Bioelectrical impedance analysis of body composition

Applications in sports science

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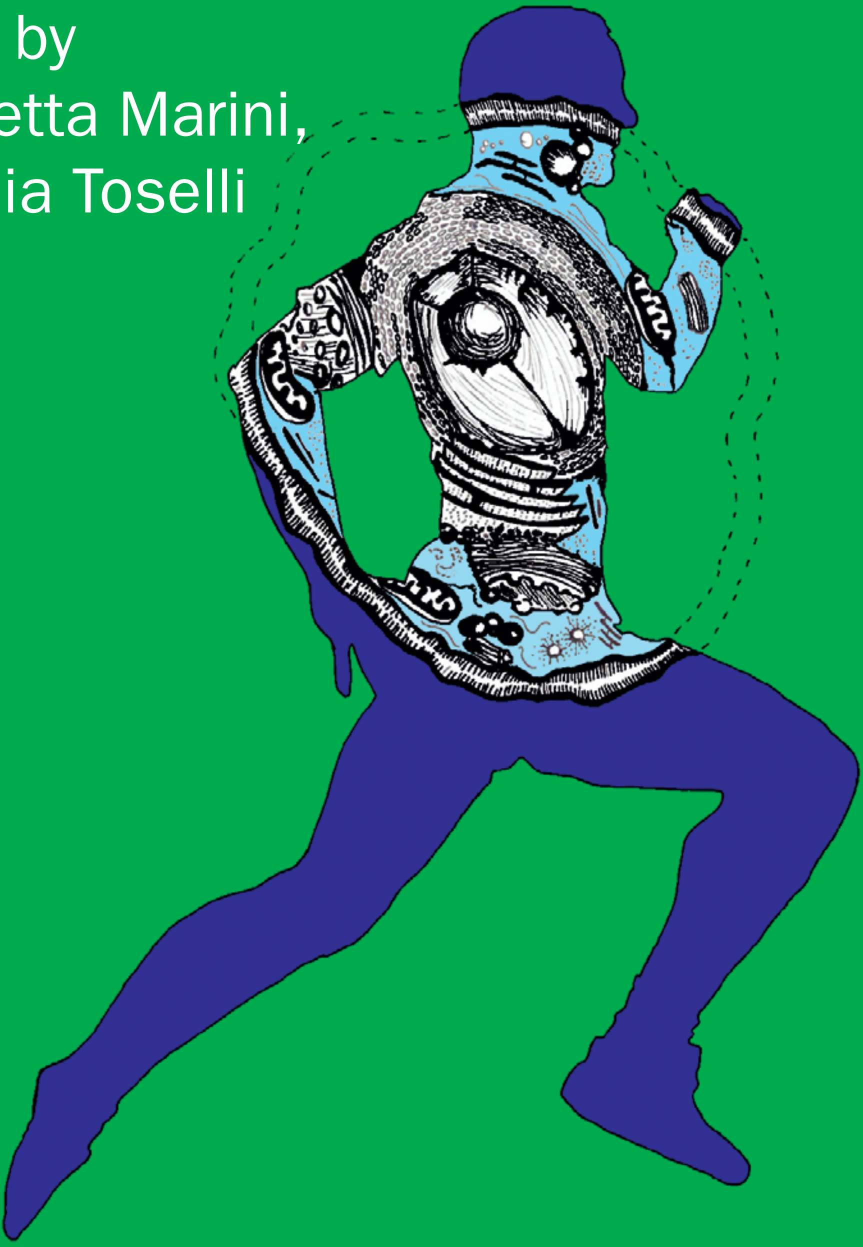
Edited by
Elisabetta Marini,
Stefania Toselli



This book represents the result of an interdisciplinary and international effort to provide an updated overview of the theoretical and methodological principles of bioimpedance, and their applications in sports science.
The structure and the writing style are intended to be useful for scholars and students in a range of academic disciplines, as well as for professionals wishing to expand and consolidate their knowledge in the field.

The first chapter, entitled "*Body composition*", describes the variability of body composition in the two sexes, during the life cycle, and in different populations, considering the relationship to life style and health status. It also summarises the relevant models for the analysis of body composition.
The second chapter "*Bioelectrical impedance analysis: principles, instruments, and measurement methods*" is centred on bioimpedance – the theoretical principles, the advantages and limitations, the measurements and analytical procedures –, illustrating both the conventional procedure based on predictive equations and the alternative ones, such as phase angle and bioelectrical impedance vector analysis (BIVA).
The third chapter "*Bioelectrical impedance analysis: Research on body composition in sports science*" discusses the more recent applications of bioimpedance, and of BIVA in particular, in the field of physical training and sport.

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Presentations

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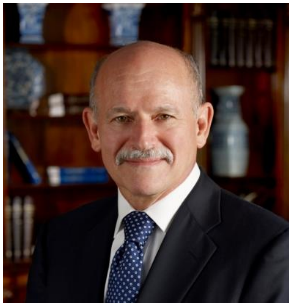

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Part 1 – Body composition

Intra-population and inter-population variability of body composition

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The human body is composed of a variety of different tissues (e.g. skeletal, muscle, adipose) and its variability reflects differences not only on the amount or relative proportion of these tissues (body composition), but also on the distribution of the different tissues that constitute body morphology. Body composition refers to all components (elements, cells, tissues and organs) that provide mass, shape, and function to all living beings. Wang et al. (1992) defined body composition as "the branch of human biology dealing with the in vivo quantification of body components, quantitative relations between components, and quantitative changes in components related to various influencing factors". The influence of body composition on health and sports performance has made this area of research one of the most relevant in the study of human biology. Its evaluation is of interest in many scientific disciplines, including physical anthropology, medicine, sports science, epidemiology and nutrition, and is therefore considered a multidisciplinary field of study.

Throughout the 150 years of development of this area of study, many methods have been validated and applied not only in the laboratory and clinic, but above all in the field research. Currently, the determination of body composition is focused on the validation of body composition models and developing specific equations according to sex and age, ethnicity and health status, on the analysis of the genetic factors involved, and on the study of the dynamic relationships between the components of the body and its functions. Its assessment in different population groups is fundamental for the functional evaluation of the human body, since it has been demonstrated that changes in body composition are closely related to various diseases and morbid conditions, whose higher or lower risk depends not only on individual but also on population features.

Body composition assessment

The human body can be quantified at several levels, depending on clinical and research concerns. The central model in body composition research is the five-level model, which characterizes the human body in terms of five increasingly complex levels (Wang et al., 1992): atomic, molecular, cellular, tissue-system, and whole body. Although each level and its multiple compartments are distinct, biochemical and physiological connections exist such that the model is consistent and functions as a whole (Wang et al., 1992).

The main elements at atomic level are four, oxygen, carbon, hydrogen, and nitrogen with an additional seven, namely calcium, phosphorous, potassium, sodium and chlorine (all these account for over 99.5% of the total body mass); at molecular level, water, lipids (fats), proteins, minerals, and to a lesser extent carbohydrates (i.e. glycogen) are the essential chemical compounds; cell mass (body cell mass and fat), extracellular fluid and extracellular solids are the three main components at cellular level; the main tissue-system level components are adipose tissue, skeletal muscle, bone, visceral organs and brain; finally, the whole body level concerns to body size, shape, and external and physical characteristics. It can be divided into appendages, trunk and head. The sum of all components at each level is equivalent to the total body mass.

One of the most frequently applied models in clinical practice and in epidemiology research is the two-compartment model (2-C), in which body is divided in two components, fat mass (FM) and fat-free mass (FFM). FM is the water-free body component; the remaining body components (skeletal muscle, internal organs, and interstitial fat tissue) are included in the FFM, being water the main component. Common body composition assessment techniques such anthropometry and bioelectrical impedance analysis (BIA) are based on 2-C model. Anthropometry describes body mass, size, shape, and level of fatness through the measurement of body dimensions such as lengths and breadths, weight, skinfold thicknesses and circumferences; these measurements can be combined to obtain some indices and variables of epidemiological interest (e.g. Body Mass Index (BMI kg/m^2), Waist-

Hip Ratio (WHR), body fat percentage (%FM), etc.). BIA measures the electrical properties of body tissue and estimates body composition parameters as total body water (TBW) and FFM. Multi-compartment models have also been developed to individually assess the varying components of FFM. Such models are suitable when comparing groups in which the composition of the FFM differs, for example among children, adults and the elderly, between women and men, and in different ethnic groups.

The fat-free mass (FFM) has not received as much attention as FM, partly because the variability in total body mass between individuals, but also within an individual over time, is due mainly to differences of FM, being inter-individual variability of FFM smaller. In addition, is well established that an excessive amount of body fat, which often leads to overweight and obesity, is related to increased morbidity and mortality. Most of the body fat is stored in adipose tissue, but fat is found in some organs such as liver and skeletal muscle. It is also known that the metabolic risk related to fat accumulation is strongly dependent on its distribution. Central obesity and, in particular, visceral and ectopic fat accumulation, are related to increased atherosclerosis, cardio-metabolic risk, hepatic disease and cancer. However, independent of body fat, which is an indicator of long-term energy storage and the primary source of energy, FFM is of great relevance. The increased prevalence of obesity together with chronic illnesses associated with fat-free mass loss will result in an increased prevalence of a “new category “of obesity named “sarcopenic obesity” (see Stenholm et al., 2008). This variant of sarcopenia is characterized by increased FM and reduced FFM with a normal or high body weight, and associated with functional decline and disability, especially mobility, as well as higher levels of cardiovascular risk factors, and an increased risk of mortality in general population (He et al., 2018).

Variability of body composition

The human body presents notable morphological differences with respect to other primates. Apart from a much larger brain and a specialised pelvis, the long lower limbs of humans, adapted to bipedal locomotion, contrast with the relatively short lower limbs of quadruped African apes (Zhilman and Bolter, 2015). Comparative studies based on primates as close genetically to *Homo sapiens* as Bonobos (*Pan* *paniscus*) indicate differences in the amount and distribution of FM and FFM, linked to locomotion (e.g. the regional distribution of muscle in our species contrasts significantly with the regional distribution of muscle in *Pan paniscus* and other apes), but not only. Throughout our evolutionary history selective pressures increased the capacity to store fat to address nutritional requirements for successful reproduction and for maintenance of a high level of activity. For females, storing fat enhanced effective pregnancy and lactation to nourish larger brained infants (Zhilman and Bolter, 2015). Currently a wide variability in body composition can be observed, both individually and between different populations. Sex and age play an important role. Genetic factors together with environmental which includes ecological-climatic factors (e.g. rural-urban environment, temperature and altitude), socioeconomics and cultural conditions, lifestyle (mainly diet and physical activity), bio-demographic factors (e.g. maternal age at birth and parity), and health status, influence in different ways the intra and inter-population variability of body composition.

Intra-population variability of body composition

Age and sex

Human body varies in size, shape, and composition as growths, maturates, and develops. The rapid growth of infants, particularly during the first year of life, is accompanied by large changes in body composition; body weight and length may increase by about 200% and 50%, respectively (Bogin, 2001). FM at birth is greater in girls than in boys and its percentage in relation to total body weight (%FM) increases to 25-30% by around 6 months. Thereafter, FFM begins to accumulate preferentially. A "plateau" in growth velocity occurring during childhood (3-7 years) is finished by a growth spurt called the "mid growth or juvenile growth spurt", that is relatively small in height but larger and more pronounced in dimensions relating to volume such as weight or fat mass. During childhood, although %FM decreases in both sexes, sex differences remain apparent. During the juvenile period (7-10 years in girls and 7 to 12 years in boys) a pronounced but short-lived decrease in rate of growth is observed (Bogin, 2001).

Growth at adolescence (10-19 years in girls and 12-20 years in boys) is characterized by the presence of the "pubertal growth spurt" which affects body composition. FM and FFM change in absolute amount, relative proportion, and anatomic distribution. Girls show a rapid increase of body mass at 12 or 13 years of age, associated with an accumulation of fat mass in the breasts and around the hips. Boys show an increase of physical dimensions and acquire FFM at a greater rate (kg/year) and for a longer period than girls. In adolescence, girls continue to gain absolute FM, however, boys maintain a relatively fixed absolute FM and thus present a decrease in %FM. Stable values of FFM are attained by approximately 15-16 years of age in girls and 2-3 years later in boys (Bogin, 2001).

Adults, like children and adolescents, change in body composition, but changes during adulthood are slower. Many women and men continue to gain weight throughout adulthood due to an increase of the total amount of adipose tissue (FM). Some studies have pointed to the existence of different patterns of change in body composition in aging men and women. In men, gains in body weight are followed by a moderate decrease after age 50, and have been attributed to a decline in FFM rather than a decrease in FM. In women, the greatest rate of decline in FFM may occur in the perimenopausal years, followed by a more gradual decline thereafter (Hughes et al., 2002). Often, there is also an age-related FFM loss, with a decrease of muscle mass and a reduction of bone mass, associated with impaired mobility and physical disability (sarcopenia). Changes that occur in muscle tissue during ageing may be aggravated by both hormonal and inflammatory changes, metabolic agents and nutritional status, and decreased level of physical activity.

