kg	6.7 ± 1.3	6.3 ± 1.1	7.0 ± 1.4	0.098	0.56
Protein, Kg	2.86 ± 0.43	2.75 ± 0.36	2.98 ± 0.48	0.096	0.55
Minerals, Kg	0.87 ± 0.18	0.86 ± 0.19	0.87 ± 0.18	0.954	0.05
Segmented FFM					
FFM of right arm, Kg	0.70 ± 0.13	0.65 ± 0.09	0.74 ± 0.16	0.040	0.70
FFM of left arm, Kg	0.68 ± 0.14	0.66 ± 0.10	0.74 ± 0.16	0.053	0.61
FFM of trunk, Kg	6.46 ± 1.21	6.09 ± 0.90	6.86 ± 1.39	0.040	0.67
FFM of right leg, Kg	1.82 ± 0.30	1.73 ± 0.23	1.91 ± 0.35	0.063	0.61
FFM of left leg, Kg	1.81 ± 0.31	1.72 ± 0.24	1.91 ± 0.35	0.054	0.64
Segmented water content					
Right arm water, liters	0.54 ± 0.10	0.51 ± 0.07	0.58 ± 0.12	0.062	0.72
Left arm water, liters	0.54 ± 0.11	0.51 ± 0.08	0.58 ± 0.13	0.066	0.67
Trunk water, liters	5.04 ± 0.94	4.75 ± 0.72	5.33 ± 1.06	0.053	0.65
Right leg water, liters	1.41 ± 0.24	1.35 ± 0.18	1.48 ± 0.27	0.075	0.57
Left leg water, liters	1.41 ± 0.24	1.34 ± 0.19	1.49 ± 0.27	0.066	0.65
Additional data					
TBW/FFM, %	74.3 (73.9–74.5)	74.1 (73.8–74.6)	74.2 (74.1–74.5)	0.440	–
Basal metabolic rate, Kcal	686 ± 49	677 ± 47	695 ± 51	0.255	0.37
Visceral fat area, cm ²	13.7 (9–17.1)	13.4 (9.3–16.0)	13.9 (8.6–18.7)	0.705	–
Cellular body mass, Kg	9.5 ± 1.4	9.2 ± 1.2	9.9 ± 1.5	0.098	0.52
Arm circumference, cm	19.2 ± 2.6	18.8 ± 2.06	19.7 ± 3.1	0.322	0.35
Arm muscle circumference, cm	15.2 ± 1.9	14.9 ± 1.4	15.6 ± 2.3	0.281	0.37
Full body phase angle (50 kHz)	4.6 (4.3–4.8)	4.4 (4.1–4.6)	4.7 (4.4–5.3)	0.111	–

Data presented as mean ± S.D., Median (P25–75), or absolute number (*n*) and proportion (%).

Parametric variable: independent sample *t*-test. Non-parametric variable: Mann-Whitney *U*-test.

TBW/FFM ratio, Total Body Water/Fat-free mass.

Discussion

For this study, we investigated the body composition of preterm VLBW children at preschool age who were subjected to a protocol of skin-to-skin care and global stimulation in a home-based program for 18 months. This investigation observed that early intervention may increase FFM in the body segments. On the other hand, despite stimulation implementation and closer family monitoring, no change in fat mass was observed. Nonetheless, this protocol may present a positive effect on reducing the relationship between visceral fat mass and insulin resistance.

Early life exposures to certain environmental factors during critical periods of development and growth may have significant short- and long-term consequences on an individual's health: for that reason, early intervention is a strategy to improve growth and developmental outcomes (32, 33). In preterm infants, studies have shown that early intervention can improve cognition, increases growth and global development (34, 35), and attenuate the decrease in bone strength that may reduce the risk of osteopenia (36). Moreover, it presents a positive effect on motor skills through environmental enrichment (37). However, there are few early interventions implemented in the

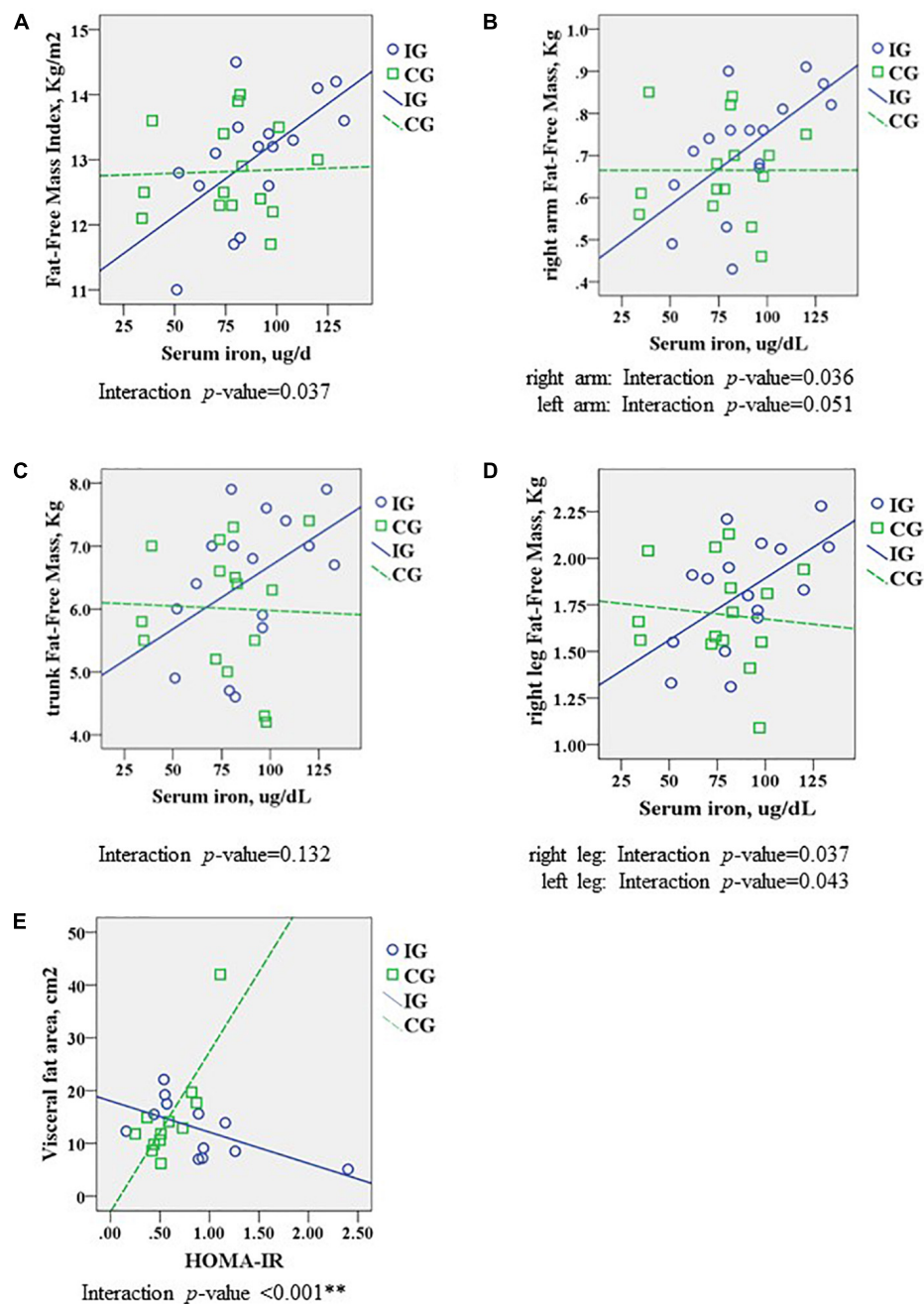


FIGURE 2

Comparison of body composition analysis at preschool age between interventional group (IG) and conventional group (CG), analyzing the interaction with biochemical blood results (blood exam at 24 months of corrected age), adjusted by gender. Association of (A) FFM Index and serum iron, (B) right arm FFM and serum iron, (C) trunk FFM and serum iron, (D) right leg FFM and serum iron, and (E) visceral fat area and HOMA-IR. Data were analyzed using ANCOVA, presenting interaction significant with a p -value < 0.05. FFM, fat-free mass; HOMA-IR, homeostatic model assessment for insulin resistance. **Removing the two outliers, interaction is still significant ($p = 0.005$).

“first 1,000 days” and limited data on these effects in VLBW children regarding body composition across childhood.

Growth assessment is generally based on anthropometric measurements, which gives insufficient attention to growth quality. Thus, the assessment of body composition through BIA

provides additional information on the relationship between growth and development (12). In a cohort study that compared extremely preterm children with full-term, in whose body composition was measured by DEXA, it was observed that preterm children presented the same height and weight as

full-term. However, the preterm group presented lower values for muscle mass [0.9 kg (95%CI: 0.3–1.5)], total bone mineral density *z*-score [0.30 units (95%CI: 0.13–0.52)] and fat mass ratio [0.14 units (95%CI 0.06–0.21)] (13). In our study, the early stimulation protocol was performed anticipating, by a month, the motor and cognitive acquisition steps expected for corrected age, approaching near-term development; the usual growth evaluations (BMI, body circumferences, and skinfolds) did not present differences over the first year between intervention and conventional groups. Nonetheless, BIA showed that the early intervention program caused a small, but significant increase of segmented FFM, a body component comprised of skeletal muscle mass, body cell mass, total body water, connective tissue, and bone mineral mass. The use of a multi-frequency BIA provides a more direct portrayal of water compartments, increasing results reliability (38). Skeletal muscle accounts for a large proportion of the FFM, and it is known that physical activity positively affects FFM accretion from birth onward (39). Loss of muscle mass is associated with poor prognosis, reduced quality of life, and increased mortality, thus highlighting the muscle as an important component of whole-body metabolism, glucose homeostasis, as well as overall health and wellbeing (40). A systematic review showed that preterm infants present less lean tissue but a similar fat mass than full-term infants (11). Muscle growth can be activated by mechanical, oxidative, and energetic distress, and influenced by the availability of nutrients, growth factors, and cytokines (41). Moreover, an experimental study showed that mechano-signaling pathways stimulated by passive movements can control myofibrillar protein synthesis (42). Our finding showed that early intervention increased FFM, corroborating a previous investigation in which motor physical therapy in preterm in NICU increased lean mass (43). The preterm infants from the intervention group presented more FFM, despite performing less physical activity during the week compared to CG. The evaluation was done by physical questionnaire and supported our hypothesis that early intervention may contribute to an increase in the components of FFM. Moreover, an observational study showed that the gain of FFM in the first 4 years of the life of preterm children was associated with higher full-scale IQ and processing speed performance, which may enhance preschool cognitive performance (16).

Evidence from the classical birth cohorts from Pelotas, South Brazil showed that prematurity was associated with decreased total body fat and FFM, but with higher fat mass in adulthood (in male) (44). Higher fat mass is associated with an increased risk for metabolic syndrome in the preterm population (45). The early intervention protocol did not impact fat mass or bone mineral content within our intervention group. The metabolic bone mineral disease of prematurity is highly prevalent in VLBW preterm as it may occur due to loss of mineral transfer from the placental in the latest trimester and the reduced mechanical stimulation from the fetus against the

uterine wall. This deficiency could be prevented by minerals and vitamin D intake, as well as physical activity (46–48). We did not observe changes in bone mineral content in response to early intervention protocol. However, a study with motor stimulation in preterm infants (26–34 weeks), with birth weight < 1,600 g, was able to increase bone mineral content evaluated by DEXA (43). A clinical study with extreme preterm-born young adults presented reduced area bone mineral density when compared to sex- and age-matched full-term controls, showing the long-term consequences of bone health (14).

Biochemical analysis performed in the follow-up clinic, at 24 months of corrected age, may support the structural findings observed in response to the early stimulation protocol. A significant relationship between iron content and FFM was observed only in IG, suggesting that the mechanical and global stimulus positively affects the components of FFM, such as the skeletal muscle which is increased by 700 g on average when compared to the CG group. Iron is an essential component of hemoglobin and myoglobin, in which iron supports muscle metabolism and healthy connective tissue, and is essential for physical growth, neurological development, and cellular function (49). Since iron is a micronutrient necessary for early development, it could be postulated that implementing an early intervention protocol of 18 months could improve muscle function, thus promoting iron homeostasis in the muscle system (50). Although no difference was observed in iron content between groups, a recent experimental study of a neurological disease demonstrated that regular physical exercise modulates iron homeostasis, in which dysregulation of iron metabolism leads to pathophysiological pathways (51).

Clinical studies indicate that preterm individuals have physiological disease pathways that differ from those born at full-term (52). In this same context of different mechanisms between preterm and full-term individuals, we observed that the hydration factor (TBW/FFM) in our preschool preterm children was 74.3%, a higher percentage than the assumed value for euhydrated individuals, which is set as 73.2%. A systematic review showed that preterm newborns present a higher TBW percentage compared to full-term individuals (73.8%). TBW of preterm reached up to 90% at 26 weeks of gestation, dropping to 75% at 36 weeks of gestation, and dropping 1.44% per week after birth (53). Estimation of total body water by the $2H_2O$ dilution method from healthy individuals (children to adults) showed that prepubescent children have a higher aqueous fraction of their fat-free body mass when compared to young adults ($72.7 \pm 1.6\%$ vs. $70.8 \pm 1.2\%$; $p < 0.01$) (54). Lohman et al. described the chemical composition of FFM changes during childhood and they were both ages- and sex-specific (55). Therefore, our data contribute to characterizing the TBW/FFM in VLBW preterm, since there is still a paucity of data in this population during growth *ex utero* (53).

Another interesting finding of early stimulation in the VLBW preterm population was the relationship between visceral fat area and insulin resistance, showing that CG presented higher insulin resistance with more visceral fat, a relation not observed in the IG group. Prematurity has been considered a risk factor for cardiovascular and metabolic diseases (5, 56), in which preterm-born individuals presented a higher incidence of hypertension (57), glucose intolerance (52, 58), as well as metabolic syndromes (59, 60). The inverse association observed in our study agrees with the observation that young adults born extremely preterm present a higher number of risk factors for cardiometabolic disorders unrelated to each other as observed in the control term group (52). Although our results are not strong enough to infer many interpretations, we believe that the protective response observed in IG could be related to the light increase of FFM components, such as skeletal muscle mass, which is insulin sensitive and regulates glucose metabolism, and contributes in the prevention of cardiometabolic disorders (61). Since early stimulation can positively affect the central nervous system, thus improving neurodevelopment, it can also positively affect the other systems, as is observed in exercising: it can improve whole-body glucose tolerance, lipid handling, and insulin sensitivity in humans (62), as well as in rodents (63).

Our follow-up study presents limitations. Although we have been using in our cohort of children born preterm the research grade InBody 770®, a multi-frequency BIA that presents more reliable results of water compartments (38), we should be careful with the data interpretation. However, many studies have shown that BIA results correlate well with the gold standard method DEXA scan. This implies that BIA demonstrated a strong accuracy and reliability when compared to DEXA (64, 65) which has been recently used in children of preschool age (66–68). Moreover, the use of this equipment is advantageous for clinical use and large-scale epidemiological studies since it is simple, rapid, non-invasive, accessible, reliable, and requires little training. This will allow an appropriate follow-up of the preterm population growth and development from our cohort (69). A study with the purpose to examine the validity of body composition showed that skeletal muscle mass was reliably measured using a multi-frequency BIA method in preschool children (70). Using BIA in preschool-aged children can be difficult due to the child's movement during measurement; the evaluation was considered complete if the child was able to stay still for 60 s over the scale holding the electrodes. Further studies from our preterm cohort population and additional follow-up evaluations, at both preschool and school ages, will contribute to confirming these preliminary results. Another limitation of this study was the small sample size due to unreachable subjects, as well as the disinterest of the legal guardians (parents) to participate in the study due to the pandemic and socio-economic-related factors.

Conclusion

This study was developed to investigate if an early intervention program could positively affect body composition in VLBW preterm children. The intervention indicates that it can probably increase FFM and modify the relationship between fat and the endocrine system, which may contribute to better health with advancing age in VLBW preterm children. Nonetheless, further longitudinal studies and follow-ups of these preterm children are required to establish the clinical significance and prolonged impact of early intervention in this population.

Data availability statement

The data that support the findings of this study are available on request from the corresponding authors JB, jbernardi@hcpa.edu.br and RF, rolfernandes@hcpa.edu.br. The data are not publicly available due to their containing information that could compromise the privacy of research participants.

Ethics statement

The ethical approval to conduct this study was granted by the Research Ethics Committee of the Hospital de Clínicas de Porto Alegre (number HCPA: 2019-0809; Certificate of Presentation for Ethical Appreciation (CAAE): 27358019.1.0000.5327). The guardians of the participants who met the eligibility criteria were invited to participate in the study and were included after signing the informed consent form.

Author contributions

RF and JB conceptualized and designed the study, performed data analyses, and wrote the manuscript. JF and FG participated in data collection and conducted the study. RP and RS conceptualized and designed the study, reviewed critically the manuscript, and obtained funding. All authors revised and finalized the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Effect of aquatic resistance interval training and dietary education program on physical and psychological health in older women: Randomized controlled trial

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Due to demographic changes, the world's population is progressively aging. The physiological deterioration of the older adult may lead to reduced balance capacity and increased risk of falls, among others, due to the prevalence of degenerative diseases. Physical exercise can be effective in reducing the risk of disease and slowing functional decline in older people. The aim of the research is to test the effects of aquatic resistance training and dietary education on health indicators, strength, balance, functional autonomy, perception of satisfaction with life. Thirty-four participants aged 69 ± 4 years were randomly assigned into two groups: experimental (aquatic resistance interval training) and control group (no intervention). The intervention consisted of resistance training in an aquatic environment carried out for 14 weeks (three sessions per week: 60 min each). All variables were analyzed twice; pre - post intervention. Aquatic resistance training has positive effects on strength ($p < 0.001$), functional self-sufficiency ($p < 0.001$) and aerobic capacity ($p < 0.001$), however, no significant differences were observed in the perception of satisfaction with life and balance. Research results suggest that older women who engage in regular, scheduled aquatic resistance training have greater autonomy in performing activities of daily living, agility, gait control, and body composition variables (lower fat compartment and greater muscle mass).

KEYWORDS

body composition, strength, geriatric rehabilitation, ageing, aquatic resistance training, physical performance, older adults

Introduction

Aging is linked to a functional deterioration in human beings and can become a serious problem due to its prevalence of degenerative diseases (1). Therefore, physical exercise can be effective to reduce the risk of disease and slow functional deterioration in the older people (2–4). An aspect of extraordinary importance in adults and the older people is the decline in functional capacity with advancing age, a predictable phenomenon that can be halted or slowed down by paying special attention to the level of physical fitness (body composition, strength, endurance, flexibility, etc.) and physical activity.

This functional decline can affect the physical, cognitive, and psychological functions of older adults, as well as life satisfaction. In a observational study carried out between 1994 and 2014 (5) they observed that the effect of physical health on later mental health is greater than the effect of mental health on later physical health in later life. In addition, a clear positive association between psychological well-being and short- and long-term health outcomes and quality of life has been found (6). Many adults and older people, due to their sedentary lifestyles, are dangerously close to their maximum capacity level, just performing normal activities of daily living (7). A small decrease in the level of physical activity in these individuals could result in the transition from a state of independence to a state of disability, which will be characterized by the need for assistance in performing co-occurring activities of daily living. Therefore, prevention of dependence takes on a special dimension in order to avoid the deterioration of quality of life and dependence in the older people (8).

