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Bioelectrical impedance analysis in the assessment of body composition – methodological considerations

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Abbreviations

3C	3-compartment
4C	4-compartment
ADP	air displacement plethysmography
ALST	appendicular lean soft tissue
ASMM	appendicular skeletal muscle mass
BCC	body composition chart
BIA	bioelectrical impedance analysis
BIVA	bioelectrical impedance vector analysis
BMC	bone mineral content
BMI	body mass index
D_2O	deuterium oxide
DXA	dual X-ray absorptiometry
ECW	extracellular water
FFM	fat free mass
FFMI	fat free mass index
FM	fat mass
FMI	fat mass index
HIV	human immunodeficiency virus
Ht	body height
ICW	intracellular water
mBCA	medical Body Composition Analyzer
MRI	magnetic resonance imaging
NaBr	sodium bromide
PhA	phase angle
R	resistance
SMI	skeletal muscle index
SMM	skeletal muscle mass
TBW	total body water
VAT	visceral adipose tissue
WHO	World Health Organization
Xc	reactance

Chapter I

General introduction and objectives

Bioelectrical impedance analysis in the assessment of body composition

Bioelectrical impedance analysis (BIA) has become a widely used method for measuring body composition, because it is a fast, non-invasive, and inexpensive method. Body compartments like fat mass (FM), visceral adipose tissue (VAT), fat-free mass (FFM), or skeletal muscle mass (SMM) can be assessed in clinical practice and measurements can be repeated frequently (Norman et al. 2012, Gonzalez et al. 2016, Bosy-Westphal et al. 2013, 2017).

BIA is based on the measurement of the electrical conductivity of the human body. Body water with solute ions serves as conductor; therefore, body water is the primary compartment that is estimated by BIA. An imperceptible alternating electrical current between hand and foot is used to measure the impedance which consists of two components: The resistance R that is inversely related to body water and the reactance Xc caused by capacitive effects of isolating cell membranes. Depending on the frequency of the current, either total body water (TBW) or extracellular water (ECW) can be calculated by using the resistance R, while the reactance Xc gives additional information about body cell mass (Foster and Lukaski 1996, Kyle et al. 2004, Kushner et al. 1992, Norman et al. 2012).

For the calculation of body compartments by BIA, prediction equations are required. In addition to the impedance, such equations use body height and further parameters like weight, age, or sex for the estimation of body compartments. Prediction equations were derived in various validation studies by comparing BIA measurements with reference methods in the respective study population. For equations used in this thesis, the 4-compartment (4C) model, as a gold standard, was used as reference method for FM and FFM, because the combination of methods in the 4C-model reduces limitations of two compartment methods, while magnetic resonance imaging (MRI) was used as reference for SMM and VAT (Bosy-Westphal et al. 2013, 2017, Fuller et al. 1992, Kyle et al. 2004).

The body mass index (BMI = weight / height²) is the primary indicator in the diagnosis of obesity and malnutrition, but information on body composition, like FM, VAT, FFM, or SMM, results in an improved diagnosis (Blundell et al. 2014, Cederholm et al. 2019). Overweight and obesity are defined by the WHO as a BMI of 25–29.9 kg/m² and ≥30 kg/m² respectively and are used as risk factors for type 2 diabetes and cardiovascular disease (WHO Expert Consultation 2004, WHO 1997). An increase in body weight is however not always associated with adiposity. The BMI has therefore limitations as an index of increased fat mass. In addition, the distribution of fat is of importance. Especially

VAT contributes to cardiometabolic risk (Müller et al. 2016). The BMI only serves as an easy to measure, but indirect, proxy of body fat. The BMI can neither distinguish between FM and FFM, nor between different fat distributions. Hence FM and VAT are more direct indicators for health risks due to overweight and obesity (Blundell et al. 2014, WHO 1997, Müller et al. 2016).

On the other hand, altered body composition due to malnutrition is characterized by a reduced muscle mass (Cederholm et al. 2019). Malnutrition and the related concepts cachexia and sarcopenia are associated with impaired clinical outcome from disease (Cederholm et al. 2015). Unintended weight loss and a low BMI are good indicators for malnutrition (Cederholm et al. 2019). But Malnutrition can also occur in combination with obesity (hidden cachexia or sarcopenic obesity) or with a retention of body water (Cederholm et al. 2017). In these cases, malnutrition cannot be diagnosed based on body weight, because the reduced muscle mass is masked by a high amount of body fat or an increasing volume of body water (Cederholm et al. 2017). Therefore, the measurement of SMM or FFM, is part of the diagnostic criteria for malnutrition (Cederholm et al. 2019).

For subjects with disturbed hydration or altered distribution between extra- and intra-cellular water, BIA prediction equations can become inaccurate (Norman et al. 2012). The equations are only valid if subjects are comparable to the study population used for equation generation. In these cases, impedance raw data like the phase angle or the bioelectrical impedance vector analysis (BIVA) can be used as an indicator of body cell mass, hydration and integrity of the cell membranes (Norman et al. 2012).

There are, however, challenges that can impair the assessment of BIA measurements. The following methodological considerations are addressed in this thesis.

Ethnic variations of body composition

Ethnical differences in body composition lead to differences in obesity related health risks (Gasevic et al. 2015, Ntuk et al. 2014). For a given BMI, some Asian populations, for example, have a higher percentage of FM and a higher risk for cardiovascular disease and type 2 diabetes (WHO Expert Consultation 2004). The WHO expert consultation therefore proposed additional BMI cut-offs for Asian populations that account for these differences (WHO Expert Consultation 2004). But ethnical differences also occur in muscle mass, which differ for example between African-American and Caucasian men and women (Gallagher et al. 1997). Cederholm et al. (2015) therefore recommended ethnic specific BMI cut-offs as diagnostic criteria for malnutrition (Cederholm et al. 2015, 2019). The

relationship between BMI and body composition, therefore, is an important basis for the ethnic-specific definition of BMI cut-offs.

In the first part of this thesis, ethnical differences in body composition among Germans, Japanese and Mexicans were investigated for standard BMI cut-offs 18.5, 25 and 30 kg/m². In addition to differences in body composition, ethnicities also differ in body proportions (Deurenberg et al. 2002). Impedance raw data depend on body composition as well as on body geometry (Foster and Lukaski 1996) and hence is affected by such differences. Therefore, differences in phase angle and BIVA among Germans, Japanese, and Mexicans were additionally investigated in the first part of this thesis.

Challenges of body composition measurements in obesity

In obesity, the measurement of body composition is of interest, because FM and VAT can be used for the assessment of energy balance and cardiometabolic risk, while SMM, or FFM help to identify patients with sarcopenic obesity (Blundell et al. 2014, Cederholm et al. 2017, Cruz-Jentoft et al. 2010).

There are, however, challenges for body composition measurements in obese subjects. A higher hydration of the fat free compartment of adipose tissue in comparison to lean tissue (Wang et al. 2000) and a lower bone mineral density in subjects with a higher percentage of FM (Dolan et al. 2017) can be sources of measurement errors in all two compartment methods that rely on certain assumptions. For the measurement of FM and FFM with air displacement plethysmography (ADP), a constant density of these two compartments is assumed, which in turn relies on a constant hydration and bone mineral density of FFM (Ellis 2000). A constant hydration of FFM must also be assumed for the calculation of FFM (and FM as difference to weight) from TBW measured with dilution with labeled water (e.g. deuterium oxide, D_2O) (Ellis 2000). FM and FFM measured by dual X-ray absorptiometry (DXA), on the other hand, relies on different X-ray absorption coefficients that are affected by differences in mineral content between FM and FFM (Tylavsky et al. 2003). Because minerals in lean soft tissue are dissolved in body water, differences in hydration can lead to measurement errors in FM and FFM. Additionally, in fan-beam DXA devices, that offer faster scans than pencil beam devices, magnification errors lead to an underestimation of trunk fat (Salamone et al. 2000, Tylavsky et al. 2003, Schoeller et al. 2005). Differences in hydration can also affect BIA measurements, because body water is the primary parameter measured with BIA (Ellis 2000).

In the second part of this thesis, the bias of FM and FFM measurements with the 2-compartment methods ADP, DXA, D_2O dilution, and BIA is investigated in comparison to a 3-compartment (3C) and a 4C model that avoid these assumptions (Fuller et al. 1992, Ellis 2000). The bias is analyzed for obese subjects and corrections for BIA equations are provided.

The increase of adipose tissue with obesity impairs the quality of FFM (Bosy-Westphal et al. 2017) and therefore challenges the use of FFM as a proxy for SMM. As a secondary objective of the second part of this thesis, FFM measured by the 4C-model is compared to measurements of SMM by MRI in order to investigate the quality of FFM as a proxy for SMM.

The phase angle in bioelectrical impedance analysis

In order to avoid limitations of BIA prediction equations, impedance raw data can be interpreted directly. The most established parameter is the phase angle that is calculated from impedance measured at a frequency of 50 kHz by:

$$phase\ angle = arctan\left(\frac{reactance}{resistance}\right) \cdot \frac{180°}{\pi}$$

A low phase angle correlates with a high ratio of extracellular to intracellular water and indicates a low amount and quality of soft tissue (Barbosa-Silva et al. 2005, Norman et al. 2012, Gonzalez et al. 2016). In disease related malnutrition a characteristic increase of the ratio of extracellular to intracellular water occurs that is reflected by a low phase angle (Norman et al. 2012). Therefore, a low phase angle can be used as a marker for malnutrition (Norman et al. 2012). It has been shown that a low phase angle is an indicator of impaired outcome in catabolic diseases like cancer, human immunodeficiency virus (HIV) infection, or liver cirrhosis (Grundmann et al. 2015, Kyle et al. 2013, Schwenk et al. 2000). The phase angle, however, is an abstract value that can only be interpreted in comparison to reference values. In cases where no individual baseline value exists as a reference, population specific normal ranges are required. Such normal ranges where, for example, acquired in German and US-American populations using BIA devices for measurements in supine position (Barbosa-Silva et al. 2005, Bosy-Westphal et al. 2006, Kuchnia et al. 2017).

As a further development of the phase angle, the BIVA was introduced by Piccoli et al. (1994). The phase angle contains only part of the information of resistance and reactance, namely the angle between these values when drawn as a vector in a coordinate system. The length of this vector contains additional information about TBW. The BIVA displays both information, phase angle and vector length, in one graphic together with ellipses that represent the distribution of vectors in a reference population (Piccoli et al. 1994, Piccoli and Pastori 2002).

There are, however, limitations for the application of the phase angle. Normal ranges for phase angle differ among ethnicities (Kuchnia et al. 2017). Also, different BIA devices use different measurement configurations that may result in differences in phase angle. Measurements in standing compared to lying position, for example, result in lower impedance values (Rush et al. 2006) and, therefore, the phase angle is likely to be influenced by the posture of the subject too. Additionally, the phase angle may be different when measured on the right side or the left side of the body or it may vary when using different positions for the electrodes used by different BIA devices. Variations in phase angle based on methodological differences are important for the selection of normal ranges that are used for the interpretation of the phase angle. Therefore, differences in phase angle due to different measurement configurations were analyzed in the third part of this thesis, while ethnical differences of phase angle were investigated in the first part.

Objectives

Based on this background and addressing the above-mentioned methodological challenges, the objectives of this thesis are:

- To analyze ethnic differences in FM, VAT, FFM and SMM for subjects with the same BMI as well as ethnical differences in phase angle and BIVA.
- To compare different methods for the estimation of FFM in obese subjects and to improve BIA equations for the use in obesity.
- To analyze differences in phase angle assessed with different measurement configurations (standing vs. lying, right side vs. left side and different electrode position).

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Chapter II

Ethnic differences in fat and muscle mass and their implication for interpretation of bioelectrical impedance vector analysis

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Abstract

According to the World Health Organization Expert Consultation, current body mass index (BMI) cut-offs should be retained as an international classification. However, there are ethnic differences in BMI-associated health risks that may be caused by differences in body fat or skeletal muscle mass and these may affect the interpretation of phase angle and bioelectrical impedance vector analysis (BIVA). Therefore, the aim of this study was to compare body composition measured by bioelectrical impedance analysis among 1048 German, 1026 Mexican, and 995 Japanese adults encompassing a wide range of ages and BMIs (18–78 years; BMI, 13.9–44.3 kg/m^2). Regression analyses between body composition parameters and BMI were used to predict ethnic-specific reference values at the standard BMI cut-offs of 18.5, 25, and 30 kg/m^2. German men and women had a higher fat-free mass per fat mass compared with Mexicans. Normal-weight Japanese were similar to Mexicans but approached the German phenotype with increasing BMI. The skeletal muscle index (SMI, kg/m^2) was highest in Germans, whereas in BIVA, the Mexican group had the longest vector, and the Japanese group had the lowest phase angle and the highest extracellular/total body water ratio. Ethnic differences in regional partitioning of fat and muscle mass at the trunk and the extremities contribute to differences in BIVA and phase angle. In conclusion, not only the relationship between BMI and adiposity is ethnic specific; in addition, fat distribution, SMI, and muscle mass distribution vary at the same BMI. These results emphasize the need for ethnic-specific normal values in the diagnosis of obesity and sarcopenia.

Introduction

Obesity-associated health risks differ greatly across ethnic groups (Gasevic et al. 2015, Ntuk et al. 2014). The proposed body mass index (BMI) cut-off points used to predict clinical outcomes are manifold and even vary among Asian populations (WHO/IASO/IOTF 2000, James et al. 2002). The World Health Organization (WHO) Expert Consultation recommended that the current WHO BMI cut-off points be retained as the international classification (WHO Expert Consultation 2004). Despite a growing debate about the need for ethnic-specific BMI cut-off points, the rationale for population-specific health risks at the WHO BMI thresholds remains insufficiently justified. The percentage of body fat varies significantly, even at the same BMI (Buffa et al. 2017). Differences across populations due to genetic and environmental effects affect not only the relationship between BMI and percentage of body fat but also the associations between BMI and body fat distribution, skeletal muscle mass (SMM), and composition of lean mass (Heymsfield et al. 2016). BMI is limited as an index of body composition in certain diseases that contribute to a loss in fat-free mass (FFM), whereas fat mass (FM) may be unchanged or even elevated (Cederholm et al. 2015). Hence, it is not sufficient to introduce ethnic-specific BMI cut-off points that are based solely on adiposity as the reference. It is also important to consider ethnic differences in other clinically meaningful body composition parameters at these cut-offs. In addition to BMI, waist circumference is recommended for health risk assessment (NIH 2000). However, at the same waist circumference, there are ethnic differences in visceral adipose tissue (VAT) (Sumner et al. 2011), which should be considered.

To facilitate this, there is a need for practical phenotypic measures, beyond BMI, that are reliable, noninvasive, easy to perform, and cost effective and can be applied in clinical routine. Bioelectrical impedance analysis (BIA) not only meets these requirements, but also offers body composition phenotyping that is related to physiologic function and is of prognostic value in patients. Clinical outcome parameters by BIA include total and regional SMM for diagnosis of sarcopenia (Chien et al. 2008, Cruz-Jentoft et al. 2010); phase angle as a predictor of mortality (Wirth et al. 2010, Norman et al. 2010); and bioelectrical impedance vector analysis (BIVA), which can be used to assess hydration status and catabolic states (e.g., in critically ill patients and in those with malnutrition and wasting diseases) (Piccoli et al. 1994, Fassini et al. 2016, Castillo-Martínez et al. 2012, Nicoletti et al. 2014). Ethnic differences in body composition may affect the interpretation of these important clinical outcome parameters.

The aim of this observational study was to analyze ethnic differences in BIA outcome measures that assess body fat and SMM at the standard BMI cut-offs of 18.5, 25, and 30 kg/m^2 among Caucasian (German), Mexican (Mexican-Mestizos), and Asian (Japanese) adults.

Because body composition differs among these ethnicities, differences can also be expected for phase angle and BIVA, which are derived from the raw data of the BIA measurement. Therefore, as a secondary objective of this study, normal ranges for phase angle and BIVA were compared among the 3 ethnicities.

Materials and methods

Body composition was measured in a cross-sectional study in 3069 healthy adults from 3 study centers in Germany, Japan, and Mexico between 2011 and 2016.

Between March and May 2014, 996 healthy Japanese adults (497 women and 499 men) 21–87 years of age (BMI, 13.9–41.2 kg/m^2) were examined at the University of Tokyo Hospital (Japan) on a seca medical Body Composition Analyzer (mBCA) 515 (seca gmbh and co. kg, Hamburg, Germany) with the handrail height adjusted for an Asian population. Measurements were taken in the morning between 1000 h and 1230 h and in the afternoon between 1400 h and 1700 h. Data from 1 subject 87 years of age were excluded because of the older age being an outlier in the study population, which had an age range of 21–78 years.

Between April 2015 and April 2016, 1026 healthy Mexican-Mestizos (503 women and 523 men) 18–67 years of age (BMI, 17.1–44.3 kg/m^2) were examined at the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (National Institute of Medical Sciences and Nutrition Salvador Zubirán, Mexico). Healthy adults with Mexican ancestors back to the second generation (all 4 grandparents) were considered Mexican-Mestizo and were eligible for the study. Only subjects who qualified for blood donation according to the Mexican official guidelines for blood donors (Norma Oficial Mexicana NOM-253-SSA1-2012 2012) were included. Measurements were taken between 0800 h and 1300 h.

Between October 2011 and January 2012, 1050 healthy Germans (518 women and 532 men) 18–65 years of age (BMI, 18.2–42.6 kg/m^2) were examined at the Institute for Transfusion Medicine at the University Hospital Hamburg-Eppendorf (Germany). All adult blood donors under the age of 65 years were generally eligible for the study. Subjects were included in the study and considered to be healthy if they qualified as blood donors according to the German guidelines for blood donors (Bundesärztekammer 2010). All BIA

measurements were taken before blood donation to avoid fluid shifts. Measurements were taken between 0730 h and 1900 h. Data from 2 subjects (1 woman, 1 man) were omitted because of missing waist circumference values. Normal values for BIA outcome parameters in the German study group have been published previously (Peine et al. 2013). Blood donors in Germany and Mexico were expected to be healthy and were obliged to answer an extensive questionnaire regarding their health and any chronic or acute diseases. Blood donors in Germany were strongly advised not to perform vigorous physical activity, to drink about half a liter, and to eat a small meal 1–2 h before donation; in Mexico, they were advised to drink, but an 8-h fast was required. Female donors in Germany were strongly advised not to donate during or within 1 week after menstrual bleeding; however, if their hemoglobin level was above 12.5 g/dL, Germans and Mexicans were allowed to donate regardless of their menstrual cycle.

Additional exclusion criteria were chronic diseases that affect fluid homeostasis (e.g., hypertension, heart or kidney failure, patients treated with diuretics); amputation of limbs; implants such as a cardiac pacemaker, insulin pumps, artificial joints, and metallic implants (with the exception of tooth implants); and pregnancy or breastfeeding period; also excluded were subjects who could not complete an informed consent form by themselves and subjects who might have been dependent on the sponsor or the investigation site. In Germany and Mexico, additional exclusion criteria were extensive tattoos and ankle edema, which were assessed by inspection. All subjects provided their fully informed and written consent before participation. The studies were approved individually by the responsible ethical committee in each institution and were performed in accordance with the ethical standards laid down in the 1964 *Declaration of Helsinki* and its later amendments.