Along with changes in FM and FFM quantities over the life cycle, there are also changes in the anatomical distribution of the major components of the body mass that shape human body in a different way between women and men. Adipose tissue can be differentiated into visceral (VAT) and subcutaneous (SAT). After birth infants have almost equal thicknesses of SAT on the trunk and extremities. The accumulation of SAT on the extremities decreases gradually to 13 years in each sex, being the difference between both sexes small. During adolescence and into adulthood men gain proportionally more SAT on the trunk than extremities, while women gain relatively similar amounts of SAT on the trunk and extremities. Aging is associated with progressive changes in regional fat distribution, with a preferential increase in abdominal fat, in particular VAT, combined with a decrease in lower body subcutaneous fat.

On average, pre-menopausal women accumulate more SAT fat in the gluteus femoral depot whereas men have more abdominal VAT (Frank et al., 2019). Similar to men, postmenopausal women tend to accumulate more abdominal VAT, due partly to a decline in ovarian production of oestrogens. Other factors such as age *per se* and total body weight gain also contribute to age-related adipose tissue redistribution (Wells, 2007; Frank et al., 2019). Abdominal VAT accumulation is known as "android" fat distribution and is related with mortality, cardiovascular disease (CVD), and the Metabolic Syndrome. "Gynoid" or peripheral fat distribution is defined as fat deposited in the limbs and hips, particularly in the lower body. In summary, sexual dimorphism in human body composition emerges primarily during puberty (Wells, 2007), and it is due to a great extent to the action of sex steroid hormones, especially during pubertal development. Body fat distribution is also sexually dimorphic, with women and men having differential distribution of adipose tissues.

Genetic factors

The research based on twins, family and adoption studies has revealed that genetic factors determine a substantial proportion of the variance in both overall obesity and the distribution of subcutaneous fat, and other body components. An important genetic contribution to obesity risk has been demonstrated, with heritability estimates ranging from 40% to 70% depending on the obesity phenotype. Twin studies have shown that genetic factors explain a considerable proportion of BMI variation over the life course and genome-wide association studies (GWAS) have identified the role of genetic variants behind BMI variation (Silventoinen et al., 2019). Other genetic and hormonal factors have effects on body composition (e.g. excess of androgens, growth hormones, leptin circulating levels, etc.), acting on muscle mass, amount of body fat (FM) and %FM. More than 700 SNPs predisposing to obesity have been identified in candidate gene studies and GWAS. Most of these studies primarily use Body Mass Index (BMI) to characterize the obesity phenotype, but other obesity related traits such as Waist Circumference (WC), WHR, and the FM percentage (%FM) have been analysed (Frank et al., 2019). Cross sectional evidence does suggest that DNA methylation and measures of body fat distribution are associated. So, epigenetic mechanisms, may also contribute to body fat distribution (Frank et al., 2019).

Environmental factors: socioeconomic status, lifestyle and diseases

Environmental factors, such as socio-economic status (SES), largely determine the morphology and composition of the body, both at the individual and population level. In Western industrialised societies, tall stature, low BMI and peripheral fat distribution are associated with higher SES. In contrast, individuals with lower SES are, on average, lower, with a higher BMI, and have less muscle and skeletal mass than those with higher SES. One possible explanation may be access to a high quality diet and increased physical activity in the former. Some bio-demographic factors may also be related; early feeding experiences (e.g. foetal malnutrition) have a great influence on later body size and composition (Silventoinen et al., 2019). Body fat distribution also differs between SES groups, with individuals of lower SES presenting more of their fat on the trunk and less of their fat on the extremities as well as a higher abdominal distribution in relation to hip than high SES individuals.

An inadequate diet and inactivity can also cause some conditions like obesity and chronic diseases. Food habits are conditioned by many factors, although those that have the greatest influence on the type of food consumed are food availability, economic resources and choice. The socio-economic and geographical characteristics of each country have a significant influence on the quantity and variety of food available. There is a wide variation in human diet; some studies have revealed signs of genetic adaptation to local environments such as cold climates, linked to diet. The Inuit show genetic and physiological adaptations to diets rich in proteins and fatty acids, especially omega-3 polyunsaturated fatty acids (PUFAs), associated with multiple metabolic and anthropometric phenotypes as weight, height, and body composition (Fumagalli et al., 2015).

Improvement in physical fitness helps to prevent obesity and other related diseases; physical activity is inversely related to the BMI, and it would attenuate losses of FFM and gains of adipose tissue over time. Sedentary lifestyle plays a role in the age-associated increase in FM since exercise is associated with a decrease in FM in the elderly.

However, exercise attenuates but does not totally prevent the age-associated increase in body fat, although preferentially reduced the disproportionate increase in abdominal fat.

The effects of disease on body composition are generally similar to those of malnutrition, by inducing a disturbance of energy balance that may influence body composition, leading in many cases to obesity. Some chronic conditions (e.g. cancer) and infectious diseases can lead to a permanent child stunting affecting body composition. In turn, diverse alterations in body composition such as decreased bone density and dehydration in the elderly, excessive fat accumulation and its unfavourable distribution in children and adults, or reduced FM and FFM due to malnutrition, influence health status. The role played by the body's lean tissue reserves, mainly skeletal muscle, in the response to injury and illness, is of relevance to nutritional status (Earthman, 2015).

Inter-population variability of body composition

Consideration of genetic ancestry of populations, geographic origin or ethnicity has acquired special importance in biomedical studies, and also in those related to body composition, as this last is strongly related to health. There are a growing number of studies on body composition in populations from different regions of the world as Asian and Pacific groups (e.g. Chinese, Indians, Japanese, Polynesians), African (e.g. Ethiopians, Nigerians); Black Americans, white South Africans, and South American (e.g. Mexican, Brazilian) and Hispanic-Americans (Deurenberg and Deurenberg-Yap, 2003; Rush et al. 2007, 2009; Marini et al., 2020a), although most research has been conducted on populations of Caucasian origin (e.g. Americans, Australians and Europeans). Data on body composition variables such TBW, FFM, %FM and obesity-related ones of children and adults by sex and ethnicity, are available from the third National Health and Nutrition Examination Survey (NHANES III) and the CDC National Center for Health Statistics (NCHS) site (www.cdc.gov/nchs) (Chumlea et al., 2002, and Duren et al., 2008).

Data from Caucasian populations made it possible to develop sex- and age-based references and to obtain cut-off points for universal use for different variables related to body composition as BMI, WC and %FM. Barba et al. (2004) have discussed the need to establish population-specific cut-off points for BMI, concluding they should be maintained. However, Deurenberg and Deurenberg-Yap (2003), have pointed to the need for "more multi-ethnic studies that could help in redefining BMI cut-off values for obesity and underweight when combined with data on risk factors such as cardiovascular disease. Cut-off points for FM distribution (based on WC, WHR, or skinfold thickness ratios) should be validated and if needed redefined in different ethnic groups".

Table 1. Remarks on ethnicity variability of Body Composition. These observations correspond to the results of the authors mentioned (*) or to those of other authors cited by them in their papers.

African and African-American *vs.* Caucasians (based on comparisons between "black" and "white" people)	**Asians *vs.* Caucasians (based on comparisons between Asian Indians, other Asian populations, and Caucasians)**
1. There are differences of both fat and muscle mass distribution between "black" and "white" people (a). 2. "Black" people show a greater amount of fat on their shoulders and backs, whereas "white" have more fat on the abdomen and thighs (a). 3. The weight of the dry fat-free skeleton may be up to 20% greater in "black" than in "white" subjects (a). 4. The bones of African-American subjects may have a higher content of protein and/or non-osseous mineral than in Caucasians (a). 5. The density of lean tissue in "black" people is slightly higher (1.110-1.113 g/cm^3) that the standard figure of 1.100 g/cm^3 (a). 6. African-Americans have higher bone density and bone mineral con-	1. The Chinese from Singapore, Malays and Indians show small differences in the composition of FFM (water, mineral and protein) (b). 2. Asian populations have different associations between BMI, %FM and health risks than do European populations (c). 3. The BMI cut-off point for observed risk in different Asian populations varies from 22 kg/m^2 to 25 kg/m^2; for high risk it varies from 26 kg/m^2 to 31 kg/m^2 (c). 4. The proportion of Asian with a high risk of type 2 diabetes and cardiovascular disease is substantial at BMIs lower than the existing WHO cut-off point for overweight (25 kg/m^2) (c). 5. Compared with European men of similar weight, height and age,

tents, as well as higher muscle mass than Caucasians, although the amount of water in the FFM can be similar between both groups (b). 7. African-Americans (both children and adults) have less visceral adipose tissue than their "white" counterparts (b). 8. American Caucasians have a lower %FM at the same BMI than do Caucasians in Europe (b). 9. American "blacks "and "whites" differ in their metabolic relationships with body fat and body fat distribution (b).	Asian Indians have significantly less skeletal muscle in the limbs. (d). 6. Whole body bone mineral content is lower in Asian Indian women than in Pacific and Maori women (d). 7. In Asian populations, for the same %FM, mean inter-ethnic BMI range by more than 10 units, with the greatest difference between the Pacific people and the Asian Indians (d). 8. Polynesians (Pacific and Maori) have higher bone mass and muscle mass than Europeans and Asians of the same size (d). 9. A higher %FM at a lower BMI has been observed in the Asian Indian compared with European populations (d). 10. As BMI rises, the %FM increases more rapidly in the European New Zealanders than in the Maori, Pacific people and in Asian Indians (d).

(*) From the texts of: (a) Shepard (1991), (b) Deurenberg and Deurenberg-Yap (2003), (c) Barba et al. (2004), and (d) Rush et al. (2009).

Table 1 summarises some observations on the inter-population variability of body composition. This variability can be due to environmental factors (energy intake, physical activity), but also to adaptations to geographic and climate conditions, with a genetic basis, and related to the plasticity during growth and development as part of the organism's "adaptability" to environmental signals (Wells et al., 2019). Such adaptations determine body morphology as well as body build (or frame) and proportionality of individuals, and can impact both the different components of body composition and the relationship between BMI and %FM (Wells et al., 2019). An example is the unusual arm length of some Africans, the shortness of the legs in circumpolar populations, or the propensity to Caucasians to deposit more fat on the upper limbs than Afro-Americans, Asians or Amerindians. Relative leg length is one of the reasons for the underestimation of %FM (data obtained through BIA), as Asians have relatively short legs compared to Caucasians (Deurenberg and Deurenberg-Yap, 2003). But differences in proportionality can also be found between groups considered as Asian (Chinese *vs.* Indians) or African (Ethiopians *vs.* West African groups) (Deurenberg and Deurenberg-Yap, 2003).

The remarks shown in Table 1, and a full reading of the papers from which those were drawn, indicate that:
i) FM and FFM distribution vary at the same BMI according to ethnicity,
ii) The relationship between %FM and BMI vary among different ethnic groups; for example, Asian populations have a lower mean BMI compared with European or American ones, but they generally have a higher %FM than Caucasians of the same age, sex, and BMI,
iii) The current WHO cut-off points for Caucasians do not provide an adequate basis for taking action on risks related to overweight and obesity in many populations in Asia,
iv) As previously stated, differences in relative leg lengths among ethnic groups are related to different body compositions and can alter the validity of some indicators as BMI as predictor of %FM,
v) %FM predicted from BMI underestimates the percentage of fat in individuals with a relatively slender body build or frame. This can be due to differences in muscularity and bone mineral content.

It is important to point out that under the denominations of Caucasian, Asian or African, populations with different lifestyles, different degree of genetic admixture and particular genetic backgrounds are grouped together. Therefore, it is common to find for some body composition phenotypes a greater intra-population than inter-population variability.

Research Highlights

- Body composition is among the most obvious ways humans vary, both within and among populations, due to a wide range of biological (including genetic heritage), behavioural, and environmental factors (involving ecological conditions), and their complex interactions.
- Body composition is a dynamic characteristic closely related to sex and age, as well as to the lifestyle of the individual; it is an essential feature of the health and physical fitness of individuals and populations.
- The differences in body composition between both sexes are observed from the early stages of life (at birth, the thickness of subcutaneous fat are greater in girls and boys have a greater overall body mass), become markedly accentuated during puberty and tend to decrease during ageing.
- Body composition is affected by the ethnic origin of individuals. This can have important public health consequences, related to a differential risk for non-communicable diseases among different geographical populations.
- From an evolutionary perspective, sexual dimorphism in human body composition can be considered as the result of differential selective pressures on each sex, with implications for survival and reproduction.

Life style, body composition and health status

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Health status of a person is not only dependent upon the physical well-being of a human but it also depends upon mental and social well-being and good nutrition as well. All these parameters are strictly connected.

Nowadays malnutrition is a growing public health problem. Historically, the use of the term malnutrition has been associated with a state of undernutrition, but both undernutrition and overnutrition are forms of malnutrition. Millions of children and adults face premature death due to both undernutrition and the chronic diseases associated with overnutrition (Tanumihardjo et al., 2007). Malnutrition is usually associated with poverty and food insecurity, and it is defined as poorly or wrongly fed and having a poor or inadequate diet. However, a new paradox links poverty, food insecurity, and malnutrition to overnutrition and obesity.

Monitoring the nutritional status of a person is fundamental to plan for effective actions, and it can be done using the anthropometric method, which consists of the measurements of human body. At this purpose, the following indicators are suggested:

Body Mass Index (BMI). It can be calculated by this formula:

BMI = Weight (kg)/ Height^2 (m)

If BMI is more than 40, then the person is suffering from a 3-grade obesity, if BMI is between 35.0-39.9 then, it is a 2-grade obesity, if BMI is between 30.0- 39.9 then it is a 1-grade, if it is between 25.0-29.9 then it is in the pre-obesity class, if it is between 18.5-24.9 the person is the normal weight class, and if it is less than 18.5, BMI indicates that person is underweight.