As age increases, moreover, the rate of falls can increase by as much as 60% (1, 9). Three of the most common modifiable intrinsic (subject-related) fall risk factors are muscle weakness (relative risk ratio/odds ratio 4.4), balance deficit (relative risk ratio/odds ratio 2.9) and gait instability (relative risk ratio/odds ratio 2.9) (10–14). In addition to balance, muscle strength/power is required (1). The general causes of age-related skeletal muscle mass loss (i.e., sarcopenia) are multiple (e.g., cellular, neural, metabolic, and hormonal contributors).

Physical exercise is considered one of the most important factors in improving quality of life in older people, due to improved functional capacity, decreased risk of falls, and improved gait ability, balance, cardiorespiratory capacity, and muscle strength development (15–18). Despite the numerous benefits, regular exercise is difficult to achieve for many older adults, as participation levels often decline with age (19). Decreased participation levels vary due to a myriad of factors such as type of physical activity, age, health problems, pain, and perception of disability (19). As per recommendation by the American College of Sports Medicine and the American Heart Association, water-based exercise is a safe and useful

alternative to land-based exercise for older adults or individuals with limited tolerance for weight-bearing activities (20).

In particular, strength exercise in an aquatic environment has been related to improved balance (21–24), as the buoyancy force of the water and hydrostatic pressure/density help participants to slow down movement, and the additional sensory cues provided by the viscosity of the water facilitate the timing of muscle recruitment (25). Thus, aquatic exercise can ameliorate the negative physiological effects of aging, which are modifiable risk factors and predisposing factors for fall (26). On the other hand, interval training has been related as an alternative method for health improvement (27), so it could be related to increased performance and improvement of blood pressure, lipid profile, improved metabolic condition and strength gain (28, 29). Also, it should be noted that there is an indirect relationship between fitness factors and some components of body composition, such as fat mass (30). In this regard, the importance of adequate nutritional education should also be emphasized, since sufficient protein intake has been shown to counteract the effects of sarcopenia in older adults, noting that, although energy requirements are lower in old age, the requirements for many other nutrients may not change or may even increase. Changes in the balance of nutrient-rich versus less nutrient-dense foods have been shown to occur, contributing to lower protein and micronutrient intakes (31).

To date, there is no research that studies the effect of interval resistance training in an aquatic environment together with nutritional education on both physical and psychological variables in older women. In this context, a water-based interval resistance training intervention was chosen as a potential candidate to provide improvements in functional capacity, balance, strength, body composition, flexibility, aerobic capacity, and satisfaction with life in women over 65 years of age. For that, the aim of the research was to find out the effects of aquatic resistance training and dietary education on physical and psychological health in older women.

Materials and methods

Study design

In this randomized controlled trial study, participants were assigned to an experimental group (EG = aquatic resistance interval training and nutritional education) and control group (CG = only nutritional education) to determine the efficacy of aquatic resistance interval training on the variables of on the variables of strength, functional autonomy, body composition, static standing balance, flexibility, aerobic endurance, and satisfaction with life. Allocation was electronically randomized by two-arm block design using online computer software, as indicated by published recommendations (32). An investigator who was not involved in the interventions or assessments

in this study performed this procedure. Previously, sports centers in the province were contacted and an invitation to participate in the research was sent out. An informative talk was given in which they were informed of the objective and their collaboration was requested.

Participants

Only female older adults participated in the investigation. Thirty-four women chose to participate; 17 were in the experimental group (69.4 ± 4.9 years old) and 17 in the control group (67.7 ± 3.6 years old). Being over 65 years of age; not having undergone surgery in the last year; not having musculoskeletal, neurological, or orthopedic diseases that could affect the ability to perform the tests and being able to walk independently without orthopedic assistance were the established inclusion criteria. After the initial assessment, one participant refused to take part in the research and four others had mobility problems. Forty women were randomly assigned, however, during the intervention six women withdrew from the study for personal reasons. Thirty-four women were included in the analysis. This procedure was established according to “CONSORT” statement¹, as displayed in the flowchart in [Figure 1](#).

Declarations: Ethical approval, consent to participate, and consent for publication

The norms of the Declaration of Helsinki were considered in the development of the research. The University Human Research Ethics Committee of Alicante University (Spain) approved the research; code UA- 2018-10-22. This trial was registered at clinicaltrials.gov as NCT05052164². All participants signed an informed consent form after being informed of the benefits, risk, and detailed description of this research. All data were coded to maintain the confidentiality of the study participants.

Intervention

The water exercise was conducted and supervised by master in sport science specialist also in accordance with physical exercise prescription for older adults established by the American College of Sports Medicine (33).

The participants completed 14 weeks of water exercise training program three times a week (42 sessions), with

60 mins per session which was comprised by 15 mins of warm-up (10 mins of aerobic and resistance exercises and 5 mins of stretching), 30 mins of water interval resistance training ([Table 1](#)), followed by 15 mins of cool down (5 mins of stretching and 10 of relaxation exercises). This intervallic training consisted of four sets of 5 min of training with 2 min rest between sets. During the 5 mins of exercise there was no rest. In each session, the same exercises (pectoral/back, hip flexor/extensor, biceps/triceps, knee flexor/extensor, shoulder, and core) were performed for 1 min consecutively, with intervals of 30, 20, and 10 s, and at low, moderate and high perceived intensity, respectively. Finally, relaxation exercises (10 min) and stretching of all muscle groups (5 min) were performed. All women in the experimental group participated in each of the sessions. The Borg scale was used in each of the sessions to measure the perception of effort (34), as well as to indicate whether they should perform the exercises at low, moderate or high intensity. After each training session, they were asked about their fatigue state. To meet 85% adherence the subjects were informed that they could not miss more than six sessions. Session attendance was recorded by the sport science specialist.

Physical activity was measured by means of the IPAQ questionnaire (35) at the beginning and end of the study. Complementarily, all participants received the same nutritional education, based on the Mediterranean diet, divided into four 60-min theoretical-practical workshops over 14 weeks, with the aim of providing updated information on the benefits of following a proper dietary pattern. Trained dietitians led the sessions. In addition, these dietitians were in contact with the sport science specialist, who led the training sessions. In this way, if any questions arose, they were resolved in these workshops. The PREDIMED Mediterranean diet adherence questionnaire was used to control dietary habits of the participants. Participation was 100%, i.e., all patients attended all workshops. These workshops were conducted to prevent eating habits from being a potential confounding factor in the results obtained. Both the water resistance training intervention and the nutritional workshops took place at the Catholic University of Murcia, during the last months of 2020 and early 2021.

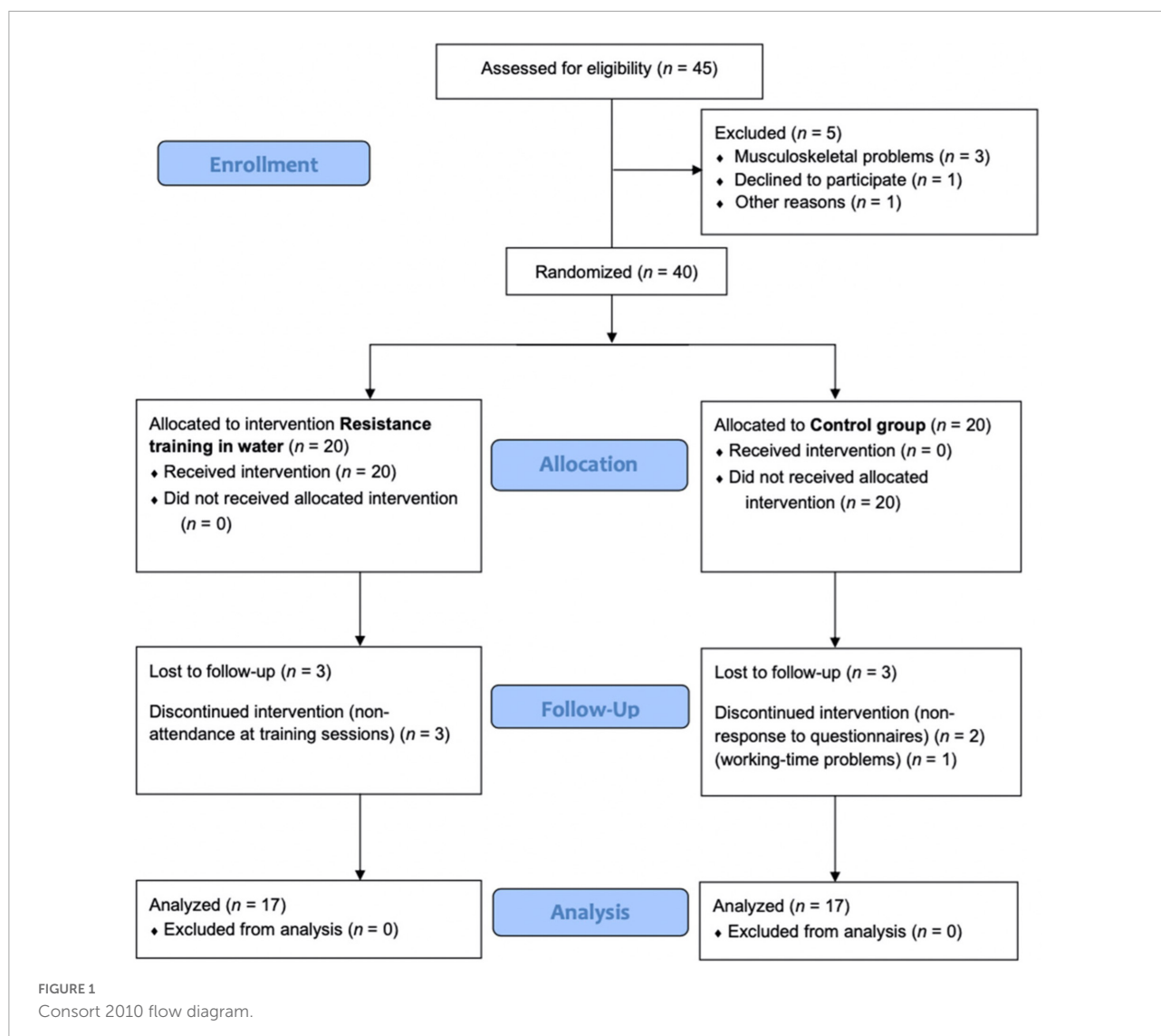
Outcome measurements

Body composition

The weight and height of all participants were measured with high-quality calibrated electronic scales and a mobile anthropometer (Seca 213, SECA Deutschland, Hamburg, Germany), respectively. The participants were dressed in light clothing and without shoes. Using weight in kilograms and height in centimeters, body mass index (BMI) was

¹ <http://www.consort-statement.org>

² www.clinicaltrials.gov



calculated as $\text{weight}/\text{height}^2$ (kg/m^2). Body perimeters were measured in triplicate (with subsequent averaging) with an anthropometric tape. Skinfolts (subscapular, tricipital, bicipital, iliac crest, supraspinal, abdominal, anterior thigh, and middle leg) were obtained with a Holtain Skinfold Caliper. Three bone diameters were also measured, using a small bone diameter pachymeter (Smartmet, Jalisco, Mexico; accuracy, 1 mm). The mean technical error for, circumferences, lengths and heights was less than 1% and for skinfolts less than 5%. All these measurements were performed by anthropometrists accredited by the International Society for the Advancement of Kinanthropometry (ISAK) level 2, according to ISAK guidelines (36). The sum of 6 folds was calculated from the tricipital, subscapular, supraspinal, abdominal, thigh, and leg folds. The rest of the measurements were used to calculate muscle mass, based on the five-component model proposed by Kerry Ross (37).

TABLE 1 Aquatic resistance interval training.

Weeks	Volume × Intensity Hate-rate × sec	Total, time Total, exercise
1–4	4 × [5 min (30 sec 30%; 20 sec 60%; 10 sec 90%) + 2 min rest] 30 sec (45.6 ± 4.94); 20 sec (91.2 ± 4.94); 10 sec (136.8 ± 4.94)	30 min 20
5–14	4 × [5 min (30 sec 30%; 20 sec 60%; 10 sec 90–100%) + 2 min rest] 30 sec (45.6 ± 4.94); 20 sec (91.2 ± 4.94); 10 sec ($136.8/152 \pm 4.94$)	30 min 20

%, heart rate; min, minutes; sec, seconds; rest, recovery between sets.

Functional autonomy

The protocol of the Group of Latin-American Development for Maturity (GDLAM) was used to evaluate functional autonomy (38) in its validated version for the Spanish

population (28). It defines functional autonomy covering three aspects: autonomy of action that relates to the notion of physical independence; autonomy refers to the possibility of self-determination and autonomy than allows the person to judge any situation. GDLAM protocol is composed by the following five tests:

(1) 10 m walk (W10 m); the purpose of this test is to evaluate the individual's speed to travel 10 m.

(2) Getting up from a seated position (GSP); the test is intended to evaluate the functional capacity of the lower extremity. The individual, starting from a sitting position on a chair, without the support of the arms, with the seat at 50 cm from the floor, stands up and sits down five consecutive times.

(3) Getting up from the prone position (GPP); the purpose of this test is to evaluate the individual's ability to get up from the floor. Starting from the initial position of ventral decubitus, with the arms along the body, after giving the command, the subject must get up and stand up as quickly as possible.

(4) Getting up from a chair and movement around the house (GCMH); the objective is to evaluate the individual's ability, in relation to agility and balance, in common day-to-day situations. With a fixed chair on the floor, two cones should be placed diagonally and behind the chair, at 4 m to the back and to the right and left sides of the chair. The individual begins the test sitting on the chair, with feet raised off the floor and, at the observer's command, stands up, goes to the right, circles the cone, returns to the chair, sits down and lifts both feet off the floor. Without hardly resting, he does the same movement to the left.

(5) To put on and take off a T-shirt (PTS); the individual should be standing with arms along the body and a T-shirt in one of the hands. At the voice signal of "Go," the individual should put on the shirt and immediately take it off, returning to the starting position. This test is intended to measure the agility and coordination of the upper limb.

All tests were individually conducted and repeated two different times with a minimum of 5 min intervals, the lowest time of the two trials was recorded. After performing this battery of tests, the GDLAM index (GI) is calculated, where the lower the value of the score, the better the result, using the following formula: $GI = [(W10\ m + GSP + GPP + PTS) \times 2] + GCMH / 4$. All the tests were measured using time in seconds. It classifies the subjects as: weak GDLAM, with figures of > 28.54 ; fair, between 28.54 and 25.25; good, between 25.24 and 22.18, and very good, below 22.18 points.

Maximal isometric hand grip force test

The manual grip strength of the upper limbs was measured by means of a dynamometer adjusted for each type of hand, and on a scale from 0 to 100 kg. The individual was in the orthostatic (standing and upright) position with the arm to the side of the body and with the dominant side performed the

grip (39). The participants performed one repetition in each hand to familiarize themselves with the device and the test. Each participant was asked to squeeze the grip with maximal strength for 3 s with the dominant hand. The highest peak strength (kg) recorded between the three attempts was considered for analysis. A digital grip strength dynamometer was used for this (TKK 5401; Takei Scientific Instruments Co., Ltd., Tokyo, Japan). Dividing the value of the best hand grip strength score by BMI (kg/m^2) obtains a field muscle quality index (MQI) (40).

Isometric strength

Isometric strength of the quadriceps was performed with the load cell maximal isometric strength test (41, 42). To determine the maximal isometric knee extension strength, the participants were assessed while seated with a knee and hip angle of 90° . Participants seated in a knee extension machine were instructed to push as strong as possible for 3 s while provided with verbal encouragement. The extension test was assessed with a load cell force transducer (Musclelab, Ergotest, Norway) sampling at 1,000 Hz. The subjects performed three IKE tests with 2 min of rest between tests. The maximum peak force in Newton (Nw) was collected.

Postural stability tests

The static standing balance test aims to maintain the verticality of the body in static situations. The way to evaluate balance in older people is the one proposed by Onambele et al. (43). Three types of balance were performed: (1) bipedal stance, (2) single-leg stance, and (3) tandem stance, all of them with eyes open. A force platform (MuscleLab force plate, 200 Hz/1 kHz, Ergotest Technology a.s., Stathelle, Norway) was used, acting as a switch as they are very useful to record contact times between supports. Subjects were barefoot throughout the whole exercise and were asked to stand quietly with hands hanging freely at either side, looking straight ahead at a target (black circle 15 cm in diameter against a white background) placed at eye level, ~ 3 m away.

Aerobic endurance

The 6-min walk test (44) was used. The women walked (without running) the longest possible distance for 6 mins, in a 45.72 m course marked in segments. It was performed in an enclosed, well-lit room on a non-slip surface. The women who needed it stopped to rest and resumed the test. The evaluator warned when there were 3-, 2-, and 1-mins left. The result was recorded as total meters walked.

Flexibility

The Chair Sit and Reach flexibility test (44) in its adapted and validated version for older adults (45) was used to measure flexibility. A 45 cm high (17 inch) chair with a backrest and attached to the wall to prevent it from moving was used, as well

as a measuring tape. Participants sat on the edge of the chair resting one foot on the floor with the leg flexed at hip width and the other leg straight with the foot in 90° dorsal flexion. They stretched their arms in front of the straight leg with one hand on top of the other and palms down, trying to touch or overlap the tip of their toes with the middle finger, maintaining the maximum trunk flexion position for 2 s, keeping the spine as straight as possible and the head in normal alignment with the spine (not cramped).

Satisfaction with life

The satisfaction with life scale (SWLS) is a 5-item scale that assesses life satisfaction (46). The responses are classified in a 7-point Likert scale. This scale has been found to have favorable psychometric properties, including high internal consistency and reliability, and has been consistently used to measure life satisfaction in several countries (47, 48).

Statistical analyses

Jamovi 1.1.3.0 software was used to perform all statistical analyses. Descriptive statistics (mean \pm standard deviation) were calculated for all the variables and the normality distribution was tested using the Shapiro-Wilk test. For equality of variances, Levene's test was performed, and analysis of covariance (ANCOVA) was applied (general linear model; time \times group) with BMI as a covariate to analyze the effects of the intervention on the assessments. For time \times group interaction effects, omega squared effect sizes were calculated. If significant main effects were found, *post hoc* (Bonferroni) tests were performed. Moreover, to set up connections between the variables of the study, the Pearson's correlation test was used in the correlations to determine the effect size (small: 0.10, medium: 0.30 and high: 0.50) (49, 50), with 95% confidence intervals. The level of statistical significance was set at $p \leq 0.05$.

Results

The baseline data of the sample are presented in Table 2. Statistically significant differences were observed between the experimental (water) group and the control group in terms of height and weight, being greater in both cases in the water group. Regarding the possible confounding variables, physical activity and adherence to the Mediterranean diet, no differences were observed in the control group for physical activity. In the experimental group there was an increase due to the intervention. Adherence to the Mediterranean diet did not change significantly in the experimental group (5.7 ± 2.0 vs. 5.9 ± 2.36 ; $p = 1.000$) and in the control group (6.1 ± 2.1 vs. 5.5 ± 2.3 ; $p = 0.75$).

TABLE 2 Baseline characteristics of study participants.