Anthropometry

Body weight was measured using the seca mBCA 514/515. In Germany and Japan, the seca 515 device was used; it includes an approved scale with an accuracy of 50 g up to 100 kg. In Mexico, the seca 514 device was used; it includes a scale with an accuracy of ±0.3%. Body height (Ht) in Germany was obtained with a seca 217 stadiometer with an accuracy of ±5 mm. In Japan and Mexico, a seca 274 digital stadiometer with an accuracy of ±2 mm was used. BMI was calculated as BMI = weight/Ht^2; subjects were classified as underweight (BMI <18.5 kg/m^2), normal weight (BMI ≥18.5, <25 kg/m^2), overweight (BMI ≥25, <30 kg/m^2), or obese (BMI ≥30 kg/m^2). Waist circumference was measured

midway between the lowest rib and the uppermost boarder of the iliac crest in the medial axillary line and at the end of normal expiration using a seca 201 nonstretchable measuring tape.

BIA

The seca mBCA 514/515 consists of a platform with an integrated scale and a handrail system. Each side of the ascending handrail carries 6 electrodes, of which 2 were chosen depending on the person's height. To obtain the correct grip position, the subject had to stand upright with outstretched arms. Another 2 pairs of electrodes were connected to the feet. This 8-electrode technique enables segmental impedance measurement. Details of the device have been described previously (Bosy-Westphal et al. 2013). The devices mBCA 514 and mBCA 515 are of identical construction and differ only in the approval of the integrated scale, which is required by law in Germany and Japan and explains the different accuracy specifications of the scales. The accuracy of measurements of the right and left body side at frequencies of 5 and 50 kHz is 5 Ω for the impedance and 0.5° for the phase angle. The prediction equations for FFM, total body water (TBW), and extracellular water (ECW) were validated by Bosy-Westphal and colleagues (2013), and FM was calculated as the difference between body weight and FFM. The reproducibility of FM measurement is 0.221 kg (Bosy-Westphal et al. 2013). The prediction equations for SMM and VAT were validated by Bosy-Westphal and colleagues (2017). Resistance (*R*) and reactance (*Xc*) values obtained at 5 and 50 kHz for different body segments were used in the prediction equations. Corrections for ethnical differences in prediction equations were implemented in the devices as described by Bosy-Westphal and colleagues (2017). Listed values for R_{50kHz}/Ht, Xc_{50kHz}/Ht, and phase angle are mean values of both sides of the body. The mBCA device used in Japan was a device for the Asian market, which has a 10-cm-lower handrail compared with the devices used in Germany and Mexico. A comparison of these devices in a subgroup of 199 subjects in Japan (102 women and 97 men) 21–78 years of age (BMI, 15.9–32.7 kg/m^2) showed a higher resistance of the arms for the device with the low handrail because of the slightly different positioning of the arms. This leads to higher impedance measurements (R_{50kHz}/Ht: +0.077 ± 0.036 Ω/cm, $p < 0.0001$ and Xc_{50kHz}/Ht: +0.0011 ± 0.0041 Ω/cm, $p < 0.001$) and a lower phase angle (–0.086° ± 0.067°, $p < 0.0001$), which explains 11%–23% of the differences in the raw data between Japanese and Germans shown in **Table 1**. The BIA-prediction equations include corrections for these differences.

Statistics

Data analyses were performed with R software, version 3.0.1 (R Foundation for Statistical Computing, Vienna, Austria). Descriptive statistics are presented as means ± SD. For the calculation of the 95% CIs of regression lines, normal distribution and homoscedasticity were assumed. Differences among independent samples of the 3 ethnic groups were analyzed using ANOVA with Bonferroni post hoc tests. Differences between Pearson correlation coefficients *r* were evaluated by using a z-transformation according to R.A. Fischer (Hedderich and Sachs 2012). A p value <0.05 was considered significant.

The fat mass index (FMI), fat-free mass index (FFMI), skeletal muscle index (SMI), and VAT in **Tables 2** and **3** were calculated by linear regression versus BMI. The percentage value of FM was calculated from the FMI as FM = 100% × FMI/BMI using the respective BMI values. Negative results for VAT at a BMI of 18.5 kg/m^2 were omitted. Calculations in **Supplementary Table S2** were performed for subgroups with at least 25 subjects; results for subgroups with fewer subjects were omitted.

For BIVA graphs, the 50% tolerance ellipses were calculated from R_{50kHz}/Ht and Xc_{50kHz}/Ht as explained in detail by Piccoli and colleagues (1994). The ellipses describe the area into which the measurements of 50% of all subjects fall. Vector displacements parallel to the major axis indicate tissue hydration (less TBW leading to longer vectors), and vector displacements parallel to the minor axis indicate cell mass (less cell mass leading to a down-sloping of the vector) (Piccoli and Pastori 2002). The 3 indices FMI, FFMI, and SMI represent the FM, FFM, and SMM normalized by Ht squared:

$$FMI = \frac{FM}{Ht^2} \qquad FFMI = \frac{FFM}{Ht^2} \qquad SMI = \frac{SMM}{Ht^2}$$

FMI versus FFMI is displayed in the body composition chart (BCC), which is based on a chart from Hattori (1991) and uses 50% tolerance ellipses that indicate the area that contains 50% of the measurements. Like the BIVA, these ellipses were calculated according to Piccoli and colleagues (1994).

Percentile curves for phase angle were calculated with the R package VGAM using lambda, mu, sigma (LMS) quantile regression with a Box-Cox transformation to normality. The M value was modelled with 2 degrees of freedom (df) for age dependency. The L and S values were modeled as intercept only. When 1 df for height dependency was added for the M value, values of –0.0197°/cm and –0.0223°/cm were found for German women and men, respectively, –0.0196°/cm and –0.0189°/cm for Japanese women and

men, respectively, and –0.0261°/cm and –0.0236°/cm for Mexican women and men, respectively.

Table 1. Characterization of the study groups.

	women			**men**		
	German	**Japanese**	**Mexican**	**German**	**Japanese**	**Mexican**
	n = 517	n = 497	n = 503	n = 531	n = 498	n = 523
Age, y	38.6 ±13.4	40.7 ±13.3[a]	39.5 ±11.9	39.3 ±13.2	42.1 ±15.9[a]	39.1 ±12.3[c]
Weight, kg	69.6 ±12.2	52.7 ±7.8[a]	66.8 ±11.4[b,c]	86.4 ±12.5	67.3 ±11.5[a]	80.2 ±13.6[b,c]
Height, cm	168 ±7	158 ±6[a]	157 ±6[b]	181 ±7	171 ±6[a]	170 ±7[b]
BMI, kg/m²	24.7 ±4.2	21.0 ±3.0[a]	27.0 ±4.5[b,c]	26.2 ±3.4	23.1 ±3.6[a]	27.8 ±4.1[b,c]
R_{50kHz}/Ht, Ω/cm	3.94 ±0.40	4.41 ±0.48[a]	4.39 ±0.50[b]	3.02 ±0.32	3.36 ±0.38[a]	3.33 ±0.37[b]
Xc_{50kHz}/Ht, Ω/cm	0.348 ±0.046	0.358 ±0.048[a]	0.399 ±0.050[b,c]	0.312 ±0.045	0.320 ±0.045[a]	0.352 ±0.048[b,c]
Phase angle, °	5.05 ±0.47	4.65 ±0.44[a]	5.21 ±0.48[b,c]	5.88 ± 0.51	5.46 ± 0.58[a]	6.04 ± 0.50[b,c]
FMI, kg/m²	8.5 ±3.2	6.4 ±2.2[a]	10.7 ±3.4[b,c]	6.4 ±2.5	4.8 ±2.5[a]	8.1 ±2.9[b,c]
FFMI, kg/m²	16.3 ±1.4	14.6 ±1.3[a]	16.3 ±1.5[c]	19.8 ±1.5	18.3 ±1.7[a]	19.7 ±1.6[c]
SMI, kg/m²	7.50 ±0.82	6.16 ±0.80[a]	7.12 ±0.88[b,c]	9.80 ±0.83	8.66 ±1.02[a]	9.43 ±0.91[b,c]
VAT, l	1.11 ±0.83	1.40 ±0.39[a]	2.19 ±0.81[b,c]	2.54 ±1.45	2.27 ±1.09[a]	3.61 ±1.49[b,c]
Age <40 y	n = 264	n = 248	n = 251	n = 265	n = 248	n = 265
BMI, kg/m²	23.9 ±3.6	20.8 ±3.1[a]	26.0 ±4.5[b,c]	25.2 ±3.2	22.4 ±3.5[a]	27.3 ±4.5[b,c]
Underweight	2	41	0	0	16	3
Normal weight	192	190	130	139	189	84
Overweight	50	14	77	111	32	108
Obesity	20	3	44	15	11	70
Age ≥40 y	n = 253	n = 249	n = 252	n = 266	n = 250	n = 258
BMI, kg/m²	25.6 ±4.5	21.2 ±3.0[a]	28.0 ±4.2[b,c]	27.3 ±3.4	23.8 ±3.6[a]	28.4 ±3.6[b,c]
Underweight	0	34	1	0	9	0
Normal weight	133	191	65	66	159	38
Overweight	82	19	112	153	69	144
Obesity	38	5	74	47	13	76

Note: Data are presented as n or means ± SD. Underweight: BMI <18.5 kg/m²; normal weight: BMI ≥18.5, <25 kg/m²; overweight: BMI ≥25, <30 kg/m²; obese: BMI ≥30 kg/m². Different letters represent significant differences (a, Japanese vs. Germans; b, Mexicans vs. Germans; c, Mexicans vs. Japanese); ANOVA with Bonferroni post hoc test. BMI, body mass index; FFMI, fat free mass index; FMI, fat mass index; Ht, body height; R, resistance; SMI, skeletal muscle index; VAT, visceral adipose tissue; Xc, reactance.

Results

Characteristics of the study groups stratified into the 3 ethnic groups are given in **Table 1**. The Japanese group was slightly older than the German group (men and women) and Mexican group (men only). Larger differences between subgroups were observed in BMI, with the lowest values in Japanese and the highest in Mexicans. Dividing each subgroup into 2 age groups (<40 and ≥40 years) revealed that the majority of the younger groups were normal weight, with the exception of Mexican men, who had a greater proportion of overweight subjects, similar to Mexican men and women ≥40 years and German men ≥40 years. The Japanese group had the greatest prevalence of underweight and the lowest prevalence of obesity.

Sex and ethnic-specific body composition for different WHO BMI cut-offs is given in **Table 2** for the whole study population and in **Table 3** as stratified by age groups. Adults with the same BMI but differing in ethnic group have different levels of adiposity, with the lowest values for FM percentage, FMI, and VAT in Germans and the highest values in Mexicans. As an exception to this rule, underweight Japanese subjects had the highest amount of VAT. However, this was not statistically significant for underweight Japanese men ≥40 years. Additional exceptions were not significant. An inverse pattern was observed for FFMI and total SMI, with the highest values in Germans and the lowest in Mexicans.

Partitioning of fat and lean mass therefore differs among ethnicities. For a given BMI, Germans have a higher FFMI and a lower FMI than do Mexicans. For normal-weight Japanese, the partitioning is comparable to that of Mexicans and approaches the partitioning of Germans with increasing BMI, resulting in more similar obese phenotypes in Japanese and German groups (**Table 2**, **Supplementary Fig. S1**). These patterns are also observed in subgroups of younger and older adults (**Table 3**).

The regional distribution of SMM differs among ethnicities as well. The SMI of the trunk and the arms was highest in Germans and lowest in Japanese (**Table 2**). In contrast, the SMI of the legs was lowest in Mexicans and was similar in Germans and Japanese, with a stronger BMI dependency in Japanese women and, to a smaller extent, in Japanese men (**Supplementary Fig. S2**).

Table 2. Fat mass (FM), fat mass index (FMI), fat-free mass index (FFMI), skeletal muscle index (SMI), and visceral adipose tissue (VAT).

	Women			Men		
BMI, kg/m²	**18.5**	**25**	**30**	**18.5**	**25**	**30**
FM, %						
German	20.6	34.6	41.3	6.6	22.3	29.8
Japanese	25.3[a]	36.2[a]	41.4	10.2[a]	23.9[a]	30.5
Mexican	24.4[b]	37.1[b]	43.1[b,c]	9.7[b]	24.8[b,c]	32.0[b,c]
FMI, kg/m²						
German	3.8	8.7	12.4	1.2	5.6	8.9
Japanese	4.7[a]	9.1[a]	12.4	1.9[a]	6.0[a]	9.1
Mexican	4.5[b]	9.3[b]	12.9[b,c]	1.8[b]	6.2[b,c]	9.6[b,c]
FFMI, kg/m²						
German	14.7	16.3	17.6	17.3	19.4	21.1
Japanese	13.8[a]	15.9[a]	17.6	16.6[a]	19.0[a]	20.9
Mexican	14.0[b]	15.7[b]	17.1[b,c]	16.7[b]	18.8[b,c]	20.4[b,c]
SMI, kg/m²						
German	6.56	7.55	8.30	8.34	9.57	10.51
Japanese	5.67[a]	6.93[a]	7.89[a]	7.67[a]	9.07[a]	10.15[a]
Mexican	5.84[b]	6.82[b]	7.57[b,c]	7.76[b]	8.93[b,c]	9.82[b,c]
SMI_{trunk}, kg/m²						
German	2.77	3.23	3.59	3.89	4.42	4.82
Japanese	2.29[a]	2.80[a]	3.19[a]	3.48[a]	4.04[a]	4.46[a]
Mexican	2.55[b,c]	3.01[b,c]	3.38[b,c]	3.81[c]	4.30[b,c]	4.67[b,c]
SMI_{arms}, kg/m²						
German	0.82	0.88	0.92	1.07	1.21	1.32
Japanese	0.64[a]	0.73[a]	0.81[a]	0.92[a]	1.08[a]	1.20[a]
Mexican	0.73[b,c]	0.80[b,c]	0.86[b,c]	1.02[b,c]	1.14[b,c]	1.23[b]
SMI_{legs}, kg/m²						
German	2.97	3.44	3.79	3.37	3.94	4.38
Japanese	2.75[a]	3.40	3.90	3.27	3.96	4.48[a]
Mexican	2.57[b,c]	3.00[b,c]	3.33[b,c]	2.94[b,c]	3.49[b,c]	3.92[b,c]
VAT, l						
German	0.16	1.15	1.92		2.12	3.83
Japanese	1.23[a]	1.67[a]	2.01	1.10[a]	2.74[a]	4.01
Mexican	0.94[b,c]	1.90[b,c]	2.64[b,c]	0.75[c]	2.74[b]	4.28[b,c]

Note: Data are calculated from the World Health Organization reference values for body mass index (BMI) (WHO Expert Consultation 2004). All values except for FM in % are calculated as linear regression to BMI. FM in % is calculated from the FMI by 100% × FMI/BMI. Negative results for VAT for BMI = 18.5 kg/m² are omitted. Underweight: BMI <18.5 kg/m²; normal weight: BMI ≥18.5, <25 kg/m²; overweight: BMI ≥25, <30 kg/m²; obese: BMI ≥30 kg/m². Different letters represent significant differences (a, Japanese vs. Germans; b, Mexicans vs. Germans; c, Mexicans vs. Japanese).

Table 3. Fat mass (FM), fat mass index (FMI), fat-free mass index (FFMI), skeletal muscle index (SMI), and visceral adipose tissue (VAT) given separately for younger adults (<40 y) and older adults (≥40 y).

	women			men		
BMI, kg/m²	**18.5**	**25**	**30**	**18.5**	**25**	**30**
Age <40 y						
FM, %						
German	20.0	33.4	39.8	4.9	21.6	29.6
Japanese	23.8[a]	35.3[a]	40.7	8.6[a]	22.4	28.9
Mexican	22.8[b]	35.5[b]	41.6[b]	7.7[b]	23.7[b,c]	31.3[b,c]
FMI, kg/m²						
German	3.7	8.4	11.9	0.9	5.4	8.9
Japanese	4.4[a]	8.8[a]	12.2	1.6[a]	5.6	8.7
Mexican	4.2[b]	8.9[b]	12.5[b]	1.4[b]	5.9[b,c]	9.4[b,c]
FFMI, kg/m²						
German	14.8	16.6	18.1	17.6	19.6	21.1
Japanese	14.1[a]	16.2[a]	17.8	16.9[a]	19.4	21.3
Mexican	14.3[b]	16.1[b]	17.5[b]	17.1[b]	19.1[b,c]	20.6[b,c]
SMI, kg/m²						
German	6.60	7.73	8.60	8.50	9.69	10.61
Japanese	5.83[a]	7.06[a]	8.01[a]	7.90[a]	9.39[a]	10.54
Mexican	5.99[b]	7.04[b]	7.84[b]	7.98[b]	9.13[b,c]	10.00[b,c]
VAT, l						
German	0.07	0.88	1.50		1.80	3.37
Japanese	1.07[a]	1.47[a]	1.78[a]	0.99[a]	2.39[a]	3.47
Mexican	0.86[b,c]	1.74[b,c]	2.41[b,c]	0.61[c]	2.47[b]	3.91[b,c]
Age ≥40 y						
FM, %						
German	22.3	35.8	42.2	9.8	23.4	29.9
Japanese	27.0[a]	37.0[a]	41.8	12.8	25.2[a]	31.1
Mexican	28.1[b]	39.0[b,c]	44.2[b,c]	13.4[b]	26.4[b,c]	32.6[b,c]
FMI, kg/m²						
German	4.1	8.9	12.7	1.8	5.9	9.0
Japanese	5.0[a]	9.2[a]	12.5	2.4	6.3[a]	9.3
Mexican	5.2[b]	9.7[b,c]	13.3[b,c]	2.5[b]	6.6[b,c]	9.8[b,c]
FFMI, kg/m²						
German	14.4	16.1	17.3	16.7	19.1	21.0
Japanese	13.5[a]	15.8[a]	17.5	16.1	18.7[a]	20.7
Mexican	13.3[b]	15.3[b,c]	16.7[b,c]	16.0[b]	18.4[b,c]	20.2[b,c]
SMI, kg/m²						
German	6.40	7.38	8.14	7.98	9.38	10.46
Japanese	5.49[a]	6.82[a]	7.84	7.29[a]	8.81[a]	9.99[a]
Mexican	5.47[b]	6.54[b,c]	7.36[b,c]	7.35[b]	8.66[b]	9.66[b,c]
VAT, l						
German	0.44	1.41	2.16	0.48	2.51	4.06
Japanese	1.41[a]	1.84[a]	2.17	1.33[a]	3.00[a]	4.29
Mexican	1.17[b,c]	2.09[b,c]	2.80[b,c]	1.04[b]	3.05[b]	4.60[b,c]

Note: Data are calculated from the World Health Organization reference values for body mass index (BMI) (WHO Expert Consultation 2004). All values except for FM in % are calculated as linear regression to BMI. FM in % is calculated from the FMI by 100% × FMI/BMI. Negative results for VAT for BMI = 18.5 kg/m² are omitted. Underweight: BMI <18.5 kg/m²; normal weight: BMI ≥18.5, <25 kg/m²; overweight: BMI ≥25, <30 kg/m²; obese: BMI ≥30 kg/m². Different letters represent significant differences (a, Japanese vs. Germans; b, Mexicans vs. Germans; c, Mexicans vs. Japanese).

Differences in body composition in ethnic groups are displayed in **Fig. 1** in the form of a BCC. In addition to the highest BMI, Mexican men and women have the highest FMI, whereas their FFMI is comparable to that of Germans. Japanese men and women have the lowest FMI and FFMI.