BMI can give an indication regarding the nutritional status of a subject or population, and since it is easy to measure, it is safe and its cost is low, it is commonly used in epidemiological studies. Nevertheless, the measurement of body composition (BC) represents a more valuable tool to assess nutritional status in health and disease, since it allows to value the weight components (fat mass, fat free mass, total body water, etc.). Both anthropometric and bioelectrical impedance techniques represent simple solutions to estimate body composition parameters and are preferred in different contexts. In this sense, anthropometry, including skinfolds and circumferences, may be a low cost and easy to use alternative method in epidemiological contexts. In addition, also bioelectrical impedance analysis (BIA) is a broadly applied approach used in body composition measurements and healthcare assessment systems. It is an inexpensive tool, which can provide estimation of body composition in field setting. For a proper interpretation of bioelectrical data some anthropometric measurements are required. In particular, most of the known prediction methods, based on equations, rely on the relation between water volume and the ratio between squared height (Ht) to resistance (Ht^2/R). The bioimpedance vector analysis method (BIVA) is a more recent approach established essentially by Piccoli et al. (1994) which use height indexed resistance (R/H) and reactance (Xc/H) data (R-Xc graph) from bioimpedance measurements. A *specific* BIVA method has been proposed by Marini et al. (2013) to neutralize the bias due to body size. The *specific* BIVA method uses a resistivity-reactivity graph that is constructed using information and results collected from multiplication of resistance and reactance by ratio of length and cross section area (determined by body circumferences) (L/A).

The evaluation of all the above mentioned parameters (weight, BMI and body composition parameters) is useful in order to value the health status of an individual and his health risk, and to keep prevention programs. In addition, another important information regards fat distribution. In fact, not all the sites of fat deposition have the same health risk. Abdominal visceral fat is the most dangerous, since it exerts its deleterious effects through the production and secretion of various proinflammatory cytokines (Conte et al., 2020), that promote the development of diabetes mellitus and insulin resistance, which is a fundamental characteristic of metabolic syndrome. The chronic inflammatory state produced by visceral obesity is associated with a pro-atherogenic alteration of the lipid profile, which contributes to increased cardiovascular risk also through an increase low density lipoproteins.

A simple way to value fat distribution is the use of:

Waist Circumference (measured in the midpoint between the lower margin of the last palpable rib and the top of the iliac crest). The cut-off points (WHO) for substantially increased risk of metabolic complication are: for men > 102 cm; for women > 88 cm

Waist-to-hip ratio (WHR) = Waist circumference (cm)/ Hip circumference (cm) (waist circumference should be taken as previously indicated; hip circumference measurement should be taken around the widest portion of the buttocks). The cut-off points (WHO) for substantially increased risk of metabolic complication are: For men > 0.90 cm; For women > 0.85 cm. The cut-off are not defined for children.

Waist-to-height ratio (WHTR) = Waist circumference (cm)/ Height (cm). A boundary value of WHTR = 0.5 indicates increased risk for men and women. WHTR may allow the same boundary values for children and adults.

Underweight among children and adolescents is associated with higher risk of infectious diseases, and for girls of childbearing age, is associated with adverse pregnancy outcomes including maternal mortality, delivery complications, preterm birth, and intrauterine growth retardation.

On the other side, also gaining excess weight in childhood and adolescence has serious consequences. First of all, it is likely to lead to lifelong overweight and obesity, second, being overweight in childhood and adolescence is associated with greater risk and earlier onset of chronic disorders (NCD Risk Factor Collaboration, 2017).

In this chapter we will address the issues related to overweight and obesity.

Obesity is a growing public health problem. In 2016, more than 1·9 billion adults worldwide were overweight and 650 million were obese, vastly outnumbering those who were normal weight. The chronic conditions that present a clear association with overweight and obesity will be outlined below.

Overweight and obesity are very strongly correlated with Type 2 diabetes (T2D) and obesity is the most important culprit of insulin resistance, which appears early in the disease, and is primarily compensated by hyperinsulinaemia (Chobot et al., 2018). In the comparison with normal weight people, obese had a higher chance to develop T2D. This is also confirmed by longitudinal studies: patients who had T2D were mostly obese compared with individuals with normal BMI. This is true for the population from different countries.

Obesity is an independent risk factor for cardiovascular disease (CVD), including coronary heart disease, myocardial infarction, angina pectoris, congestive heart failure, stroke, hypertension, and atrial fibrillation. Overall, results from large perspective and observational studies confirm the marked adverse effects of obesity on CVD. A combination of commonly associated cardiovascular risk factors is known as metabolic syndrome. Metabolic syndrome represents a group of cardiometabolic risk factors that include abdominal obesity combined with elevated blood pressure, fasting plasma glucose, and triglycerides, and reduced high-density lipoprotein cholesterol levels. Metabolic syndrome is associated with an increased risk of cardiovascular mortality (Pi-Sunyer, 2009).

A number of large-scale, prospective studies have confirmed a significant association between obesity and several types of cancer, especially with respect to tumors of the gastrointestinal tract, where being overweight carries a 1.5–2.4-fold increase in cancer risk (Stone, 2018). Body mass index was significantly associated with higher rates of death due to cancer of the esophagus, colon and rectum, liver, gallbladder, pancreas, kidney, non-Hodgkin lymphoma, and multiple myeloma (Pi-Sunyer, 2009).

Osteoarthritis (OA) has a major impact on patient mobility, disability, lost productivity, and patients may become disabled from OA early in life. It can contribute to inactivity with ageing, secondary to pain and reduced function, thus ultimately impairing quality of life. Obesity is strongly associated with an increased risk of OA, and the awareness of its effect is important for planning for weight loss and rehabilitation interventions.

Gallbladder disease is a common cause of hospitalization, especially among women, and has a considerable impact on health care costs

(Liu 2018). In particular, abdominal obesity is a more important risk factor for stones formation than general obesity. The possible pathogenesis for the close association between obesity and Gallstone disease are complex and not fully understood. However, obese patients frequently have supersaturated gallbladder bile and show a larger and gallbladder volume.

Obesity is also closely associated with acute pancreatitis, and a number of studies have shown that obesity increases the severity of and mortality from acute pancreatitis, being a primary risk factor for local complications, organ failure, and death from acute pancreatitis.

In addition, pulmonary complications are determined by obesity and a linear correlation between obesity and obstructive sleep apnea (OSA) has been observed. OSA potentially results in a number of complications including pulmonary hypertension, right heart failure, drug-resistant hypertension, stroke, and arrhythmias (Pi-Sunyer, 2009). OSA is characterized by upper airway obstruction that occurs as repetitive episodes during sleep. In obese people, fat deposits in the upper respiratory tract narrow the airway, and the decrease in muscle activity in this region leads to hypoxic and apneic episodes, which determines in sleep apnea. These hypoxia/apnea episodes lead to a decrease in oxygen that is available in body tissues and blood vessels. The decreased oxygenation causes tissue hypoxia, which is the main contributing factor to atherosclerosis, the main risk factor for cardiovascular diseases.

As regards psychosocial domain, an association between obesity and major depressive disorder has been observed, even if a causal association is uncertain. The association between obesity and depression is greater in adolescents, particularly in females (Blaine, 2008). Importantly, many antidepressant drugs are associated with weight gain. BMI is significantly associated with mood, anxiety, and personality disorders.

Obesity also has a significant impact on quality-adjusted life years and reduces years of life. Compared with normal weight, both mild and severe obesity are associated with a significant reduction in disease-free years between 40 years and 75 years: mildly obese individuals lose one in ten and the severely obese one in four potential disease-free life-years during middle and later adulthood (Nyberg et al., 2018).

The economic costs of obesity are substantial, and significant benefits could be expected from interventions that prevent or reduce obesity. Particular attention should be paid to children, since the transitional period between childhood and adulthood affects energy balance leading to weight gain (Štefan et al., 2017), and, as already reported, obesity in childhood has a positive predictive value for predicting the presence of obesity at later ages.

Since the prevalence of obesity in children, adolescents and adults is relatively high, factors influencing it need to be identified. Lifestyle is a good predictor of health, and the factors involved regard in large part poor diet, physical inactivity, and the consumption of alcohol and tobacco. Lifestyle intervention is the most common treatment strategy in subjects with obesity (Galan-Lopez et al., 2018).

Overweight/obese individuals perform less physical activity (PA) and spend more time each day sitting (Štefan et al., 2017). PA is a vital part of a healthy lifestyle and has been extensively documented and associated with health benefits at all ages. It has been observed that PA is inversely related with BMI, waist circumference (WC), waist to height ratio (WHTR) and fat-mass percentage and positively related with fat-free mass percentage. According to the WHO, children and adolescents, aged 5-17, are recommended to engage in at least 60 minutes of moderate-to-vigorous PA (MVPA) per day in order to avoid the risk of metabolic and cardiovascular diseases. However, despite these recommendations, more than half of children and adolescents worldwide do not meet the recommendation of 60 minutes of MVPA per day (Konstanbel et al., 2014). Some epidemiological evidence suggest that PA has the potential to counteract the detrimental effects of obesity: physically active individuals have a reduced risk of cardiometabolic outcomes regardless of BMI. In addition, leisure time physical activity significantly altered the risk related to a high body fatness on colon cancer (Nunez et al., 2018). Some of the benefits of PA include reductions in blood cholesterol, hypertension, metabolic syndrome, obesity, and associated health problems such as diabetes mellitus type 2, cardiovascular diseases, or bone health problems (Štefan et al., 2017).

Sedentary behavior has been associated with negative health outcomes in all age groups. Sedentary behavior is not synonymous with physical inactivity. This behavior is common in present-day societies, particularly in high income countries. In recent years, this behavior has emerged as an additional potential risk factor associated with adverse cardio-metabolic profile, premature mortality and various types of cancer. The increased cancer risk associated with sedentary behavior seems to be independent of PA. A reduction of time for sitting can improve health, and reduce obesity consequences, irrespective of the level of the individual's physical activity (Martínez-Ramos et a al. 2018).

Another important factor is nutrition: food intake in adolescence is a significant predictor of intake in adulthood (Galan-Lopez et al., 2018). The Mediterranean Diet (MD) has been accepted as one of the healthiest dietary patterns in the world, showing significant protection concerning mortality and morbidity and being associated with physical benefits and high levels of health-related quality of life. It reduces cardiovascular diseases, type 2 diabetes, certain types of cancer, and some neurodegenerative diseases. Some of its components, such as fruits, vegetables and olive oil, are mainly responsible for the protection against hypertension and cardiovascular diseases. Moreover, the high consumption of products rich with minerals and plant foods rich with antioxidants contribute to the health of vascular system and reduction of high arterial blood pressure. MD is rich with omega-3 fatty acids, which can potentially reduce heart rate and suppress the automaticity of cardiac contraction. Bacopoulou et al. (2017) determined that the increase in adherence to MD was associated with a decrease in waist circumference, indicating a potential for school interventions to fight against abdominal obesity in adolescents.

Smoking and alcohol consumption have also negative consequences on body composition parameters: heavy smokers and drinkers have greater body weight and BMI status. In addition, common stressful events are often followed by higher smoking and cigarette consumption, which potentially lead to increased fat-mass percentage. In adolescents, smoking at least 10 cigarettes per day increases the risk of adult abdominal obesity. Smoking causes an acute increase in both blood pressure and heart rate, due to a nicotine act as an adrenergic agonist, which may release vasopressin. Analogously, alcohol consumption increases BMI, WHR, WC and fat-mass percentage. Alcohol serves as a suppressor for fat oxidation and can increase fat synthesis. Physiologically, ethanol affects the transcription of genes involved in muscle hyperthrophy, where inhibits testosteron and increases cortisol levels.

Another potential factor related with body composition is self-rated health (SRH). Poor SRH is related with both underweight and overweight/obese nutritional status: being underweight and obese increase the odds of medium and high psychological distress in a general population (Štefan et al., 2017). Stress level, often characterized by negative life events and problems, is related with overall and central adiposity in children. A dysregulation of the stress system and the production of stress hormones, such as cortisol often caused changes in metabolism, i.e., increased visceral fat.

There is growing evidence that health behaviors are grouped. For example, the combination of regular PA and healthy eating habits helps to maintain and improve health and physical and mental well-being (Galan-Lopez et al., 2018). In addition, there are independent and combined associations between physical fitness, physical activity, and adherence to the MD with quality of life related to health in children, adolescents, and adults, with significant improvements in joint interventions.

However, despite all the benefits mentioned above, the current data show unhealthy patterns of eating and PA during the transition from childhood to adolescence, substantially contributing to the global burden of morbidity, mortality, and disability, and increasing the prevalence of overweight and obesity at those ages. PA and nutrition are shown as fundamental pillars in the prevention and control of obesity.

Recognition of the association between obesity and comorbidities is critical for patient diagnosis and management by primary care physicians. Physicians need to be aware of comorbidities and their implications for outcomes and patient management of the obese patient.

Global efforts to control obesity and minimize factors that contribute to obesity are essential to improve health status and life expectancy worldwide.

Research Highlights

- Evaluation of nutritional status is important in order to value the health status of an individual and his health risk.
- Overweight and obesity are associated with greater risk and earlier onset of chronic disorders.
- Physicians need to be aware of the comorbidities to plan prevention programs at all ages but with particular attention to children and adolescents.
- Lifestyle interventions are the most efficacious strategy in subject with obesity.