	Experimental		Control	
	Mean	SD	Mean	SD
Height (cm)	161*	7.95	154*	5.47
Weight (kg)	75.4*	12.4	66.9*	10.2
Age (years)	69.6	5.01	67.7	3.60

cm, centimeters; kg, kilograms; SD, standard deviation. *Mean differences were significant at $p < 0.05$.

Body composition

Table 3 show the body composition variables from EG and CG, respectively, at the beginning or end of the study for women. Statistically significant differences were observed in the summary of folds and muscle mass variables. For the sum of folds, a decrease in the sum of folds was observed in the experimental group ($p < 0.001$) and an increase in the control group ($p < 0.001$). In addition, there are significant differences between both groups after the intervention ($p = 0.014$). Muscle mass increases significantly in the experimental group ($p < 0.001$) and after the intervention they present significantly higher values than women in the control group ($p = 0.016$). No significant differences were observed for waist, hip and thigh circumferences.

Functional autonomy

For the level of functional autonomy, both groups presented fair to good functional autonomy according to the reference values (Table 4). After the intervention, it was observed that the experimental group improved its functional capacity significantly ($p < 0.001$). Furthermore, after the intervention, significantly lower values were observed in the experimental group ($p = 0.001$) and therefore a higher degree of functional autonomy. Considering that the GI was designed to represent the degree of functional autonomy in the older people, and that healthy aging depends on the level of functional status, it seems that physical exercise increases this capacity.

Isometric strength

For the handgrip test (Table 4), significant differences were observed for both dominant and non-dominant hands, both over time and between groups. In the experimental group, a significant increase was observed in both the dominant ($p < 0.001$) and non-dominant ($p < 0.001$) hands after the intervention. In addition, significant differences were observed after the intervention between groups, with significantly higher

TABLE 3 Body composition variables on pre- and post-training moments of the resistance training and control groups.

	Experimental group				Control group				Effect time			Effect time × Group		
	Baseline		Post		Baseline		Post		F	p	ω^2	F	p	ω^2
	Mean	SD	Mean	SD	Mean	SD	Mean	SD						
Waist (cm)	88.5	11.5	88.3	11.5	89.6	8.91	88.7	10.2	1.256	0.270	0.006	0.646	0.427	−0.008
Hip (cm)	104	11.4	104	11.7	103	6.48	104	7.59	0.207	0.652	−0.002	2.126	0.154	0.027
Thigh (cm)	49.3	5.68	50.4	5.64	49.2	5.03	49.2	5.48	2.52	0.122	0.036	2.18	0.149	0.028
Σ 6 skinfolds	128	39.7	113	35.8	140	29.7	151	31.6	1.86	0.182	0.021	25.36	<0.001	0.375
Muscular mass (Kg)	30.4	5.41	32.5	6.1	27.8	4.71	26.6	4.45	1.36	0.253	0.008	60.05	<0.001	0.593
Muscle/bone index	2.83	0.373	3.24	1.09	2.79	0.359	2.68	0.374	1.53	0.225	0.013	4.77	0.036	0.085

cm, centimeters; kg, kilograms; SD, standard deviation; ω^2 , omega squared.

TABLE 4 Functional capacity and muscular strength variables on pre- and post-training moments of the resistance training and control groups.

	Experimental group				Control group				Effect time			Effect time × Group		
	Baseline		Post		Baseline		Post		F	p	ω ²	F	p	ω ²
	Mean	SD	Mean	SD	Mean	SD	Mean	SD						
GDLAM														
10 mW (sec)	6.25	1.15	5.59	1.06	7.26	1.35	7.39	1.17	6.34	0.017	0.116	13.88	<0.001	0.241
GSP (sec)	8.95	1.69	8.77	1.78	12.1	2.64	12.8	2.57	1.17	0.287	0.041	3.45	0.072	0.057
GPP (sec)	3.71	1.02	3.62	0.984	6.14	2.70	6.08	2.97	0.20	0.655	−0.020	0.01	0.912	−0.024
GCMH (sec)	51.9	9.67	41.3	5.15	56.3	7.45	53.3	8.06	58.5	<0.001	0.586	17.8	<0.001	0.293
PTS (sec)	12.2	3.38	11.2	3.21	14.5	5.00	15.6	6.29	9.61	0.975	0.175	4.66	0.038	0.083
GI GDLAM	28.6	3.97	24.7	2.75	34.1	6.40	34.2	7.27	23.0	<0.001	0.352	25.4	<0.001	0.376
Handgrip														
HG D (kg)	25.5	5.96	29.1	7.25	23.4	3.12	22.5	3.75	16.6	<0.001	0.278	40.3	<0.001	0.492
HG ND (kg)	24.2	6.44	26.8	6.97	21.4	3.91	20.8	3.84	16.6	<0.001	0.278	40.3	<0.001	0.492
MQI	0.89	0.26	1.03	0.29	0.84	0.15	0.81	0.16	0.686	0.414	0.007	29.9	<0.001	0.416
Load cell quadriceps extension														
Max. force (N)	221	96.7	301	113	202	70.9	178	61.9	16.3	<0.001	0.274	55.2	<0.001	0.572
Time (sec)	4.45	1.73	4.40	1.75	4.53	2.00	4.02	2.14	0.491	0.488	−0.012	0.31	0.578	−0.011

SD, standard deviation; HG, handgrip; D, dominant; ND, no dominant; MQI, muscle quality index [handgrip strength (kg)/BMI (kg/m²)]; 10 M, 10 m walk; GSP, to get up from the sitting position; GPP, to get up from the ventral decubitus position; GCMH, getting up from a chair and movement around the house; PTS, put on and take off a T-shirt; sec, seconds; Max, maximum; N, newton; ω^2 , omega squared.

values in the experimental group ($p = 0.011$ and $p = 0.022$, dominant and non-dominant hand, respectively). For the muscle quality index (Table 4), significant differences were observed in the experimental group before and after the intervention ($p < 0.001$), with a higher value after the intervention. In addition, the experimental group presented a significantly higher value than the control group at the post-intervention time ($p = 0.026$). Regarding the maximum force in Newton (Nm) measured with a load cell force transducer (Musclelab, Ergotest, Norway), it was observed that there were significant differences in the intervention group after the intervention ($p < 0.001$) and between both groups at the time post ($p = 0.002$).

Postural stability tests

In none of the three postural stability tests (Figures 2A,B) were significant differences found in either group.

Flexibility and aerobic endurance

After determining the flexibility of the participants and analysing the results, it was observed that at the initial moment there were no significant differences (Figure 3A). Only in the experimental group there is a significant increase ($p = 0.003$) after the intervention. As for endurance (Figure 3B), prior to

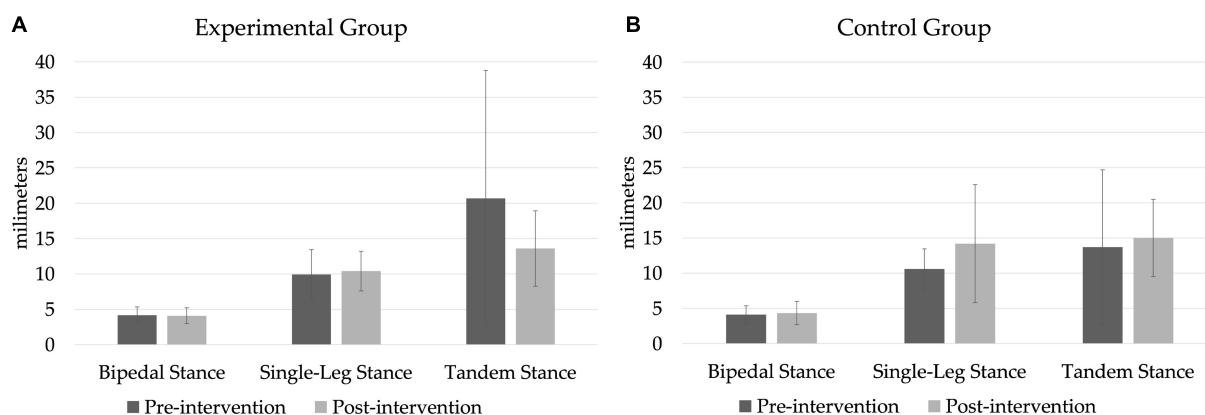


FIGURE 2
Postural balance tests on a force platform. (A) Experimental group, (B) control group.

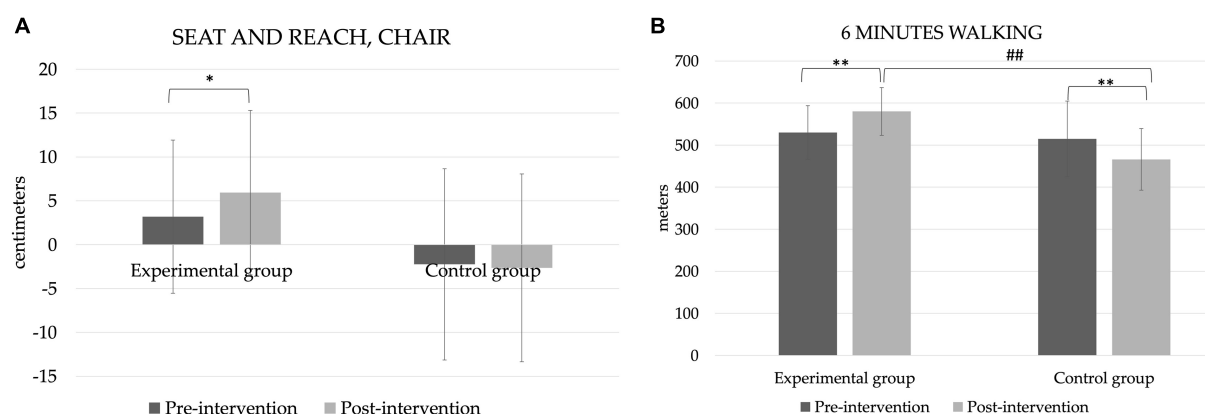


FIGURE 3
Flexibility and resistance. * $p < 0.05$ and ** $p < 0.01$ by intragroup analysis. ## $p < 0.01$ by intergroup analysis. (A) Seat and reach, chair, (B) 6 mins walking.

the intervention the results were 530 ± 63.9 and 515 ± 89.9 m, for the experimental and control groups, respectively. After the intervention, a significant increase was observed in the experimental group (580 ± 56.7 ; $p < 0.001$) and a decrease in the control group (466 ± 73.1 ; $p < 0.001$). There were also differences between the two groups at post moments ($p < 0.001$).

Satisfaction with life

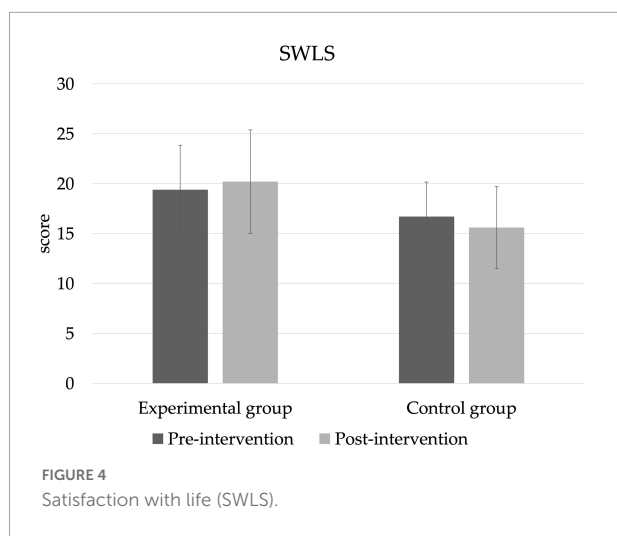
The SWLS scores can be seen in Figure 4. There are no significant differences in scores between groups or at different points in time; however, in the experimental group the score goes up slightly, while in the control group it goes down.

The analysis of the correlations between all participants and each variable are shown in the Table 5. Statistically significant positive correlations are observed between the variables sit and reach and walking 6 mins ($p = 0.044$), as well as with the

subscales of the GDLAM, walking 10 m ($p < 0.001$), GSP ($p = 0.12$), GPP ($p = 0.002$), GCMH ($p < 0.001$), and the total index ($p < 0.001$). As for the life satisfaction questionnaire, it is positively related both with the handgrip results ($p = 0.002$) and with the maximum strength result in loading platform ($p = 0.006$) and muscle mass ($p = 0.010$), in a significant way. Finally, in the case of stability, significant correlations were found between the tandem variable with some of the GDLAM subscales and with the GI ($p = 0.003$), as was the case with the single-leg stance variable ($p = 0.013$).

Discussion

The aim of this study was to analyze the efficacy of the addition of aquatic resistance interval training program and dietary education on body composition, functional capacity,



balance, strength, aerobic capacity, flexibility, and satisfaction with life in women over 65 years of age. Performing training in an aquatic environment in older population allows providing a light and low-impact environment where people can exercise safely (26), where buoyancy, pressure, resistance, and water temperature maximize the effectiveness of aquatic exercise, allowing light and safe body movements (51).

The present study reveals several findings: aquatic resistance interval training for 14 weeks improves body composition, upper and lower body strength, increases functional autonomy, muscle mass, flexibility, and aerobic capacity. However, the stability variables showed no statistically significant differences. Regarding body composition, in the training group, significant improvements were observed in the variables of fold sum and muscle mass, coinciding with results already published (23, 52, 53), in which programmed training caused decreases in body fat. Muscle mass has a fundamental role in the older population, since there is evidence that, without intervention, sarcopenia, and frailty often lead to disability, falls and a decrease in quality of life (54).

The results of the present investigation are in agreement with previous findings, where aquatic exercise has been shown to elicit positive responses on FA in the population between 50 and 80 years of age (55). After the intervention, the FA of older people who underwent aquatic resistance interval training improved significantly, with no improvement in CG. In addition, FA was positively correlated with stability values, so it seems that women with greater stability and muscle mass have greater functional autonomy. In this sense, it is confirmed that systematic and controlled training, widely evidences ostensible improvements in older adults, especially in functional autonomy (56).

Stability is considered one of the most important variables when designing a training program for fall prevention. Research has shown that resistance training in an older people population

can improve static balance (57, 58), however, not all of them always get these same conclusions (26). In the present study, no significant differences were observed between groups in terms of stability variables. The lack of significant results may be due to the lack of specificity of the training to improve this variable (26).

The properties of water provide unique opportunities for rehabilitation through the hydrostatic and hydrodynamic principles of buoyancy and resistance (59), which consequently contributes to reduced pain, stiffness, and difficulty in physical functions. Consequently, physically active older adults across the lifespan have higher levels in terms of physical and cognitive function, mobility, less musculoskeletal pain, lower risk of falls and fractures, depression, and better quality of life. Therefore, the results systematized by Fuentes et al. (55) are in line with the results obtained after the present investigation.

After the intervention, a significant increase in isometric strength of both upper and lower limbs was observed. Previous findings show that limited mobility during aging is associated with the loss of strength and/or function that characterizes sarcopenia (60). In particular, when training is performed in an aquatic environment, it should be taken into account that the density of water is an important characteristic since it can generate an increase in muscle strength because movement in water offers 900 times greater resistance than in air (61). In the correlations, it is also observed that women who have more upper and lower body strength and greater muscle mass show higher values in the satisfaction with life questionnaire.

In the review conducted by Martínez-Carbonell (26) included six studies that evaluated flexibility as an outcome after aquatic training, in all cases, they observed that flexibility improved significantly from pre-test to post-test. This study confirms these results, as significant improvements were only observed between pre- and post-test in the experimental group. It seems that this may be due to decreased stiffness in the pelvic muscles, improving gait and decreasing the risk of falls (26). It has been shown (26) that in order to observe significant changes in flexibility in older adults, training should last a minimum of 12 weeks, with two to three sessions per week of about 60 mins duration. The intervention performed in the present investigation met the requirements. Finally, after the intervention, the distance walked in 6 mins improved in the experimental group. This corroborates current findings, which show that resistance training improves aerobic capacity (62), as interval resistance training is also effective in improving cardiac, respiratory and metabolic function in an older adult population.

In terms of life satisfaction, improvements were observed, although not significant in the experimental group. Positive effects on life satisfaction have previously been found after both shorter (e.g., 12 weeks) and longer (e.g., 8 months) interventions

TABLE 5 Correlations between variables increments (post-intervention–basal).

	HG D	HG ND	Sit and reach	6 min W	Max. Force (N)	Time (sec)	Bipedal stance	Tandem stance	Single-leg stance	10 mW	GSP	GPP	GCMH	PTS	IG	SWLS	THIGH	Skinfolds	MM
HG D	–																		
HG ND	0.925**	–																	
Sit and reach	0.088	0.149	–																
6 min W	0.562**	0.566**	0.343*	–															
Max. force (N)	0.724**	0.707**	0.098	0.602**	–														
Time (sec)	–0.098	0.013	–0.090	–0.136	–0.017	–													
Bipedal stance	0.091	0.146	0.007	–0.159	–0.139	0.068	–												
Tandem stance	0.013	0.005	–0.275	–0.320	–0.124	0.294	0.439*	–											
Single-leg stance	–0.013	–0.094	–0.528*	–0.386*	0.009	0.109	0.046	0.309	–										
10 mW	–0.486*	–0.501*	–0.590**	–0.686**	–0.492**	0.071	–0.050	0.174	0.418*	–									
GSP	–0.479*	–0.463*	–0.420*	–0.539**	–0.563**	–0.010	0.032	0.311	0.112	0.604**	–								
GPP	–0.445*	–0.481*	–0.509*	–0.659*	–0.408*	0.294	0.141	0.415*	0.541**	0.659**	0.701**	–							
GCMH	–0.504*	–0.542**	–0.539**	–0.760**	–0.541**	–0.027	0.185	0.365*	0.385*	0.777**	0.777**	0.805**	–						
PTS	–0.071	–0.056	–0.492*	–0.448*	–0.217	0.237	0.377*	0.600**	0.428*	0.511*	0.492*	0.663**	0.610**	–					
GDLAM IG	–0.406*	–0.413*	–0.583**	–0.690**	–0.481*	0.130	0.229	0.495*	0.414*	0.759**	0.827**	0.881**	0.917**	0.832**	–				
SWLS	0.516*	0.449*	–0.020	0.326	0.459*	0.172	0.191	–0.047	0.177	–0.395*	–0.393*	–0.151	–0.287	–0.144	–0.296	–			
Thigh	0.113	0.034	0.057	–0.119	–0.070	–0.020	–0.069	–0.026	0.037	0.027	0.043	–0.002	0.057	–0.058	–0.012	0.015	–		
Skinfolds	–0.166	–0.215	–0.033	–0.494*	–0.356*	–0.090	–0.116	0.203	0.074	0.197	0.288	0.236	0.306	0.187	0.280	–0.148	0.547**	–	
MM	0.522*	0.426*	0.062	0.209	0.242	–0.028	0.153	–0.109	–0.004	–0.292	–0.395*	–0.318	–0.293	–0.186	–0.339*	0.427*	0.668**	0.121	–

P*-value < 0.05; *p* value < 0.01. HG, handgrip; D, dominant; ND, no dominant; min, minutes; W, walking; N, Newtons; max., maximum; 10 M, 10 m walk; GSP, to get up from the sitting position; GPP, to get up from the ventral decubitus position; GCMH, getting up from a chair and movement around the house; PTS, put on and take off a T-shirt; SWLS, satisfaction with life; MM, muscular mass.