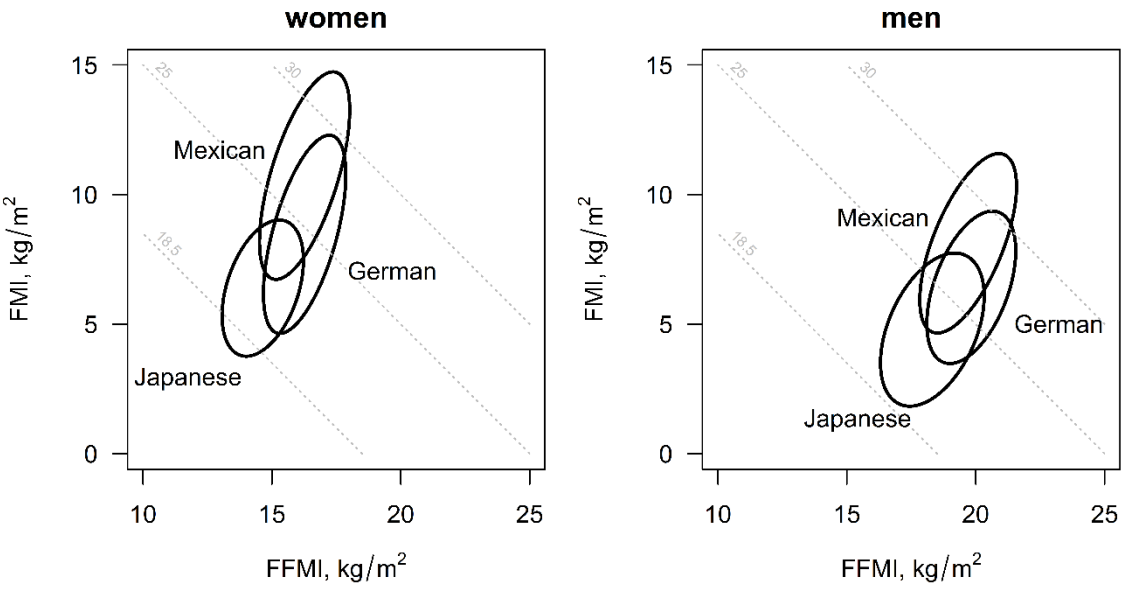

Fig 1. Body composition chart with 50% tolerance ellipsis for Germans, Japanese, and Mexicans. The dashed lines indicate body mass index values of 18.5, 25, and 30 kg/m². Statistical significance of differences are described in **Supplementary Table S1**. FMI, fat mass index; FFMI, fat-free mass index.

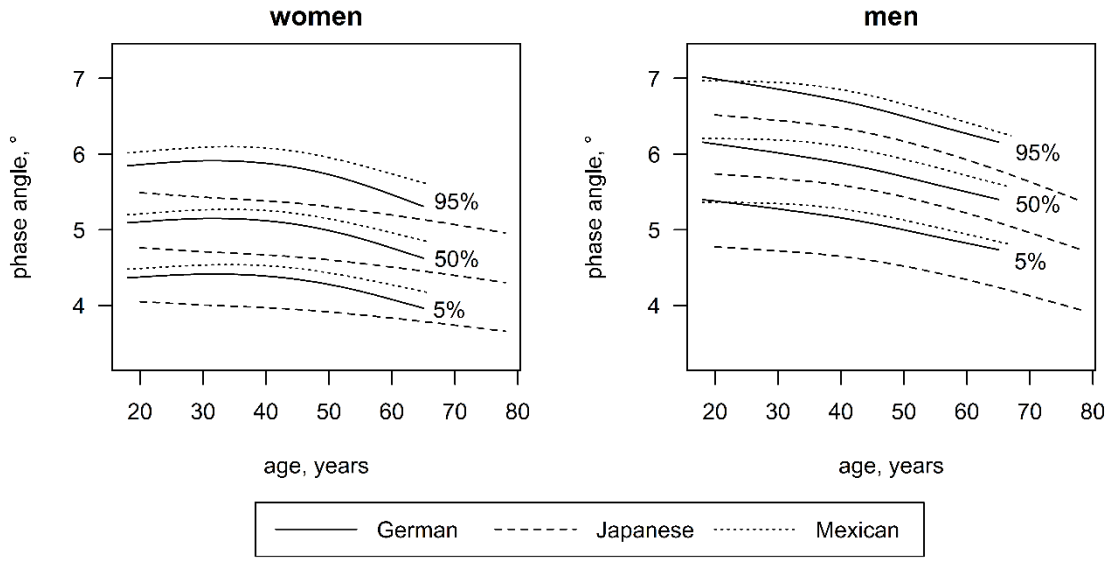

Fig 2. Comparison of 5th, 50th, and 95th percentiles of phase angle among Germans, Japanese, and Mexicans.

Phase angle percentiles are lower in Japanese than in German and Mexican groups (**Fig. 2**). This is also reflected by a higher ECW/TBW ratio in Japanese compared with Germans and Mexicans (**Table 4**) and is also consistent with a lower SMI in the Japanese group compared with the German group.

Table 4. Extracellular water as a percentage of total body water for subjects with normal weight (body mass index ≥18.5, <25 kg/m²) stratified by age range and ethnicity.

	women	**men**
Age <40 years		
German	43.3 ± 1.3	39.8 ± 1.1
Japanese	44.9 ± 1.7[a]	40.2 ± 1.4[a]
Mexican	42.9 ± 1.6[b,c]	39.1 ± 1.2[b,c]
Age ≥40 years		
German	45.0 ± 1.6	41.4 ± 1.2
Japanese	47.0 ± 2.0[a]	42.8 ± 1.9[a]
Mexican	45.3 ± 1.7[c]	41.1 ± 1.2[c]

Note: Data are presented as means ± SD.
Different letters represent significant differences (a, Japanese vs. Germans; b, Mexicans vs. Germans; c, Mexicans vs. Japanese); ANOVA with Bonferroni post hoc test.

The BIVA reveals additional ethnic differences in body composition related to tissue composition (hydration and cellularity). In **Fig. 3**, BIVA-tolerance ellipses for normal-weight subjects are compared among ethnic groups. Consistent with the low FFM, the longest vectors are observed in Mexicans. However, vector length was similar between German and Japanese groups despite a lower FFMI and higher FMI and VAT in Japanese compared with German subjects (**Table 2**). The lower phase angle of the Japanese group (**Fig. 2**) is represented by a shift of the ellipses to the bottom right of the graph. Databases for BCC and BIVA charts are given in **Supplementary Tables S1** and **S2**.

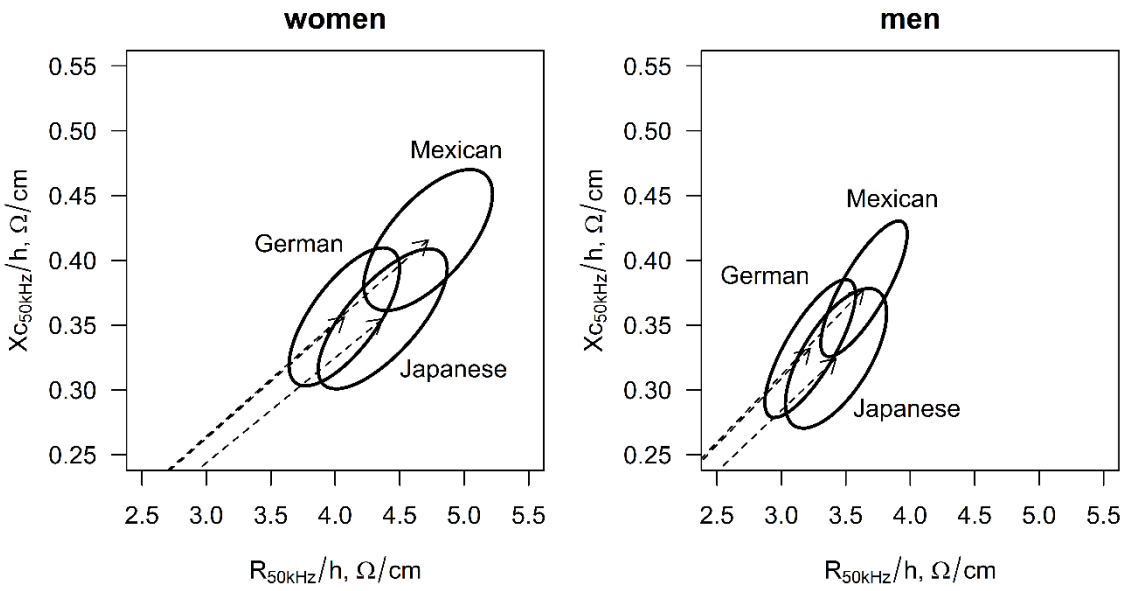

Fig. 3. Bioelectrical impedance vector analysis with 50% tolerance ellipsis for normal-weight (body mass index ≥18.5, <25 kg/m²) subgroups (means ± SD in Ohm per centimeter). German women: R = 4.07 ± 0.36, Xc = 0.356 ± 0.045; German men: R = 3.22 ± 0.30, Xc = 0.332 ± 0.045; Japanese women: R = 4.37 ± 0.42, Xc = 0.355 ± 0.046; Japanese men: R = 3.42 ± 0.33, Xc = 0.324 ± 0.046; Mexican women: R = 4.72 ± 0.42, Xc = 0.416 ± 0.046; Mexican men: R = 3.64 ± 0.28, Xc = 0.378 ± 0.044. Significance of differences and correlation coefficients are described in **Supplementary Table S2**.
R, resistance; Xc, reactance.

Discussion

The primary aim of the current study was to analyze differences in clinically relevant outcome parameters of body composition at standard BMI cut-offs across 3 ethnic groups. We found profound differences among ethnicities in percentage FM at the same BMI. Therefore, it appears that ethnic differences contribute significantly to the well-known interindividual variance in percentage FM at the same BMI. We have shown that ethnic-specific relationships between BMI and body composition depend on weight status. For a given BMI, Mexicans have higher FM and VAT when compared with Germans. Normal-weight Japanese are similar to Mexicans, whereas overweight Japanese are more similar to Germans (**Table 2**, **Supplementary Fig. S1**). The relationship between FMI and BMI had a similar pattern and slope in younger (<40 years) and older (≥40 years) subgroups (**Table 3**). By contrast, results from the National Health and Nutrition Examination Survey (NHANES) have shown that ethnic differences in body shape and composition are less apparent in older (≥70 years) Mexican-American and non-Hispanic white and black populations (Heymsfield et al. 2016).

The lower increase of the fat-to-lean partitioning with increasing BMI in Japanese compared with German and Mexican groups is unlikely to be explained by ethnic differences in body proportions (i.e., a higher trunk mass in the Japanese group). With weight gain, a disproportional higher gain in FM at the trunk in men and at the extremities in women was observed in Germans (Schautz et al. 2012). A lower increase in total body fat with weight gain would therefore have required longer legs in men and a gynoid fat distribution in women; however, both are unlikely phenotypes in the Japanese population and disagree with the finding of a lower ratio of leg length to trunk length in Asian populations (Deurenberg et al. 2002).

The results of the current study demonstrate ethnic differences in the raw data of BIA presented as phase angle (**Fig. 2**) and BIVA charts (**Fig. 3**). The lower phase angle in Japanese compared with Germans in all age groups is likely a result of a higher ECW/TBW ratio (**Table 4**) and is also compatible with a lower SMM/FFM (i.e., 0.41 vs. 0.45 in underweight and 0.45 vs. 0.47 in obese Japanese vs. German women or 0.46 vs 0.48 in underweight and 0.49 vs. 0.50 in obese Japanese vs. German men, as calculated from data in **Table 2**). In addition, the lower phase angle and the higher ECW/TBW ratio in the Japanese group are explained by ethnic differences in partitioning of fat and lean mass, with a lower FFMI and a higher FMI in underweight and normal-weight BMI subgroups (**Table 2**). A higher phase angle for Mexicans in comparison with Germans is in accordance with differences in Ht (**Table 1**) and the height dependency of the phase angle in all groups. Phase angle measured with the seca mBCA 515 is lower in comparison with values measured by BIA 2000-S, (Data Input, Data Input GmbH, Pöcking, Germany) (Bosy-Westphal et al. 2006), because of differences between the devices, the measurement while standing versus in the supine position, and the different type and placement of electrodes (unpublished data).

In BIVA analysis, longer vectors at the same BMI were observed in Mexicans compared with Germans. This is in accordance with a lower FFMI at the same BMI for Mexicans compared with Germans (**Table 2**, **Supplementary Fig. S1**), which results in lower TBW per weight for the same BMI (data not shown) and therefore in higher impedances per body length. This result is supported by NHANES data showing less FFM and muscle mass per BMI with a concomitant higher FM percentage in Mexican-American men and women compared with the corresponding groups of non-Hispanic whites or blacks (Heymsfield et al. 2016, Heo et al. 2012).

In addition to a lower phase angle with the corresponding down-sloping of the BIVA vector, Japanese men and women have slightly longer vectors compared with Germans. A lower FFMI and SMI for normal-weight Japanese (**Table 2**) can explain longer vectors because of lower TBW per weight. However, mean BIVA vectors were shorter in the Japanese group than in Mexicans, despite a similarly low FFMI and high FMI for normal-weight subjects (**Table 2**, **Supplementary Fig. S1**). This result was likely a result of a higher SMI_{legs} in relation to SMI_{trunk} in Japanese men and women (**Table 2**, **Supplementary Fig. S2**) and a lower ratio of leg length to trunk length (Deurenberg et al. 2002). After normalization of sex and BMI, differences in body shape (i.e., the distribution of lean mass) are often overlooked as an important additional confounder for the interpretation of BIVA. Because legs have a small diameter relative to their length, when compared with the trunk, they contribute to approximately one-half of total body resistance, whereas the trunk contributes only 9% (Foster and Lukaski 1996). A higher SMI_{leg} in Japanese men and women compared with Mexicans, as well as a shorter leg length, therefore leads to better conductivity of the legs and, hence, to a shortening of vectors (**Fig. 3**, **Supplementary Table S2**).

When compared with Germans, for a given BMI, a lower SMM at the trunk and a similar SMM at the legs were found for Japanese (**Table 2**, **Supplementary Fig. S2**). This leads to a higher SMI_{legs}/SMI_{trunk} ratio and therefore, together with shorter legs, to a shortening of vectors, which counteracts longer vectors because of lower FFMI. This finding is in accordance with a higher leg/trunk ratio of muscle thickness measured with ultrasound in Japanese compared with American women (Ishida et al. 1992).

The relationship between SMM at the legs and trunk was also slightly lower in Mexican men and women when compared with Germans (**Table 2**, **Supplementary Fig. S2**). This adds to the longer vectors in Mexicans. By contrast, no significant differences in the ratio of leg length to trunk length were found between American whites (who are assumed to be similar to Germans) and Mexicans (Heymsfield et al. 2005).

In summary, longer vectors in Mexicans compared with Germans may be explained by a higher FMI and a lower FFMI per BMI and a lower SMI_{leg} in relation to SMI_{trunk} (**Table 2**), whereas the shorter vectors in the Japanese group compared with the Mexican group might be explained by (*i*) a higher SMI_{legs} in relation to SMI_{trunk}, (*ii*) a higher trunk length/height, and (*iii*) a lower leg length/height, which unfortunately were not measured in the current study. Differences in vector length between Japanese and Germans are small because of opposing effects.

It has been shown by Marini and colleagues (2013) in elderly Italians and by Buffa and colleagues (2013) in adults in the United States that classic BIVA, in contrast to specific BIVA, does not recognize differences in the percentage of FM. Classic BIVA has limitations in the interpretation of body composition because values are not corrected for cross-sectional areas. For classic BIVA, a negative correlation between FM% by dual-energy X-ray absorptiometry and vector length was reported by Buffa and colleagues (2013), whereas we found a positive correlation between FM% and vector length at the same BMI. This discrepancy can be explained by the fact that we compared FM% at the same BMI. Because of negative correlations that were also found between BMI and the vector length of classic BIVA (Buffa et al. 2013) and because of the correlation between BMI and FM%, the vector displacement caused by a higher BMI can counteract the displacement caused by higher percentages of FM at the same BMI. Therefore, the comparison of subjects with the same BMI helps avoid the limitations of classic BIVA.

As a limitation of our study, weight status differed among ethnic groups. Subgroups with underweight or obesity are underrepresented in certain ethnic groups (**Table 1**, **Fig. 1**). These differences were accounted for by comparing subjects with similar BMI in the BMI range of 18.5 to 30 kg/m^2, which was represented in all groups. To get comparable groups for all 3 ethnicities, similar inclusion and exclusion criteria were applied, and people who were eligible for blood donation were considered to be healthy. In addition, a lower phase angle in the Japanese group as seen in the BIVA could be explained by the older age in this group. However, adjusting *Xc* per height for age did not change the BIVA pattern (data not shown).

The consequences of the observed ethnic differences in the relationships between BMI and different body composition parameters for BMI-associated health risks deserve more attention. Of note, ethnic differences in body composition contribute to differences in the metabolic clearance rate of insulin (i.e., lower clearance rate in Hispanics compared with non-Hispanic whites) and thus to differences in insulin sensitivity across ethnic groups (Lorenzo et al. 2013). Ethnic variations in adipokine levels and metabolic risk factors have been shown to persist after adjustment for BMI (Morimoto et al. 2014) and need to be investigated with regard to ethnicity specifics in body composition to understand the cause of these differences in obesity-associated health risk.

In conclusion, the relationship between BMI and adiposity is ethnic specific; in addition, fat distribution, SMI, and muscle mass distribution vary at the same BMI according to ethnicity and lead to profound differences in BCC, phase angle, and BIVA. Ethnic-specific

normal values are therefore required for BCC, phase angle, and BIVA. The developed ethnic-specific reference values for different WHO BMI cut-off points can serve as a useful research tool.

Conflict of interest statement: This work was supported by a grant from seca gmbh and co. kg., Germany. Björn Jensen and Michael J. Maisch are employed by seca. Anja Bosy-Westphal serves as a consultant for seca. Takashi Moritoyo, Martha Kaufer-Horwitz, Sven Peine, Kristina Norman, Aya Matsumoto, Yuka Masui, Antonio Velázquez-González, Jannet Domínguez-García, Elizabeth Fonz-Enríquez, and Saori G Salgado-Moctezuma declare that they have no conflicts of interest.

Author contributions: B. Jensen and A. Bosy-Westphal designed the research, analyzed the data, and wrote the paper. T. Moritoyo, A. Matsumoto, Y. Masui, and the Trial Group of P1 Unit of the University of Tokyo Hospital conducted research for the Japanese population. M. Kaufer-Horwitz, A. Velázquez-González, J. Domínguez-García, E. Fonz-Enríquez, and S.G. Salgado-Moctezuma conducted research for the Mexican population. S. Peine conducted research for the German population. K. Norman and M.J. Maisch contributed to the discussion of the data. T. Moritoyo and M. Kaufer-Horwitz contributed to the critical review and intellectual content of the manuscript. All authors read and approved the final manuscript.