Assessing fat and fat-free mass: two-, three-, and four-compartment models at the molecular level

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Considering the recognized impact of several body components in health, research in human body composition has also increased in relevance. Therefore, a proposal was advanced to organize body composition into three distinct but interconnected areas (Wang et al, 1992): body composition rules (relatively constant relationships between body components and/or their measurable properties), body composition methodology (in vivo methods), and body composition alterations (growth, weight loss).

In 1992, Wang et al. helped define human body composition as a branch of human biology by developing a structure for body composition research, namely by the proposal of the five-level model (Wang et al., 1992) (Figure 1).

The main body components are organized into five distinct levels of increasing complexity: atomic, molecular, cellular, tissue-system, and whole-body (Wang et al., 1992). By adding all components at each level total body weight is achieved. An important detail of this model is that the components of successively higher body composition levels include the lower-level components.

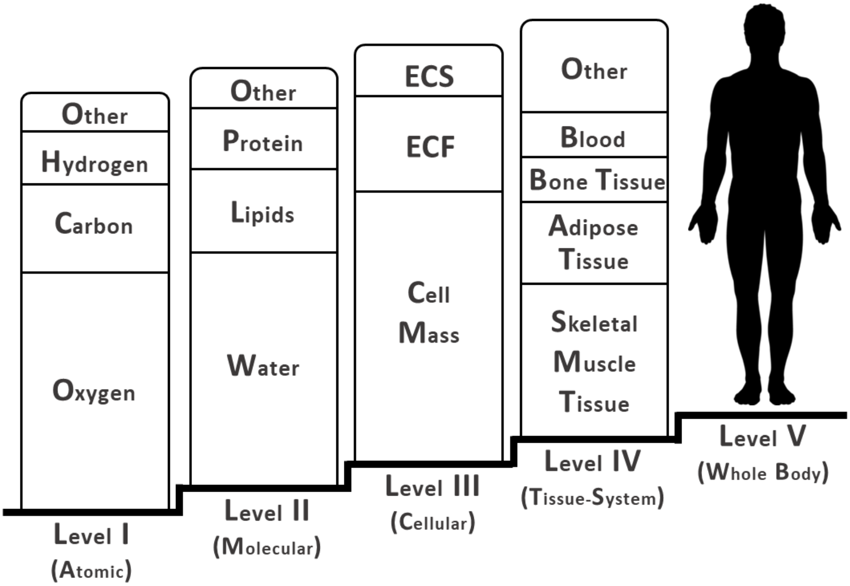

Figure 1. The five-level model of human body composition analysis, adapted from Wang et al., 1992. Abbreviations: ECS, Extra-cellular solids; ECF, Extra-cellular fluids.

Molecular Models for Body Composition Assessment

In this section the most important methods will be highlighted to determine body composition at the molecular level, ranging from the basic two-compartment models (2C) to the reference method, the four-compartment model (4C), including dual-energy x-ray absorptiometry (DXA), a three-compartment model that given its widespread will be further discussed.

The molecular level of body composition analysis consists of five major components: total body water (TBW), protein, carbohydrates (glycogen), minerals (bone and soft tissue minerals), and lipids, as illustrated in Figure 2.

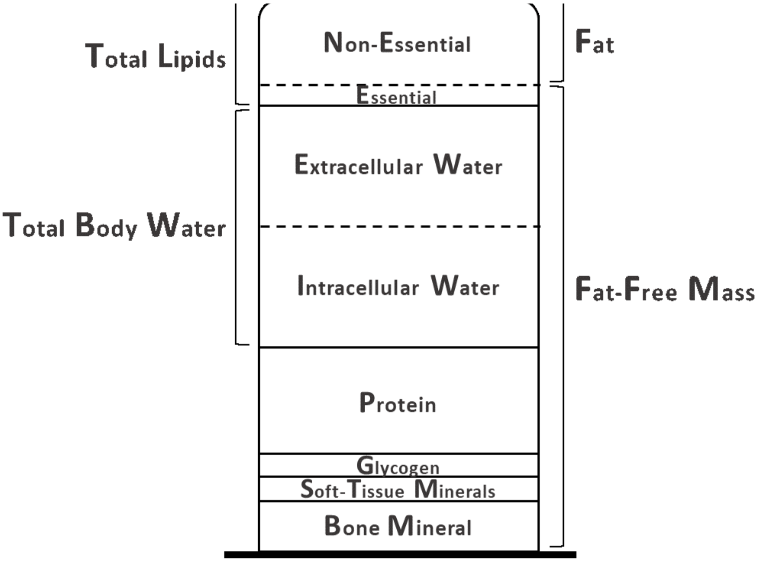

Figure 2. Components of the molecular level, adapted from Wang et al., 1992.

Many body composition models are based on relationships between body components observed at the molecular level. One example is the physical density of the molecular level components. In this sense, by assuming relatively stable proportions among the various constituent chemical components, the density of combined components, such as fat-free mass (FFM), can be calculated (Behnke, 1942).

Two-Compartment Models

On the basis of limited data from chemical analysis of animal carcasses and human cadavers (Brozek et al., 1963), more detailed densitometric values have been estimated for fat mass (FM), mineral, and protein. Behnke (Behnke, 1942) introduced the classic 2C body composition model which divides body weight into FM with a density of 0.9007 g/cm^3 and FFM with a density of 1.100 g/cm^3. Thus, the recognition that FM is a more homogeneous chemical compound than FFM and the implications from assuming the adult constant value for FFM density (1.1 g/cm^3), compromises the correct estimation of FM in different population groups, particularly children. Hydrometric methods are also considered 2C models, as weight is divided in TBW and a residual mass (FM, proteins, minerals). Assuming that TBW represents 73.2% of FFM, FM can be obtained as body mass minus FFM, though a large individual variation in the assumed constant is observed (Wang et al., 1999).

Below are described the main densitometric and hydrometric techniques.

Densitometric methods

Underwater weighting (UWW) or air displacement plethysmography (ADP) are the most common and valid approaches to determine body density (BD). The UWW involves body immersion with body mass during submersion recorded by a platform force transducers during a 5-s maximal expiration. After a series of practice trials, usually 10 runs are performed and BD results are averaged. Corrections in BD are made for residual lung volume using a closed-circuit oxygen-dilution system described by Wilmore (Wilmore, 1969). Residual volume is measured immediately after the UWW procedure. The UWW was progressively replaced by ADP, where instead of immersed in water a person is placed in a close air-filled chamber. The ADP system consist of a single structure that contains two chambers: the front chamber is where the participant is tested while the rear chamber is where the instrumentation is housed and serves as a reference volume, as described in detail elsewhere (Dempster and Aitkens, 1995). During measurements, the subject use a bathing suit and an acrylic bathing cap and the body mass is measured to the nearest 0.01 kg using the ADP system's electronic scale. Prior to an individual evaluation, a two-point chamber calibration is performed using the empty chamber and a 50.01281 L calibration cylinder. For each person, two trials of body volume determination are performed and the measured volumes are averaged (if within 150 mL or 0.2%). The subject's thoracic gas volume is estimated using the BOD POD® breathing circuit system and a final body volume is computed based on the initial body volume corrected for thoracic gas volume and a surface area artifact.

Hydrometric methods

The principle behind hydrometric models is that lipids are hydrophobic and thus free of water, therefore water is restricted to the FFM compartment. TBW measurement using heavy water was proposed in 1934 but the method was refined later using mass spectrometry (Schoeller, 1985) enabling safe measurements in human throughout the life span. Deuterium (^2H), tritium (^3H), and oxygen-18 (^{18}O) are among the most recognized tracers though tritium involves radiation being less used. Nevertheless deuterium is used more as its close to ideal as a water tracer (Edelman et al., 1952). A rigorous protocol should be followed, including the participant's preparation, dosing and sampling collection, and isotope analysis. The isotope dose should be administered in fasting condition, preferably in the morning. The isotope reaches the equilibration time, within 3 to 4 hours after the ingestion of the dose, and biological samples, commonly urine, are collected before dose and after tracer equilibration (Schoeller, 1985). Generally, the calculation of the isotope dilution space (N) involves the application of the dilution principle. To finalize the process of determining TBW the isotope dilution space should be corrected for exchange with the nonaqueous compartments, as deuterium overestimates the body water pool by 4.2% in adults and children (Schoeller, 1985).

Three-Compartment Models

The assumption of a relative stability for the FFM density (1.1 g/cm^3) was crucial for developing methods to assess FM, such as hydrostatic weighing or air displacement plethysmography (where the density is determined as a result of dividing body mass by body volume), based on a 2C model. It has been observed that growth, weight loss and the training process related changes affects the FFM composition and therefore the respective density value, which may be lower or higher than the value of 1.1 g/cm^3 (Silva et al., 2006). Thus, to avoid the use of assumptions in the FM determination it is crucial to evaluate more than two components. The assessment of TBW in addition to the estimation of body density obtained by densitometry methods, allows the division of body weight into 3 compartments (3C), FM, TBW and residual mass (protein and mineral). The assessment of TBW, the major molecular component of FFM, considerably reduces the assumptions used in FM calculation, since the biological variability of this component is controlled. Thus, the transition of a 2C to a 3C model substantially improves the validity of molecular models in FM estimation, overcoming the limitations of measuring density alone.

Dual-energy X-ray absorptiometry (DXA)

The DXA method can be regarded as a three-compartment model at the molecular level of body composition analysis, as based on their X-ray attenuation properties body mass is divided into FM, bone mineral and fat plus bone free masses (commonly referred to lean-soft tissue). Theoretically, assessing three unknown compartments requires measurement at three different photon energies. Nonetheless, in practice, DXA can only resolve the fractional masses of a two-component mixture. Indeed, DXA is based on the attenuation rates each body component shows when passed through X-rays of two different levels of energy, low and high (typically 40 and 70 keV, correspondingly).

The attenuation rates of each component are related to its elemental composition (specifically density and thickness). The attenuation of the photon energy is a function of the initial photon energy of the X-ray beam, the mass per unit area of the absorber material, and the mass attenuation coefficient of the absorber (Pietrobelli et al., 1996). When photons at two different energies (namely lower and higher) drive through an absorber, attenuation at the lower energy can be expressed as a ratio (R) to attenuation observed at the higher energy. As described by Pietrobelli et al. (1996), R is a function of mass attenuation coefficient and mass fraction of each component, considering a homogeneous absorber. Consequently, each element has a characteristic mass attenuation coefficient and an R-value at a given energy. For example, bone presents relevant attenuating minerals (Ca and P), that allow its distinction from soft tissues (Pietrobelli et al., 1996).

DXA first separates pixels into those with soft tissue only (FM and fat plus bone free masses) and those with soft-tissue plus bone mineral (FFM), based on the two different photon energies. This means that estimating the soft tissue composition in bone containing pixels is complex and requires some assumptions. As indicated by Pietrobelli et al. (1996) a typical approach is to assume a similar soft tissue composition over bone as for the average of all of the surrounding soft tissue. Normally, 40% to 45% of the whole-body scan contains bone in addition to soft tissue. Hence, a systematic individual error is introduced, as there might be deviations in uniformity in soft tissue distribution (e.g., thorax and appendicular regions). The aforementioned error can be further increased when tracking body composition compartments is required.

Considering DXA's good precision, large availability, and low radiation dose its measurement presents several advantages over other laboratory methods (Toombs et al., 2012). In the early 1990s, the replacement of the original pencil-beam densitometers by the fan-beam devices allowed for better resolution and faster scans while maintaining the accuracy, without increasing considerably the radiation dose. Thus, the burden of use of DXA for both patient and clinicians was eased (Toombs et al., 2012). However, caution must be taken when using DXA on multiple occasions, not only due to the cumulative radiation dose, but also due to the error of measurement, which limits the ability to detect small body composition changes over time, leading to misinterpreting data (Santos et al., 2010; Toombs et al., 2012).

As underlined by Toombs et al. (2012), despite DXA's accuracy, precision, reliability, high speed, and non-invasive estimates with minimal radiation exposure, some limitations should be mentioned including: i) algorithms calculations differ between manufacturers and are not published; ii) pencil and fan-beam densitometers accuracy differ; iii) limited active scan area. This last constraint is particularly relevant for athletes involved in sports where height is a major factor of performance (e.g., basketball and volleyball) or obese individuals who are broader than the DXA scan area. Thus, alternative procedures are required to allow complete whole body scans for people taller and/or larger than the DXA scan area (Silva et al., 2013). Another relevant issue is the reliability of DXA in assessing body composition, in particular in very active populations. To address this issue, Nana et al. (2015) have extensively reviewed the literature about the reliability of DXA estimations in athletic and very active populations and provided a best practice scan protocol likely to serve the interests of researchers, coaches, and athletes.

Four-compartment model

In addition, the classic Siri (1961) model can be extended to a 4C model by adding bone mineral (Mo) (Baumgartner et al., 1991; Friedl et al., 1992; Fuller et al., 1992; Heymsfield et al., 1990; Selinger, 1977; Siconolfi et al., 1995; Wang et al., 2002; Withers et al., 1998).

The formula for the 4C model, which controls for the biological variability in body TBW, Mo, and residual can be generated using the same concept as for the 2C and 3C models:

$$\frac{1}{Db} = \frac{FM}{FM_D} + \frac{TBW}{TBW_D} + \frac{Mo}{Mo_D} + \frac{Res}{R_D} \qquad (1)$$

where Db is body density, FM is fat mass, TBW is total-body water, Mo is bone mineral, res, is residual, and D is density.