(63). It appears that these improvements may be since resistance training influences some areas of psychological functioning, in addition to improving physical function, increasing the ability to perform activities of daily living and decreasing pain.

The present study has some limitations that should be considered when interpreting or applying our results. First, the sample is only composed of women. It is unclear whether male participants would receive similar benefits from aquatic training, and more research needs to be conducted in this cohort of participants. Secondly, this trial included only a small number of participants, whereas a larger sample size would have helped to quantify the changes resulting from this exercise training more accurately. Furthermore, the observed adaptations are limited to the duration of our intervention; a longer intervention could have resulted in greater adaptations. It should also be considered that there are differences in the biometric variables at the beginning of the investigation, probably related to the randomization process. Another limitation is that a third training group that did not receive nutrition education was not created because we preferred that the larger sample benefit from this program. The Borg scale is a subjective and qualitative measurement tool; therefore, it is not global; intensity should also be measured with objective data. Finally, it should be noted that the CG did not perform any structured physical activity during the 14 weeks of intervention, which may have contributed to their deterioration.

Future research should measure body composition with the standard reference model; dual-energy X-ray absorptiometry (DXA). In addition, more consistent methods of analysis could be used to obtain more representative information for the study population. The variable sleep quality of the participants also should be controlled. It has previously been observed that more than 50% of people aged 65 years or older have sleep disorders. These sleep disorders are associated with decreased cognitive function, increased falls, worsened health status, and increased mortality (64). In addition, a stress test with gas analyser should be performed. Always trying to obtain a larger sample size.

Conclusion

In conclusion, the key observation of this study is that, in addition to the known physical benefits for older populations such as strength, functional capacity and flexibility, resistance training in an aquatic environment, along with nutritional education are beneficial for improving body composition and life satisfaction. Future research should consider the frequency and duration of training in relation to psychological functioning to identify when changes occur, thus more accurately defining the duration and intensity of training needed to obtain benefits. Under supervised conditions,

the intervention is safe and, based on the results, should be further investigated in a larger cohort of male and female participants.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Alicante University Ethical Committee. The patients/participants provided their written informed consent to participate in this study.

Author contributions

AM-R and PM-P: conceptualization, validation, and supervision. AM-R, JG, RY-S, and PM-P: methodology. AM-R, RY-S, and JG: software. AM-R, BC-C, and RY-S: formal analysis. AM-R, BC-C, RY-S, JG, and PM-P: investigation. BC-C and PM-P: resources. BC-C, RY-S, and JG: data curation. AM-R, BC-C, and PM-P: writing—original draft preparation. PM-P: writing—review and editing. AM-R and RY-S: visualization. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Comparison of body fat percentage assessments by bioelectrical impedance analysis, anthropometrical prediction equations, and dual-energy X-ray absorptiometry in older women

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Background: Individuals with high body fat have a higher risk of mortality. Numerous anthropometric-based predictive equations are available for body composition assessments; furthermore, bioelectrical impedance analysis (BIA) estimates are available. However, in older adults, the validity of body fat estimates requires further investigation.

Objective: To assess the agreement between percentage body fat (BF%) estimates by BIA and five predictive equations based on anthropometric characteristics using dual X-ray absorptiometry (DXA) as reference method. A secondary objective was to identify whether excluding short-stature women improves the agreement of BF% estimates in a group of community-dwelling, older Mexican women.

Methods: A concordance analysis of BF% was performed. A total of 121 older women participated in the study. Anthropometric information, BIA, and DXA body composition estimates were obtained. Five equations using anthropometric data were evaluated in order to determine body fat percentage (BF%) using DXA as reference method. Paired *t*-test comparisons and standard error of estimates (SEE) were obtained. The Bland-Altman plot with 95% limits of agreement and the concordance correlation coefficient (CCC) were used to evaluate the BF% prediction equations and BIA estimates.

Results: The mean age of the study participants was 73.7 (± 5.8) years old. BIA and the anthropometric based equations examined showed mean significant differences when tested in the entire sample. For the taller women (height > 145 cm), no significant difference in the paired comparison was found between DXA and BIA of BF% estimates. The mean BF% was 40.3 (± 4.8) and 40.7 (± 6.2) for DXA and BIA, respectively. The concordance between methods was good (CCC 0.814), (SEE 2.62). Also, in the taller women subset, the Woolcott equation using waist-to-height ratio presented no significant difference in the paired comparison; however, the error of the estimates was high (SEE 3.37) and the concordance was moderate (CCC 0.693).

Conclusion: This study found that BIA yielded good results in the estimation of BF% among women with heights over 145 cm. Also, in this group, the Woolcott predictive equation based on waist circumference and height ratio showed no significant differences compared to DXA in the paired comparison; however, the large error of estimates observed may limit its application. In older women, short stature may impact the validity of the body fat percentage estimates of anthropometric-based predictive equations.

KEYWORDS

aging, body fat, anthropometric, bioelectrical impedance, DXA (dual X-ray absorptiometry), validation studies

Introduction

In 2019, the global population of older adults aged 60 and over was nearly 1 billion people, representing 13.2% of the total population. By 2050, their number is expected to reach 2.1 billion, 2.5 times more than in 1980 (382 million). By 2050, United Nations projections estimate that there will be twice as many older adults as children under the age of five. Most older adults live in middle-income countries (1). With an aging population, it is particularly difficult to adequately respond to related epidemiological changes, such as the increasing rate of chronic non-communicable diseases (NCDs). According to Global Burden of Disease (GBD) estimates in 2015, 28.9% of GBD was attributable to people over 60 years of age, and NCDs accounted for 86.8% of the total burden of disease (2, 3). In European countries the prevalence of obesity has increased rapidly in the last 40 years, particularly among adults aged 60–74 years (4). The results of a study in China showed that more than half of the Chinese aged 70 years or older have obesity-associated multimorbidity, which has become a major public health problem in this country (5, 6). Older adults will develop obesity and multiple chronic diseases (Type-2 diabetes mellitus, cardiovascular and cerebrovascular diseases, high blood pressure, dyslipidemias, metabolic syndrome, and abdominal adiposity) generating a reduction in the quality of life. In addition, obesity is also associated with greater disability and worsening of non-communicable chronic diseases (NCDs)

(7, 8). In Mexico there is a high prevalence of obesity, mainly, in sectors with greater poverty and vulnerability. According to the Mexican National Survey of Health and Nutrition (ENSANUT), in the range of women aged 60–69 years, there was an increase in the prevalence of obesity from 41.0% in 2012 to 45.9% in 2018 (9).

The method most widely used to estimate obesity is the body mass index (BMI) (kg/m^2), because it is simple and inexpensive and is the basis for the World Health Organization (WHO) criteria of overweight ($25 \leq \text{BMI} < 30$) and obesity ($\text{BMI} \geq 30$) (10). However, for a given BMI, body fat percentage changes with age, and the form of this change is different according to sex, ethnicity, and individual differences (11). Changes in body composition due to aging have led to the proposal of different cut-off points for defining underweight and overweight in older adults. Lipschitz considers that older adults with $\text{BMI} \leq 22 \text{ kg}/\text{m}^2$ are underweight and those with $\text{BMI} > 27 \text{ kg}/\text{m}^2$ are overweight or obese (12).

Additionally, height plays a very important role in determining BMI. Changes in height that occur during aging will impact the BF estimates using BMI (13). The decrease in height occurs mainly due to the following factors: reduction of the plantar arch, increase in the curvature of the spine, vertebral compression, shape of the vertebral discs, loss of muscle tone and inadequate posture habits as well as due to injuries and diseases that affect the joints and the musculoskeletal system (14). After 50 years of age, men's height decreases between 0.08%

and 0.10%, while women's height declines between 0.12 and 0.14% per year, sharpening after 70 years of age (14, 15). In China, the height of women decreases by 3.8 cm every 10 years from the age of 40, while in Indonesia it decreases by 0.6 cm per year for women 60 years and older (16). Age-related changes in height have been associated with health problems (17, 18). Mexican women of short stature and over 50 years of age had an increased risk of obesity (OR = 1.84) compared to women without this condition (19).

To obtain a complete nutritional evaluation of older adults, body composition should be considered for both the nutritional diagnosis (risk of malnutrition or malnutrition) (20, 21) as well as to determine the different body compartments and assess more precisely if the patient presents obesity (22), sarcopenia (decrease in appendicular skeletal muscle mass) (23) or osteoporosis (decrease in bone mineral density) (24).

Dual X-ray absorptiometry (DXA) is frequently used as the gold standard for evaluating body composition prediction methods. DXA estimates are at a molecular level and identify three body components: bone mineral content (BMC), lean mass (LM), and fat mass (FM). This technique has shown good agreement compared with more sophisticated techniques (25).

Bioelectrical impedance analysis (BIA) is increasingly used to evaluate body composition. BIA safe, BMI is simple to apply, non-invasive, and inexpensive as it avoids radiation exposure (26). Based on the electrical properties of the body, BIA determines the resistance resulting from an electrical current passing through the body. It considers the subject's weight, height, and age to estimate, the total body's water, and applies specific equations (Siri or Brosek) (27, 28) in order to determine the BF%. BIA is a doubly indirect method of assessing body composition. Since it is based on factors such as type of device, water distribution, hydration status, weight, and height, BIA estimates may vary (29).

Studies of comparison of DXA and BIA for body composition assessment are scarce in older adults. There is considerable interest in the field of body composition for developing and properly validating equations based on anthropometric measurements so as to determine lean body mass, fat mass percentage, and fat content in wide population groups without having to use technologies such as DXA (30). Currently, in older adults, there is no agreement on whether equations based on anthropometric data can successfully be used in clinical practice or public health settings. There are conflicting results on the validity of predicting equations available based on anthropometric characteristics (31). Some studies have shown good agreement while others found low concordance and biased estimates when comparing predictive equations with DXA (32, 33).

The objective of the study was to assess the agreement between percentage body fat (BF%) estimates by BIA and five

predictive equations based on anthropometric characteristics using DXA as reference method. A secondary objective was to identify whether excluding short-stature women improves the agreement of BF% estimates in a group of community-dwelling, older Mexican women.

Materials and methods

Study design

The current study has a cross-sectional design. The study group was selected from attendees of a sports and social entertainment facility in Southeast Mexico City, between April and July of 2019. This facility has governmental support and is free of charge for people over 60 years old. There are several activities that the attendees can engage in, such as dancing classes, needle knitting, and singing lessons (chorus). Also, a gym is available, and attendees may participate in gymnasia, physical conditioning, spinning, yoga, Tai Chi, and similar classes.

To enroll participants in the study, we placed an ad at the entrance and registered those who were interested in receiving nutritional assessment and have a DXA evaluation. All the procedures were free of charge for the facility members. The eligibility criteria of the study were the following: women over 60 years old, capable of independent mobility (not using a wheelchair), who were under medical treatment and supervision if they had NCDs. The women who were willing to participate in the study signed an informed consent letter. Among the exclusion criteria were women who have a recent history of falls and fractures or recent hospital admissions (within the last 6 months), those with serious medical conditions (cardiovascular or cerebrovascular disease, respiratory failure, liver failure, Parkinson's disease, advanced diabetic neuropathy, rheumatoid arthritis, and cognitive impairment) were also excluded from the study as well as those with signs of edema, physical disability, and those wearing an orthopedic prosthesis that could alter their body composition results. The study's goals and evaluation procedures were individually described in a detailed form to each participant. All subjects signed an informed consent letter in which the goals and procedures of the study were fully described. This study was conducted in accordance with the ethical standards of the Helsinki Declaration of Ethical Principles for Medical Research Involving Human Subjects. Recruitment and data collection took place between April and July 2019. The protocol was registered and approved by the Division of Biological and Health Sciences and the Ethics Committee of the Universidad Autónoma Metropolitana Unidad Xochimilco (DCBS.CD, approval CD.52.17).

TABLE 1 Selected equations presenting age, body mass index, body fat percentage, coefficient of determination and measurement error in women.

References	Equations	<i>n</i>	Age (years) mean/range	BMI (kg/m ²) mean/range	Body fat %	<i>R</i> ²	SEE/ RMSE
Deurenberg et al. (54)	$%BF = 1.20 \times BMI + 0.23 \times age - 10.8 \times sex - 5.4$	708	7–83	13.9–40.9 kg/m ²	Group A: 24.7 Group B ^a : 25.7	0.79	4.1
Gallagher et al. (11)	$%BF = 64.5 - 848 \times \left(\frac{1}{BMI}\right) + 0.79 \times age$	1,013	African American: 56.2 ± 16.8 White: 48.8 ± 17.6 Asian: 39.3 ± 15.9	African American: 27.1 ± 4.3 White: 24.5 ± 4.5 Asian: 23.2 ± 3.9	African American: BMI < 18.5: 23% BMI ≥ 25: 35% BMI ≥ 30: 41% White: BMI < 18.5: 25% BMI ≥ 25: 38% BMI ≥ 30: 43% Asian: BMI < 18.5: 26% BMI ≥ 25: 36% BMI ≥ 30: 41%	0.86	4.98
Woolcott and Bergman (55)	$%BF = 64 - 20 \times \left(\frac{height}{waist}\right) + 12 \times sex$	NHANES 1999–2004 6,261 NHANES 2005–2006 1,700	NHANES 1999–2004 47.2 ± 0.3 NHANES 2005–2006 43.3 ± 0.3	NHANES 1999–2004 28.2 ± 0.1 NHANES 2005–2006 28.7 ± 0.3	NHANES 1999–2004 30.8 ± 0.3 NHANES 2005–2006 31.2 ± 0.6	0.70–0.49	3.91–4.01
Woolcott and Bergman waist/height (55)	$%BF = -5 + 58 \times \left(\frac{waist}{height}\right) + 11 \times sex$	NHANES 1999–2004 6,261 NHANES 2005–2006 1,700	NHANES 1999–2004 47.2 ± 0.3 NHANES 2005–2006 43.3 ± 0.3	NHANES 1999–2004 28.2 ± 0.1 NHANES 2005–2006 28.7 ± 0.3	NHANES 1999–2004 30.8 ± 0.3 NHANES 2005–2006 31.2 ± 0.6	0.67–0.48	4.12–4.07
Woolcott and Bergman height ³ / waist × weight (55)	$%BF = 44 - 230 \times \left(\frac{height^3}{waist \times weight}\right) + 12 \times sex$	NHANES 1999–2004 6,261 NHANES 2005–2006 1,700	NHANES 1999–2004 47.2 ± 0.3 NHANES 2005–2006 43.3 ± 0.3	NHANES 1999–2004 28.2 ± 0.1 NHANES 2005–2006 28.7 ± 0.3	NHANES 1999–2004 30.8 ± 0.3 NHANES 2005–2006 31.2 ± 0.6	0.75–0.61	3.60–3.51

^aGroup B: cross-validation group. BMI, body mass index; %BF, percentage of body fat; *R*², coefficient of determination; SEE, standard error of the estimate; RMSE, root mean squared error; NHANES, National Health and Nutrition Examination Survey.

Anthropometry

Body weight and height measurements were taken by a certified dietitian (International Certification in Kinanthropometry, Isak Level 1) using the recommended techniques and procedures (34). A senior researcher supervised the anthropometric evaluation. Body weight and height were measured using a portable, electronic digital scale, equipped with a built-in stadiometer with a resolution of 0.1 kg and 0.1 cm, respectively, according with Lohman et al.'s specifications (35). The waist circumference was measured with a fiberglass tape and was reported in centimeters. The anatomic landmarks used to measure waist circumference were the midpoint between the lower rib and the iliac crest T, crest. This is considered the ideal place to perform the procedure. BMI was calculated dividing body weight (kg) by the square of height (m) and expressed in kg/m². Using the WHO criteria, the participants were classified according to BMI in four groups: low (BMI < 18.5 kg/m²); normal (BMI

20–24.9 kg/m²); overweight (BMI 25.0–29.9 kg/m²) and obese (BMI > 30 kg/m²) (36). Additionally, a BMI classification especially design for older adults was applied in the study group. The Lipschitz cut-off points proposed for individuals older than 65 years were also applied: underweight (BMI < 22 kg/m²), eutrophic BMI (22–27 kg/m²) and excess weight (BMI > 27 kg/m²) (12).

Body composition assessment

Dual-energy X-ray absorptiometry (DXA)

Participants were required to wear light sport clothing free of metal zippers and metal decorations, jewelry (watches, earrings, necklaces, and rings), hairpins and coins, keys, to avoid interference with DXA measurements. Whole body DXA scans were carried out following the manufacturer's instructions by a laboratory technician with experience, using the Hologic Discovery QDR Series DXA equipment. The

technician inspected each scan image and performed the necessary corrections to ensure reliable and high-quality results. The DXA equipment was calibrated daily in the morning with a phantom prior to the actual measurements. Values of total BF expressed in grams and percentage, as well as fat free mass (in grams) were determined directly with DXA. To perform the scan, each participant was asked to lay down on the equipment table in a supine position along their longitudinal axis, using the middle line as a reference. Each participant was asked to keep the toe tips in close contact while the scans were performed. The women's hands were kept in a prone position within the scan field of the equipment. While the body scan was being performed, participants were asked to stay still. Whole body scans had a mean length of 6 min per person.

Bioelectric impedance analysis (BIA)

A multiple frequency equipment with a current between 100 and 500 μ A was used. The device was equipped with eight tactile electrodes (four in the platform, to make feet connect, and four on each of the two handles, to connect the hand fingers in order to ensure passage of the electric current. The women fasted 8–12 h prior to each BIA or DXA measurement. The evaluations were performed in the morning. Each person was told to avoid over-hydration and to avoid performing strenuous exercise. Each participant emptied their bladder prior to the BIA or DXA test. Participants were asked to take off their shoes and to maintain an orthostatic position (standing up) during BIA measurements.

Equations to predict body fat percentage

The number of equations available in the literature to estimate body fat is large; therefore, it is not practical to test all of them. We selected five equations for the prediction of BF%. The criteria for selecting these equations were: (1) Equations developed including adults and older adults in the study group; (2) Equations including white or Hispanic ethnic groups in the development process; (3) An adequate sample size; (4) Only requiring anthropometric, sex, and age data to obtain the BF% estimates; (4) Whether these equations were used in other tests with good results (cross-validation study). **Table 1** presents the characteristics the five equations selected.

Sample size

The sample size was calculated for a type I error at $\alpha = 0.05$ and power = 90% (type II error $\beta = 0.10$) and an expected Pearson Correlation Coefficient $r = 0.40$, which is considered a moderate correlation, the sample size obtained was 62. The Pearson Correlation Coefficient is frequently used as part of the evaluation of reliability of body composition equations (37). The authorities of the facilities visited wished to include as many participants as possible. The number of participants was scaled up to 132 of these 125 fulfilled the inclusion criteria, 4 did not attend the appointment for DXA examination. In the end, the

data of 121 participants was analyzed. **Figure 1** presents a flow chart of the participant's selection process.