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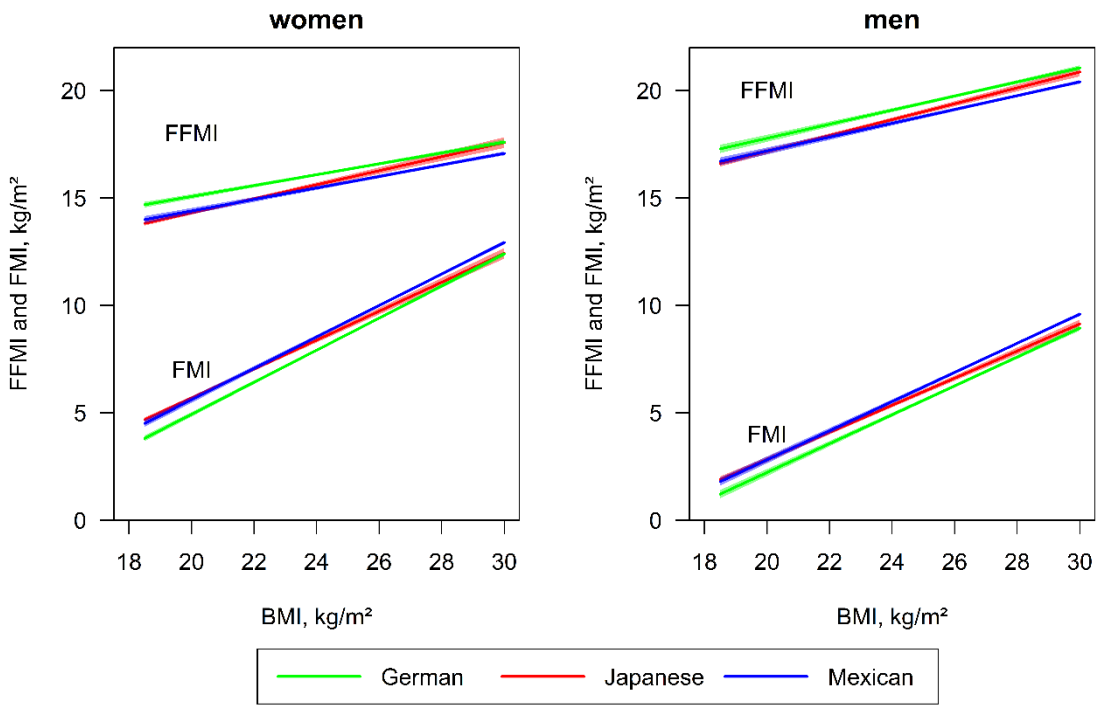

Figure S1 Relationship between fat free mass index (FFMI) and fat mass index (FMI) versus BMI for normal weight and overweight Germans, Japanese and Mexicans. The shaded areas indicate 95% confidence intervals of the regression lines

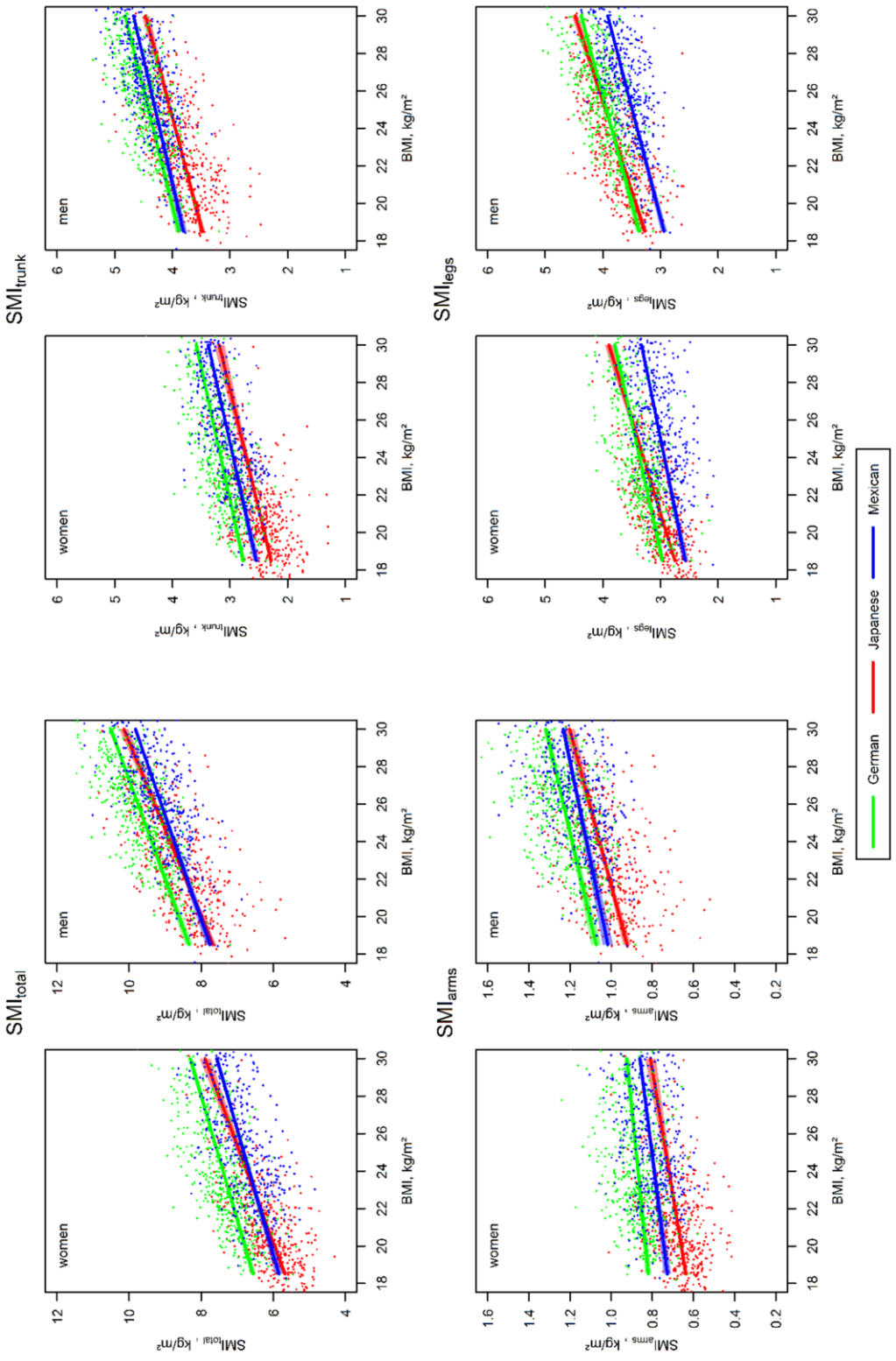

Figure S2 Relationship between the skeletal muscle index (SMI) of the total body, trunk, arms and legs versus BMI for normal weight and overweight Germans, Japanese and Mexicans, stratified by sex. The shaded areas indicate 95% confidence intervals of the regression lines

Table S1 The components fat free mass index (FFMI) and fat mass index (FMI) of the body composition chart (BCC) (means ± SD and correlation coefficients, r for FFMI and FMI) stratified by ethnicity, sex and age range

	FFMI, kg/m²	FMI, kg/m²	r
women, total population			
German	16.3 ± 1.4	8.5 ± 3.2	0.60
Japanese	14.6 ± 1.3[a]	6.4 ± 2.2[a]	0.41[a]
Mexican	16.3 ± 1.5[c]	10.7 ± 3.4[b,c]	0.63[c]
women, <40 years			
German	16.3 ±1.3	7.5 ±2.7	0.63
Japanese	14.8 ±1.3[a]	6.0 ±2.2[a]	0.52
Mexican	16.4 ±1.5[c]	9.6 ±3.3[b,c]	0.73[c]
women, ≥40 years			
German	16.2 ±1.4	9.4 ±3.5	0.64
Japanese	14.5 ±1.4[a]	6.8 ±2.2[a]	0.37[a]
Mexican	16.2 ±1.5[c]	11.9 ±3.1[b,c]	0.68[c]
men, total population			
German	19.8 ± 1.5	6.4 ± 2.5	0.48
Japanese	18.3 ± 1.7[a]	4.8 ± 2.5[a]	0.43
Mexican	19.7 ± 1.6[c]	8.1 ± 2.9[b,c]	0.63[b,c]
men, <40 years			
German	19.7 ±1.3	5.6 ±2.4	0.44
Japanese	18.4 ±1.6[a]	4.0 ±2.3[a]	0.52
Mexican	19.8 ±1.6[c]	7.5 ±3.2[b,c]	0.70[b,c]
men, ≥40 years			
German	20.0 ±1.6	7.3 ±2.3	0.51
Japanese	18.2 ±1.8[a]	5.6 ±2.4[a]	0.43
Mexican	19.6 ±1.6[b,c]	8.7 ±2.5[b,c]	0.62[c]

Significant differences between [a]Japanese vs. Germans, [b]Mexicans vs. Germans, [c]Mexicans vs. Japanese, FFMI and FMI are tested by ANOVA with Bonferroni post hoc test, r-values are tested after ż-transformation according to R.A. Fischer with Bonferroni correction

Table S2 Components of the bioelectrical impedance vector analysis (BIVA) (mean ± SD and correlation coefficients, r for R/Ht and Xc/Ht) stratified by ethnicity, sex, age range and BMI

BMI, kg/m²	**<18.5**			**≥18.5, <25**			**≥25, <30**			**≥30**		
	R/Ht, Ω/cm	Xc/Ht, Ω/cm	r	R/Ht, Ω/cm	Xc/Ht, Ω/cm	r	R/Ht, Ω/cm	Xc/Ht, Ω/cm	r	R/Ht, Ω/cm	Xc/Ht, Ω/cm	r
women, total population												
German				4.07 ±0.36	0.356 ±0.045	0.70	3.79 ±0.33	0.340 ±0.042	0.64	3.51 ±0.32	0.315 ±0.042	0.68
Japanese	4.86 ±0.40	0.381 ±0.052	0.70	4.37 ±0.42[a]	0.355 ±0.046	0.72	4.07 ±0.44[a]	0.355 ±0.048	0.79			
Mexican				4.72 ±0.42[b,c]	0.416 ±0.046[b,c]	0.65	4.32 ±0.38[b,c]	0.401 ±0.049[b,c]	0.69	3.95 ±0.41[b]	0.370 ±0.046[b]	0.65
women, <40 years												
German				4.11 ±0.35	0.364 ±0.042	0.67	3.83 ±0.34	0.352 ± 0.043	0.69			
Japanese	4.97 ±0.42	0.396 ±0.050	0.77	4.47 ±0.43[a]	0.368 ±0.043	0.69						
Mexican				4.76 ±0.43[b,c]	0.425 ±0.043[b,c]	0.60	4.32 ±0.43[b]	0.411 ± 0.051[b]	0.77	3.91 ±0.44	0.370 ±0.051	0.60
women, ≥40 years												
German				4.01 ±0.37	0.345 ±0.047	0.73	3.76 ±0.33	0.333 ± 0.040	0.60	3.49 ±0.32	0.304 ±0.039	0.67
Japanese	4.72 ±0.31	0.362 ±0.048	0.49	4.27 ±0.39[a]	0.342 ±0.045	0.72						
Mexican				4.64 ±0.40[b,c]	0.396 ±0.046[b,c]	0.74	4.32 ±0.35[b]	0.394 ± 0.046[b]	0.64	3.97 ±0.39[b]	0.370 ±0.044[b]	0.69
men, total population												
German				3.22 ±0.30	0.332 ±0.045	0.78	2.95 ±0.25	0.304 ±0.041	0.73	2.70 ±0.25	0.277 ±0.034	0.77
Japanese	3.88 ±0.41	0.341 ±0.054	0.82	3.42 ±0.33[a]	0.324 ±0.046	0.65[a]	3.12 ±0.22[a]	0.306 ±0.037	0.48[a]			
Mexican				3.64 ±0.28[b,c]	0.378 ±0.044[b,c]	0.81[c]	3.34 ±0.30[b,c]	0.354 ±0.043[b,c]	0.75[c]	3.03 ±0.29[b]	0.325 ±0.045[b]	0.73
men, <40 years												
German				3.27 ±0.30	0.344 ±0.042	0.78	2.98 ±0.24	0.323 ± 0.038	0.72			
Japanese				3.47 ±0.32[a]	0.341 ±0.041	0.62[a]	3.11 ±0.18[a]	0.313 ± 0.028	0.64			
Mexican				3.67 ±0.29[b,c]	0.388 ±0.043[b,c]	0.83[c]	3.38 ±0.33[b,c]	0.369 ± 0.046[b,c]	0.77	3.03 ±0.27	0.332 ±0.046	0.77
men, ≥40 years												
German				3.11 ±0.27	0.307 ±0.040	0.75	2.92 ± 0.26	0.291 ± 0.037	0.76	2.65 ±0.23	0.273 ±0.034	0.76
Japanese				3.37 ±0.34[a]	0.304 ±0.043	0.69	3.12 ± 0.24[a]	0.302 ± 0.041	0.45[a]			
Mexican				3.57 ±0.24[b,c]	0.356 ±0.039[b,c]	0.74	3.31 ± 0.27[b,c]	0.343 ± 0.037[b,c]	0.74[c]	3.02 ±0.30[b]	0.319 ±0.043[b]	0.70

Significant differences between [a]Japanese vs. Germans, [b]Mexicans vs. Germans, [c]Mexicans vs. Japanese, R/Ht and Xc/Ht are tested by ANOVA with Bonferroni post hoc test, r-values are tested after ż-transformation according to R.A. Fischer ($p < 0.05 / 3$ when values are available for three ethnicities, $p < 0.05$ for two ethnicities). Values are only calculated for subgroups with $n \geq 25$.

Underweight: BMI <18.5 kg/m², normal weight: BMI ≥18.5, <25 kg/m², overweight: BMI ≥25, <30 kg/m², obesity: BMI ≥30 kg/m².

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Chapter III
Limitations of Fat-Free Mass for the Assessment of Muscle Mass in Obesity

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Abstract

Background: A high amount of adipose tissue limits the accuracy of methods for body composition analysis in obesity.

Objectives: The aim was to quantify and explain differences in fat-free mass (FFM) (as an index of skeletal muscle mass, SMM) measured with bioelectrical impedance analysis (BIA), dual energy X-ray absorptiometry (DXA), air displacement plethysmography (ADP), and deuterium dilution in comparison to multicompartment models, and to improve the results of BIA for obese subjects.

Methods: In 175 healthy subjects (87 men and 88 women, BMI 20–43.3 kg/m², 18–65 years), FFM measured by these methods was compared with results from a 3- (3C) and a 4-compartment (4C) model. FFM_{4C} was compared with SMM measured by magnetic resonance imaging.

Results: BIA and DXA overestimated and ADP underestimated FFM in comparison to 3C and 4C models with increasing BMI (all $p < 0.001$). Differences were largest for DXA. In obesity, BIA results were improved: $value_{corrected} = value_{uncorrected} - a \cdot (BMI - 30 kg/m^2)$, $a = 0.256$ for FFM and $a = 0.298$ for SMM. SMM accounts for 45% of FFM in women and 49% in men.

Conclusions: In obesity, the use of FFM is limited by a systematic error of reference methods. In addition, SMM accounts for about 50% of FFM only. Corrected measurement of SMM by BIA can overcome these drawbacks.

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Introduction

Measurement of body composition is especially important in obesity because a low muscle mass can be obscured by a high amount of adipose tissue. “Hidden cachexia” and “hidden sarcopenia” have been increasingly recognized as high-risk phenotypes associated with adverse health outcomes like asthma and high cholesterol levels (Xiao et al. 2018), and they also limit therapeutic success and affect patient prognosis (Fearon et al. 2013, Prado et al. 2008). Identification of muscle loss at an early stage as well as monitoring of body composition during therapeutic interventions are therefore critical and require repeat measurements with noninvasive and clinically accessible technology. In this regard, bioelectrical impedance analysis (BIA) has gained importance because of technological advances and improved validation of outcome measures (Bosy-Westphal et al. 2017). This is supported by the growing number of publications that provide impedance-based reference data for fat-free mass (FFM) or skeletal muscle mass (SMM) (Chiplonkar et al. 2017, Chumlea et al. 2002, Dey et al. 2003, Franssen et al. 2014, Kudsk et al. 2017, Schutz et al. 2002).

The validity of BIA for body composition analysis in obesity is challenged by the assumption of a constant hydration of FFM that is violated by the higher hydration of FFM in adipose tissue and the higher ratio of extracellular (ECW) to intracellular water (ICW) in the adipose tissue part of connective tissue (Wang et al. 2000). This drawback however applies to all body composition techniques that are based on a 2-compartment model that divides the body into fat and fat-free mass, and can only be ruled out by a 3-compartment (3C) model that avoids the assumption of a constant hydration by measuring the water content of FFM (Das 2005). Additional limitations come from the assumption of a constant mineral content of FFM because a higher percentage of fat mass (FM) was associated with a lower bone mineral density and could thus decrease the mineral content of FFM (Dolan et al. 2017). A 4-compartment (4C) model independently measures the water and mineral content of FFM, and thus provides the most accurate tool for FM and FFM measurements in obesity (Fuller et al. 1992). A 4C model that requires the combination of densitometry by air displacement plethysmography (ADP) or underwater weighing, deuterium (D_2O) dilution for measurement of total body water (TBW), and dual energy X-ray absorptiometry (DXA) for measurement of bone mineral content (BMC) is however cumbersome and not suitable for clinical practice. Therefore, the aim of the present study was (i) to quantify the impact of adiposity on the systematic error of BIA and other 2-

compartment methods for the assessment of FFM by comparison versus a 3C and 4C model and (ii) to mathematically correct BIA equations for the systematic error with increasing adiposity. To evaluate the necessity of the additional DXA measurement required by the 4C model, we evaluated both, the 3C and the 4C model, as a reference.

Finally, the increase in connective tissue (i.e., adipose tissue) with obesity impairs the "metabolic quality" of FFM (Bosy-Westphal et al. 2017). As a secondary aim of the study, the relationship between FFM and SMM was analyzed in order to evaluate the use of FFM as a proxy for SMM in obesity.

Subjects and methods

In a first phase of this study, 153 Caucasian men and women with a BMI <35 kg/m² were recruited from the area of Kiel, Germany, and FFM and SMM prediction equations for the seca medical body composition analyzer (mBCA) devices were developed (Bosy-Westphal et al. 2017, 2013). A seca mBCA 515 device (seca gmbh & co. kg., Hamburg, Germany) was used for BIA measurements, and a 4C model based on D_2O dilution, DXA, and ADP was used as reference for FFM, whereas whole-body magnetic resonance imaging (MRI) and sodium bromide (NaBr) dilution were used as references for SMM and ECW, respectively. Details of the study protocol were described previously (Bosy-Westphal et al. 2017, 2013). In a second phase, 35 obese Caucasian men and women with a BMI ≥30 kg/m² were examined using the same study protocol. Ten subjects of the first phase and 3 of the second phase had to be excluded from the study due to missing or implausible reference measurements. The results of the remaining 175 men and women aged 18–65 years were analyzed.

The subjects were asked to fast overnight and come to the study center between 07:00 and 07:30 in the morning. Whole-body MRI measurements took place at a separate appointment not more than 4 days apart. Subjects were excluded from the study if they fulfilled one of the following criteria: acute and/or chronic diseases (e.g., hypertension, renal and cardiac insufficiency), regular intake of medications (except for contraceptives), amputation of limbs, electrical implants as cardiac pacemaker, metallic implants (except for tooth implants), pregnancy or breastfeeding period, current alcohol abuse, and extensive tattoos at the arms or legs. Edema of the ankles were excluded by inspection and manual compression if appropriate.

Anthropometrics

Body height and weight were obtained on a measuring station (seca 285) with an accuracy of ±50 g up to 100 kg and ±75 g up to 150 kg for the scale and ±2 mm for the stadiometer. BMI was classified as normal weight (BMI ≥18.5, <25 kg/m²), overweight (BMI ≥25, <30 kg/m²) and obesity (BMI ≥30 kg/m²) with obesity class I (BMI ≥30, <35 kg/m²), obesity class II (BMI ≥35, <40 kg/m²), and obesity class III (BMI ≥40 kg/m²).

3.2. Bioelectrical impedance analysis

The seca mBCA 515 device consists of a platform with an integrated scale and a handrail system. Each side of the ascending handrail carries 6 electrodes, of which 2 were chosen depending on the person's height. To get the right choice of grip position, the subject has to stand upright with outstretched arms. Another 2 pairs of electrodes contact the feet. This 8-electrode technique enables segmental impedance measurements. Details of the device were previously described (Bosy-Westphal et al. 2013). The accuracy for measurements of the right and left body side at frequencies of 5 and 50 kHz is 5 Ω for the impedance and 0.5° for the phase angle. Prediction equations use BIA values obtained at 5 and 50 kHz (Bosy-Westphal et al. 2013).