By assuming the densities of the molecular components, it is possible to derivate the following equation:

$$BV = \frac{FM}{0.9007} + \frac{TBW}{0.99371} + \frac{Mo}{2.982} + \frac{Res}{1.404} \qquad (2)$$

where BV is body volume, FM is fat mass, TBW is total-body water, Mo is bone mineral, and res, is residual.

Although multi-component models share assumed constant densities for FM, TBW, and Mo, two main strategies are applied in developing these models. In one approach, residual mass (Res) is assumed to be protein and soft tissue minerals of known densities after subtracting FM, TBW, and Mo. The other approach is to assume a combined residual mass (i.e. protein, soft tissue mineral, and other) of known density (Wang et al., 2005). In equation 2, a value of 1.404 g/cm^3 is assumed for the residual mass density (Allen et al., 1959).

Generally, these models were developed from simultaneous equations, which may include two or more unknown components, and/or the measurable property. Body density is assessed by densitometric techniques (underwater weighting or air-displacement plethysmography), TBW by isotope dilution and Mo from DXA.

Table 1. Examples of body composition molecular models to estimate FM

	Author	**Equation for FM (kg) estimation**	**Main assumptions**	**Methods and measures**
2-COMPARTMENT	Behnke, 1942; Brozek et al., 1963	4.57 x BV – 4.12 x BM	FFMD = 1.10 g/cm^3 Constant proportions of TBW, protein, and mineral in FFM	UWW/ADP: BV
	Siri, 1961	4.95 x BV – 4.50 x BM		
	Pace and Rathbun, 1945	BM – 1.3661 x TBW	TBW/FFM = 0.732	Isotope dilution: TBW
3-COMPARTMENT	Siri, 1961	2.057 x BV – 0.786 x TBW – 1.286 X BM	M/Prot = 0.351 M to Prot = 1.565	Isotope dilution: TBW UWW/ADP: BV
	Withers et al., 1998	2.115 x BV – 0.78 x TBW – 1.348 X BM	M/Prot = 0.354 M to Prot = 1.569	
	Lohman, 1986	6.386 x BV + 3.961 x M – 6.09 x BM	TBW/protein = 3.80 $(TBW+Prot)_D$ = 1.0486	UWW/ADP: BV
4-COMPARTMENT	Selinger, 1977	2.747 x BV – 0.714 x TBW + 1.129 x Mo – 2.037 x BM	Ms = 0.0105 x BW	Isotope dilution: TBW UWW/ADP: BV DXA: M/Mo/TBBA
	Heymsfield et al., 1990	2.748 x BV – 0.6744 x TBW + 1.4746 x TBBA – 2.051 x BM	Ms = TBBA x 0.235	
	Baumgartner et al., 1991	2.747 x BV – 0.7175 x TBW + 1.148 x M – 2.05 x BM	Ms = 0.235 x Mo	

	Fuller et al., 1992	2.747 x BV – 0.710 x TBW + 1.460 x TBBA – 2.05 x BM	Ms = TBBA x 0.23048	
	Friedl et al., 1992	2.559 x BC – 0.734 x TBW + 0.983 x Mo – 1.841 x BM	ResD = 1.39 (Res = Prot + Ms + G)	
	Withers et al., 1998	2.513 x BV – 0.739 x TBW + 0.947 x Mo – 1.790 x BM	ResD = 1.404 (Res = Prot + Ms + G)	
	Siconolfi et al., 1995	2.7474 x BV – 0.7145 x TBW + 1.1457 x M – 2.0503 x ~BM[#]	M = TBAA / 0.824	
	Wang et al., 2002	2.748 x BV – 0.699 x TBW + 1.129 x Mo - 2.051 x BM	Ms = 0.0129 x TBW	

Model developed considering 3.037 as the total mineral density;
* Model obtained considering the density of FFM and fat at 37ºC; Abbreviations: BM, body mass; FM, fat-mass (kg); BV, body volume; TBW, total body water; M, total mineral; Mo, bone mineral; TBBA, total body bone mineral; FFM, fat-free mass; FFMD, FFM density; Prot, protein; M/ProtD, total mineral + protein density; TBW/ProtD, total body TBW + protein density; Ms, soft mineral; ResD, residual density; DXA, dual-energy X-ray absorptiometry; UWW, underwater weighting; ADP, air-displacement plethysmography.

General discussion and conclusion

This contribution reviewed the several methods that are available to assess body composition at the molecular level. Two, three and four compartment molecular models were reviewed, and methodological issues were detailed.

Although not yet a reference method at the molecular level, DXA is becoming more accessible and widespread as a technique to assess body composition by providing total and regional estimates of FM, bone mineral and fat plus bone free masses (3C model). Nevertheless, to improve DXA reliability of body composition measurements, studies should follow the recommendations proposed by Nana et al. (2015) regarding the scan protocol. Additionally, to overcome the methodological constraints due to the limited DXA scan area, alternative procedures should be adopted to determine fat and FFM in taller or broader individuals (Silva et al., 2013).

The 4C model is considered the state-of-the-art method for body composition measurement as it includes the evaluation of the main FFM components, thus reducing the effect of biological variability. This model allows the assessment of various assumptions central to 2C models, as FFM density and hydration, respectively using densitometric or hydrometric techniques. Such assumptions invalid FM estimation, as physiological processes (such as growth, aging, exercise training, disease states) affect the proportions of the main FFM components.

Although the 4C model is considered the reference method for determining body composition at the molecular level, it is time consuming, costly, difficult to apply in certain population groups, and involves sophisticated analytical techniques that are impractical in non-research facilities. Additionally, reliability of the 4C model may be offset by the propagation of measurement errors associated with the determination of BV, TBW, and Mo, particularly in determining small body composition changes. However, its reliability is not compromised if technical errors are relatively small. Recent technological advances highlight opportunities to expand model applications to new groups and measured components (Heymsfield et al., 2015a).

Research Highlights

- The 4C model is considered the state-of-the-art method for assessing fat and FFM at the molecular level of body composition analysis.
- Implementation of the 4C model is less practical in non-research facilities given the high cost and technological complexity.
- If recommendations regarding the scan protocol are adopted, DXA, a 3C model at the molecular level, provides precise and accurate measures of fat and FFM and is becoming more accessible in non-laboratorial settings.

Part II – Bioelectrical impedance analysis: principles, instruments, and measurement methods

Principles, advantages and limitations of bioimpedance analysis

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Body composition is one component of the physiological profile of an athlete (Lukaski, 2017). Fat-free mass (FFM), lean soft tissue mass (LSTM; FFM – bone mineral content) and muscle mass (MM) are functional body components that are related to strength, power and performance. Hydration, including total body water (TBW) and its distribution [intra- and extra-cellular water (ICW and ECW)], also directly impacts physical training, recovery and performance. In contrast, fat mass (FM) is non-functional, although a small, ill-defined, amount is required for the health of an individual. FM is mechanically and metabolically detrimental to sport performance and adversely affects thermoregulation. Bioimpedance analysis (BIA) is a safe, practical and valid method to assess and monitor body hydration and certain aspects of functional mass.

Principles

BIA provides direct, uncomplicated, noninvasive measurements of bioelectrical properties of the body that allow interpretations of soft tissue hydration, a fundamental component of LSTM and MM, and membrane integrity of an individual (Lukaski et al., 2019). BIA determines the impedance (Z) or the hindrance of an applied alternating electrical current that is related to water and electrolytes in body fluids and tissues, and ascertains the delay of alternating current penetration at cell membranes and tissue interfaces (points of contact between contiguous tissues).

Application of BIA to sport requires empirical models that enable the interpretation of passive bioelectrical measurements as related to specific body components. BIA considers the human body as a network of resistors (R), consisting of physiological fluids (ICF and ECF), and capacitors (C) comprised of cell membranes and connections among adjacent tissues (Lukaski et al., 2019). Early biophysical models simplified the body as a single cell in fluid and described the conductivity of alternating current as R and C in a parallel array consisting of extracellular and intracellular conductive pathways (Figure 1).

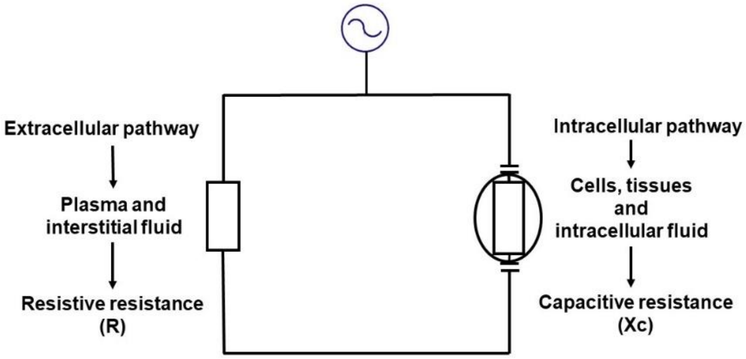

Figure 1. Characterization of a cell with extracellular and intracellular electrical pathways including resistive (R) and capacitive (Xc) resistance components.

A more relevant physiological model depicts the body as tissues and ionic fluids in a parallel RC equivalent circuit (Figure 2). An applied low-frequency alternating current divides into resistive (fluid and electrolytes) and capacitive (cell membranes and tissue interfaces) pathways. The lower resistivity of body fluids and tissues containing water and electrolytes, compared to intact lipid-containing cell membranes, enables frequency-dependent impedance measurements. The flow of alternating current in the body depends on conductivity, which is the opposite of resistivity, of individual fluids and tissues (Figure 3). Among the various conductors in the body and arranged in parallel, current flows mainly through the best conductor. The overall R of a parallel array of resistive conductors is $R = 1/R_{blood} +$

$1/R_{ECF} + 1/R_{LSTM} + 1/R_{adipose\ tissue} + 1/R_{fat} + 1/R_{connective\ tissue} + 1/R_{bone}$. Thus, body components with low resistivity are good conductors.

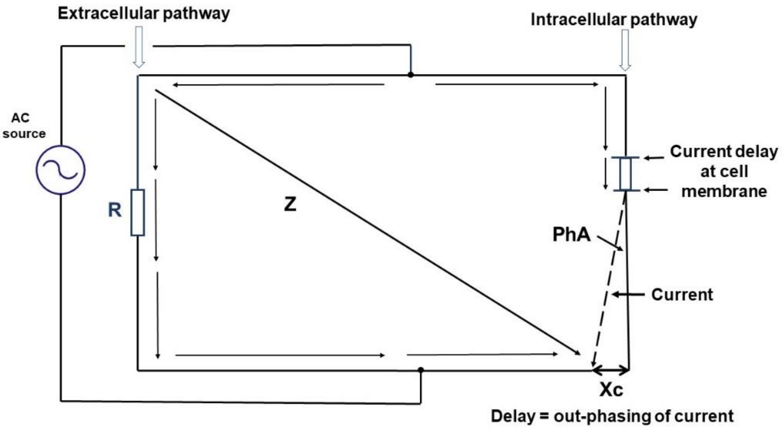

Figure 2. Representation of the body as a parallel resistor-capacitor (RC) network with the cell membrane capacitance causing an out-phasing of current and voltage delay resulting in reactance (Xc), phase angle (PhA) and a shift in impedance (Z). Abbreviated line indicates current delay (out-phase) at cell membrane.

The low-level alternating current passes mostly in-phase (without significant delay) mainly through the extracellular resistive component but is delayed (temporarily stored) by seemingly capacitive elements, such as cell membranes separating ECF and ICF. A variable amount of very low-frequency current, regardless of the frequency, however, can penetrate the membranes of muscle cells, particularly when the current is parallel to the muscle fiber. Cell membranes are capacitive elements surrounding the ICF in a series circuit that exists in parallel with the water-containing interstitial gel of ECF. Any alternating current will penetrate the capacitive component of the cell membrane in proportion to the frequency of the applied current and result in capacitive resistance of the ICF.

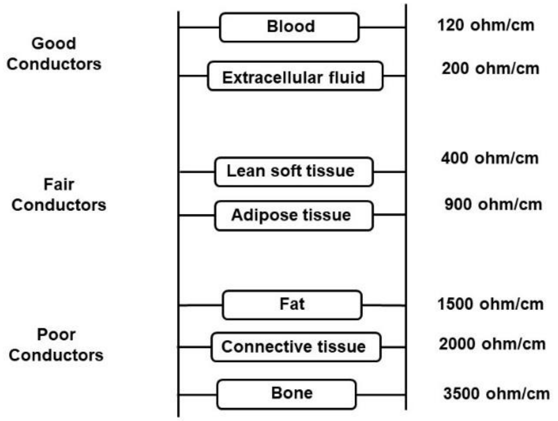

Figure 3. Illustration of the resistivity of the physiological components of the mammalian body. Adapted from Geddes and Baker (1967).

Capacitance (CAP) is the ability of a system or circuit to store an electrical charge. Reactance (Xc) is inversely related to frequency (f) and CAP (Farad): Xc (ohm) = 1/[(2Π x f x CAP)]. Cell membranes and tissue contact areas cause an out-phasing (delay) of the current and a voltage drop. Importantly, CAP, or more appropriately Xc, when viewed in relation to the resistive component, contributes to the phase angle (PhA), which is an angular index of the delay between current and voltage at the cell membrane: PhA (degree) = arc tan [Xc/R] · [180°/Π]. BIA devices display series Z, R, and Xc values.