Statistical analysis

The description of the data included means and standard deviations (\pm sd) for the continuous variables. Categorical data was presented as percentages. DXA BF% estimates were used as reference values to compare BIA and the five different equations that were tested. The normality of the main variable distributions was assessed using the Shapiro Wilkins test. As part of the accuracy evaluation paired *t*-tests were performed to identify differences in BF% estimations between methods. Simple linear regression models were fitted, and the Coefficient of Determination (R^2) and Standard Error of Estimates (SEE) were reported. Lin's concordance correlation coefficient (CCC) and the 95% confidence interval (95%CI) were obtained. The CCC corresponding graph representing the line of perfect concordance (45-degree line in the Cartesian axes) and the reduced major axis line of the methods being compared were constructed. The reduced major axis regression method has the advantage over simple linear regression to allow error in the measurement of both the independent and the dependent variables (38). This is appropriate considering that DXA body composition measurements have several sources of error. The CCC combines the assessment of precision and accuracy in relation to the perfect concordance line, as observed in the formula ($CCC = r * C_b$): where *r* is the Pearson Correlation Coefficient, which measures how far the observations deviate from the line of perfect concordance and is considered a measure of precision, and *C_b* is the bias correction factor that uses measurements of dispersion to estimate the differences of data points with respect to the line of perfect agreement as a measure of accuracy (39). A CCC = 1 indicates perfect concordance between measurements. According to Hinkle et al. (40), the CCC could be classified as follows: $0 \leq CCC < 0.10$ negligible, $0.10 \leq CCC < 0.39$ weak, $0.39 \leq CCC < 0.69$ moderate, $0.69 \leq CCC < 0.89$ strong and $0.90 \leq CCC$ very strong. A bootstrap method (1,000 repetitions) was used to obtain 95% confidence intervals of the CCC (95%CI). Additionally, systematic differences (bias) between the tested equations and DXA were evaluated using the Bland-Altman plot, identifying differences between methods and the Limits of Agreement (LoA). The statistical hypotheses tested were considered significant at a *p*-value < 0.05. The statistical analysis was performed using Stata V16 package (Stata Corp. LP, College Station TX).

Results

A total of 121 older women participated in the study. Their mean age was 73.7 (± 5.8), ranging from (65–88) years old.

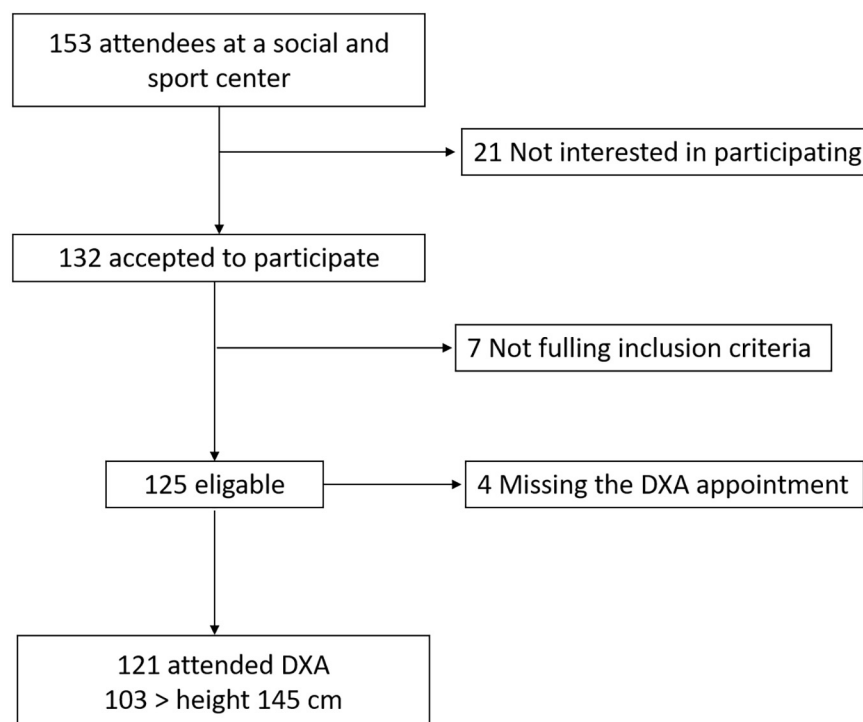


FIGURE 1
Flow chart of participant recruitment.

Table 2 presents the anthropometric characteristics of the study group. The mean weight and height were 61.4 kg (± 8.8) and 151 cm (± 6.0), respectively. The percentage of women shorter than 1.50 was 39.7% and the number of women shorter than 145 cm was 18 (14.9%); the height of 145 cm lies approximately in -1 standard deviation of the height distribution. This subset encompassed 103 women, excluding those with the lowest stature. The mean BMI was 26.9 (± 3.6), range (19.4–37.3). None of the women were classified as underweight, and approximately one-fifth were classified as obese according to the WHO criteria. However, using Lipschitz criteria (BMI < 22) 9.9% of the women were underweight and 46.3% (BMI > 27) were overweight or obese.

Table 3 presents the mean of BF% estimates by DXA and BIA in the entire group ($n = 121$), and in a subset of taller women (≥ 145 cm). BF% based on DXA was 40.3% (± 4.7) while the BIA mean value was 40.9% (± 6.1) [difference -0.7 , (± 3.4)], suggesting an overestimation of BF% by BIA in the study group. The paired t -test results indicated a significant difference between measurements ($p = 0.035$) upon the comparison of these two methods. However, in the subset of taller women, the difference between these methods was lower (0.40) and not statistically significant ($p = 0.228$) based on the paired t -test results. The SEE was 2.58 percentage points in the entire group and slightly higher in the subset (2.62 percentage points).

Table 4 presents Lin's CCC and bias results for DXA and BIA. Satisfactory results were obtained in bias by BIA in both the entire group ($C_b = 0.961$) and in the subset of the taller women

TABLE 2 Anthropometric characteristics and body mass index categories of participating older women ($n = 121$).

Characteristic	Mean (\pm sd)
Age (years)	73.7 (± 5.8)
Height (cm)	151 (± 6.0)
Weight (kg)	61.4 (± 8.8)
Waist circumference (cm)	91.7 (± 9.9)
Hip circumference (cm)	103.6 (± 8.8)
Body mass index (BMI) (kg/m ²)	26.9 (± 3.6)
BMI, WHO categories	n (%)
Underweight (BMI < 18.5)	0 (0)
Normal weight (18.5 < BMI \leq 24.9)	36 (29.7)
Overweight (25 < BMI \leq 29.9)	58 (47.9)
Obese (BMI > 30)	27 (22.3)
BMI, Lipschitz categories	n (%)
Underweight (BMI < 22)	12 (9.9)
Eutrophy (22 \leq BMI \leq 27)	53 (43.8)
Excess weight (BMI > 27)	56 (46.3)

TABLE 3 Mean percentage body fat (%BF) estimated by DXA, BIA and equations using anthropometric characteristics for the entire group ($n = 121$) and a subgroup of women with heights greater than 145 cm ($n = 103$) and the results of regression models and paired t -test.

%BF estimation method/equation	Mean (SD) $n = 121$	* R^2	**SEE	P -value for paired t -test	Mean (SD) $n = 103$	* R^2	**SEE	P -value for paired t -test
DXA	40.3 (4.7)	Ref [§]	Ref	Ref	40.3 (4.8)	Ref	Ref	Ref
BIA	40.9 (6.1)	0.703	2.58	0.035	40.7 (6.2)	0.709	2.62	0.228
Deurenberg et al. (54)	43.8 (4.5)	0.463	3.46	<0.001	43.7 (4.6)	0.467	3.55	<0.001
Gallagher et al. (11)	38.2 (4.4)	0.541	3.21	<0.001	38.1 (4.5)	0.538	3.30	<0.001
Woolcott and Bergman height/waist (55)	42.7 (3.6)	0.352	3.81	<0.001	42.5 (3.6)	0.339	3.95	<0.001
Woolcott and Bergman (height ³ /waist, weight) (55)	41.3 (3.4)	0.509	3.31	<0.001	41.2 (3.5)	0.498	3.44	<0.001
Woolcott and Bergman waist/height (55)	41.1 (3.9)	0.515	3.32	0.011	40.9 (3.8)	0.501	3.37	0.074

* R^2 Coefficient of determination. **SEE Standard error of estimate. [§]ref, standard reference.

TABLE 4 Bias and concordance correlation coefficient for percentage body fat using BIA and equations based on anthropometric measurements in the entire group (121) and a subgroup.

Method/equation	Bias $n = 121$	CCC ^a (95% CI)	Bias $n = 103$	CCC ^a
BIA ^b	0.961	0.805 (0.750, 0.858)	0.966	0.814 (0.745 0.865)
Deurenberg et al. (54)	0.771	0.525 (0.443, 0.605)	0.795	0.543 (0.423 0.644)
Gallagher et al. (11)	0.902	0.663 (0.576, 0.732)	0.896	0.657 (0.546 0.746)
Woolcott and Bergman (55) height/waist	0.830	0.492 (0.379, 0.603)	0.843	0.491 (0.357 0.605)
Woolcott and Bergman (55) (height ³ /waist, weight)	0.921	0.657 (0.559, 0.744)	0.925	0.653 (0.542 0.742)
Woolcott and Bergman (55) waist/height	0.961	0.637 (0.525, 0.728)	0.959	0.638 (0.517 0.735)

^aLind's concordance correlation coefficient. ^bBioelectrical impedance analysis (MF Inbody 720 equipment).

group ($C_b = 0.966$). The CCC between DXA and BIA was 0.805 and 0.814 in the entire and in the subset groups, respectively. Those CCC are considered to indicate a strong concordance. **Figure 2A** presents the reduced major axis line and the line of perfect concordance for DXA and BIA of BF% in women with a height over or equal to 145 cm. The data points were distributed tightly along the line of perfect agreement. Moreover, the Bland-Altman plot (**Figure 2B**) of DXA and BIA results suggest a slight overestimation of BF% (0.40) by BIA, proportional bias was significant and wide LoA ($-7.03, 6.22$) were observed.

The difference between DXA and Deurenberg's estimates indicated an overestimated BF% [difference 3.36, (± 3.7)] by this equation in the entire group (**Table 3**), ($p < 0.001$). Similarly, in the subset of taller women, significant differences were found in the paired t -test comparison showing an overestimation of Deurenberg estimates ($p < 0.001$). The SEE was higher than three percentage points in the entire group and the subset of taller women.

In both the entire group and the subset of taller women, moderate concordance was observed (**Table 4**). **Figure 2C** presents the CCC plot depicting the reduced major axis line and the line of perfect concordance of DXA and the Deurenberg equation. Results of the CCC indicated a moderate concordance. Additionally, in the subset of taller women, the Bland-Altman

plot (**Figure 2D**) BF% shows wide LoA, indicating low precision in the BF% results.

The Gallagher equation underestimated BF% [difference 2.19, (± 3.3)] in the women studied; the differences were significant in both the entire group and the subset of taller women ($p < 0.001$) (**Table 3**). **Figure 3A** displays the reduced major axis line, and the line of perfect concordance for DXA and Gallagher estimates of BF% in women with a height of more than or equal to 145 cm. The CCC was 0.657, suggesting a moderate concordance between methods (**Table 4**). The Bland-Altman plot displays the difference of BF% between Gallagher and DXA, where underestimation of BF% was observed (**Figure 3B**). Bias results showed satisfactory values (**Table 4**). The findings suggested a slightly higher agreement using Gallagher compared to Deurenberg estimates of BF%.

The three Woolcott equations selected showed a statistically significant difference in the paired t -test results in the entire sample ($p < 0.001$) (**Table 3**). However, in the subset of taller women (height ≥ 145 cm), Woolcott's waist-to-height equation showed no statistically significant difference [difference -0.65 , (± 3.7), $p = 0.074$]. In the paired t -test results for the other two equations the difference remained significant (**Table 3**). As well, in the Woolcott waist/height

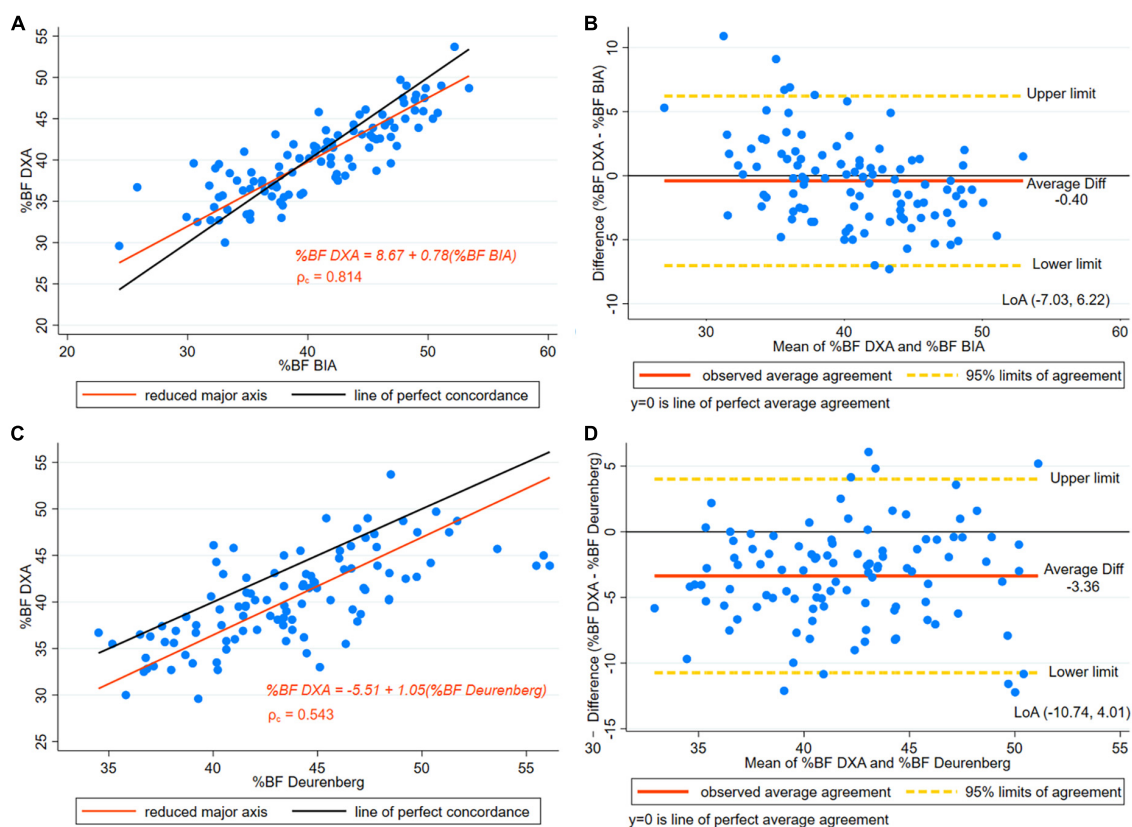


FIGURE 2

(A) Concordance plot of body fat percentage (BF%) estimated by DXA and MF-BIA (multi-frequency InBody 720 equipment). Pearson's correlation coefficient (r) and Lin's concordance correlation coefficient (ρ_c) for women with height > 145 cm. (B) The Bland-Altman plot is presented along with the Limits of Agreement (LoA) of BF% estimated by DXA and MF-BIA (Multi-Frequency BIA InBody 720) for women with height > 145 cm. (C) Concordance plot for BF% estimated by DXA and Deurenberg's equation in women with height > 145 cm. (D) The Bland-Altman plot is presented along with the LoA for BF% estimated by DXA and Deurenberg's equation in women with height > 145 cm.

equation, in both the subset and the entire group, the R^2 was slightly higher than 0.5. As for the remaining Woolcott equations, both groups had a R^2 of below 0.50, which represents a low coefficient (Table 3). SEE exceeded three points in BF%. A moderate level of CCC was found in all of the three Woolcott equations studied (Table 4 and Figures 3C, 4A, C).

Figure 3D presents the Bland-Altman plot of Woolcott equation using height-to-waist ratio showing a 2.21 percentage points overestimation of BF% compared to DXA and wide LoA (-10.45, 5.64). Figure 4B presents the Bland-Altman plot showing an overestimation of BF% using Woolcott cubic height, waist circumference, and weight equation; the mean difference was around one percentage point (0.91). The graphs displayed LoA (-7.62, 5.79), which appears to be narrower than the LoA obtained by the Woolcott equation using the height/waist ratio. Lastly, Figure 4D presents the Bland-Altman plot of the Woolcott equation using waist/height ratio, a difference of less than one percentage point (0.65) compared to DXA was found.

However, proportional bias was significant, and wide LoA (-7.80, 6.50) of BF% were observed in women with heights of 145 cm or higher.

Discussion

This study aimed to compare the BF% assessment by BIA and five different prediction equations based on anthropometric characteristics, using DXA as the reference method in older women. A significant difference was observed in the mean BF% obtained by BIA and DXA. However, the difference between the mean estimates of BF% with BIA compared to DXA was less than one percentage point. Accordingly, a retrospective study in French adults reported a lack of agreement between BIA and DXA at individual level and good agreement at the population level. Achamrah et al. (32) have suggested that BIA and DXA are interchangeable methods for estimating BF at the population level; nevertheless, at individual level differences were significant. Other studies have reported similar results

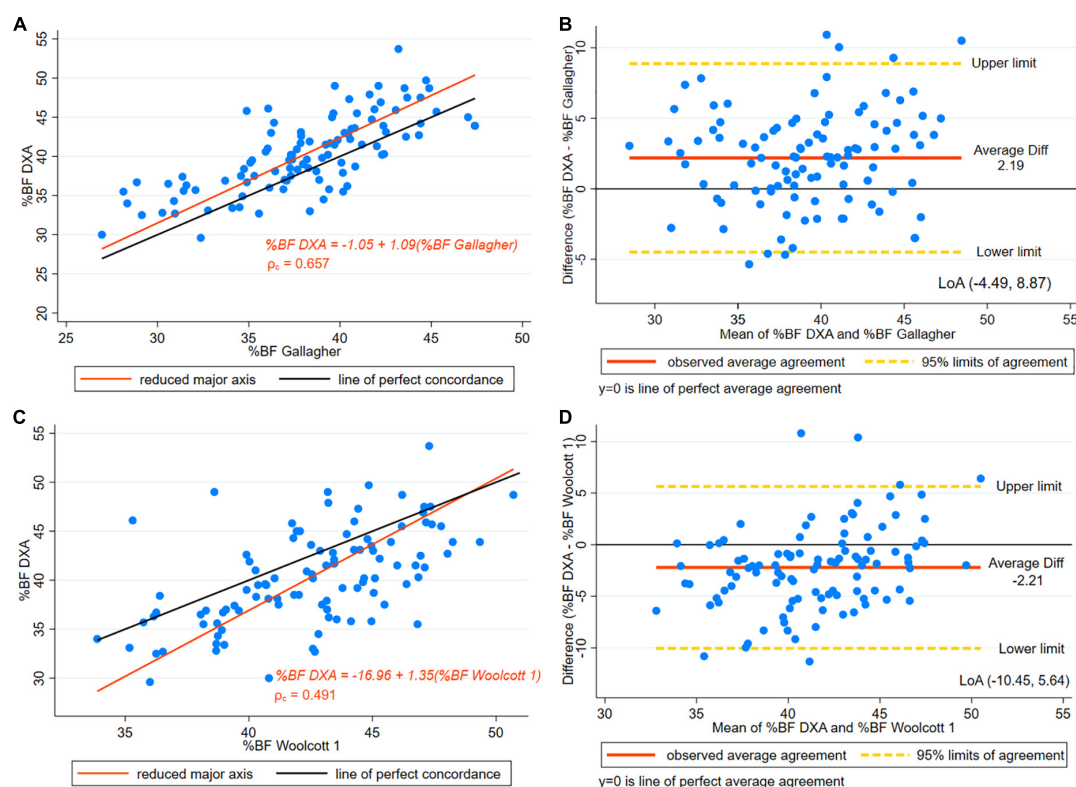


FIGURE 3

(A) Concordance plot of body fat percentage (BF%) estimated by DXA and Gallagher's equation. Pearson's correlation coefficient (r) and Lin's concordance correlation coefficient (ρ_c) for women with height > 145 cm. (B) The Bland-Altman plot is presented along with the Limits of Agreement (LoA) for BF% estimated by DXA and Gallagher's for women with height > 145 cm. (C) Concordance plot for BF% estimated by DXA and Height/Waist ratio Woolcott's equation (Woolcott equation 1) in women with height > 145 cm. (D) The Bland-Altman plot is presented along with the LoA for BF% estimated by DXA and height/waist ratio Woolcott's equation (Woolcott equation 1) in women with height > 145 cm.

across categories of BMI, but other authors have suggested that BIA underestimates BF% (30, 37).