Participants were asked not to exercise within 12 h and drink alcohol within 24 h before the impedance measurement. The duration of each BIA measurement was 75 s.

Reference methods

FM was calculated using a 3C model and a 4C model that include body volume (by ADP), TBW (by D_2O dilution), BMC (by DXA), and weight using the following equations (Fuller et al. 1992):

$$FM_{3C}\ (kg) = 2.220 \times body\ volume\ (l) - 0.764 \times TBW\ (l) - 1.465 \times weight\ (kg)$$

$$FM_{4C}\ (kg) = 2.7474 \times body\ volume\ (l) - 0.7100 \times TBW\ (l) + 1.4599 \times BMC\ (kg) - 2.0503 \times weight\ (kg)$$

FFM_{3C} and FFM_{4C} were calculated as the difference between body weight and FM.

Body volume was measured with ADP using the BOD POD™ device (Cosmed, Italy). FM_{ADP} was calculated from body density using Siri's (Siri 1993) equation, and FFM_{ADP} was calculated as the difference to body weight. A whole-body DXA scan was performed to measure BMC, FFM_{DXA}, and lean soft tissue using a Hologic Discovery A densitometer and the whole-body software 12.6.1:3 (Hologic, Inc., Bedford, MA, USA). D_2O dilution

was used to measure TBW and NaBr dilution to assess ECW. FFM_{D2O} was calculated by $FFM_{D2O}\ (kg) = TBW\ (l)/0.732$. ICW was calculated as the difference between TBW and ECW. Details of these reference methods were described previously (Bosy-Westphal et al. 2013).

Total SMM (excluding head and neck) and visceral adipose tissue were measured by MRI using a Magnetom Avanto 1.5-T scanner (Siemens Medical Systems, Erlangen, Germany). Details were described previously (Bosy-Westphal et al. 2017).

Statistics

Data analyses were performed with R software, version 3.0.1 (R Foundation for Statistical Computing, Vienna, Austria). Descriptive statistics and errors are presented as means ± SD. Differences between women and men as well as significant errors were analyzed by *t* test. The FM index (FMI) was calculated using the following formula: $FMI = FM/height^2$. Significance of the Pearson correlation coefficients is based on Fisher's ż-transformation. For correction equations, a linear regression was calculated using the uncorrected value from subjects with BMI ≥30 kg/m². BMI – 30 kg/m² was used as independent variable, and the intercept was fixed to zero at BMI = 30 kg/m² to enforce continuity between uncorrected and corrected values at the threshold of BMI = 30 kg/m². Regressions were calculated for FFM, SMM (total and segmental), TBW, ECW, and visceral adipose tissue. Significance of coefficients was assessed by *t* test, and equations were corrected for BMI ≥30 kg/m² using these coefficients. The pure error was calculated as:

$$Pure\ error = \sqrt{\frac{\sum(predicted\ value - reference\ value)^2}{number\ of\ observations}}$$

A *p* value <0.05 was considered significant, values <0.01 and <0.001 are indicated.

Results

Characteristics of the study population stratified by gender are given in **Table 1**. Men were heavier and taller than women and also had higher amounts of SMM and FFM, whereas FMI was higher in women. The BMI ranged from 20 to 43.3 kg/m², and the prevalence of

normal weight, overweight, and obesity class I–III was 49, 24, 13, 10, and 4%, respectively.

Table 1. Characteristics of the study population

	Women (n = 88)	**Men** (n = 87)	**All** (n = 175)
Age, years	38.5 ± 13.0	38.6 ± 11.6	38.5 ± 12.3
Weight, kg	75.8 ± 20.9	88.8 ± 16.7***	82.3 ± 20.0
Height, cm	168.4 ± 6.6	179.8 ± 5.8***	174.1 ± 8.4
BMI, kg/m²	26.6 ± 6.5	27.5 ± 5.2	27.0 ± 5.9
FMI_{4C}, kg/m²	9.8 ± 4.9	7.1 ± 4.1***	8.4 ± 4.7
FFM_{4C}, kg	47.8 ± 7.3	66.0 ± 7.2***	56.9 ± 11.7
SMM_{MRI}, kg	21.7 ± 3.8	32.5 ± 4.0***	27.1 ± 6.7

FMI, fat mass index; FFM, fat-free mass; SMM, skeletal muscle mass; 4C, 4-compartment model; MRI, magnetic resonance imaging. ***$p < 0.001$ vs. women (t-test).

Table 2 shows the error of FFM for normal-weight, overweight, and obese subjects measured using BIA, DXA, ADP, or D_2O dilution, and the 3C and the 4C model as a reference. BIA overestimated FFM in obese subjects for both models (3C and 4C). DXA overestimated FFM in all BMI groups and for both models; the systematic error increased with BMI (**Tables 2**, **3**) and was largest in obese subjects. By contrast, ADP underestimates FFM in all BMI groups and for both models with the highest negative systematic error in obese subjects. No significant systematic error was found for D_2O dilution. The highest systematic error was found for both models when FFM was measured with DXA in obese subjects. Significant differences between the 3C and the 4C model were only found in normal-weight subjects.

The error of all methods increased with increasing FMI (the negative error in case of ADP; **Table 3**). In addition, the error of FFM_{ADP}, FFM_{DXA} and FFM_{BIA} also correlated with water and mineral content of FFM as well as the ECW/ICW ratio.

Table 2. Error of fat-free mass (FFM) measured by bioelectrical impedance analysis (BIA), dual-energy X-ray absorption (DXA), air displacement plethysmography (ADP), and deuterium (D2O) dilution in comparison with a 3-compartment (3C) and a 4-compartment (4C) model, stratified by normal weight, overweight, and obesity

	Normal weight (n = 86)	**Overweight** (n = 42)	**Obesity** (n = 47)
FFM_{BIA} - FFM_{3C} , kg	-0.25 ± 1.83	-0.26 ± 1.92	1.39 ± 2.41***
FFM_{DXA} - FFM_{3C} , kg	1.09 ± 1.34***	2.21 ± 1.95***	5.25 ± 2.10***
FFM_{ADP} - FFM_{3C} , kg	-0.90 ± 1.17***	-1.07 ± 1.38***	-1.53 ± 1.33***
FFM_{D2O} - FFM_{3C} , kg	-0.11 ± 0.97	-0.14 ± 1.15	0.26 ± 1.04
FFM_{BIA} - FFM_{4C} , kg	-0.02 ± 1.83	-0.25 ± 1.82	1.34 ± 2.40***
FFM_{DXA} - FFM_{4C} , kg	1.32 ± 1.33***	2.22 ± 1.97***	5.19 ± 2.05***
FFM_{ADP} - FFM_{4C} , kg	-0.67 ± 1.10***	-1.06 ± 1.38***	-1.58 ± 1.48***
FFM_{D2O} - FFM_{4C} , kg	0.12 ± 1.15	-0.13 ± 1.28	0.20 ± 1.03
FFM_{3C} - FFM_{4C} , kg	0.23 ± 0.33***	0.01 ± 0.37	-0.06 ± 0.37

***$p < 0.001$: error significantly different from zero by t-test.

Table 3. Correlations between the error of fat-free mass (FFM) assessed by different methods and potential determinants

	FFM_{BIA} - FFM_{4C}	**FFM_{DXA} - FFM_{4C}**	**FFM_{ADP} - FFM_{4C}**	**FFM_{D2O} - FFM_{4C}**
BMI	0.36***	0.73***	-0.31***	0.03
FMI_{4C}	0.46***	0.77***	-0.23**	0.18*
BMC_{DXA} / FFM_{4C}	-0.16*	-0.29***	0.60***	0.06
TBW_{D2O} / FFM_{4C}	0.20**	0.19*	-0.70***	n.c.
ECW_{NaBr} / $ICW_{D2O, NaBr}$	0.21**	0.28***	0.23**	0.01

BIA, bioelectrical impedance analysis; 4C, 4-compartment model; DXA, dual energy x-ray absorptiometry; ADP, air displacement plethysmography; D2O, deuterium dilution; FMI, fat mass index; BMC, bone mineral content; TBW, total body water; ECW, extracellular water; ICW, intracellular water; NaBr, sodium bromide dilution. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$: correlation coefficients significantly different from zero.

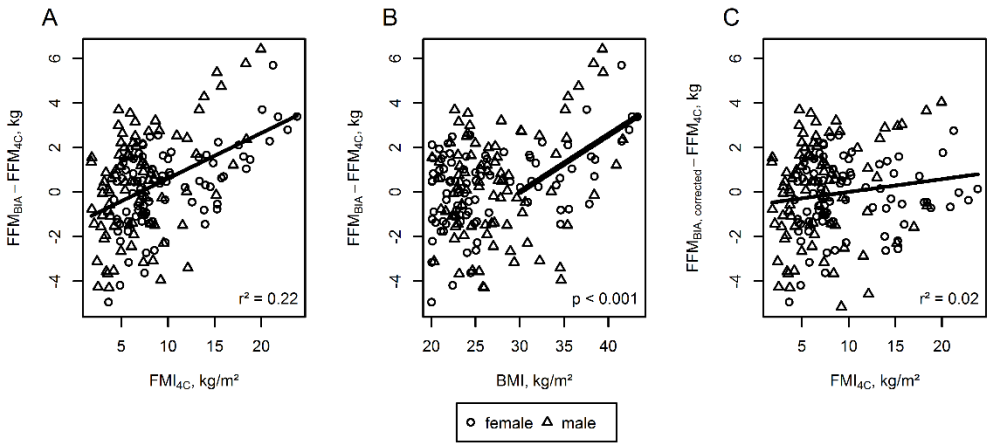

Figure 1. Development of correction of fat-free mass (FFM) measured with bioelectrical impedance analysis (BIA). **A** Fat mass index (FMI) dependency of the error of uncorrected FFM in comparison to a 4-compartment (4C) model. **B** BMI dependency of the error for BMI ≥30 kg/m² which is used as correction, $FFM_{BIA} - FFM_{4C} = 0.256\ (BMI - 30\ kg/m^2)$. **C** Error of corrected FFM vs. FMI.

Figure 1 explains how BIA results for FFM were corrected for obese subjects. Without correction, BIA overestimates FFM at high FMI, and 22% of the variance of the difference between FFM_{BIA} and FFM_{4C} are explained by FMI (**Fig. 1A**). Instead of FMI, BMI was used for correcting FFM because it is independent of impedance measurements and can be used as a proxy for adiposity. **Figure 1B** shows that a systematic overestimation of FFM_{BIA} only occurs when BMI exceeds 30 kg/m². A linear regression including subjects with BMI ≥30 kg/m² was therefore used for the correction of FFM_{BIA} in obese subjects. To enforce continuity, the correction is zero at BMI = 30 kg/m² (**Fig. 1B**). FFM was corrected by the following formula:

$$FFM_{BIA,\ corrected} = FFM_{BIA,\ uncorrected} - 0.256 \times (BMI - 30\ \mathrm{kg/m^2})$$

No correction was applied for subjects with a BMI <30 kg/m². This correction reduces the overestimation of FFM by BIA in obese subjects from 1.34 ± 2.40 to –0.06 ± 2.15 kg (**suppl. Table S1**), and the variance in the difference between FFM_{BIA} and FFM_{4C} explained by FMI is reduced from 22 to 2% (**Fig. 1C**). A similar correction can be applied to SMM, which reduces the error by BIA in obese subjects from 1.63 ± 2.40 to 0.01 ± 2.11 kg:

$$SMM_{BIA,\ corrected} = SMM_{BIA,\ uncorrected} - 0.298 \times (BMI - 30\ \mathrm{kg/m^2})$$

Further corrections can be applied to other BIA equations using the correction factors listed in **supplemental Table S1**.

A linear relationship was found between FFM according to the 4C model and SMM measured by MRI (**suppl. Fig. 1**). SMM accounts for 45% of FFM in women and for 49% of FFM in men. A significant correlation between the corresponding residuals and BMI was found in men ($r = 0.22$, $p < 0.05$) but not in women. As a parameter describing the "quality" of FFM, the ECW/ICW ratio correlates with the residuals with $r = –0.26$ ($p < 0.05$) in men and $r = –0.54$ ($p < 0.001$) in women. In contrast to BIA measurements of appendicular SMM (based on MRI as reference), SMM will be overestimated when appendicular lean soft tissue measured by DXA is used as a proxy (**suppl. Fig. S2**).

Measuring the FFM with BIA using the FFM correction formula and calculating the SMM from FFM using the equations presented in **supplemental Figure 1** leads to a pure error for SMM of 1.50 kg for all subjects and 2.14 kg for obese subjects when compared to SMM measured by MRI, whereas measuring the SMM with BIA using the SMM correction formula leads to a pure error of 1.41 kg for all subjects and 2.09 kg for obese subjects.

Discussion

The primary aim of the present study was to quantify the impact of obesity on the systematic error of FFM measurements by BIA, DXA, ADP, and D_2O dilution in comparison to a 3C and a 4C model and to correct BIA equations. We found that the error of all 2-compartment methods correlated with FMI (**Table 3**). BIA and DXA overestimated and ADP underestimated FFM with increasing obesity (**Table 2**). The overestimation of FFM in obesity by BIA equations was partly explained by a higher hydration of FFM (**Table 3**). In obesity, a higher hydration of FFM is due to an increased water fraction of FFM in adipose tissue (Wang et al. 1999). For obesity, these effects were not adequately accounted for in the original BIA equations (Bosy-Westphal et al. 2017, 2013), since inclusion criteria for the reference population used to generate the BIA algorithm were limited to BMI <35 kg/m² (Bosy-Westphal et al. 2013). On the other hand, overestimation of FFM by DXA can be explained by an underestimation of trunk FM by fan beam DXA devices (Salamone et al. 2000). Since mineral content of the TBW is largely responsible for X-ray attenuation and differentiation between FM and FFM (Bosy-Westphal and Müller 2015, Tylavsky et al. 2003), a higher hydration of the FFM in adipose

tissue also adds to the overestimation of FFM by DXA in obesity. However, the error of FFM measured by BIA or DXA was more dependent on BMI and FMI than on differences in hydration (**Table 3**). The accuracy of FFM measured by ADP depends on the assumption of a constant density of FFM (Ellis 2000). A higher hydration or a lower BMC of the FFM in obesity therefore leads to a lower density and hence an underestimation of FFM, which is in accordance with the correlations given in **Table 3**.

The systematic error of FFM measured by D_2O dilution was not significant in any BMI group, neither for the 3C nor for the 4C model. Therefore, D_2O dilution is a valid method for measuring FFM in obesity, but is too time consuming for clinical practice. Differences between the 3C and the 4C model were only found in normal-weight subjects, suggesting that a 3C model might be sufficient in overweight and obese subjects if DXA measurements are not suitable.

The present study shows that the BIA equations can be improved for measurements in obese persons when a correction term for subjects with BMI ≥30 kg/m² is used (**Fig. 1**; **suppl. Table S1**). This correction leaves results for nonobese subjects unchanged and avoids abrupt changes of results with increasing BMI. Correction factors were statistically significant ($p < 0.001$) for FFM, total SMM, SMM of the legs, TBW, and visceral adipose tissue, but not for SMM of the arms and ECW (**suppl. Table S1**). In our study population (which was partly also used for the generation of BIA equations), the systematic error of the corrected BIA equation for FFM is lower than the systematic error of uncorrected DXA and ADP results. However, the standard deviation of the error in obesity is smaller in DXA and ADP (**Table 2**; **suppl. Table S1**), which indicates a higher precision of those methods. As an alternative approach for the correction of BIA equations, we used a nonlinear (quadratic) correction over the complete BMI range. This approach resulted in a similar pure error compared to the linear correction beginning at BMI = 30 kg/m².

A linear relationship was found between FFM according to the 4C model and SMM assessed by MRI (**suppl. Fig. S1**). FFM can therefore be used for the prediction of SMM. However, SMM accounts for only 45–49% of FFM in women and men. This relationship also explains why appendicular muscle mass is overestimated when measured by DXA (**suppl. Fig. S2**). While muscle tissue volume is measured with MRI, the results of DXA represent lean soft tissue mass which is FFM without bone. Therefore, lean soft tissue of the extremities as a proxy for SMM leads to an overestimation of muscularity (Cruz-Jentoft et al. 2010). Definitions of a low muscle mass that are based on different methods therefore

differ between guidelines from the European Group on Sarcopenia in Older People and the International Consensus for Cancer Cachexia (Gonzalez and Heymsfield 2017). The prevalence of low muscle mass in patients with cancer may depend on the method of muscle measurement (Blauwhoff-Buskermolen et al. 2017). Using the seca mBCA 515 device, the prediction of SMM has advantages over the prediction of FFM because the pure error in comparison to MRI is smaller for the corrected SMM equation than for SMM calculated from corrected FFM (1.41 vs. 1.50 kg for all and 2.09 vs. 2.14 kg for obese subjects).

As a limitation to our study, participants were healthy, apart from being obese, and results may not be valid in subjects with diseases leading to disturbed hydration. Also, corrections were calculated for BIA equations implemented in the seca mBCA 515 device and cannot be applied to other BIA instruments. Furthermore, the 3C and 4C models as a reference were not independent because they included information derived from ADP, D_2O dilution, and DXA.

In conclusion, FFM measured by the reference methods DXA and ADP has a systematic error in obesity. These methods are therefore no appropriate standards for body composition in obesity. In clinical practice, the use of corrected BIA measurements for FFM or SMM can be a suitable alternative as long as BIA equations are validated versus a 4C or 3C model or MRI, like the equations implemented in the seca mBCA 515 device.

Statements

Statement of Ethics

This study was approved by the Medical Ethics Committee of the Christian Albrechts University Kiel, Germany, and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All subjects provided their fully informed and written consent before participation.

Disclosure Statement

Björn Jensen and Kristin Klückmann are employed by seca. Anja Bosy-Westphal serves and Manfred J. Müller served as consultant for seca. Wiebke Braun, Corinna Geisler, and Markus Both declare to have no conflict of interest.

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Author Contributions

B. Jensen and A. Bosy-Westphal designed research, analyzed data, and wrote the paper. W. Braun, C. Geisler, and M. Both conducted research. K. Klückmann designed and coordinated the research. M.J. Müller designed the research and contributed to the critical review and intellectual content of the manuscript. All authors read and approved the final manuscript.

Table S1. Correction factors[a] and error for BIA equations for fat-free mass (FFM), skeletal muscle mass (SMM), total body water (TBW), extracellular water (ECW) and visceral adipose tissue (VAT) before and after correction. Error stratified by normal weight, overweight and obesity.

		error before correction			error after correction
	a	**normal weight**	**overweight**	**obesity**	**obesity**
FFM, kg	0.256***	-0.02 ± 1.83	-0.25 ± 1.82	1.34 ± 2.40†††	-0.06 ± 2.15
SMM, kg	0.298***	0.07 ± 0.94	0.01 ± 1.30	1.63 ± 2.40†††	0.01 ± 2.11
SMM$_{\text{right arm}}$, kg	0.001	0.01 ± 0.12	0.00 ± 0.19	0.00 ± 0.20	-0.01 ± 0.20
SMM$_{\text{left arm}}$, kg	0.001	0.01 ± 0.11	-0.01 ± 0.20	0.01 ± 0.19	0.01 ± 0.19
SMM$_{\text{right leg}}$, kg	0.120***	-0.01 ± 0.32	0.03 ± 0.43	0.63 ± 0.76†††	-0.03 ± 0.58
SMM$_{\text{left leg}}$, kg	0.119***	-0.02 ± 0.31	0.02 ± 0.45	0.63 ± 0.76†††	-0.02 ± 0.58
TBW, l	0.239***	-0.04 ± 1.35	-0.11 ± 1.20	1.30 ± 1.90†††	0.00 ± 1.66
ECW, l	0.028	-0.08 ± 0.72	-0.06 ± 0.81	0.12 ± 0.72	-0.03 ± 0.71
VAT, l	0.136***	-0.01 ± 0.43	0.06 ± 0.67	0.54 ± 1.66†	-0.20 ± 1.47

[a]Correction for BMI >30 kg/m² according to:

corrected value = uncorrected value - a (BMI – 30 kg/m²)

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$: Significance of coefficient by t-statistic.