The parallel RC network model shows resistive (ECF) and capacitive (ICF) resistance. These parallel conductors improve conductivity and, hence, decrease Z, which is consistent with experimental findings of an inverse relationship between Z and TBW (Lukaski et al., 2019). They also contribute to a negative PhA that BIA devices report as a positive value.

Physiological Correlates of BIA Measurements

Physiological interpretation of BIA measurements obtained with an alternating current at 50 kHz and a phase-sensitive device depends on the biophysical model employed. In a series model, R and Z refer to ECW (ECF) and TBW, respectively, and are highly correlated ($r > 0.95$). Series Xc relates to cells and ICF collectively. Thus, series Xc provides an index of cell population density (cells in water and electrolytes) and may be interpreted qualitatively as cell mass and as a biomarker of cell integrity or quality.

The parallel array model provides additional associative fluid and cellular information. Similar to the series model, parallel R and Z indicate ECW (ECF) and TBW, respectively. Parallel Xc, however, describes cells and ICF separately, and allows quantification of body cell mass (Talluri, 1998).

The bioelectrical model impacts the magnitude of R, Xc and Z values. Series Z is defined as $Z^2 = R^2 + Xc^2$, so that changes in series R and Xc directly affect the value of Z. For example, an increase in R or Xc will increase the magnitude (value) of series Z. In contrast, changes in parallel R and Xc values indirectly affect parallel Z values: $1/Z^2 = 1/R^2 + 1/Xc^2$. Thus, an increase in parallel R or Xc decreases the value of parallel Z.

An example shows the potential value of parallel BIA measurements to track fluid changes in sport. Table 1 describes the effects of a sport season on changes in mean measured fluid volumes and corresponding average reported raw series and calculated parallel BIA values. An increase in ICW (1.7 L, 5.9%) and TBW (2.4 L, 4.9%) with little change in ECW (0.7 L, 3.4%) was detected by parallel compared to series Xc (-10.9 vs 0%, respectively) and CAP (-12.2%). In contrast, a decrease in ICW (-1.5 L, -4.7%) and TBW (-2.3 L, -4.4%) and negligible loss in ECW (-0.7 L, 3.4%) was revealed similarly with series and parallel R (2.9 and 2.6%) but improved with series compared to parallel Xc (5 vs 1%, respectively) with a negligible change in CAP (<1%). This preliminary analysis reveals the potential value of parallel BIA values and CAP to identify and track changes in ICW and hence body cell mass. Future research should determine the sensitivity and specificity of parallel BIA to identify fluid changes by using height-adjusted R and Xc values to account for inter-individual differences in body geometry (conductor length).

Table 1. Summary of series (s) and parallel (p) BIA measurements relative to changes in measured fluid volumes of athletes. TBW = total body water, ECW = extracellular water, ICW = intracellular water, R = resistance, Xc = reactance, CAP = capacitance. Adapted from Campa et al. (2019a).

Mean data of male athletes who gained TBW

	Pre	Post	Δ	%Δ
TBW, L	49.2	51.6	+2.4	+4.9
ECW, L	20.3	21	+0.7	+3.4
ICW, L	28.9	30.6	+1.7	+5.9
R-s, ohm	491	463	-28	-5.7
R-p, ohm	498	471	-27	-5.4
Xc-s, ohm	60	60	0	0
Xc-p, ohm	4078	3633	-445	-10.9
CAP, pFarad	781	876	-95	-12.2
Z-s, ohm	495	467	-28	-5.7
Z-p, ohm	444	416	-27	-6.1

Mean data of male athletes who lost TBW

	Pre	Post	Δ	%Δ
TBW, L	52.1	49.8	-2.3	-4.4
ECW, L	20.4	19.7	-0.7	-3.4
ICW, L	31.7	30.2	-1.5	-4.7
R-s, ohm	448	461	+13	+2.9
R-p, ohm	456	468	+12	+2.6
Xc-s, ohm	60	63	+3	+5
Xc-p, ohm	3405	3436	+31	+0.9
CAP, pFarad	935	926	-9	-0.9
Z-s, ohm	452	465	+13	+2.9
Z-p, ohm	417	402	+15	+3.6

Advantages of BIA

BIA is a safe, practical, and reliable laboratory and field method with emerging applications in sport. Its principal value is as a real time, non-invasive alternative to isotope dilution to characterize fluid volumes and monitor changes in fluid distribution after physical activity. Observational and longitudinal studies demonstrate that 50 kHz phase-sensitive BIA measurements are significantly related to reference (isotope-dilution determined) fluid volume measurements (Campa et al., 2019a; Francisco et al., 2020), and confirm R/H and Z/H significantly correlated with TBW and ICW, and PhA significantly correlated with gender-specific fluid distribution (ECW/ICW) in diverse groups of athletes.

BIA identifies changes in body fluid distribution and composition associated with adaptations in function subsequent to training and sport participation. Resistance training of young adults and older women significantly increased PhA (5 and 8%, respectively), independent of gender, with smaller increases in FFM (2%), whereas detraining significantly decreased PhA (-8%) with minimal loss of FFM (-2%) (Sardinha, 2018). The training-induced increase in PhA depended on simultaneous changes in Xc and R; a significant increase in Xc and a smaller decrease in R, confirmed with tracer dilution determinations of increases in ICW and TBW. An increase in muscle cell volume, using ICW as a surrogate, due to hypertrophy was proposed to explain the increase in Xc, and hence PhA. Also, independent of FFM, PhA was significantly related to maximal power (r = 0.66) and inversely related to fatigue (r = -0.7) in soccer players (Nabucco et al., 2019). Thus, PhA can be a categorical variable to monitor the impact of physical training on various measures of muscle quality and function. All interpretations of PhA, however, should concurrently assess hydration based on vector length (Z/H) and position on the RXc graph (Lukaski et al, 2017).

BIA detects small changes, acutely and chronically, in hydration (Lukaski and Piccoli, 2012) and, thus, is amenable to track fluid distribution changes in responses to sport training and competition. Significant vector lengthening with increases in R/H and Xc/H occurred with a 2% decrease in body weight and significant increases in plasma osmolality after an acute bout of prolonged exercise in the heat (Gatterer et al., 2014). Importantly, Xc was significantly and inversely related to increases in osmolarity suggesting an isotonic hypohydration due to a decrease in ECF. Similar findings were reported after a single bout of training and sport competitions with recovery and characterized by significant increases in Z/H, R/H and Xc/H (Castizo-Olier et al., 2018a). Thus, phase-sensitive BIA measurements can detect a pattern of fluid loss acutely after a bout of physical training or a competitive event.

A novel use of BIA measurements is to detect deleterious changes in fluid distribution associated with increased risk of acute kidney injury (AKI; Nescolarde et al., 2020). Ultramarathon runners with significantly elevated, compared to normal, post-race serum creatinine levels, diagnostic of AKI, had significant shortening of vector lengths and reduced Xc/H values with significantly increased blood levels of biomarkers of inflammation and muscle injury 48-h after the race. Thus, BIA revealed inflammation-related expansion of ECF. Forty-eight hours after the race, runners with low Xc/H (< 30.5 ohm/m) had 87% sensitivity and 92% specificity of risk of AKI. These findings identify a new role of BIA to detect fluid disturbances associated with muscle injury and inflammation as a prognostic biomarker for increased risk of acute renal damage.

Limitations of BIA

BIA relies on passive bioelectrical measurements of the body to detect alterations in fluid distribution. Uncontrolled factors directly and predictably affect BIA measurements and are sources of error that can lead to misinterpretation of physiological status.

Ohm's Law is the physical basis of BIA. It states the volume of a conductor (fluid and electrolytes) depends on the length and cross-sectional area of a cylindrical conductor, and assumes constant geometry and uniform resistivity due to constant hydration and composition. The human body, however, consists of five cylinders (arms, legs and trunk) with different lengths, cross-sectional areas and composition. Whole-body BIA measurements also presume a constant upper limb-to-trunk-to lower limb ratio (Organ et al, 1994). BIA measurements on the lower leg and arm contribute the greatest proportion of R (25 to 35%) but contain the least conductor volume (lean soft tissue mass; 1 to 2%) (Figure 4). Differences in limb length and cross-sectional area may explain this discrepancy. Thus, whole-body BIA measurements have random error due to inter-individual differences in body size and geometry resulting in non-uniform conductor homogeneity.

Limited research has examined the effect of correcting BIA measurements for individual assessments of regional conductor size and shape. Marini et al. (2020b) compared relationships between whole-body BIA measurements normalized for length (height) and adjusted for cross-sectional area, which was estimated using circumferences of the arm, waist and mid-calf, and length with estimated fluid volumes in athletes. Traditional Z/H explained significantly more variance in TBW than Z corrected for body shape and length (~70 vs 3%). These data are consistent with the notion that whole-body BIA measurements normalized for height are a reasonable surrogate for bioelectrical conductor volume. However, further research is needed to ascertain the value of traditional and geometry-individualized BIA measurements subsequent to changes in fluid volume and distribution with physical training. Additionally, critical evaluation of the contribution of site-specific differences in size and shape to regional BIA measurements might accommodate inter-individual differences in resistivity for regional fluid distribution and muscle assessments.

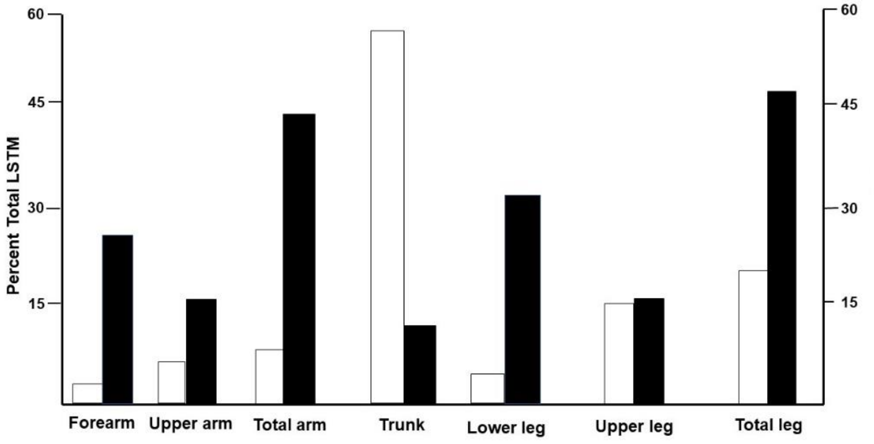

Figure 4. Relative contribution (% total) of body lean soft tissue mass (LSTM) (open bars) and resistance at 50 kHz (closed bars) of 10 athletes. Adapted from Lukaski and Scheltinga (1994).

Another concern is the contribution of water in adipose tissue (AT) to bioelectrical conductor volume. Chemical analyses of excised AT reveal the approximate water content of mammalian AT is 20% (DiGirolamo and Owens, 1976). Inter-individual differences in volume of AT and changes in amount of AT with interventions directly affect BIA measurements.

Physical and technical factors affect BIA measurements (Gonzalez-Correa and Eraso, 2012). Some concerns include the environment (temperature, humidity and measurement surface), subject presentation (hydration state, recent food intake, physical activity), skin conditions (sweat, temperature), breathing rate, limb position, electrode characteristics (composition, size, spacing, placement sites), and BIA instrument properties (current, phase-sensitivity, frequency; measurement accuracy, reproducibility). Standardization of these factors is needed to optimize the quality of BIA measurements.

Research Highlights

- Series Xc relates to cells and ICF collectively, describes cell density and may be interpreted as cell quality. Parallel Xc describes cells and ICF separately. CAP may be a useful, single, independent indicator of body cell mass.
- Changes in fluid volumes (ICF and ECF) result in concurrent changes in series R and Xc measurements (e.g., hypohydration = ↑ Xc and ↑ R).
- Interpretation of PhA values should consider vector length (Z/H) as an indication of TBW.
- Series BIA measurements significantly predict TBW and ECF of athletes and identify group changes in fluid distributions with training and competition. Confirmation with reference dilution methods is needed.
- Standardization of BIA test conditions is necessary to expand use of BIA in sport applications.

Bioimpedance devices and measurement procedures

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As previously presented, the assessment of body composition in scientific or clinical settings has been proposed for the analysis of the behavior of body components, such as body fat, lean body mass and total body water, throughout the life cycle.

Although methods considered highly valid for the assessment of the components are proposed, such as dual energy x-ray absorptiometry (DXA), magnetic resonance imaging, computed tomography and isotope dilution methods, they are more expensive, with complex methods that cannot be reproduced frequently, either in the laboratory or in an epidemiological way.

As a valid alternative, the bioelectrical impedance analysis is a non-invasive, relatively inexpensive, and low complexity method, which has been used with accepted validity for the body composition assessment, especially in the evaluation of body fluids, lean body mass and fat mass. However, with less validity than the aforementioned methods, greater attention should be given to the procedures and equipment used, in order to reduce inter and intra-evaluator errors.

In this sense, in addition to the knowledge of the biophysical principles that support the assumptions of bioimpedance analysis for the assessment of body composition in humans, it is also necessary to know and understand the equipment and methods of assessment, to maintain the validity of the method throughout the evaluation process.