The lack of agreement between BIA and DXA body composition estimates may be due to several factors, such as body density and sample selection (age, sex, ethnic group, body density, fat distribution, and body proportions) (41).

A second aim of the present study was to identify the impact of excluding short stature women in the concordance assessment of BIA and DXA. Approximately 40% of the participating Mexican women presented short stature, defined as height under or equal to 150 cm, and 15% presented a height lower than 145 cm. In the current study, in addition to the analysis of the entire group, an analysis was performed in a subset where the shortest women were excluded (those with a height under 145 cm). In the subset of taller women, BIA presented no statistically significant difference in the paired comparisons of BF% with DXA. A good CCC between methods was found, and the SEE was lower than three percentage points. An improvement in the performance of BF% estimates was observed regarding lower mean differences excluding the shortest women.

The effect of short stature in body composition has not been fully elucidated. A high prevalence of short stature has been identified in Mexico (19), Latin-American countries (42), and other areas in the world (43). A study in Mexican adults detected high BF% in short-stature individuals. Short-stature participants (44) with a BMI ≥ 25 presented a 4.2% higher BF% compared to those with normal stature. Furthermore, the influence of short stature on body composition was studied in a group of children using a case-control design matched on age and sex comparing short-stature children with their average-stature counterparts. Differences in body composition were identified, lower fat-free mass was observed in the children with short stature (45). Height is a long-term indicator of growth associated with nutritional status during growing stages. Certain diseases, health behaviors, and socioeconomic conditions may affect height. Genes have a key role in height; recently, the list of genes associated with short stature has increased (46–48).

Short stature is considered a risk marker for mortality. In a systematic review and meta-analysis of longitudinal studies, a U-shape relationship was observed between height and the risk of death (49). Further studies on the body fat of adults with

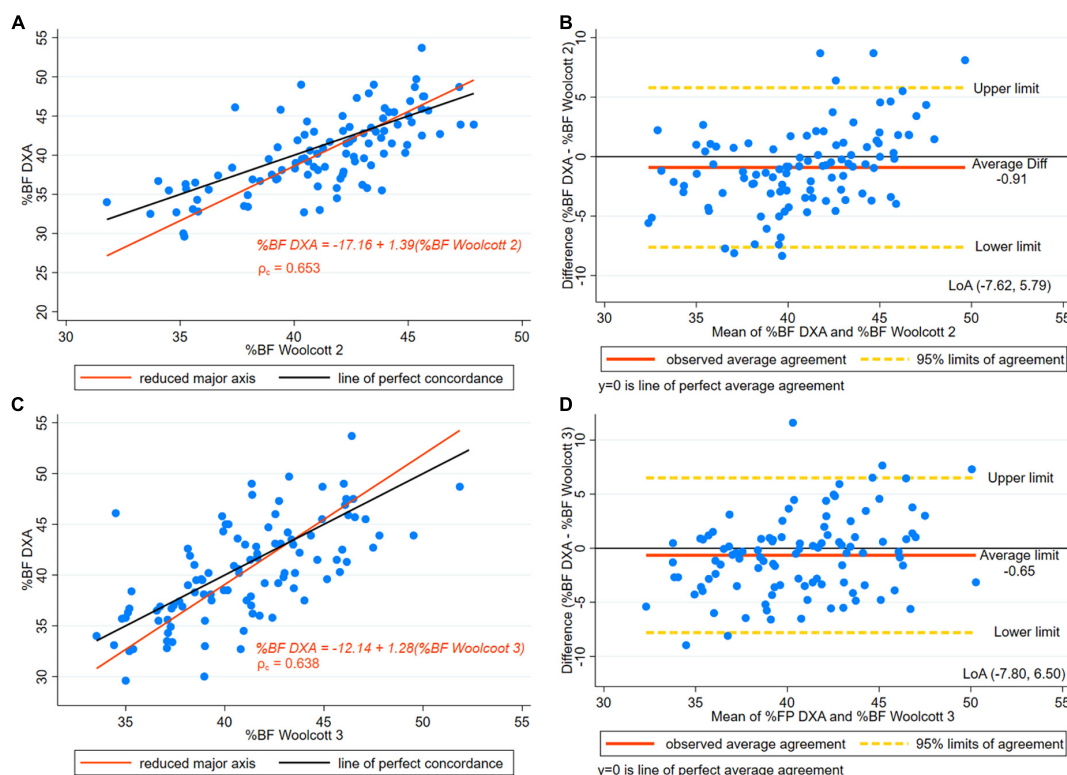


FIGURE 4

(A) Concordance plot of body fat percentage (BF%) estimated by DXA and Height³/Waist x Weight Woolcott's equation (Woolcott Equation 2). Pearson's correlation coefficient (r) and Lin's concordance correlation coefficient (ρ_c) for women with height > 145 cm. (B) The Bland-Altman plot is presented along with the Limits of Agreement (LoA) for BF% estimated by DXA and Height³/Waist x Weight Woolcott's (Woolcott Equation 2) for women with height > 145 cm. (C) Concordance plot for BF% estimated by DXA and Waist/Height Woolcott's equation (Woolcott Equation 3) in women with height > 145 cm. (D) The Bland-Altman plot is presented along with the LoA for BF% estimated by DXA and Waist/Height Woolcott's equation (Woolcott Equation 3) in women with height > 145 cm.

short stature are warranted to improve the estimation of body composition considering the anthropometric characteristics of this population group.

Studies in individuals under 60 years of age have shown good concordance between BIA and DXA (50, 51). A study in older adults comparing BIA (InBody 720) and DXA found favorable estimates of body composition. However, an equation was developed to lower the error so as to improve the BF% and FFM estimates. In the present study, a multi-frequency BIA equipment was used. The multi-frequency BIA (InBody 720) was found to be superior to a single-frequency BIA (Tanita BC-418) in terms of accuracy in the estimation of fat mass and fat-free mass (52). Results of the PREVED cohort study of the association between body fat and cardiovascular risk found that BIA estimates predict cardiovascular risk better than BMI and waist circumference (53).

In the current study, considering all participants, significant differences were observed between the five anthropometric-based predictive equations and DXA in BF% results. Applying the Deurenberg's equation (54) led to an overestimation BF% being detected (more than three percentage points).

Additionally, the limits of agreement were wide. This equation was derived from a sample of the Netherlands, and the age range of the participants was 7–83 years old, and the prediction formula included BMI. Deurenberg et al. constructed specific equations for both children and adults. It is possible that differences in the age range and ethnicity may contribute to the BF% discrepancies observed between Deurenberg's equation and the results of DXA for the older Mexican women who were studied. In contrast to our results, a Brazilian study found an adequate prediction of BF% using the Deurenberg's equation in older women (37). As in the present study, in the Brazilian group, BF% was overestimated with Deurenberg's equation, and SEE was higher than three percentage points. This error is considered high for clinical practice applications (33).

The Gallagher et al. (11) equation uses 1/BMI, age, and sex for BF% estimates. The results indicated that the predictive equation underestimated BF% in approximately two percentage points compared to DXA in the Mexican older women. Gallagher's equation was constructed from an international sample including individuals from UK, Japan, and the US. The sample excluded adults with a BMI ≥ 35 . In the present study,

however, older women with high BMI were included. Similarly to our results, a study in French adults detected a significant difference in the estimated BF% and BF (kg) applying the Deurenberg's and Gallagher's equations (33). In contrast, a study in older UK men reported satisfactory results in the validation of Gallagher's equation for the prediction of lean body mass (31). It is likely that differences in ethnicity and inclusion criteria contributed to the low accuracy of these prediction equations in the Mexican women examined.

In the current study, the Woolcott equation (55) that includes the height/waist ratio overestimated BF% and showed significant difference compared to DXA, and low precision with a large error margin ($SEE > 3$). These results were similar in the three Woolcott equations that were previously tested. However, in the subset of older Mexican women with a height of more than or equal to 145 cm, the Woolcott equation using the waist / height ratio showed no significant difference to DXA in the paired comparisons. This equation had a moderate concordance according to Lin's CCC results.

Woolcott and Bergman created (55) body composition prediction equations from the data of NHANES 1999–2004 ($n = 12,581$), and cross-validation was performed with NHANES 2005–2006 ($n = 3,456$). This study applied DXA as reference method. More than 350 different anthropometric, empirical equations were constructed and tested. The best fitted equations were obtained using waist circumference and height and showed better results than the equations based on BMI. In this study, the equation using (cubic height/ waist \times weight) had the highest correlation among women in the estimation of total BF%. The results of this equation were close to those of the best anthropometric equation identified in the preset study, which used the waist/height ratio; the former equation performed better than the Deurenberg and Gallagher equations that were based on BMI. This suggests that height and waist circumference may be suitable anthropometric characteristics to be used in equations estimating the BF% in older women. Nevertheless, in the present study, the Woolcott equations that were analyzed showed a $SEE > 3$ percentage points, and the limits of agreement were also large, showing low precision of the BF% results. Woolcott et al. identified a decline in weight, height, and FFM after the age of 50, additionally, fat mass and waist circumference decreased after age 70. The authors suggested that the lower predicting ability of the different equations analyzed in older age groups may be related with the anthropometric and body composition changes experienced during aging.

The evidence of the validity of body fat estimates through BIA and prediction equations using anthropometry in the older adults is scarce. Some studies in individuals under 60 years of age have shown good concordance between BIA and DXA (50, 51). In older adults, it has been suggested that the use of prediction equations using BIA information improves the validity of body composition estimates. Accurate evaluation of BF% is particularly important considering the high prevalence of obesity and the high burden of NCDs, associated with obesity.

Additionally, obese older adults showed higher risk of mortality when they developed infectious diseases compared to those in the normal weight group (56).

In the present study, more than 45% of the Mexican women were overweight or obese based on the Lipschitz criteria for older adults. The Mexican National Health and Nutrition Survey (ENSANUT 2018–19) results indicated that the prevalence of this condition continues to increase (19). It is important to emphasize that there is an increase in obesity prevalence worldwide, which has been described as an epidemic (57). In Mexico, for more than two decades, obesity has been identified as a serious public health problem (58).

Increased obesity prevalence will result in growing of obesity-related chronic diseases. This relationship has been extensively investigated in terms of its effect on disability and mortality among older adults (59).

The impact of obesity on older adults goes beyond their inability to remain independent but also increases the burden on their families, their care givers, and their communities in general (60). Obesity prevention and management programs at the clinical and public health levels for older Mexican people are required.

Additionally, it was found that approximately 10% of the participating women were underweight based on the Lipschitz BMI classification. Low weight is associated with a precarious socioeconomic status, other factors that may favor low weight are a pro-inflammatory state, depressive symptoms, or cognitive disorders (61, 62). Unintentional weight loss or low body-mass index may be an indicator of malnutrition in the elderly because it may reflect energy and nutrient deficiencies, which are difficult to detect in the older adults (63).

Strengths and limitations

As far as it was possible to investigate, this is the first study that assessed the concordance of BIA (InBody 720) using DXA as a reference method in older Mexican women. Older Mexican women share anthropometric characteristics with women of Latin American and other countries in the world (64). It is important to notice that no significant differences were observed in women taller than 145 cm between BIA and DXA, with the SEE being 2.6 percentage points of the BF%. BIA is a simple technique and available in many settings; thus, the finding of a satisfactory agreement between BIA and DXA supports the use of these devices in the nutritional assessment of older adults; however, improving its precision is desirable considering the large LoA observed. Additionally, the Woolcott predictive equation based on the waist /height ratio showed no significant mean differences compared to DXA when the shortest women were excluded. Utilizing anthropometric measures in order to obtain body composition is useful when resources are limited and DXA is unavailable. The study included women 60 years old and older, active, and living in the community; therefore,

the results may not be extrapolated to populations with severe illness and disabilities or those that are institutionalized. There are limitations when using BIA; this method is affected by the hydration status and dehydration is difficult to diagnose in older adults. Additionally, DXA was used as the gold standard, yet there may be errors in estimations of body composition using this technique, regarding body thickness and adiposity, and limitations in the assessment of lean and fat tissue overlying bone structures. Additionally, DXA results may change when using different software or equipment. However, it is frequently used as a reference method in body composition studies and has advantages such as the facts that it is not invasive and that it has good concordance with more advanced techniques in the evaluation of body composition (26).

Conclusion

In summary, significant differences of BF% estimates were observed between BIA and DXA and between the anthropometric based prediction equations and DXA. In older women who were 145-cm-tall or taller, BIA estimates were closer to the DXA results, and the concordance was good. Additionally, Woolcott's equation based on the waist/height ratio showed no significant mean difference in BF% estimates from DXA in this group of taller women. Thus, excluding the women with the lowest height decreased the mean difference between methods. The mean difference between BIA and Woolcott's equation and DXA was less than one percentage point. Nevertheless, the concordance of the Wolcott prediction equation was only moderate. The results indicated that BIA BF% estimates may be more accurate than the five anthropometric-based prediction equations that were tested. The results of this study may assist healthcare professionals who are working with older women in selecting the appropriate methods for BF% estimations.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

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Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the Metropolitan Autonomous University, Unit Xochimilco (Division of Biological and Health Sciences, DCBS/52-17-20) at Mexico City. The patients/participants provided their written informed consent to participate in this study.

Author contributions

MV-A, MI-C, and MZ-Z: conceptualization and formal analysis. MV-A, IR-C, and MI-C: data curation, supervision, and investigation. MV-A and MI-C: funding acquisition. MV-A, IR-C, AC-S, JF-F, and MI-C: methodology. MV-A, IA-C, IR-C, LM-G, and MI-C: resources. MZ-Z and MI-C: formal analysis. MV-A, MZ-Z, IA-C, IR-C, LM-G, AC-S, JF-F, RG-J, and MI-C: writing—original draft and review and editing. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Optimal cut-off value of waist circumference-to-height ratio to predict central obesity in children and adolescents: A systematic review and meta-analysis of diagnostic studies

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Introduction: Waist circumference-to-height ratio (WHtR) is a simple anthropometric index with good screening power and fast interpretation for early detection of childhood abdominal obesity. This systematic review and meta-analysis aims to determine the best cut-off value of WHtR to use in clinical setting.

Methods: Comprehensive searches were conducted in PubMed, Scopus, and Web of Science by the end of March 2021. Observational studies investigated the best WHtR cut-off to detect abdominal obesity in children and adolescents were included. Thirteen articles ($n = 180,119$) were included in this systematic review and eight documents were included in the meta-analysis.

Results: The overall optimal cut-off was 0.49 with pooled sensitivity, specificity and diagnostic odds ratio (DOR) of 0.93 (95% confidence interval (CI): 0.93–0.96), 0.88 (95% CI: 0.85–0.91) and 102.6 (95% CI: 50.7–207.5),

respectively. The optimal WHtR cut-off to predict abdominal obesity in girls and boys were both 0.49.

Discussion: The current study shows that we could use this cut-off as a simple index for predicting abdominal obesity in children and adolescents without the need for any charts in practice.

KEYWORDS

waist to height ratio, central obesity, abdominal obesity, children, adolescents

1. Introduction

Nowadays, the increasing prevalence of childhood obesity has become a worldwide issue (1). Obesity has increased five times in children and adolescents since 1975 in the world, especially in developed countries (2). According to the latest the United Nations International Children's Emergency Fund (UNICEF)/World Health Organization (WHO)/World Bank Group Joint Child Malnutrition Estimates, there are 38.3 million overweight children globally, an 8 million increase from 2000 to 2020 (2). Obesity is a multifactorial disease involving biological and environmental interactions such as physical activity, food consumption, sleep duration, etc. (3). Potentially, childhood obesity can lead to many devastating complications in adulthood, and central fat distribution is related to metabolic and cardiovascular diseases, particularly (1, 4).

It is helpful to identify an index with good screening power, easy measurement, and fast interpretation for early detection and management of childhood obesity (5). Body mass index (BMI) and waist circumference (WC) are the most commonly used measures for defining general and central obesity in clinical practices, respectively (6, 7). However, both measures are age, sex, and ethnicity dependent (8). After measuring WC or calculating BMI, both indices need standard growth charts matched for gender, age, and ethnicity, to assess the child's anthropometric condition. It is well accepted that central obesity increases the risk for cardiometabolic disease in adults and children independent of general obesity measured by BMI, which cannot distinguish fat distribution (7). Although WC is strongly correlated with abdominal fat, it may over or under-evaluate the risk for the tall or short individual with the

same WC (7) besides relying on age and sex-specific cut-off values (4).

In recent years, waist circumference-to-height ratio (WHtR) has been suggested as an alternative anthropometric indicator for screening central obesity. WHtR is a simple index without the need for age and gender-specific charts for interpretation (3). The National Institute for Health and Care Excellence (NICE) recommended WHtR as a simple index which could be measured by people themselves easily, and they can interpret the result whether they are at high health risk or not (9).

Prior studies found that WHtR is more sensitive, cheaper, and easier to measure and calculate than BMI and WC and can be used for both genders (10). Waist circumference changes with puberty, so we cannot propose the same cut-off for central obesity, but WHtR changes slightly with age, and its variations between boys and girls are not significant (11). A considerable number of studies have recommended using the WHtR cut-off value of 0.5 as a marker for screening central obesity in children and adolescents (7) with a simple health message "keep your waist circumference to less than half of your height" (12). However, some studies showed different thresholds with more sensitivity and specificity in various ethnicities (11, 13–20). A recently published meta-analysis reported different WHtR cut-offs for different regions as a screening tool for cardiometabolic risks in children and adolescents (1, 21).

Although many studies have investigated the utility of the WHtR as an index to screen central obesity and cardiometabolic risk among children and adolescents, there has been no systematic approach to identify a pooled cut-off of WHtR. The NICE committee noted that WHtR is the best measure for central obesity, but the evidence identified on boundary values for children and adolescents is not as sufficient as the evidence for adults. Considering that it is necessary to invoke a cut-off or boundary value for an index to use in public health for screening (7), the purpose of this systematic review and meta-analysis is to sum up the evidence and to assist NICE by finding the best cut-off value with high sensitivity and specificity for WHtR, a simple and easy indicator to screen central obesity in children and adolescents.

Abbreviations: WHtR, waist circumference-to-height ratio; DOR, diagnostic odds ratio; CI, confidence intervals; UNICEF, the United Nations International Children's Emergency Fund; WHO, World Health Organization; BMI, body mass index; WC, waist circumference; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta Analyses; AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value; NOQAS, the adapted Newcastle-Ottawa Quality Assessment Scale; PLR, positive likelihood ratio; NLR, negative likelihood ratio; SROC, summary receiver operator characteristic; DXA, dual-energy X-ray absorptiometry.