$^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$: Error significantly different from zero by t-test.

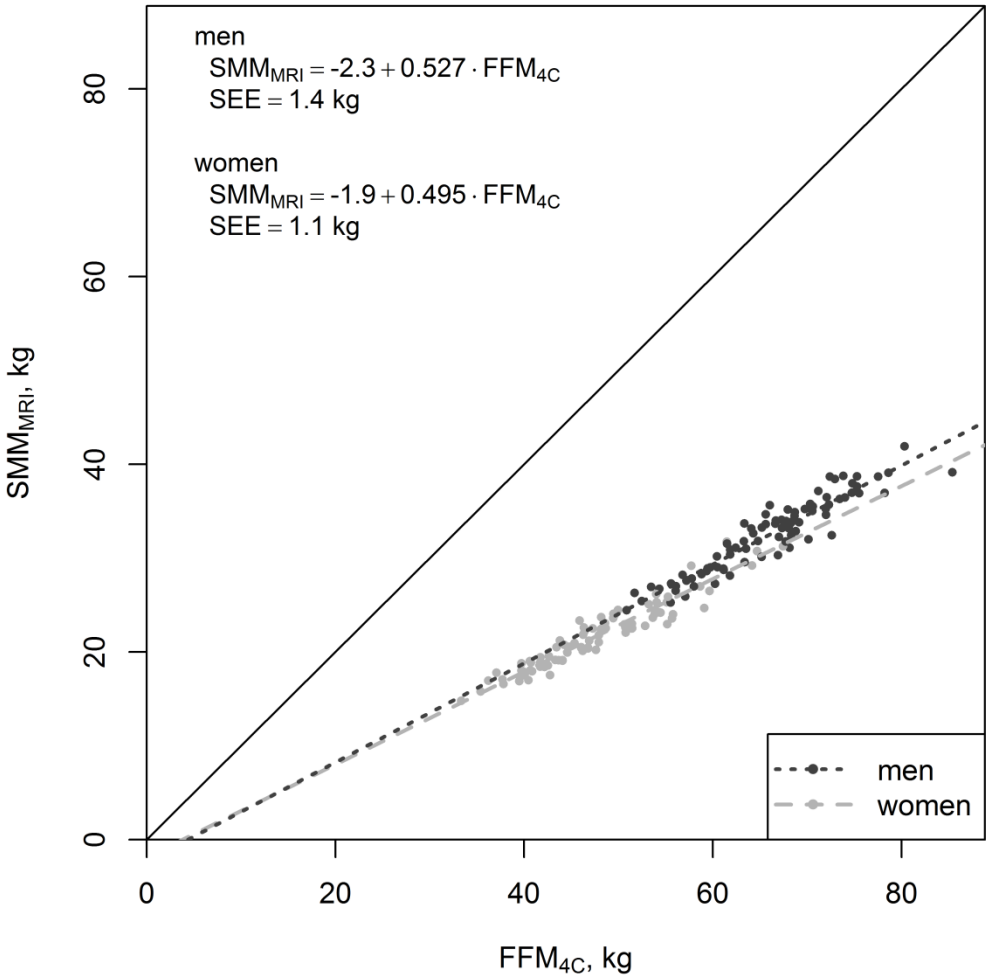

Figure S1. Relationship between skeletal muscle mass (SMM) measured with MRI and fat-free mass (FFM) according to the 4-compartment (4C) model for men and women

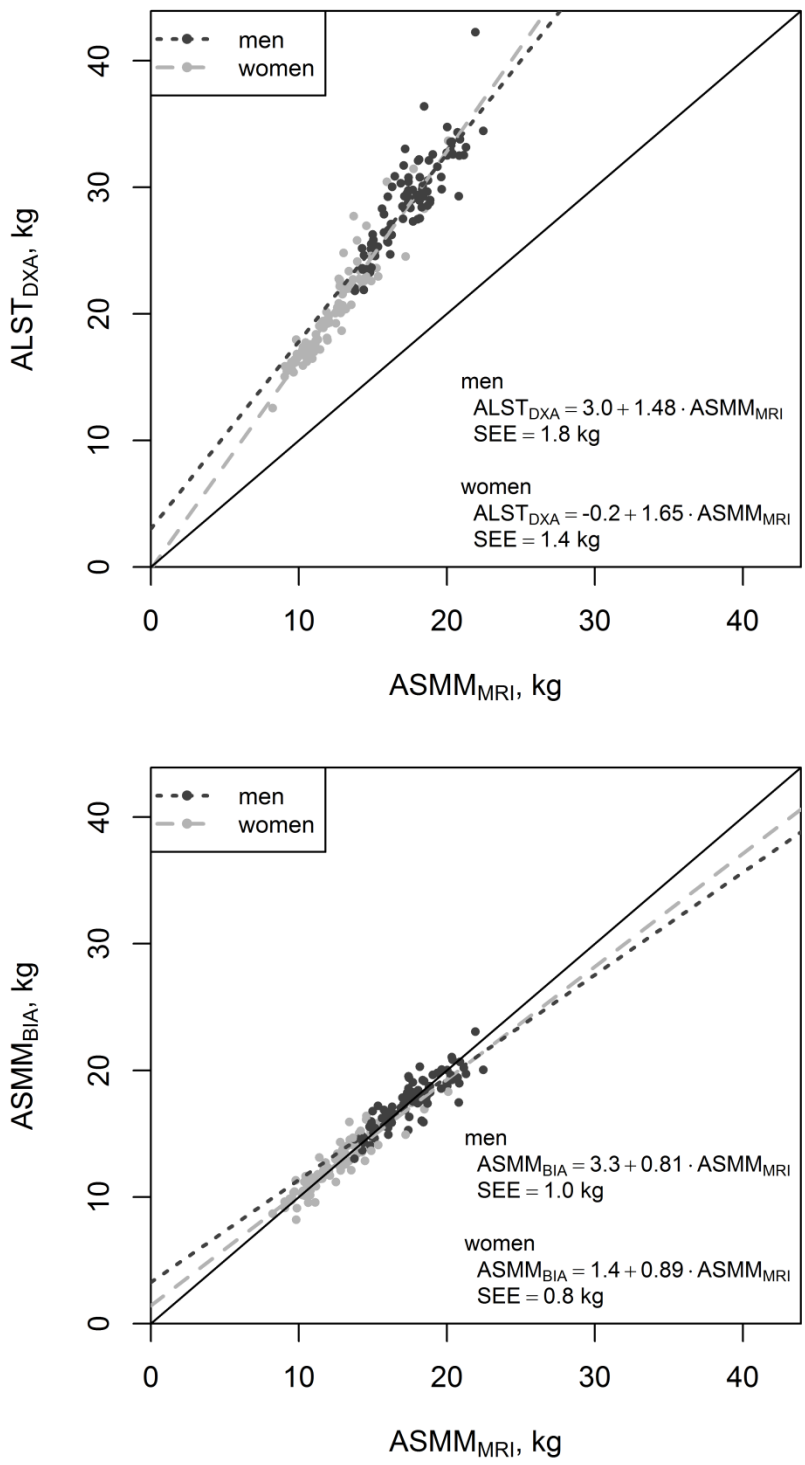

Figure S2. Relationship between appendicular lean soft tissue (ALST) measured with DXA (top) and appendicular skeletal muscle mass (ASMM) measured with BIA (bottom), including BIA corrections for the muscle mass of the legs, in comparison to ASMM measured with MRI for men and women

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Chapter IV

Configuration of bioelectrical impedance measurements affects results for phase angle

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Abstract

Objective: Phase angle (PhA) obtained by bioelectrical impedance analysis is a well-established predictor of malnutrition that reflects the amount and quality of soft tissue. PhA results may however depend on configurations of the measurement that differ between devices.

Approach: In a cross-sectional study, differences in PhA were analyzed comparing supine vs. standing positions, metal vs. adhesive electrodes and the right vs. left side of the body in 302 multi-ethnic adults (18 – 65y) and 1298 Mexican children and adolescents (4 – 20y).

Main results: PhA was higher in supine than in standing position (0.71° ± 0.22° in children – 0.97° ± 0.25° in adults; all $p < 0.001$) with approximately fifty percent of observed differences explained by electrode placement. PhA differences increased with increasing PhA and decreased with age in adults, but increased with PhA, age and height in children. In adults, PhA was higher on the right side of the body ($p < 0.001$). PhA differences in posture, electrode position and body side were independent of ethnicity.

Significance: Phase angle results are influenced by posture and electrode placement. Measurement configuration must be considered when phase angle values are compared between different devices or with literature values.

Trial Registrations: ClinicalTrials.gov NCT01368640, NCT03779932, NCT01471938

Introduction

Bioelectrical impedance analysis (BIA) is a fast, inexpensive and non-invasive tool for the assessment of malnutrition. Fat-free mass or skeletal muscle mass are important outcome parameters for the diagnosis of malnutrition that can be measured by BIA (Bosy-Westphal et al. 2017, 2013, Cederholm et al. 2019), but the required prediction equations have limitations in patients with diseases leading to an inaccurate estimate of hydration. As an alternative, the raw impedance values can be interpreted directly to overcome this drawback. Impedance raw data consist of two components: Resistance that is inversely related to body water and reactance caused by the capacitive effect of isolating cell membranes. The most established parameter for the assessment of malnutrition using raw impedance values is the phase angle that is calculated by:

$$\text{phase angle} = \arctan(\text{reactance} / \text{resistance}) * 180° / \pi$$

The phase angle decreases with an increasing ratio of extracellular to intracellular water and indicates the amount and "quality" of soft tissue, because of a higher ratio of extra- to intracellular water in connective tissue (e.g. adipose tissue) compared to skeletal muscle or organ mass. A low phase angle therefore is an indicator of malnutrition (Norman et al. 2012, Gonzalez et al. 2016, Barbosa-Silva et al. 2005). Phase angle is of prognostic value, due to a loss of cell mass or integrity of cell membranes in catabolic disease or chemotherapy toxicity or due to fluid overload in heart or kidney failure (Grundmann et al. 2015, Kyle et al. 2013, Norman et al. 2012, Schwenk et al. 2000, Selberg and Selberg 2002).

For the evaluation of the phase angle in children or adults, normal values are required. Published normal values have been acquired with different BIA devices, with measurements obtained on one side of the subject in the supine position using adhesive electrodes at the wrist and ankle (Barbosa-Silva et al. 2005, Bosy-Westphal et al. 2006, Kuchnia et al. 2017). This measurement configuration is, however, not consistently used for all BIA measurements. In contrast to the above described configuration for measurements in supine position, the BIA device mBCA 514/515 (seca gmbh & co. kg., Germany) measures the impedance in a standing position, using metal electrodes at the fingers and beneath the feet and calculates the mean phase angle from both sides of the body (Bosy-Westphal et al. 2013). These two configurations differ not only in the position of the subject (standing vs. supine), but also in electrode placement and the analyzed body

side (mean of both sides vs. one side). Differences in resistance between supine and standing positions due to fluid shifts have been reported (Rush et al. 2006), suggesting that differences might also occur in phase angle. In addition to differences in BIA raw values between devices from different manufacturers (Norman et al. 2012), the measurement configuration used by the device is a further potential cause for deviations in phase angle. The aim of the present study was to investigate differences in phase angle between standing and supine measurements, between different electrode placements and between measurements of the phase angle on the right and left side of the body. Because phase angle depends on age, BMI, height, sex and ethnicity (Jensen et al. 2019, Kuchnia et al. 2017), the influences of these predictors on phase angle differences between configurations were also investigated.

Subjects and methods

Three existing datasets were used for this analysis. Additional measurements in the seated position were included for separation of the effects of subject position (standing vs. supine) and by electrode placement, as no measurements with the same electrode placement in standing and supine position were available.

For adults, data from two studies in Kiel, Germany and New York, USA were reevaluated. These studies were originally designed for the generation of BIA prediction equations including adjustments for obese subjects (Kiel) and for the validation of these equations in different ethnicities (New York). Details of these studies were previously described (Bosy-Westphal et al. 2017, 2013). In Kiel, 177 Caucasian men and women aged 18 – 65 years (BMI 20.0 - 45.6 kg/m²) were measured with BIA devices in standing and supine position. In New York, 125 men and women from different ethnicities (32 Caucasians, 33 Asians, 30 African-Americans, 30 Hispanics) aged 18 – 65 years (BMI 18.7 – 34.4 kg/m²) were investigated following the same protocol. For measurements in standing position, a mBCA 514/515 with handrail was used. For measurements in supine position, prototypes with adhesive electrodes connected by cables were used. For subgroups of 146 men and women in Kiel and 121 men and women in New York, additional measurements were made in standing and sitting position with prototypes with handles that were held by the subjects by the side of the body as well as a measurement in sitting position with adhesive electrodes using the prototypes for measurements in supine position. One subject in Kiel had no dominant hand and was neither classified as right- nor as left-handed.

For children and adolescents, data from a study for the generation of normal ranges in Mexico City, Mexico were analyzed (Lopez-Gonzalez et al. 2017, 2019). 1298 Mexican boys and girls aged 4 – 20 years were measured within one hour with a mBCA 514 or a prototype with handles in standing position and with a mBCA 525 (seca gmbh & co. kg., Germany) in supine position. The mbca 514 was used for children ≥130 cm, the prototype for children <130 cm.

Exclusion criteria for the studies were: acute or chronic diseases (e.g. hypertension, renal and cardiac insufficiency), regular intake of medications except for contraceptives, amputation of limbs, electrical implants like cardiac pacemakers, metallic implants except for tooth implants, pregnancy or breastfeeding period, current alcohol abuse, and extensive tattoos at arms or legs. Edema of ankles were excluded by inspection and manual compression if appropriate.

The studies were approved by the medical ethics committees of the Christian-Albrechts-University Kiel, Germany, St Luke's-Roosevelt Hospital, New York, USA, and Hospital Infantil de Mexico Federico Gomez, Mexico City, Mexico respectively. Data acquisition was performed in accordance with the ethical standards according to the 1964 Declaration of Helsinki and its later amendments. All adult subjects provided full informed and written consent before participation. Children provided their assent and the consent was provided by the parents.

Bioelectrical impedance analysis

The BIA device seca mBCA 514/515 conducts measurements in standing position and consists of a platform with a handrail and an integrated scale which is approved in the mBCA 515 as required by law in Germany. Six electrodes are positioned on either side of the ascending handrail. Two pairs of hand electrodes were chosen based on the person's height so that the subject stood upright with outstretched arms. Another two pairs of electrodes made contact with the feet. This eight-electrode configuration enables segmental impedance and phase angle measurements on the right and the left sides of the body. Details of the device were previously described (Bosy-Westphal et al. 2013). The accuracy for measurements of the right and left body sides at frequencies of 5 and 50 kHz is specified by the manufacturer with 5 Ω for impedance and 0.5° for phase angle.

For measurements with other configurations, two additional BIA device prototypes were used in Kiel and another two in New York that contained the same BIA electronic as the mBCA 515 but with no weight scale. On one of the two prototypes the handrail was

replaced by handles connected by cables which were held with outstretched arms, raised at approximately 30° angle by the side of the body. The placement of the fingers on the electrodes was similar to the handrail. On the other prototype all metal electrodes were replaced by adhesive electrodes connected by cables. Adhesive electrodes were placed on the wrists and ankles as well as on the back of the hands and feet as described by Bosy-Westphal et al. (2006).

The seca mBCA 525 is a BIA device for measurements in the supine position using 8 adhesive electrodes. Electrode placement was the same as described above. Wrist electrodes where placed on the palmar side of the wrist for children with small hands, if the distance between the electrodes would otherwise have been smaller than 5 cm. The accuracy specified by the manufacturer is similar to the mBCA 514/515.

Statistics

Data analysis was performed with R software, version 3.5.3 (R Foundation for Statistical Computing, Vienna, Austria). BMI was calculated by BMI = weight / height² and classified as normal weight (BMI ≥18.5, <25 kg/m²), overweight (BMI ≥25, <30 kg/m²) and obesity (BMI ≥30 kg/m²). Ethnic differences were analyzed by ANOVA using the data from New York. Potential determinants for the difference in phase angle between supine and standing position (including different electrode placement) were analyzed by a stepwise regression. The change in R^2 is given for each step of the regression, whereas coefficients, their significance and total R^2 are given for the complete multiple regression and the correlation coefficients r indicate the correlation for each predictor separately. For phase angle as a predictor, the mean value between right and left side and between supine and standing measurements was used. A p value <0.05 was considered significant, values <0.01 and <0.001 are indicated.

For separation of effects resulting from subject position and electrode placement, two measurements were combined in each case, as no measurements with adhesive electrodes were conducted in standing position. The effect of posture was analyzed according to i) the difference between supine and sitting position, both measured with adhesive electrodes, and ii) the difference between sitting and standing position, both measured with metal electrodes placed on handles. Summation of these differences reflects the difference between supine and standing positions, while each separate difference is based on equal electrodes. The effect of electrode placement was analyzed by summation of the difference between i) adhesive electrodes and metal electrodes placed on handles, both measured in

sitting position, and ii) metal electrodes located on handles and on a handrail, both measured in standing position.

Results

Basic characteristics of the study populations are presented in **Table 1**. For adults, the prevalence of normal weight, overweight and obesity was 55%, 23% and 22% in women and 39%, 36% and 25% in men.

In **Table 2**, phase angle measured in supine position is compared to standing measurements. In all study populations, phase angle was significantly higher in the supine compared to the standing position ($p < 0.001$). While no ethnic differences were found for this posture effect on phase angle for adults in New York, we found a larger posture effect for Caucasian adults in Kiel compared to New York when phase angle was measured on the right side, but a lower posture effect when measured on the left side (both $p < 0.001$).

Determinants of the posture effect on phase angle are analyzed in **Table 3**. The difference in phase angle between supine and standing measurements increased with increasing phase angle ($p < 0.001$) and body height ($p < 0.01$ in adults, $p < 0.001$ in children and adolescents). In children and adolescents, this posture effect also increased with age and BMI, whereas it decreased with age in adults (all $p < 0.001$). In a multiple regression analysis with all four variables, only phase angle and age independently contributed to the variance in the posture effect of phase angle in adults, whereas in children, phase angle, age and height were significant independent predictors (all $p < 0.001$).

For adults, differences in phase angle measured in supine and in standing positions are separated in **Table 4** by posture and by placement of electrodes (adhesive electrodes at wrist and ankle vs. metal electrodes at fingers and beneath feet). Phase angle was higher in the supine compared to standing position when the effect of different electrode placement is removed ($p < 0.001$). Phase angle was higher using adhesive electrodes at the wrist and ankle, compared to metal electrodes at the fingers and beneath the feet ($p < 0.001$). Approximately fifty percent of the difference in phase angle between supine and standing measurements can be explained by electrode placement (**Table 4**). In contrast to the effect of electrode placement, the effect due to posture significantly differed between adults in Kiel and New York ($p < 0.001$), and no ethnic differences were found.