Biophysical Principles of Bioelectrical Impedance

As already presented in detail in previous chapters, the biophysical principles of bioimpedance analysis for the assessment of body composition are based, in a very brief way, on the assumption that the human body behaves as a geometric cylinder. Once the electric current emitted by the bioelectrical impedance analyzer is emitted through the body, two impedance parameters, depending on the frequency, can be evaluated: resistance (R) and reactance (Xc) (Mulasi et al., 2015). The resistance is the opposition generated by the different tissues to the current flow when passing through the body, and reactance is the delay in electrical conduction caused by cell membranes of different types of cells, tissue interfaces and non-ionic substances (Lukaski, 1996; Matthie, 2008). Such delay creates a phase shift, also called a phase angle (PhA), calculated mathematically as the ratio of the tangent arc of the reactance to the resistance (tangent arc [Xc / R] · [180°/Π]).

Hence, in body composition assessment in healthy people, it is assumed that body components with a higher level of hydration (also called conductors components), the lower the resistance offered to the electric current, the lower the R values presented by the analyzer. The inverse condition is also assumed, since higher R values indicate greater resistance to electric current, due to the greater amount (absolute and/or relative) of insulating or hydrophobic tissue.

Likewise, higher Xc values indicate cell membranes with higher integrity. In other words, cells considered healthier have more integer membranes, which cause greater resistivity to the electric current, and therefore, present in the BIA assessment, higher Xc values. On the other hand, less intact membranes, due to lower cell health, either due to different clinical conditions, such as the presence of chronic diseases or injuries, for example, or due to lower global/systemic functionality, will provide lower values of Xc.

Finally, the understanding of the operations of the different types of equipment is important to manage them to different assessments with distinct goals.

Electrical currents emitted at very low frequencies does not permeate cell membranes, with a higher capacitance, promoted by the double layer of impermeable lipids in the membrane. In this case, the device will only quantify the extracellular water of the body. Electric currents emitted at higher frequencies, especially above 50 kHz, are greater than the capacitance of the cell membrane, and thus permeate into the cell internal space. In this case, the device will be able also to measure total body water (Earthman et al., 2007; Matthie, 2008).

Devices with low and high intensity electrical current emission, concomitantly, consequently allows the quantification of both extracellular water (at low frequencies) and total body water (at high frequencies). By the mathematical difference between total body water and extracellular water, it is then possible to estimate intracellular body water. From the quantification of intracellular water, it is theoretically possible to estimate body cell mass, based on the assumption that body cells mass is composed of ~70% of water (Moore, Boyden, 1963).

With such assumptions, the potential applications available for the assessment of body composition through bioelectrical impedance analysis depend on the nature of the device used, including the number of frequencies and frequency range, as well as the software's capacity, among others. Commercially, three categories of bioelectrical impedance analyzers with different methodologies are offered: single frequency, multiple frequency and bioimpedance spectroscopy. Although single frequency analyzers were initially used for both research and clinical care, as well as being the most abundant commercially, devices with multiple frequency analysis and bioimpedance spectroscopy are becoming increasingly commercialized and used in the clinical and scientific environment.

Single frequency bioelectrical impedance analysis

Normally, single frequency bioelectrical impedance analyzers (SF-BIA) are presented with the single frequency option of 50 kHz, in the tetrapolar model (with placement of 4 contact electrodes on the wrist and ankle). These equipment models are the most widely used bioimpedance approach to estimate total body composition. Impedance data measured at a single frequency at 50 kHz is used to estimate body components using predictive equations of body composition, and also for bioelectrical impedance vector analysis (BIVA).

The specific biophysical principle of the SF-BIA model assumes that the human body is a uniform conductor with constant geometry and composition. In this condition, R is directly related to the product of the resistivity of a given body segment (ϱ, ohm m) and the conductor length (L, cm), and indirectly related to the area of the conductor cross section (A, cm^2). Thus, it is possible to express R from the mathematical expression $R = \varrho (L/A)$. An adjustment, based on the ratio of the squared height by R (Ht^2 / R), is made, which provides the estimation of the total body water volume (V, cm^3), from the mathematical equation $V = \varrho (Ht^2 / R)$ (Kyle et al., 2004a; Lukasky, 2013).

The main limitations of SF-BIA concern the consideration that the human body is assumed to be a geometric and symmetrical cylinder, with homogeneous composition and uniform cross-sectional area, which implies some methodological problems, in view of the knowledge that, physiologically, such approach is theoretical. In this condition, the best approach for the biophysical explanation would be an approach with five distinct cylinders: four cylinders for upper and lower right and left limbs and one cylinder for the trunk (Ellis et al., 1999)

It is also important to mention that both volume and mass presented by SF-BIA come from predictive equations (the majority developed in specific populations, with variables such as weight, height, age, in addition to BIA variables, such as impedance, resistance, and to a lesser extent, the reactance). Such equations, in large part, were developed and validated in healthy people, with normal weight and highly controlled conditions (Kyle et al., 2004a). Thus, for a better results of body components and water volume, the choice of predictive equations developed and validated in populations similar to the samples used in each study is extremely important. Finally, in the assessment of the area, an important problem to note is that many devices do not present Z, R or Xc values, which does not allow researchers or clinicians to choose the respective equations, as well as the equipment manufacturers do not provide the equations used for the body components in the assessment of body composition.

Although there are limitations for the analysis of bioelectrical impedance with single frequency, it is a fact that currently many scientific studies with BIA variables, with clinical and/or nutritional evaluation, have shown results with raw BIA parameters (R, Xc and PA), from equipment with a frequency of 50 kHz, without the presentation of body component values predicted by regression equations. As an alternative, the phase angle has been used especially with this specific frequency, for nutritional and in physical exercise studies, including the presentation of reference values for specific populations (Mulasi et al., 2015).

Finally, for almost 3 decades, BIVA using SF-BIA equipment at 50 kHz (Piccoli et al., 1994), has been used in different work environments in the health area, from the response to dialysis of chronic renal patients to the relationship with functional variables, such as muscle strength.

Multiple frequency bioelectrical impedance analysis

As presented before, it is possible in BIA devices the emission of frequencies with different intensities, ranging from very low to high intensities, for the evaluation of different body components. This approach is called multiple frequency bioelectrical impedance analysis (MF-BIA), and like SF-BIA, it is commonly performed with tetrapolar equipment, with electrodes placed on the wrist and ankle. The differential of the MF-BIA is the use of two or more frequencies emitted for the quantification of body components, based on predictive equations, also specific to different populations (Kyle et al., 2004a; Earthman et al., 2007).

As a rule, MF-BIA devices emit the first current at low frequency (between 1 and 10 kHz) and following, another frequency or several higher frequencies, usually from 50 kHz, up to approximately 500 kHz. In this way, and as previously presented, it is possible to calculate total body water and its intra- and extracellular fractions.

In addition to this feature of the MF-BIA in quantifying body hydration, this model has also been used to assess segmental body composition (Lorenzo and Andreoli, 2003). The segmental MF-BIA operates under the assumption that the human body is not just one but five distinct cylinders with different resistivities and impedances, as previously demonstrated. As in the SF-BIA assessment, the contribution of the trunk in the impedance measures is approximately 10%, thus significant changes in this region of volume or tissues, especially adipose tissue, may not be accurately represented in the total body composition or volume. These significant changes are frequently observed in obese patients, with weight change and with different chronic conditions. MF-BIA provided a correction, in view of the segmented assessment. Even so, the raw values of BIA should be, just as for SF-BIA, applied to predictive equations (Shafer et al., 2009).

Bioimpedance spectroscopy

Bioimpedance spectroscopy (BIS) is the third BIA approach for assessing whole body composition. BIS equipment is commercially available in smaller quantities and brands compared to SF-BIA and MF-BIA equipment; however, they have great application in clinical and scientific area.

BIS contemplates the use of impedance data measured at frequencies that normally range from 5 to 1000 kHz (Lukaski, 2013). Clinically, the analysis of frequencies for the quantification of body fluids is performed using software that accompanies the devices. These software, on most commercialized devices, are programmed to perform biophysical modeling on the impedance data, with adjustment of the spectral data to the Cole-Cole model using the adjustment of the nonlinear curve (Ward and Muller, 2013; Kyle et al., 2004b). The Cole-Cole model is a mathematical model that best describes this type of physiological data. This procedure generates the terms of the Cole-Cole model, with the resistance associated with extra-cellular water (R_e), intracellular water (R_i), the capacitance of the cell membrane (C_m) and the exponent α.

With the parameters presented, the characteristic frequency (fc), which is the frequency at which the effects of the cell membrane capacitance are maximum, is calculated (Kyle et al., 2004b). In the sequence, the Cole-Cole model terms are applied to the equations derived from the mixture theory of Hanai (Ward, 2013), which assumes that the human body is a conductive medium for water, electrolytes and lean tissue, as well as non-conductive material within it (for example, bone and fat). Unlike the MF-BIA, where intracellular water is calculated from the difference between total body water and extracellular water, in BIS, the amounts of intra- and extracellular water are calculated individually, and the total body water is presented as the sum of the two fractions.

The major difference of BIS from SF-BIA and MF-BIA is the fact that BIS is not based on predictive equations, derived statistically, for the estimation of body components, but for the volumes of intra- and extracellular water. In the BIS methodology, separate resistivity constants (derived from isotope dilution reference techniques) for each of the fluid compartments (for males and females, differently) are applied to the equations of volume, so that the amounts of intra- and extracellular water are not evenly distributed (Matthie, 2008).

There are possible limitations to the BIS methodology, especially in the fact that the constants applied to the equations are used to calculate the intra- and extracellular water volumes, which can induce potential errors in the estimates of these volumes. In the Hanai mixture theory, fixed values for resistivity of body water fractions and body densities, in addition to body shape, are applied to the equations. Such constants have been shown to be suitable for the assessment of body composition in a wide range of ages, but there is still a need for more accurate research when evaluating people with excess fat tissue, especially in the trunk region, and patients with fluid imbalance associated with injuries and diseases.

In this sense, the choice of different methodologies - SF-BIA, MF-BIA, and BIS - must be made with a lot of criteria, especially when the decision comes from health professionals who apply the results in clinical studies or scientific research. Thus, it is equally important to choose the type of analyzer to be used.

Types of Impedance Analyzers

Medical bioimpedance devices may be used in the supine and standing position. In the supine position, for the whole-body analysis, evaluations are usually performed with four gel-type electrodes, placed in pairs, on the wrist and ankle, with two tension electrodes (in the most proximal part of the body segment) and two current electrodes (in the most distal part of the body segment). For this type of equipment, SF-BIA, MF-BIA, and BIS methodologies can be used, therefore, they are frequently applied in clinical and scientific environments (Jaffrin, 2009).

In the standing position, bioelectrical impedance analyzers are found more frequently for domestic use, for the public. These analyzers are also called leg-to-leg or foot-to-foot impedance measurements and consist of a body scale with four reusable electrodes at its base, for direct contact with the feet. This equipment is usually cheaper, and evaluations are performed with patients in an upright position. They are easy to operate, and offer values of body components, fat, and lean mass, in addition to total body weight (Jaffrin, 2009).

The biggest limitation of these equipment is that the current flows only in the lower limbs and in the lower part of the trunk, and the values for the rest of the body are extrapolated, based on predictive equations of the manufacturers, almost never available to users.

Like the leg-to-leg or foot-to-foot analyzers, there are hand-to-hand analyzers, with the same proposal, but with the extrapolation of values to the lower limbs. These upper body analyzers only have electrodes for contact with the fingers and hands, and measurements are also made in the standing position, with the arms extended horizontally.

Finally, more recently, equipment for clinical and scientific use was commercially presented, with the possibility of evaluation in the vertical position, but with contact of the hands and feet of the right and left hemi bodies with the electrodes, for segmental evaluation. These devices can measure the impedances of the upper and lower limbs and trunk, based on the sequential connection of the various combinations between the eight contact points (four current electrodes and four voltage electrodes). Thus, estimates of the fat-free mass and adipose tissue of the different segments can be analyzed, using predictive equations (Jaffrin, 2009). Usually, this equipment, considerably more expensive than the others equipment, is presented in a multifrequency version. Validation studies have shown good validity of these devices for the assessment of overweight and obese patients (Jebb et al., 2007), although comparisons for referential methods, such as the 4-compartment method and DXA, have shown differences, however, smaller, compared to other equipment.

Although it is of great importance to choose the equipment for a good conduct of body composition evaluations, for an evaluation with quality, validity and reproducibility, higher attention should be given to the standardization of evaluations and protocols.

BIA Assessment Protocols and Standardization

Normally, measurements with bioelectrical impedance analyzers are made with the patient in the supine position, according to standardized protocols. Despite the possibility of placing the electrodes on the body in different arrangements, the most common proposal is placing two electrodes on the wrist region (one placed on the ulna styloid process and the other just behind the metacarpus), and two electrodes in the ankle region (one placed on the midline between the medial and lateral malleoli, and the other just behind the metatarsals), for evaluation through tetrapolar approach. The evaluation option with eight contact points is usually performed in the vertical position, as already mentioned, but with reusable electrodes, attached to the equipment. In this sense, the concern with proper placement is reduced since the equipment itself automatically adjusts the contact points with the body. Finally, there is the option of segmental, or localized BIA assessment. In this sense, the option of placing the electrodes will be in accordance with the objectives of the evaluator.