2. Materials and methods

This systematic review and meta-analysis followed the established guidelines from the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA statements) (Figure 1) (22).

2.1. Search strategy

Electronic searches were conducted in three major databases: PubMed, Scopus, and Web of Science. The search strategy included search terms for “pediatrics” OR “children” OR “adolescents” OR “students” AND “WHR” OR “waist to height ratio” OR “waist-height ratio” AND “abdominal obesity” OR “central obesity” OR “visceral obesity” OR “abdominal adiposity” OR “central adiposity” OR “abdominal fat” OR “Central fat.” Searches were limited to studies published in English by the end of September 2022. The reference list of included articles was also screened.

2.2. Eligibility criteria

We included all the cross-sectional original articles reporting a cut-off value for WHtR in children and adolescents to detect central obesity (with reporting sensitivity and specificity).

Articles were excluded if they did not evaluate central obesity or if their study population was adult. Clinical trials, review articles, conference proceedings, and book chapters also were excluded. Moreover, we excluded the studies that calculated WHtR diagnostic ability to predict central obesity in children and adolescents according to a predefined cut-off value.

2.3. Data collection

2.3.1. Selection of studies

After the electronic search, all records were imported into Endnote software version X8, and duplicates were removed. Two researchers independently reviewed all articles based on titles and abstracts, then full-text of the included studies were judged and reviewed by inclusion criteria. Any disagreement between the two researchers was resolved by discussion until reaching consensus. A total of thirteen articles met the inclusion criteria for this systematic review (3, 11, 13, 20, 23–31). E-mails were sent to corresponding authors for any supplementary data. The studies selection process is summarized in the PRISMA flow diagram (Figure 1).

2.3.2. Data extraction

Data were extracted independently from included articles by two authors according to predefined data extraction sheet. The extracted data included:

- (1) General information (authors, publication year, country, study design).
- (2) Participants' characteristics (sample size, target population, age range).
- (3) Diagnostic test for abdominal obesity.
- (4) Cut-off values, sensitivity, specificity, the area under the curve (AUC), positive predictive value (PPV), negative predictive value (NPV).

2.4. Study quality assessment

The adapted Newcastle-Ottawa Quality Assessment Scale (NOQAS) for cross-sectional studies (32) was used to appraise the methodological quality of included papers. This scale consists of seven items within three categories including selection of participants (maximum 5 score), comparability of outcomes (maximum 2 score), and assessment of outcomes (maximum 2 score). The total score which ranges from 0 to 10 is the sum of all the scores. A higher score indicates lower risk of bias. We categorized the quality assessments as follows: 0 to 4 as “unsatisfactory,” 5 and 6 as “satisfactory,” 7 and 8 as “good,” and 9 and 10 points as “very good.” Two independent investigators conducted the quality assessment and a third investigator resolved any probable discrepancies.

2.5. Statistical analysis

We carried out a diagnostic test accuracy meta-analysis using a bivariate random-effects model. In the meta-analysis, we calculated the combined sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR) and their 95% confidence intervals (CI), as summary estimates of cut-off scores accuracy based on the 2×2 tables (values of true positive, true negative, false positive, and false negative). Additionally, summary receiver operator characteristic (SROC) curves were created to assay the association between sensitivity and specificity. The heterogeneity was evaluated according to the I^2 -statistic of the pooled DOR. To find optimal cut-off score of WHtR, we performed meta-regression analysis and summarize operating sensitivity and specificity based on SROC curve. Since included studies have provided raw data of cut-off scores in the overall population and by sex, we decided to compute the optimal cut-offs into three categories: the overall optimal cut-off score, cut-off score in girls, and cut-off score in boys. We carried out

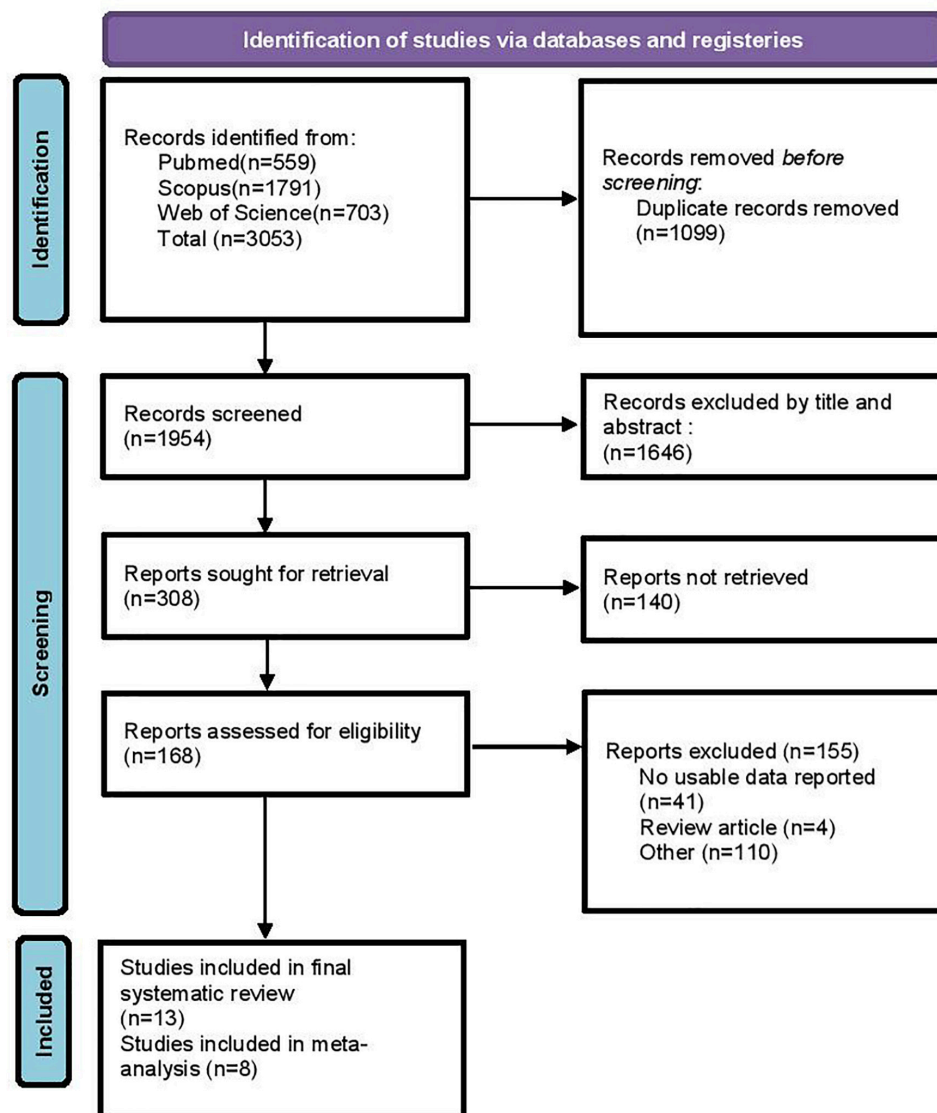


FIGURE 1
Flow chart for study identification and selection. Based on PRISMA 2020.

a sensitivity analyses by excluding study that solely conducted in 3–5 years children or children under 10 years. Publication bias was evaluated based on Deek's funnel plot analysis. When the P -value < 0.05 , significant publication bias was considered. STATA 16.0 was used for statistical analysis.

3. Results

3.1. Literature research

Electronic searches in three databases retrieved 3,053 papers, of which 1,099 were duplicates. The remaining 1,954 papers

were screened on titles and abstracts. After excluding 1,786 irrelevant papers, 168 full texts were reviewed, and 155 studies were further identified as ineligible. Finally, 13 articles were included in this systematic review (Figure 1). We could not pool data from 5 articles in the meta-analyses because of a lack of data.

3.2. Study characteristics

The general characteristics of the included studies are shown in Table 1. All the papers were cross-sectional in study design and published between 2008 and 2019. Studies originated from nine countries consisting of Brazil (three studies), Korea and

China (two studies from each country), Pakistan, Turkey, Iran, Japan, Argentina, New Zealand (one study). Most of the studies were performed in Asia ($n = 8$). The sample size ranged from 108 to 121,025, yielding a total sample of 180,119 in our systematic review. The minimum and maximum age of participants was two and nineteen years old.

3.3. Quality assessments

The overall quality assessment of included studies ranged from 5 to 10. Most of the studies had 6 to 7 points thus falling within the “satisfactory” to “good” subgroups. The quality assessment results are summarized in [Table 1](#).

3.4. General findings of the included studies

The reported cut-off values, sensitivity, specificity, AUC, and diagnostic test of abdominal obesity are summarized in [Table 2](#). The Diagnostic test for evaluating central obesity was WC percentile or dual-energy X-ray absorptiometry (DXA). Among thirteen studies, seven have reported overall optimum cut-off, and nine articles have reported cut-off values for boys and girls, separately. The maximum cut-off point was 0.54 ([29](#)), and the minimum was 0.45 for both boys and girls ([20](#)). Almost all AUCs values in studies were close to 1. The highest and the lowest reported AUC (0.990 and 0.79) was for 0.50 Cut-off value ([11](#), [31](#)).

3.5. Meta-analysis

Among 13 included studies in this systematic review, we could not pool data from five articles for the meta-analyses because we could not reach the authors for the data we needed, leaving eight articles to be included in the meta-analysis. Among the eight articles, four have indicated different overall cut-off points for diagnostic central obesity according to WHtR indicator, and eight have reported the cut-off values for both sexes.

According to the [Figure 2](#), the regression lines slopes show that accuracy of WHtR change with cut-off values, and across to characteristics of summary receiver operating characteristic curve (SROC curve), the optimal overall cut-off point was calculated at 0.49 ([Figure 2](#)). The pooled value of sensitivity and specificity of studies that have provided overall cut-off points were 0.94 (95% CI: 0.91–0.97) and 0.85 (95% CI: 0.72–0.93), respectively ([Figure 3](#)). The pooled PLR and NLR were 6.46 (95% CI: 3.23–12.93) and 0.07 (95% CI: 0.04–0.10), and the combined values of DOR was 99 (95% CI: 41.48–236.25). The studies heterogeneity according to pooled DOR was high

($I^2 = 100\%$). The forest plots were presented in a [Supplementary material](#). Considering the cut-off score as a continuous variable, we carried out a multiple thresholds model to compute the optimal cut-off point of the WHtR index to detect visceral obesity in children and adolescents.

The pooled estimates sensitivity of cut-off points in girls and boys were 0.94 (95% CI: 0.90–0.97) and 0.94 (95% CI: 0.88–0.97), respectively. Also the combined specificity was 0.83 (95% CI: 0.69–0.91) in girls and 0.88 (95% CI: 0.73–0.95) in boys. We calculated pooled likelihood ratios in both sexes. The combined DOR in girls and boys were 83.2 (95% CI: 35.6–194.7) and 109.6 (95% CI: 29.6–405.9), respectively. The heterogeneity among the studies based on pooled DOR was high. The forest plots are shown in the [Supplementary material](#). The optimal calculated cut-off scores to central obesity detection according to WHtR index was 0.49 and 0.49 in girls and boys, respectively ([Figure 4](#)).

3.6. Sensitivity analysis and publication bias

We performed sensitivity analyses by excluding the Taylor et al. study that was performed on 3–5 years children from the meta-analysis. To estimate the overall cutoff point, the summary sensitivity was 0.95 (95% CI: 0.92–0.97) and specificity 0.86 (95% CI: 0.70–0.94), and the optimal cutoff point was the same as the overall analysis. Additionally, for a finding of optimal cutoff points in different age ranges, the optimal cutoff point was calculated in studies with 10–18 years of participants, and the results of the analysis were similar to the overall cutoff score ([Supplementary material](#)).

We separately examined publication bias at the studies that had reported cut-off values in overall or both sexes. There was no asymmetry among the data points of the Deeks funnel plot of studies ($P \geq 0.05$) ([Supplementary material](#)).

4. Discussion

To the best of our knowledge, the present study is the first systematic review and meta-analysis that summarized all evidence investigating the optimal cut-off value of WHtR for predicting abdominal obesity in children and adolescents of different ethnicities. The reported cut-off values and their sensitivities and specificities were collated to provide a universal, practical, and accurate criterion for screening central obesity.

Totally, thirteen articles were included in this systematic review and meta-analysis was done on eight articles. We reached the number 0.49 as the optimum cut-off value for boys, girls and overall to predict central obesity in children and adolescents. Our findings confirmed that the same cut-off value can be used for both sexes. The maximum and minimum of the reported cut-off values among the included

TABLE 1 General characteristics of included studies.

Number	Study	Country	Study design	Population	Sample size	Diagnostic test of abdominal obesity
1	Asif et al. (23)	Pakistan	Cross-sectional	5–12 years old public places and from public and private schools (primary, secondary and higher secondary)	T:5964 B:2865 G:3099	WC \geq 90 Percentile
2	Carvalho et al. (24)	Brazil	Cross-sectional	10–18 years old adolescents of public school	T:731 B:252 G:479	Body fat with DXA
3	Choi et al. (11)	Korea	Cross-sectional	10–19 years old adolescents 15th Korean national survey	T:3057 B:1625 G:1432	WC \geq 90 Percentile
4	Filgueiras et al. (25)	Brazil	Cross-sectional	4–9 years old children Born in Maternity hospital	T:788 B:407 G:388	Android Fat \geq 90 percentile by DXA
5	Dong et al. (26)	China	Cross-sectional	7–18 years old Chinese National Survey	T:121025 B:60435 G:60590	WC \geq 90 Percentile
6	Ejtahed et al. (27)	Iran	Cross-sectional	7–18 years old National school-based surveillance study	T:14274 B:7223 G:7051	WC \geq 90 Percentile
7	Fujita et al. (28)	Japan	Cross-sectional	10 years old (Fifth grade) school children	T:466 B:226 G:196	Body fat with DXA
8	Guntsche et al. (29)	Argentina	Cross-sectional	6–16 years obese children and their siblings	T:108 B:NR G:NR	Trunk fat mass with DXA
9	Kilinc et al. (3)	Turkey	Cross-sectional	6–17 years old primary school/high school students	T: 2718 B:1467 G:1251	WC \geq 90 Percentile
10	Kim et al. (30)	Korea	Cross-sectional	6–18 years old Korea National health and nutrition examination survey	T:13257 B:6987 G:6270	WC \geq 90 Percentile
11	Sousa et al. (20)	Brazil	Cross-sectional	10–19 years old public School Children	T:516 B:152 G:364	Body fat with DXA
12	Taylor et al. (31)	New Zealand	Cross-sectional	3–5 years old predominantly white children	T: 301 B:151 G:150	Body fat with DXA
13	Zhou et al. (13)	China	Cross-sectional	7–17 years old children and adolescents from 6 regions of China	T:16914 B: 8843 G:8071	Chinese National Reference

T, total; B, boys; G, girls.

studies were 0.54 for Argentina (29) and 0.45 for Brazil (20), respectively. The variance in optimal reported cut-offs between studies may be due to differences in races and ethnicities (10). In a newly published systematic review and meta-analysis, different WHtR cut-off values were reported for populations of children and adolescents with different ethnicities as an indicator of cardiometabolic risks (21). The calculated optimal cut-off for East and Southeast Asian region and Latin American region was 0.46 and 0.54, respectively (21). Moreover, it should be noted that measurement of the indices like waist circumference

and height depends on the used protocols which may cause variability of the cut-offs between different studies. With regard to a recent systematic review, the weighted average WHtR cut-off points of 0.47 and 0.46 have been reported to predict central obesity in 6–18 years old boys and girls, respectively (33). Another systematic review and meta-analysis which published in 2021 evaluated the performance of the WHtR for identifying cardio-metabolic risks in children and adolescents and reported high heterogeneity regarding the optimal cut-off of WHtR among different ethnicities (34). In this systematic review,

TABLE 2 The list of included studies with reported cut-offs and ROC curve analysis data for predicting abdominal obesity.

Number	References	AUC	Cut off points	Sensitivity (%) overall	Specificity (%) overall	PPV (%)	NPV (%)
1	Asif et al. (23)	B:0.969 (0.959–0.979)	B:0.47	B:97%	B:84%	NR	NR
		G:0.948 (0.936–0.961)	G:0.48	G:88%	G:87%		
2	Carvalho et al. (24)	B:0.98 (0.96–1.00)	B:0.46	B:93.6%	B:94.1%	NR	NR
		G:0.98 (0.96–0.98)	G: 0.48	G:90.2%	G:92.7%		
3	Choi et al. (11)	B:0.990	B:0.50	B:97.5%	B:94.4%	NR	NR
		G:0.985	G:0.48	G:94.6%	G:94.6%		
		T:0.978	T:0.48	T:96.4%	T:90.6%		
4	Filgueiras et al. (25)	B (4–5 y):0.904 (0.830–0.954) B(6–7 y):0.980(0.937–0.997)	B(4–5 y):0.51 B(6–7 y):0.51	B(4–5 y):90% (55.5–98.3)	B(4–5 y):89.1% (80.9–94.7)	B: (4–5 y):47.4	B: (4–5 y):98.8
		B(8–9 y):0.963(0.924–0.985) G(4–5 y):0.902(0.815–0.957)	B(8–9 y):0.49 G(4–5 y):0.50	B(6–7 y):100% (73.4–100)	B(6–7 y):91.8% (85–96.2)	(6–7 y):57.1 (8–9 y):53.1	(6–7 y):100 (8–9 y):99.3
		G(6–7 y):0.835(0.749–0.900) G(8–9 y):0.937(0.893–0.966)	G(6–7 y):0.50 G(8–9 y):0.47	B(8–9 y):94% (72.6–99.1)	B(8–9 y):90.8 (85.3–94.8)%	G: (4–5 y):43.8	G: (4–5 y):98.4
		T(4–5 y):0.898(0.816–0.979)		G(4–5 y):87.5% (47.4–97.9)	G(4–5 y):87.5% (77.6–94.1)	(6–7 y):38.1	(6–7 y):97.6
		T(6–7 y):0.915(0.824–1.00)		G(6–7 y):80% (44.4–96.9)	G(6–7 y):86.2% (77.5–92.4)	(8–9 y):31.6	(8–9 y):100
		T(8–9 y):0.950(0.926–0.974)		G(8–9 y):100% (81.3–100)	G(8–9 y):78.2% (71.4–84)		
5	Dong et al. (26)	B:	0.46	B:	B:	B:	B:
		(7–9 y):0.916(0.913–0.919)		7–9 y:100%	7–9 y:84%	(7–9 y):48%	(7–9 y):100%
		(10–12 y):0.909(0.905–0.912)		10–12 y:100%	10–12 y:82%	(10–12 y):53%	(10–12 y): 100%
		(13–15 y):0.950(0.946–0.955)		13–15 y:96%	13–15 y:94%	(13–15 y):74%	(13–15 y):99%
		(16–18 y):0.950(0.945–0.954)		16–18 y:97%	16–18 y:93%	(16–18 y):68%	(16–18 y): 100%
		T:0.932(0.930–0.934)		T:98%	T:88%	T:59%	T: 100%
		G:		G:	G:	G:	G:
		(7–9 y):0.926(0.919–0.933)		7–9 y:92%	7–9 y:93%	(7–9 y):66%	(7–9 y):99%
		(10–12 y):0.922(0.915–0.928)		10–12 y:89%	10–12 y:95%	(10–12 y):74%	(10–12 y):98%