Table 1: Characterization of the study populations, stratified by study centers and ethnicity

	women / girls	men / boys	all
Caucasians, Kiel			
n	88	89	177
age, years	38.5 ± 12.9	39.6 ± 11.7	39.0 ± 12.3
weight, kg	76.5 ± 21.2	91.4 ± 18.5	84.0 ± 21.2
height, cm	168 ± 6	180 ± 6	174 ± 8
BMI, kg/m²	27.0 ± 6.7	28.3 ± 5.8	27.6 ± 6.3
right-hander	81 (92%)	82 (92%)	163 (92%)
Caucasians, New York			
n	16	16	32
age, years	42.7 ± 13.7	43.1 ± 15.7	42.9 ± 14.5
weight, kg	68.0 ± 12.0	81.8 ± 15.0	74.9 ± 15.1
height, cm	164 ± 5	175 ± 7	170 ± 8
BMI, kg/m²	25.2 ± 4.2	26.8 ± 4.6	26.0 ± 4.4
right-hander	14 (88%)	12 (75%)	26 (81%)
Asians, New York			
n	17	16	33
age, years	39.4 ± 12.2	39.6 ± 14.0	39.5 ± 12.9
weight, kg	58.2 ± 6.5	68.9 ± 10.6	63.4 ± 10.2
height, cm	161 ± 4	172 ± 6	166 ± 8
BMI, kg/m²	22.5 ± 1.9	23.3 ± 3.6	22.9 ± 2.8
right-hander	17 (100%)	16 (100%)	33 (100%)
African-Americans, New York			
n	14	16	30
age, years	36.1 ± 10.2	40.9 ± 11.7	38.7 ± 11.1
weight, kg	68.2 ± 10.3	81.3 ± 16.7	75.2 ± 15.3
height, cm	167 ± 5	176 ± 8	172 ± 8
BMI, kg/m²	24.6 ± 3.8	26.0 ± 3.8	25.3 ± 3.8
right-hander	13 (93%)	14 (88%)	27 (90%)
Hispanics, New York			
n	15	15	30
age, years	41.1 ± 13.6	39.0 ± 12.5	40.0 ± 12.9
weight, kg	69.5 ± 4.1	80.3 ± 12.1	74.9 ± 10.4
height, cm	157 ± 7	174 ± 5	165 ± 10
BMI, kg/m²	28.2 ± 2.8	26.7 ± 4.2	27.5 ± 3.6
right-hander	14 (93%)	12 (80%)	26 (87%)
Children and adolescents, Mexico City			
n	636	662	1298
age, years	12.2 ± 4.4	11.7 ± 4.3	12.0 ± 4.4
weight, kg	44.7 ± 17.2	45.7 ± 19.7	45.2 ± 18.5
height, cm	144 ± 18	148 ± 22	146 ± 20
BMI, kg/m²	20.5 ± 4.5	19.8 ± 4.4	20.1 ± 4.4

All values except prevalence of right-handedness are mean ± SD

Table 2: Differences in phase angle measured with prototype devices or mBCA 525 in supine and mBCA 514/515 in standing position. The bias of phase angle differences is presented for participants in Kiel compared to New York and for women (girls) compared to men (boys). Significance of ethnic variations in phase angle difference was calculated by ANOVA for adults in New York.

	right side	left side
adults		
PhA supine, °	6.59 ± 0.74	6.23 ± 0.73
PhA standing, °	5.62 ± 0.65	5.44 ± 0.64
PhA supine - standing, °	0.97 ± 0.25***	0.79 ± 0.25***
bias: Kiel – New York, °	0.15†††	-0.18†††
bias: women – men, °	-0.11†††	-0.04
ethnic differences	p = 0.43	p = 0.43
children and adolescents		
PhA supine, °	5.65 ± 0.79	5.66 ± 0.77
PhA standing, °	4.94 ± 0.64	4.79 ± 0.62
PhA supine - standing, °	0.71 ± 0.22***	0.87 ± 0.24***
bias: girls – boys, °	-0.01	0.05†††

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, significance of differences between supine and standing measurements by paired t-test.
$^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$, significance of bias between study centers or sex by unpaired t-test.

Table 3: Determinants of phase angle differences between supine and standing measurements (mean of right and left side). Correlation coefficients r are given for phase angle (PhA, mean between supine and standing), age, height and BMI. Coefficients are given for a multiple regression and the increase in R^2 is given for a stepwise regression.

	r	coefficient	change in R^2
adults			
PhA, °	0.419^{***}	$0.103^{\dagger\dagger\dagger}$	0.176
age, years	-0.346^{***}	$-0.00405^{\dagger\dagger\dagger}$	0.064
height, cm	0.155^{**}	-0.000217	0.000
BMI, kg/m²	0.098	0.00115	0.001
		total R^2 =	0.240
children and adolescents			
PhA, °	0.677^{***}	$0.0921^{\dagger\dagger\dagger}$	0.458
age, years	0.752^{***}	$0.0155^{\dagger\dagger\dagger}$	0.164
height, cm	0.737^{***}	$0.00289^{\dagger\dagger\dagger}$	0.016
BMI, kg/m²	0.486^{***}	0.000985	0.000
		total R^2 =	0.638

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, significance of correlation coefficients r based on Fisher's ż-transformation.
$^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$, significance of the coefficients of a multiple regression by t-statistic.

Table 4: Separation of phase angle differences between supine and standing measurements into the effects caused by posture and by electrode positioning for adults. Differences of available measurement configurations were calculated and added up to get the differences between supine and standing positioning and between adhesive electrodes (el.) and metal electrodes on a handrail. The bias of phase angle differences is analyzed for participants in Kiel compared to New York. Significance of ethnic variations in phase angle differences was calculated by ANOVA for adults in New York.

	right side	left side
Differences due to posture of subject		
sitting - standing (with handle), °	$0.07 \pm 0.12^{***}$	$0.07 \pm 0.12^{***}$
supine - sitting (with adhesive el.), °	$0.37 \pm 0.36^{***}$	$0.33 \pm 0.27^{***}$
supine - standing, °	$0.44 \pm 0.35^{***}$	$0.41 \pm 0.30^{***}$
bias: Kiel – New York, °	$0.15^{‡‡}$	$-0.18^{‡‡‡}$
ethnic differences	p = 0.45	p = 0.17
Differences due to electrode positioning		
handle - handrail (standing), °	$-0.06 \pm 0.13^{***}$	$-0.08 \pm 0.14^{***†}$
adhesive el. - handle (sitting), °	$0.58 \pm 0.34^{***}$	$0.46 \pm 0.30^{***†††}$
adhesive el. - handrail, °	$0.52 \pm 0.35^{***}$	$0.39 \pm 0.31^{***†††}$
bias: Kiel – New York, °	0.00	-0.01
ethnic differences	p = 0.44	p = 0.17

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, significance of differences between different measurement configurations by paired t-test.
$^{†}p < 0.05$, $^{††}p < 0.01$, $^{†††}p < 0.001$, significance of differences between measurements on the right and left side by paired t-test.
$^{‡}p < 0.05$, $^{‡‡}p < 0.01$, $^{‡‡‡}p < 0.001$, significance of bias between study centers by unpaired t-test.

In **Table 5**, phase angle measured on the right side of the body is compared to phase angle measured on the left side. In adults, phase angle was significantly higher when measured on the right side ($p < 0.001$). In children and adolescents, measurements on the right side were only higher when measured in standing position ($p < 0.001$), whereas supine position measurements on the left side were higher ($p < 0.01$). In adults, the phase angle difference between right and left side was significantly higher for right-handers than for left-handers (handedness was not recorded in children). While no differences for phase angle asymmetry were found between populations in Kiel and New York in the standing position, the asymmetry was larger for adults in Kiel compared to New York when the phase angle was measured in supine position ($p < 0.001$).

Table 5: Asymmetry of phase angle (right side – left side) for standing and supine measurements. The bias of phase angle asymmetry is presented for handedness and data collection site, Kiel compared to New York. Ethnic variations in phase angle asymmetry was calculated by ANOVA for adults in New York.

	standing	supine
adults		
phase angle asymmetry, °	$0.18 \pm 0.17^{***}$	$0.36 \pm 0.33^{***}$
bias: right- – left- hander, °	$0.21^{\dagger\dagger\dagger}$	$0.23^{\dagger\dagger\dagger}$
bias: Kiel – New York, °	-0.02	$0.32^{\dagger\dagger\dagger}$
ethnic differences	$p = 0.97$	$p = 0.99$
children and adolescents		
phase angle asymmetry, °	$0.15 \pm 0.15^{***}$	$-0.01 \pm 0.16^{**}$

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, significance of differences between phase angle measurements on the right compared to the left side by paired t-test.
$^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$, significance of bias between right- and left-hander or study centers by unpaired t-test.

Discussion

This study reports that phase angle measured in supine position is higher than in standing position (**Table 2**) due in part to different electrode placements (**Table 4**). Moreover, phase angle was higher when measured on the right compared to the left side of the body in adults (**Table 5**).

A lower resistance in standing compared to lying positions was reported previously (De Lorenzo et al. 1997, Kushner et al. 1996, Rush et al. 2006, Slinde et al. 2003, Zhu et al. 1998), with one exception (Andreoli et al. 2002), even though the measurement protocol was comparable to our studies. The difference in resistance between upright and supine positions can be explained by fluid shifts from the blood into the interstitium due to higher hydrostatic pressure in the limbs and reduced venous return while the subject is standing (Kushner et al. 1996, Rush et al. 2006). In men, plasma volume is reduced by ~700 ml after 10 min standing (Lundvall et al. 1996). This fluid shift likely explains differences in phase angle because a high ratio of extra- to intracellular water corresponds to a low phase angle (Gonzalez et al. 2016). When standing, more extracellular fluid accumulates in the lower extremities resulting in lower phase angle measurements in these body parts. The low cross-sectional area of the distal extremities leads to higher resistances of these body parts compared with the trunk. Therefore the limbs contribute to a disproportionately high portion of total body impedance measurements (Foster and Lukaski 1996). A higher amount of water in the legs thus leads to a lower phase angle compared to increased water in the trunk.

Differences in phase angle due to electrode placement can be explained by the metal electrodes, which in the standing position, contact the fingers and feet while the proximal adhesive electrodes used for supine measurements contact the wrist and ankle. Accordingly, in the standing position, parts of the hands and feet were included that were excluded in the supine measurements. These parts had a significant influence on impedance measurements due to the small cross-sectional area. Effects due to different types of electrodes (adhesive vs. metal) are of minor concern. Arising from the tetrapolar measurement approach, where separate electrodes for the current source and voltage detection are used, the impedance of electrodes and skin are excluded (Foster and Lukaski 1996), making the BIA measurement independent of the electrode type. Additionally, the mBCA 514/515 and mBCA 525 monitor the contact impedances to ensure proper skin-electrode contact.

The finding that the difference in phase angle between supine and standing measurements decreases with age in adults (**Table 3**) is consistent with the finding of a decreasing posture effect of resistance with age in adults (Rush et al. 2006). In our study, however, the difference in phase angle between supine and standing increased with age in children and adolescents (**Table 3**). In comparison to young adults, a lower posture difference for phase angle in young children and older adults may be due to a lower amount of muscle mass and hence a smaller volume into which fluid can shift. A decreasing phase angle with age in older adults (Jensen et al. 2019) adds to a decreasing posture effect of phase angle with age, due to the correlation between phase angle and the posture effect. This correlation also explains a larger posture effect in men compared to women, because of a higher phase angle in men (Jensen et al. 2019). Differences in phase angle due to different measurement configurations were independent of ethnicity. Therefore, even though the phase angle depends on ethnicity (Jensen et al. 2019), ethnicity must not be considered for phase angle adaptations to different measurement configurations.

A high phase angle is a marker of a high number of cells (Gonzalez et al. 2016). Therefore, a likely explanation for higher phase angle on the right compared to the left side of the body is an increased amount of muscle on the dominant side. This could also explain that phase angle asymmetry in adults corresponded to handedness (**Table 5**). The asymmetry was, however, more pronounced for right-handers, than for left handers. Handedness was unfortunately not recorded in children.

The difference in phase angle between the body sides was 0.32° higher when measured in supine position in the Kiel sample compared to the New York sample (**Table 5**). This site difference also explains the site difference for phase angle differences between supine and standing measurements (**Table 2 and 4**). The etiology of this difference between these two populations remains unclear. It could have been caused by differences in the study populations themselves, but it also could have been caused by differences in the setup and use of a prototype with long electrode cables that are prone to measurement errors. The observed differences between the study populations are however smaller than the accuracy of 0.5° for the phase angle measurement that is specified by the manufacturer.

As a limitation to our study, the prevalence of obesity in the population from Kiel was higher compared with the population from New York. However, exclusion of obese subjects with a BMI ≥35 kg/m² in the Kiel population lead to similar results (data not shown). All BIA devices in the present study were equipped with similar electronics. The effects of the measurement configuration therefore were analyzed independently of

hardware differences between manufactures. Since devices from different manufacturers differ greatly especially with respect to reactance, these discrepancies could lead to further differences in phase angle (Norman et al. 2012).

In conclusion, results for phase angle depend on posture, electrode position and body side; the measurement configuration therefore needs to be considered when phase angle values are compared between different devices or with normal values.

Statements

Disclosure Statement

Björn Jensen and Kristin Klückmann are employed by seca. Anja Bosy-Westphal serves as consultant for seca. Wiebke Braun, Markus Both, Dympna Gallagher, Patricia Clark and Desirée López González declare no conflict of interest.

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Author Contributions

B. Jensen and A. Bosy-Westphal designed research, analyzed data and wrote the paper. W. Braun and M. Both conducted research in Kiel, D. Gallagher conducted research in New York, P. Clark and D. López González conducted research in Mexico City. K. Klückmann designed and coordinated research.

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Chapter V
General discussion

BIA has become a widely used method for the assessment of body composition. Body compartments like FM, VAT, FFM or SMM can be estimated and improve the diagnosis of obesity and malnutrition (Norman et al. 2012, Blundell et al. 2014, Müller et al. 2016, Cederholm et al. 2017, 2019). There are, however, challenges that can impair the assessment of BIA measurements.

Ethnic differences in fat and muscle mass

Body composition differs between different ethnicities, leading to differences in body composition related health risks (WHO Expert Consultation 2004, Gallagher et al. 1997). In addition, ethnicities also differ in body proportions (Deurenberg et al. 2002). Since impedance measurements depend on body composition and body geometry (Foster and Lukaski 1996), differences can be expected in impedance raw data. The first objective of this thesis therefore was to analyze ethnical differences in FM, VAT, FFM, and SMM for subjects with similar BMI as well as differences in BIA raw data.

German, Japanese and Mexican men and women with the same BMI differ in their percentage of FM and FFM. In all BMI groups, Mexicans have higher FMI and VAT with a corresponding lower FFMI than Germans with similar BMI. FM percentage therefore is higher in Mexicans compared to Germans with the same BMI. In normal weight Japanese men and women, the relationship between FMI and FFMI is similar compared to Mexicans, whereas in overweight Japanese it is similar compared to Germans (**Chapter II, Table 2, Figure S1**). We found similar patterns in younger (<40 years) and older (≥40 years) subpopulations (**Chapter II, Table 3**). The results for Japanese in comparison to Germans are in accordance with published results (Gallagher et al. 2000), whereas results from NHANES (National Health and Examination Survey) show smaller ethnic differences in an older subpopulation (≥70 years) of Mexican Americans compared to non-Hispanic white Americans (Heymsfield et al. 2016).

Because of higher FM percentage and increased health risks at similar BMI in some, but not all, Asian populations compared to Europeans, the WHO proposed additional lower BMI cut-offs for these Asian populations (WHO Expert Consultation 2004). Our results suggest that these lower cut-offs are more appropriate for normal weight Japanese, but they may be too strict for obese Japanese.

We also found differences in muscle mass distribution between trunk and legs among the three ethnicities. Japanese men and women have the lowest SMI in the trunk and arms, but similar SMI in the legs compared to Germans with the same BMI (**Chapter II, Table 2,**

Figure S2). The resulting higher ratio of SMI_{legs} / SMI_{trunk} is in accordance with a higher leg/trunk ratio of muscle thickness measured with ultrasound in Japanese women compared to American women (Ishida et al. 1992). In Mexicans, the ratio between SMI of the legs and the trunk is lower compared to Germans, because of low leg SMI in Mexicans. Ethnic differences were also found for impedance raw data. A lower phase angle in Japanese compared to Germans can be explained by a higher ECW / TBW ratio which is compatible with a lower SMI / FFMI and a higher FMI / FFMI for underweight and normal weight men and women (**Chapter II, Figure 2, Table 2 and 4**). A higher phase angle in Mexicans compared to Germans, however, is in accordance with a lower body height (**Chapter II, Table 1**) and an increase of phase angle with decreasing body height (**Chapter II**, Material and methods, Statistics). Similar to our results, data from NHANES show higher phase angle in Hispanics compared to white Americans (Kuchnia et al. 2017). In BIVA analysis, longer vectors for normal weight Mexicans in comparison to Germans can be explained by a higher FM percentage and a lower SMI in the legs at a given BMI (**Chapter II, Figure 3, S1 and S2, Table 2**), both leading to less body water and hence a higher resistance and longer vectors. Shorter vectors for Japanese in comparison to Mexicans can be explained by a higher SMI in the legs and a lower ratio of leg length to trunk length (Deurenberg et al. 2002). Both causing a lower resistance that is indicated by shorter vectors. Differences in vector length between Japanese and Germans are small because of opposing effects: Higher FM percentage leading to longer vectors and higher SMI in the legs as well as a lower ratio of leg length to trunk length leading to shorter vectors.

As a limitation to the study, obese men and women were underrepresented in the Japanese population, whereas underweight subjects were underrepresented in Germans and Mexicans (**Chapter II, Table 1**). Because of these differences, men and women with similar BMI where compared for normal weight and overweight BMI groups (BMI 18.5 – 30 kg/m^2).

In conclusion, the amount and distribution of FM, FFM, and SMM at the same BMI differ between ethnicities, leading together with variations in body proportions (Deurenberg et al. 2002) to differences in phase angle and BIVA. These differences among ethnicities should be considered for the assessment of body composition. Ethnic-specific normal values are therefore required.

Limitations of fat-free mass for assessment of muscle mass in obesity

The assessment of body composition in obese subjects poses challenges, because two compartment methods like DXA, ADP, or D_2O dilution, as well as BIA, require assumptions like a constant hydration, mineral content, or density of FFM. These assumptions can be inaccurate in obese subjects (Ellis 2000, Tylavsky et al. 2003). In fan-beam DXA devices, a magnification error adds additional inaccuracies (Salamone et al. 2000, Schoeller et al. 2005). The second objective of this thesis therefore was to analyze differences between different methods for the assessment of FFM in obese subjects and to improve BIA results in this group.

FFM in obese men and women was overestimated by BIA and DXA, while it was underestimated by ADP, when compared to a 3C or a 4C model (**Chapter III, Table 2**). FM therefore was underestimated by BIA and DXA and overestimated by ADP. The bias increased with increasing obesity. The bias of ADP can largely be explained by differences in hydration and BMC of the FFM (**Chapter III, Table 3**) that are responsible for differences in the density of the FFM, which was assumed to be constant. Differences in hydration and BMC of the FFM also correlate with the bias of BIA and DXA, but for these methods, the bias was more dependent on the FMI and BMI. This is in accordance with studies that found an overestimation of FFM and an underestimation of FM by fan-beam DXA devices (Tylavsky et al. 2003, Schoeller et al. 2005). Especially additional fat located at the trunk is only partly detected by DXA (Salamone et al. 2000). Differences in mineral content of body water, that contribute to differences in X-ray attenuation (Tylavsky et al. 2003, Bosy-Westphal and Müller 2015), are therefore of minor concern.

The bias of FFM measured with D_2O dilution compared to a 3C or 4C model was not significant for any BMI group (**Chapter III, Table 2**), even though a constant hydration of the FFM was assumed. D_2O dilution therefore is a valid method for measuring FFM, but the required time of 3 – 5 hours until equilibrium (Heymsfield et al. 2005, p. 45) and expensive laboratory equipment make it impractical for clinical practice.

The BIA equations implemented in the mBCA 515 device, that was used in this study, were generated with a reference population that was limited by the inclusion criteria to a BMI range of 18.5 – 35 kg/m² (Bosy-Westphal et al. 2013, 2017). Obese subjects therefore were underrepresented, which explains a bias of these equations for obese subjects. A bias for BIA equations was found for BMI ≥30 kg/m² that increased with increasing obesity (**Chapter III, Figure 1, Table S1**). We were able to correct this bias with a linear correction for subjects with a BMI ≥30 kg/m², leaving values for subjects with BMI

<30 kg/m² unchanged, and avoiding abrupt changes at a BMI of 30 kg/m². These corrections, however, can only be applied to the BIA equations implemented in the mBCA 515 device.

Even though the bias of BIA equations (corrected and uncorrected) is lower than the bias of DXA and ADP, the standard deviation in obesity is smaller in DXA, ADP and D_2O dilution (**Chapter III, Table 2**). However, the 3C model is based on D_2O dilution and ADP, the 4C model additionally on DXA. Therefore, only the comparison between DXA and the 3C model is a compassion of independent methods. The error calculated by the comparison of the dependent methods is probably underestimated. Therefore, the lower standard deviation of DXA vs. the 3C model in comparison to BIA indicates that a bias correction for DXA would lead to a higher accuracy compared to BIA. Correcting the DXA bias in a similar way like the correction of the BIA bias, however, would counteract the advantage of DXA in comparison to BIA that it is independent of reference body composition measurements. A bias correction for ADP would have the same disadvantage.

We found a linear relationship between FFM according to the 4C model and SMM by MRI (**Chapter III, Figure S1**), where SMM accounts for 45% of FFM in women and 49% in men. Therefore, FFM can be used for the assessment of SMM, but FFM values are approximately twice as high as SMM. This explains, why appendicular lean soft tissue measured by DXA (which is FFM without bone) is higher than SMM of arms and legs measured by MRI (**Chapter III, Figure S2**, Bosy-Westphal et al. 2017). Therefore, cut-offs for sarcopenia depend on the measurement method and differ between DXA and MRI (Gonzalez and Heymsfield 2017, Cederholm et al. 2019). Since BIA prediction equations are validated against a specific reference method, it is important to take the reference method into account for BIA measurements of SMM, when published cut-off values are used for the diagnosis of sarcopenia or sarcopenic obesity.

As a limitation to the study, all participants were healthy, apart from being obese, and the results may not be valid in subjects with diseases leading to disturbed hydration.

In conclusion, for FFM measurements with DXA and ADP in obesity the bias of these methods should be considered. Corrected BIA measurements of FFM or SMM can be an alternative, as long as the equations that are used are based on a 3C or 4C model or on MRI respectively.

Influence of measurement configuration on phase angle

BIA prediction equations are adjusted to results of reference methods in a study population. These equations can be inaccurate for subjects that differ from this population. In order to avoid such limitations, impedance raw data, like the phase angle, can be interpreted directly (Norman et al. 2012). But such raw data depend on the measurement configuration used by a BIA device (Rush et al. 2006). The third objective therefore was to analyze differences in phase angle between standing vs. lying measurements, measurements on the right side vs. left side, and different electrode positions.

We found that phase angle measured in supine position is higher when compared with measurements in standing position (**Chapter IV, Table 2**). Approximately half of this difference could be explained by a different positioning of electrodes among the two measurements, while the other half of the difference was explained by the different posture of the subjects (supine vs. standing, **Chapter IV, Table 4**). This finding is in accordance with a lower resistance in lying measurements compared to standing measurements that was found by many (De Lorenzo et al. 1997, Kushner et al. 1996, Rush et al. 2006, Slinde et al. 2003, Zhu et al. 1998), but not all authors (Andreoli et al. 2002).

When the subject is standing, a raised hydrostatic pressure in the limbs and reduced venous return lead to a fluid shift from the blood into the interstitium (Kushner et al. 1996, Rush et al. 2006). After 10 minutes standing, the plasma volume is reduced by approx. 700 ml in men (Lundvall et al. 1996). When the subject is standing, a higher amount of ECW can therefore be found in the distal extremities compared to a supine position. An increasing ECW correlates with a decreasing resistance and with a decreasing phase angle (Gonzalez et al. 2016). Because of the low cross-sectional area and the resulting high resistance of the distal extremities, the total body impedance is disproportionally high influenced by these body parts (Foster and Lukaski 1996). Therefore, an increase of ECW in the distal extremities explains a lower total body resistance and phase angle in standing compared to supine measurements.

In addition to the different posture, the measurements were carried out with different electrodes placements. While the proximal electrodes contacted wrist and ankle in the supine measurement, the electrodes in the standing measurement contacted the fingers and the feet. Therefore, in the standing measurement parts of the hands and feet were included that were not measured in supine position. A low cross-sectional area of hands and feet explains a high influence of these parts on the total body impedance. Therefore, the

difference due to electrode placement can be explained by these additionally measured parts of hands and feet in standing measurements.

The difference in phase angle between supine and standing measurements increased with increasing phase angle and in addition, it increased with age in children, but decreased with age in adults (**Chapter IV, Table 3**). These correlations with phase angle and age are not independent of each other, because phase angle increases with age in children ($r = 0.50$ in girls and $r = 0.69$ in boys, both $p < 0.001$, unpublished data) and decreases with age in older adults (**Chapter II, Figure 2**). The decreasing phase angle difference with age between supine and standing measurements in adults is in line with a decreasing posture effect of resistance with age in adults (Rush et al. 2006). A possible explanation for a lower posture effect in young children and older adults could be a lower muscle mass compared to young adults and, therefore, a smaller volume into which fluid can shift while the subject is standing.

In adults, phase angle was higher when measured on the right side compared to the left side, whereas in children a higher phase angle on the right side was only found in standing measurements (**Chapter IV, Table 5**). In adults, the phase angle asymmetry corresponds to handedness that unfortunately was not recorded in children. A high phase angle corresponds to a high number of cells (Gonzalez et al. 2016) and more than 90% of the subjects were right-hander (**Chapter IV, Table 1**). A higher amount of muscle on the dominant side is therefore the likely explanation for the phase angle asymmetry and the correlation of this asymmetry to handedness.

For measurements in supine position, the phase angle asymmetry was higher for subjects measured in Kiel than for subjects measured in New York (**Chapter IV, Table 5**). This bias between study sites remains unexplained. It could have been caused by differences in the study population or by differences in the setup of the device. The observed bias is, however, smaller than the specified accuracy of the device.

The initial aim of the studies in Kiel and New York was the generation of BIA prediction equations and ethnic corrections of these equations (Bosy-Westphal et al. 2013, 2017). As an extension to the study in Kiel, additional obese subjects were examined for the analysis of the BIA prediction equations in obese subjects, as presented in **chapter III**. These additional subjects resulted in a higher prevalence of obesity in the study population from Kiel compared to the study population in New York. However, an exclusion of the additional obese subjects from the second part of the study in Kiel leads to similar results.

The BIA devices used for the comparison of different measurement configurations in this study were equipped with similar electronics and differed only in the connected electrodes. The differences therefore were analyzed independently of manufacturer specific device differences. Differences between devices from different manufacturers, however, may lead to additional differences in phase angle (Norman et al. 2012).

In conclusion, the phase angle depends on the configuration that is used for the BIA measurement. The measurement configuration must therefore be considered, when the phase angle is compared between subjects measured with different devices or when applying reference values obtained from different devices.

Conclusion

BIA has many advantages for body composition measurements in comparison to other methods. It is fast, inexpensive, non-invasive and measurements can be repeated frequently. Like all body composition methods, BIA has methodological challenges and taking such methodological considerations into account improves the results.

Body composition differs between ethnicities and these differences must be considered for the interpretation of BIA results. Ethnic specific cut-offs should therefore be used. Ethnic differences in body composition also lead to ethnic variations in percentage FM at the same BMI. Obesity related health risks may be higher in Mexicans compared to Germans with the same BMI, because of a higher FM percentage. In Japanese however, differences in health risk compared to Germans may depend on BMI, because FM percentage is only higher in normal weight, but not in obese subjects. Ethnic differences must also be considered for the evaluation of impedance raw data.

Because of the bias in obesity for FFM measured with DXA or ADP in comparison to a 3C or 4C model, a multicompartment model should be preferred as reference method for the generation of BIA prediction equations. For individual measurements of FM and FFM with DXA or ADP in obesity, the bias between the methods should be considered. In clinical practice, BIA can be a suitable alternative, if the equations were validated against a 3C or 4C model and obese subjects were included in the validation. FFM can be used as a proxy for SMM, but since SMM accounts for only half of FFM, both compartments should not be confused and the term “muscle mass” should not be used for FFM.

When phase angle is compared between different BIA devices or with published data, differences caused by discrepant measurement configurations must be considered. Especially when phase angle is measured on a BIA device for standing measurements,

normal values generated with a BIA device for supine measurements cannot be applied without adaptation.

Differences in impedance raw data among BIA devices with different measurement configurations also complicate the application of published prediction equations. Instead, prediction equations should be matched to a specific BIA device. For FM and FFM a multi-compartment model should be used as reference while MRI should be used as a reference for SMM and VAT. Under these conditions, the accuracy of BIA is comparable with other two compartment methods like DXA, ADP or D_2O-Dilution, but BIA is easier to apply in clinical practice. It is therefore important to know, how the prediction equations, which are used in a BIA device, were validated and how much agreement between prediction equation and the reference method was archived with this device. The publication of the exact prediction equation itself, which usually is a business secret of the manufacturer, is of minor importance.

In addition, ethnic-specific reference values are essential. In the case of impedance raw data, these reference values also have to be matched to the measurement configuration of the BIA device. Considering the potential ethnic diversity of patients, reference values for further ethnicities would be desirable.

Under those conditions, todays BIA devices, which were designed for medical use, allow body composition measurements in clinical practice with an accuracy comparable to much more expensive methods that are limited to few facilities.

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Summary

Bioelectrical impedance analysis (BIA) has become a widely used method for body composition analysis. Body compartments like fat mass (FM), visceral adipose tissue, fat-free mass or skeletal muscle mass can be estimated and improve the diagnosis of obesity and malnutrition. Impedance raw data, like the phase angle, give additional information on tissue quality and the prognosis of the patient. There are, however, challenges that can impair the assessment of BIA measurements.

(i) Body composition differs among ethnicities. We found a higher percentage of FM in Mexicans compared to Caucasians with the same BMI. FM percentage is similar in normal weight Japanese and Mexicans, whereas in overweight Japanese it is similar compared to Caucasians for men and women with the same BMI. These differences may lead to differences in obesity related cardiometabolic risk. We also found ethnic differences in muscle mass distribution and impedance raw data.

(ii) In obese subjects, the assessment of body composition by two compartment methods is limited, because assumptions become inaccurate with higher amount of adipose tissue. FM is therefore underestimated by BIA and dual X-ray absorptiometry and it is overestimated by air displacement plethysmography. Corrections for BIA equations were developed.

(iii) Impedance raw data are dependent on the measurement configuration used by a BIA device. Phase angle is higher when measured in supine compared to standing position and it is higher when measured on the right side of the body compared to the left side. The placement of the electrodes also has an impact on the phase angle.

In conclusion, obesity related cardiometabolic risk may vary among different ethnicities due to differences in FM percentage for subjects with the same BMI. Ethnic differences in body composition and differences in phase angle between different measurement configurations must be considered in the assessment of BIA results. In obesity, BIA equations for FM and FFM should be validated against a multi-compartment model in a reference population that includes obese subjects.

Zusammenfassung

Die bioelektrische Impedanz Analyse (BIA) hat sich zu einer weit verbreiteten Methode zur Beurteilung der Körperzusammensetzung entwickelt. Körperkompartimente wie die Fettmasse (FM), das viszerales Fettgewebe, die fettfreie Masse oder die Skeletmuskelmasse können bestimmt werden und erlauben eine verbesserte Diagnose von Adipositas und Mangelernährung. Impedanz-Rohdaten, wie der Phasenwinkel, liefern wertvolle Zusatzinformationen über die Gewebequalität und die Prognose des Patienten. Es gibt jedoch methodische Herausforderungen, die eine Beurteilung von BIA-Ergebnissen erschweren.

(i) Es gibt Unterschiede in der Körperzusammensetzung zwischen verschiedenen Ethnien. Wir haben bei Mexikanern einen höheren Anteil an FM im Vergleich zu Kaukasiern mit einem gleichen BMI gefunden. Für normalgewichtige Japaner ist der Fettanteil ähnlich, wie bei Mexikanern mit gleichem BMI, wohingegen er bei übergewichtigen Japanern vergleichbar zu kaukasischen Frauen und Männern ist. Diese Differenzen könnten zu Unterschieden im durch Übergewicht bedingten kardiometabolischen Risiko führen. Außerdem haben wir Unterschiede in der Muskelverteilung und bei den Impedanz-Rohdaten zwischen den Ethnien gefunden.

(ii) Die Bestimmung der Körperzusammensetzung von adipösen Personen mittels Zweikompartimentmethoden ist durch methodenimmanente Annahmen limitiert. Diese Annahmen sind bei hohem Körperfettanteil nicht erfüllt. Die FM wird daher bei Adipositas von BIA und DXA unterschätzt und von ADP überschätzt. Für BIA-Prädiktionsformeln wurden entsprechende Korrekturen entwickelt.

(iii) Impedanz-Rohdaten sind abhängig von der Messkonfiguration des BIA-Gerätes. Der Phasenwinkel ist höher, wenn er im Liegen gemessen wird, als im Stehen. Er ist außerdem höher, wenn er auf der rechten im Vergleich zur linken Körperseite gemessen wird. Zusätzlich beeinflusst die Positionierung der Elektroden den Phasenwinkel.

Zusammenfassend ergibt sich, dass unterschiedliche Körperfettanteile bei Personen mit gleichem BMI aber unterschiedlicher Ethnie zu Unterschieden im durch Übergewicht bedingten kardiometabolischen Risiko führen könnten. Unterschiede in der Körperzusammensetzung zwischen verschiedenen Ethnien sowie Unterschiede im Phasenwinkel aufgrund verschiedener Messkonfigurationen müssen für die Bewertung von BIA-Ergebnissen berücksichtigt werden. Für übergewichtige Patienten sollten BIA-

Prädiktionsformeln für FM und FFM nach einem Multikompartiment-Modell validiert sein und die Referenzpopulation sollte adipöse Personen einschließen.

Appendix

Reference numbers and titles of the ethical approvals for each study

Declarations of co-authorship

Reference numbers and titles of the ethical approvals for each study

Ethics Committee, Graduate School of Medicine, The University of Tokyo
Examination numbers 10323, 10323-(1)
Analysis of the body composition of Japanese adults based on bioelectrical impedance analysis (BIA), normal range generation

Comité de Ética en Investigación del Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán
REF. 1344
Generación de valores normales para analizar la composición corporal de adultos mexicanos con el Análisis de bioimpedancia eléctrica

Ethik-Kommission der Ärztekammer Hamburg
Bearb.-Nr.: PVMP3918
Generation of normal ranges to analyze body composition of adults based on Bioelectrical Impedance Analysis (BIA)

Ethik-Kommission, Medizinische Fakultät der Christian-Albrechts-Universität zu Kiel
AZ: A111/11
Generation of prediction equations to analyze body composition of adults based on Bioelectrical Impedance analysis (BIA)
AZ: A142/13
Generation of prediction equations to analyze body composition of obese adults based on Bioelectrical Impedance Analysis (BIA)

Institutional Review Board, Institute for Health Sciences, St. Luke's – Roosevelt Hospital Center
IRB# 11-079
Application and adaptation of device specific body composition formulas to various ethnic groups

Comités de Investigación, Ética y Bioseguridad, Instituto Nacional de Salud, Hospital Infantil de México Federico Gómez
HIM 2015-05

Determinación de valores de referencia de composición corporal en población pediátrica de la ciudad de México

Declarations of co-authorship

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Declaration of co-authorship

If a dissertation is based on already published or submitted co-authored articles, a declaration from each of the authors regarding the part of the work done by the doctoral candidate must be enclosed when submitting the dissertation.

1. Doctoral candidate
Name: Björn Jensen

2. This co-author declaration applies to the following article:
Ethnic differences in fat and muscle mass and their implication for interpretation of bioelectrical impedance vector analysis

The extent of the doctoral candidate's contribution to the article is assessed on the following scale:

A. Has contributed to the work (0-33%)
B. Has made a substantial contribution (34-66%)
C. Did the majority of the work independently (67-100%)

3. Declaration on the individual phases of the scientific work (A,B,C)	Extent
Concept: Formulation of the basic scientific problem based on theoretical questions which require clarification, including a summery of the general questions which, it is assumed, will be answerable via analyses or concrete experiments/investigations	B
Planning: Planning of experiments/analyses and formulation of investigative methodology, including choice of method and independent methodological development, in such a way that the scientific questions asked can be expected to be answered	B
Execution: Involvement in the analysis or the concrete experiments/investigation	C
Manuscript preparation: Presentation, interpretation and discussion of the results obtained in article form	B

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Manuscript preparation: Presentation, interpretation and discussion of the results obtained in article form	**B**

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Planning: Planning of experiments/analyses and formulation of investigative methodology, including choice of method and independent methodological development, in such a way that the scientific questions asked can be expected to be answered	**B**
Execution: Involvement in the analysis or the concrete experiments/investigation	**C**
Manuscript preparation: Presentation, interpretation and discussion of the results obtained in article form	**B**

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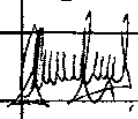
14-05-2019	Mayanin Jannet Dominguez Garcia	

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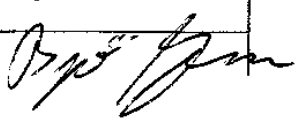
3.7.2019	Björn Jensen	

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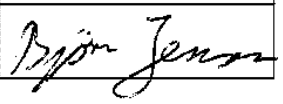
3.7.2019	Björn Jensen	

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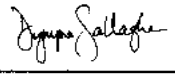
Sept 30, 2019	Dympna Gallagher	

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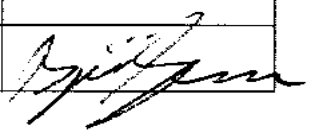
2.10.2019	Björn Jensen	

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Originalarbeiten

Peine S, Knabe S, Carrero I, Brundert M, Wilhelm J, Ewert A, Denzer U, **Jensen B**, Lilburn P. *Generation of normal ranges for measures of body composition in adults based on bioelectrical impedance analysis using the seca mBCA.* International Journal of Body Composition Research, 2013, 11, S. 67–76

Bosy-Westphal A, **Jensen B**, Braun W, Pourhassan M, Gallagher D, Müller MJ. *Quantification of whole body and segmental skeletal muscle mass using phase sensitive 8-electrode medical bioelectrical impedance devices*. European Journal of Clinical Nutrition, 2017, 71, S. 1061 – 1067, doi:10.1038/ejcn.2017.27

Jensen B, Moritoyo T, Kaufer-Horwitz M, Peine S, Norman K, Maisch MJ, Matsumoto A, Masui Y, Velázquez-González A, Domínguez-García J, Fonz-Enríquez E, Salgado-Moctezuma SG, Bosy-Westphal A. *Ethnic differences in fat and muscle mass and their implication for interpretation of bioelectrical impedance vector analysis.* Appl Physiol Nutr Metab. 2019, 44(6):619-626, doi: 10.1139/apnm-2018-0276

Jensen B, Braun W, Geisler C, Both M, Klückmann K, Müller MJ, Bosy-Westphal A. *Limitations of Fat-Free Mass for the Assessment of Muscle Mass in Obesity*. Obesity Facts. 2019 May 27;12(3):307-315. doi: 10.1159/000499607