(Continued)

TABLE 2 (Continued)

Number	References	AUC	Cut off points	Sensitivity (%) overall	Specificity (%) overall	PPV (%)	NPV (%)
		(13–15 y):0.926(0.920–0.933)		13–15 y:91%	13–15 y:94%	(13–15 y):70%	(13–15 y): 99%
		(16–18 y):0.920(0.915–0.926)		16–18 y:95%	16–18 y:90%	(16–18 y):59%	(16–18 y): 99%
		T:0.923(0.920–0.927)		T:92%	T:93%	T:67%	T: 99%
6	Ejtahed et al. (27)	B:	B:	B:	B:	NR	NR
		(7–10 y):93(91–95)	(7–10 y):0.50 (0.49–0.51)	(7–10 y):84(79–89)	(7–10 y):91(87–94)		
		(11–14 y):98(97–99)	(11–14 y):0.51 (0.50–0.53)	(11–14 y):94(91–97)	(11–14 y):93(89–95)		
		(15–18 y):98(97–99)	(15–18 y):0.51 (0.50–0.52)	(15–18 y):98(95–100)	(15–18 y):90(86–92)		
		(7–18 y):96(95–97)	(7–18 y):0.50 (0.49–0.52)	(7–18 y):93(90–96)	(7–18 y):90(87–92)		
		G:	G:	G:	G:		
		(7–10 y):95(94–97)	(7–10 y):0.50 (0.49–0.51)	(7–10 y):90(85–95)	(7–10 y):89(85–93)		
		(11–14 y):97(97–98)	(11–14 y):0.49 (0.49–0.50)	(11–14 y):97(94–98)	(11–14 y):90(88–92)		
		(15–18 y):98 (97–98)	(15–18 y):0.51 (0.50–0.52)	(15–18 y):95 (91–98)	(15–18 y):93 (89–96)		
		(7–18 y):97 (96–97)	(7–18 y):0.50 (0.49–0.500)	(7–18 y):95(93–98)	(7–18 y):88(86–90)		
		T:	T:	T:88(84–92)	T:		
		(7–10 y):94(92–95)	(7–10 y):0.50 (0.49–0.50)	(7–10 y):88(84–92)	(7–10 y):89(85–91)		
		(11–14 y):97 (97–98)	(11–14 y):0.49 (0.49–0.50)	(11–14 y):97(95–98)	(11–14 y):89(87–90)		
		(15–18 y):98 (97–98)	(15–18 y):0.51 (0.51–0.52)	(15–18 y):96(93–97)	(15–18 y):92(89–94)		
		(7–18 y):96 (96–97)	(7–18 y):0.50 (0.49–0.51)	(7–18 y): 94–90–97)	(7–18 y):89(85–92)		
7	Fujita et al. (28)	B:0.981 (0.964–0.998)	B:0.519	B:100%	B:95%	NR	NR
		G:0.992 (0.981–1.004)	G:0.499	G:100%	G:95%		
8	Guntsche et al. (29)	Pubertal children: 0.99	B:0.54	Pubertal children: 97.2%	Pubertal children: 100%	NR	NR
		Prepubertal children: 0.98	G:0.54	Prepubertal children: 93.9%	Prepubertal children: 100%		
9	Kilinc et al. (3)	B:0.940	B:0.47	B:92.58%	B:78.97%	NR	NR
		G:0.907	G:0.46	G:90.05%	G:74.76%		
		Children: 0.926	Children: 0.49	Children: 89.51%	Children: 82.61%		
		Adolescents: 0.964	Adolescents: 0.46	Adolescents: 93.19%	Adolescents: 87.93%		
		T:0.920	T:0.47	T:89.89%	T:77.44%		

(Continued)

TABLE 2 (Continued)

Number	References	AUC	Cut off points	Sensitivity (%) overall	Specificity (%) overall	PPV (%)	NPV (%)
10	Kim et al. (30)	0.985 (0.985–0.985)	0.48	97.6%	91.3%	54.7	99.7
11	Sousa et al. (20)	B:0.98 (0.97–1.00) G:0.96 (0.95–0.98)	B:0.45 G:0.45	B:98.1% G:99.1%	B: 20.2% G:30.8%	NR	NR
12	Taylor et al. (31)	B:0.81 (0.73–0.90) G:0.79 (0.67–0.91) T:0.79 (0.71–0.87)	0.5	B:57% G:74%	B:76% G:72%	NR	NR
13	Zhou et al. (13)	B for central obesity: 0.983 G for central obesity: 0.984	B for central obesity: 0.47 G for central obesity: 0.45	B for central obesity: 94.3% G for central obesity: 96.3%	B for central obesity: 92.1% G for central obesity: 90.5%	NR	NR

AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value; WC, waist circumference; DXA, dual-energy X-ray absorptiometry; Y, year; B, boys; G, girls; T, total; NR, not reported.

we have not included the studies that evaluated pre-defined WHtR cut-off (0.5). According to the NICE guideline, WHtR range of 0.4 to 0.49 indicates healthy central obesity and without increased health risk, but WHtR 0.5 and more indicates increased health risks (35). However, our final cut-off value is approximately equal to the universally accepted one (7, 36) which is certainly easier to memorize and utilize. Practitioners, parents, care-givers or youth themselves can measure the index just with a string with no need for tape.

In this systematic review and meta-analysis, the pooled sensitivity of optimal cut-off point (0.49) in girls and boys were 0.94 and 0.94, respectively. Also the combined specificity was 0.83 in girls and 0.88 in boys. In recent meta-analysis, the sensitivity and specificity of WHtR performance in screening central obesity in children and adolescents have been reported as 0.91 and 0.90, respectively (25). Besides, several studies aimed to investigate the sensitivity and specificity of the pre-defined cut-off point of 0.5 in their sample population. In one study, sensitivity of 91% and specificity of 95% were reported in Greek adolescents (37). Another study was carried out on 649 American children (2–18 years) and proposed 99 and 72% for sensitivity and specificity of pre-defined cut-off point of WHtR in predicting central obesity (38).

The pooled calculated AUC for our suggested cut-off value (0.49) is 0.96, which proves we can predict childhood central obesity with high accuracy. Our pooled AUC was very close to the previous meta-analysis which reported AUC = 0.96 for WHtR (34). Another systematic review and meta-analysis assessed the discriminatory capacity of the anthropometric indices for body fat and revealed an excellent power of WHtR in males (AUC: 0.897) and females (AUC: 0.914) (39).

In our study the pooled estimated DOR of WHtR to predict central obesity was 102 (95% CI: 50–207). This finding was concordant with previous study which reported that DOR of WHtR for predicting enteral obesity was 88 (95% CI: 40–195) (25). A little discrepancy between our estimated DOR with that study was due to this point that we estimated DOR according to our pooled estimated optimal cut-off point (0.49).

Different anthropometric indices with different strengths and limitations are used to diagnose childhood obesity. BMI and WC are the most commonly used indices as a screening tool for obesity worldwide. However, BMI cannot differentiate fat mass (33). On the other hand, WC is another anthropometric index used to diagnose central obesity. It should be noted that age and sex-specific curves are required for both indices in clinical practice. Recent studies have proposed WHtR as a new anthropometric indicator facilitating the diagnosis of obesity, specifically central obesity in children and adolescents. As evidenced in our study, WHtR is less dependent on age and sex and does not need charts for interpretation. Moreover, WHtR has the superiority of predicting health risks related to central obesity such as type 2 diabetes, hypertension, and cardiovascular disease in children and adolescents aged

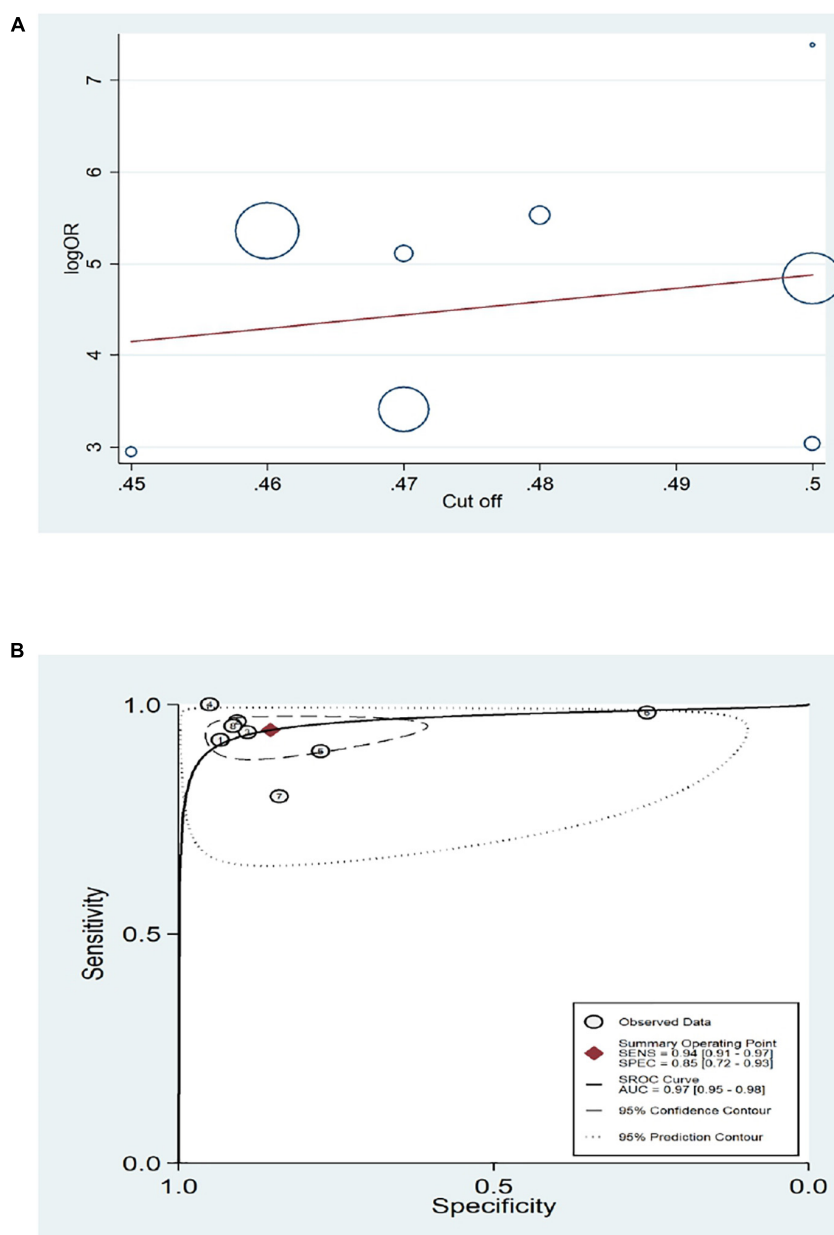


FIGURE 2

Diagnostic test accuracy meta-analysis for overall optimal cutoff score of WHtR for detecting central obesity in children and adolescence. (A) Regression lines of accuracy of WHtR for children and adolescence. (B) The optimal cutoff score 0.49 was marked as a cross in the estimated SROC curve.

five and more (35, 40). Several studies have shown that WHtR had the largest discriminatory power for metabolic disorders such as diabetes and dyslipidemia in comparison with WC, BMI and waist-to-hip ratio (41, 42). A cross-sectional study evaluated the usefulness of the WHtR in predicting cardiometabolic risks in children in five European countries. They suggested WHtR > 0.55 as an appropriate boundary value for screening young European population at high cardiometabolic risk (43). The higher WHtR value in

children and adolescents could predict high cardiometabolic risks in future life (44–46).

4.1. Strengths and limitations

The main strength of this study is that we comprehensively reviewed 13 studies and analyzed data of a large number of participants (total sample size = 180,119) and proposed an

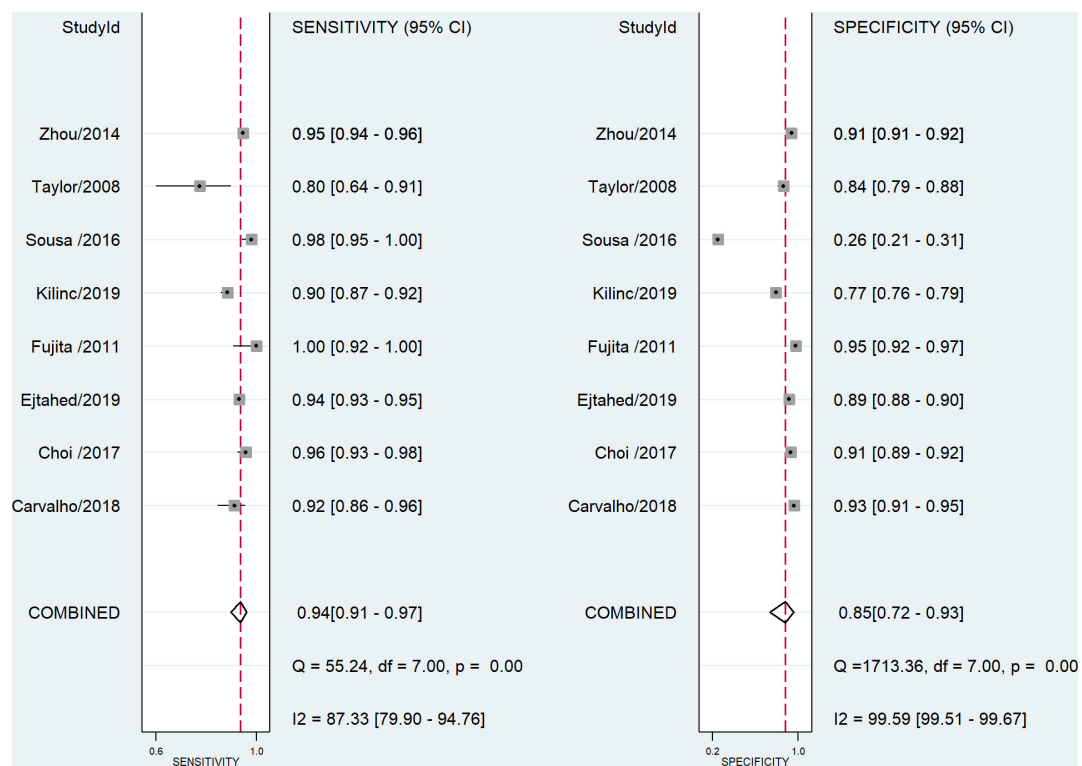


FIGURE 3
Forest plots for the diagnostic accuracy of overall cutoff point.

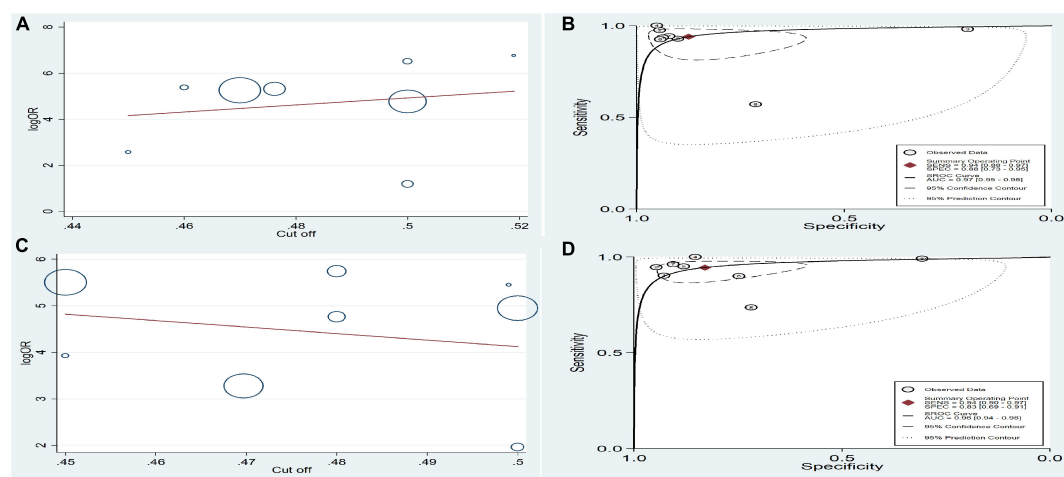


FIGURE 4
Diagnostic test accuracy meta-analysis for optimal cutoff score of WHtR for detecting central obesity in children and adolescence.
(A) Regression lines of accuracy of WHtR for boys. (B) The optimal cutoff score 0.48 was marked as a cross in the estimated SROC curve.
(C) Regression lines of accuracy of WHtR for girls. (D) The optimal cutoff score 0.49 was marked as a cross in the estimated SROC curve.

optimum cut-off value of WHtR for diagnosis of central obesity with high accuracy in children and adolescents which verify that approximately the predefined cut-off 0.5 is appropriate cut-off value in clinical setting. Moreover, some of the included studies were from national surveys. This study has some limitations.

Thirteen studies met the inclusion criteria for this systematic review. However, we could not access to the required data of five articles for the meta-analysis despite our efforts to contact the researchers (23, 25, 26, 29, 30); hence, eight articles were used in the analysis. Also, since the main goal of this study was to

estimate the optimal cut-off value, therefore we excluded studies which assessed sensitivity and specificity, AUC, DOR according to pre-defined cut-off (0.5). This exclusion criteria which we considered in our study may effect on the pooled estimated of these diagnostic criteria.

5. Conclusion

The results of the current systematic review and meta-analysis confirm that WHtR cut-off value could predict central obesity with high accuracy in children and adolescents with various races, ages, and genders. Although 0.49 is the proposed theoretical cut-off value, 0.5 is much more practical value in children and adolescents. Moreover, it can be easily communicated with the message “keep your waist to less than half your height” (35). Totally, it is recommended to use WHtR cut-off value as a simple tool to screen central obesity without the need for any charts in practice.

Data availability statement

The original contributions presented in this study are included in this article/**Supplementary material**, further inquiries can be directed to the corresponding authors.

Author contributions

MQ and H-SE came with the idea of this manuscript. FP and ME did the study search, evaluated the articles, wrote the manuscript, and extracted the data then prepared the tables. MK did the meta-analysis and wrote statistical analysis method and result section. JT reviewed the manuscript. ZE-A and KP did the final required revisions. MQ and H-SE supervised all the process and did the final proof of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnut.2022.985319/full#supplementary-material>

SUPPLEMENTARY FIGURE 1

Forest plots of NLR values on overall cut-off point.

SUPPLEMENTARY FIGURE 2

Forest plots of DOR values on overall cut-off point.

SUPPLEMENTARY FIGURE 3

Forest plots for the diagnostic accuracy of cutoff point in girls.

SUPPLEMENTARY FIGURE 4

Forest plots of NLR values on cut-off point in girls.

SUPPLEMENTARY FIGURE 5

Forest plots of DOR values on cut-off point in girls.

SUPPLEMENTARY FIGURE 6

Forest plots for the diagnostic accuracy of cutoff point in boys.

SUPPLEMENTARY FIGURE 7

Forest plots of NLR values on cut-off point in boys.

SUPPLEMENTARY FIGURE 8

Forest plots of DOR values on cut-off point in boys.

SUPPLEMENTARY FIGURE 9

Deek's funnel plot analysis (A) cutoff point in girls (B) cutoff point in boys.

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