However, the concern with the number of electrodes and form of contact is not the only one of the evaluators, clinicians, and researchers. Many other protocols and standards must be considered for the correct and adequate assessment. As previously presented, the bioelectrical impedance analysis has been studied and applied in the clinical environment for approximately 50 years, however, it was only at the end of the 1980s that there was a concerning with the presentation of guidelines for a better experimental design of studies with such equipment. But formally and officially, it was only in the mid 1990's that a document was prepared with the appropriate protocols and standards for users, prepared by the National Institutes of Health (NIH, 1996), which was updated in 1999 by Ellis et al. (1999). Currently, the updated current document is the ESPEN guideline, written by Kyle et al. (2004a).

The criteria for standardizing protocols for assessing body composition by BIA by Kyle et al. (2004a) are listed below, with some recommendations from a systematic review on standardization of bioelectrical impedance analysis for the estimation of body composition (Brantlov et al., 2017), according to each criteria of evaluation.

Instruments and Material. Concerning the instruments and material, attention should be given to the following items: generator, analyzer, cables, electrodes, stadiometer, and scale.

- Generator
    - A consistent signal of reproducible amplitude should be always present, and devices must be often calibrated.
    - Equipment should preferably work with battery to avoid interference with current variations and with autonomy for >20 measurements.
- Analyzer
    - Devices should present measures of R or impedance and Xc or phase angle; the analyzers must pass through regular calibration against known ohmmeter.
    - Automatic verification of skin resistance should be considered, to identify abnormal skin resistance, in cases of excessive resistance (e.g., pachydermia).
- Cables
    - The appropriate length should be up to 2 m.
    - The diameter/isolation of the cables must meet manufacturer's recommendation.
- Electrodes
    - The surface size of electrodes must meet instrument requirements (>4 cm^2).
    - The integrity of gel must be checked, and electrodes must be kept in sealed bag, and protected against heat.
- Stadiometer
    - Calibrated to the nearest 0.5 cm.
- Scale
    - Calibrated to the nearest 0.1 kg, cross-calibrated with other scales.

Subjects. Whenever measuring any patient, attention should be given to height, food and drinking, bladder voided, physical exercise, skin condition, electrode position, limb position, body position, environment, body shape and the ethnic group which the patient belongs.

- Height
    - Measure height (0.5 cm) and weight (0.1 kg) at the time of the BIA measurement. Self-reported height and weight are not valid.
- Food, drink, alcohol
    - Fasting/no alcohol for 48h previously to the assessment is recommended. Shorter periods of fasting may be acceptable for clinical practice.
    - Subjects should (ideally) be normally hydrated.
- Bladder voided
    - Subject should be asked to void bladder before measurement.
- Physical exercise timing
    - Time of measurement must be noted, and no exercise for >8h. For longitudinal follow-up, perform measurement at the same time of day. The menstrual cycle should be noted for assessment in women.
- Skin condition
    - Ambient temperature should be recorded.
    - No skin lesions at the sight of electrodes should be presented, and if so, change site of electrodes.
- Electrode position
    - Clean the site of electrodes with alcohol.
    - Always measure the same body side.
    - Distance between electrodes: a minimum of 5 cm between electrodes must be placed. If needed, move proximal electrode.
    - For whole-body BIA, electrodes should be placed at the dorsal surfaces of the wrist and ankle. Voltage elec-

  - trodes are applied at midline between the prominent bone ends on the wrist (ulna and radius) and the ankle (medical and lateral malleoli). Current electrodes are placed 5 cm distal to these positions (acceptable for larger subjects).
  - For segmental BIA, segmental lengths and circumference should be measured to ±0.1 cm, using a circumference measuring tape. Two voltage electrodes are placed on the exact location where circumference has been measured, and the distance between the electrodes should then be measured from center-to-center of the electrodes. Two current electrodes are located distally to the voltage electrodes.
  - In studies with localized BIA, as for the whole limb segment, composition can be predicted assuming cylindrical geometry using the mean circumference of the segment and inter-sensor electrode length as in "Segmental BIA".
- Limb position
  - Abduction of limbs: arms separated from trunk by about 30^{o} and legs separated by about 45^{o}.
- Body position
  - Body position at the supine position, except for "scale" type BIA instruments, where the vertical position is adopted. Ambulatory subjects should stay at the supine position for 5–10 min. For research protocol, note the time that subjects were supine before measurement, and if patient were confined to bed.
- Environment
  - Electrical interference must be avoided. No contact with metal frame of bed, and a neutral environment (no strong electrical or magnetic fields) should be used for the assessment.
  - Ensure that no metals are in the clothing. If this is met, only remove clothing to access the skin of the electrode sites when doing measurements.

- Body shape
    - Note body abnormalities if there is any. Note measurement validity (e.g., R or Xc outside of expected range for subject). Consider validity of measurement when interpreting results (e.g., abnormally low R suggests edema).
    - If there is any amputation, measurement should be performed at the non-affected limb. Not valid for research because measurement error is consistent but permits determination of body compartment.
    - Also, for atrophy and hemiplegia, the measurements should be performed on the non-affected side.
    - If abnormal limb or trunk (e.g., scoliosis) is verified, the abnormal condition should be noted.
    - In conditions such as dystrophy (HIV, Cushing's syndrome, etc.), there is limited validity in conditions of abnormal body compartment distribution.
    - In obesity conditions, to better adapt the subject in the bed, electricity-isolating material (e.g., towel) between arm and trunk, and between thighs should be used.
- Ethnic group
    - The ethnic group should be noted, for specific BIA equation, if applicable.

Disease conditions. When patients with different conditions, such as chronic diseases, are being assessed, attention should be given to subjects with cardiac insufficiency, liver and kidney failure, abnormal serum electrolyte concentrations and hypothyroidism. Any chronicle condition should be noted.

- Cardiac insufficiency
    - Edema interferes with measurement, so patient should be measured in stable condition.
- Liver failure

    - Ascites/edema interferes with measurement accuracy. In this case, segmental BIA measurement should be considered.
- Kidney failure
    - Edema/altered ion balance interferes with measurement.
- Abnormal serum electrolyte concentrations
    - Electrolyte concentration affects BIA measurement. BIA should be performed when serum electrolytes are within normal range, and comparisons of results should be done when serum electrolyte concentrations are similar.
- Hypothyroidism
    - In pachydermia condition, the measurement may be invalidated because of high skin resistance.

Treatments. About treatments, the assessors should consider the following conditions: intravenous (IV)/electrolyte infusions, drugs usage, dialysis, the use of orthopedics prothesis, pacemakers and defibrillators.

- IV/Electrolyte infusions
    - Peripheral edema interferes with measurement; hence measurements are invalid if patient is abnormally hydrated.
- Drugs that affect water balance
    - Patients in steroids, growth hormone and diuretics use, if they are in stable condition, measurement should be done at the same time after medication administration.
- Dialysis
    - In hemodialysis and peritoneal dialysis, special protocols with standardize measurement procedure should be applied, i.e., measurement should be performed 20–30 min post-dialysis.
- Orthopedic prosthesis/implants (metal)

    - The measurement should be performed on the non-affected body side, and the prosthesis/implants should be noted.
- Pacemakers / Defibrillators
    - Although there are no known incidents reported because of BIA measurements, the possibility cannot be eliminated that the induced field of current during the measurement could alter the pacemaker or defibrillator activity. Therefore, the patient should be monitored for cardiac activity.

Research Highlights

- Body composition assessment with bioelectrical impedance analysis has been gaining increasing acceptance in the health area, especially in nutrition, sports medicine, and exercise physiology, as well as in the clinical area.
- The possibility of choice of different equipment with different methodologies and protocols has offered clinicians and researchers specific options for different purposes.
- The monitoring of the standards and protocols for the evaluation process is necessary to guarantee the validity of the results, whether they are collected in a single moment or throughout time, along clinical, nutritional, or physical intervention programs.

Predictive equations

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Prediction equation models for total body water (TBW), extracellular water (ECW), and fat-free mass are based on weight, sex, height (H), resistance (R), reactance (Xc), or H^2/R. General prediction equations across different age, body composition, ethnic groups, and lifestyles, without prior testing of their validity should be avoided. The best bioelectric impedance analysis (BIA) equation chosen should be one that is adapted to the studied population. However, the agreement levels between the BIA analyzer and reference methods depend on the characteristics of the population and on the statistical method chosen to develop the equation. Generally, performance is assessed based on the magnitude of the correlation between body composition assessed using BIA and another method with high accuracy, such as dual-energy X-ray absorptiometry (DXA). The agreement between the methods can be observed using the limits of agreement (LOA) analysis. The prediction error (SEE) is very important for choosing an equation. The ideal values for SEE are 2.0–2.5 kg in men and 1.5–1.8 kg in women and very good 3.0 kg for men and 2.3 kg for women (Houtkooper et al., 1996).

Predictive equations for a healthy population could be found in review studies (Wang et al., 2014; Beaudart et al., 2020). Athletes tend to present higher skeletal muscle mass and hypohydration status (Silva et al., 2011; Moon, 2013) when compared with non-athlete individuals. Therefore, fat-free mass predictive equations should be developed, especially for athletes. However, the generalized equations have been validated for athletes and can be used with caution (Moon, 2013). Table 1 presents specific fat-free mass predictive equations for athletes.

Main variables considered as bias

Sex

Since birth, female babies have higher fat mass than male babies, regarding growth and development; and it continues until adulthood, even in élite athletes. Prediction equations based on BIA to assess body composition tend to include sex as one main determining factor (Kyle et al., 2004a). From late childhood to young adulthood, fat-free mass follows a growth pattern that accompanies total body mass and height. Sex differences are lower during childhood than in adolescence growth spurt, and they increase with age (Malina et al, 2015). The relationship between fat-free mass and body impedance was found to be slightly S-shaped, being identical for males and females adolescents until 13 years, after which sex differences became apparent (Deuremberg et al, 1990).

Age

Body composition changes with aging, with an increase in fat mass and a reduction in muscle tissue, TBW, and bone mass. These changes can alter hydration status and consequently impair conductivity (Khalil et al., 2014), affecting body composition results assessed using BIA.

Therefore, chronological age should be considered as an independent indispensable variable in the regression model for children and adolescents and as dispensable in models for predicting the body composition of the elderly population (Bosy-Westphal et al., 2006). However, it is necessary to understand the adolescent maturity status because adolescents at the same age vary in biological maturity status (Malina et al., 2015).

Maturity status

Regardless of maturity (early, mature, or late), growth is a process that leads to changes in body composition. Fat-free mass in male adolescents is higher than in female adolescents, while fat mass is higher in female adolescents because of hormonal differences (Malina et al., 2015). In addition, hydration status changes during growth, which can lead to significant errors in the estimation of fat-free mass in adolescents (Kyle et al., 2004a). Hypohydration status has been observed in football players and in adolescent athletes of different sports (Silva et al., 2011; Koury, et al., 2014).

Maturity status can be measured using skeletal maturity, which is more commonly used for male than female athletes, except for female artistic gymnasts that presented menstrual dysfunction. Ethnic variation in skeletal maturity should be considered when the equations are tested. For females, menarche status (menarche occurred or not) was used to assess sexual maturity (Malina et al., 2015).

Bioimpedance parameters are sensitive to changes in body composition. Buffa et al. (2002) concluded that sexual maturity, observed by menarche status, is the main cause of differences in body composition in female adolescents. Therefore, it is imperative to study the possible variations in the bioelectrical parameters in relation to sexual maturity. Montagnese et al. (2013) suggested that the main factor preventing deviation in results using a single BIA for the youth population (4–24 years old) is age or maturation. Araujo et al. (2020) found that, regardless of the method for assessing maturational status in young athletes, a positive correlation with the phase angle is maintained, indicating that biological maturation could be a factor in explaining this relationship. However, despite the recognized importance of skeletal or sexual maturity, only one regression model for prediction equations for fat-free mass included skeletal and sexual maturity for male and female athletes, respectively (Koury et al., 2019).

Anthropometric measurements

The BIA principle is based on the impedance of a conductor, which is related to its length, sectional area, and frequency of the electric current applied over it. A cylinder containing fluid can be used as a practical model to explain the impedance. If an alternating electric current is applied to the cylinder, the content is opposed to that current, which will be measured as resistance. Therefore, the resistance reflects the volume of the cylinder if its length and diameter are known (Kyle et al., 2004a).

Table 1. Specific fat-free mass predictive equations for athlete and generalized predictive equations validated for this group

Author	**Target group**	**Validation method**	**Sports**
Prichard et al, 1997	adult female	DXA	elite runners
Fornetti et al, 1999	adult female	DXA	basketball, crew, cross-country, field hockey, golf, gymnastics, soccer, softball, swimming and diving, tennis, track and field, and volleyball
Langer et al, 2016	adult male	DXA	army cadets
Koury et al, 2018	adolescent female/male	DXA	swimming, judo, badminton, athletics, soccer, volleyball, table tennis
Matias et al, 2020	adult female/male	4-Compartment model	basketball, handball, judo and wrestling, karate and taekwondo, pentathlon, rugby, sailing, soccer, swimming, tennis, track and field athletics, triathlon, and volleyball.

BIA analyzer	**Size sample; age (year)**	**Fat-free mass predictive equation**	**R^2**	**SEE**
RJL-109 and 101 analyzers (RJL Systems Inc, Chicago, IL)	n=70; 26.5±1.4	$=5.091+(0.6483*H^2/R)+0.1699$ -WT	0.94	1.76
RJL 101A analyzer; Clinton Township, MI	n= 132; 20.4±1.5	$=(0.282*HT)+(0.415*WT)-(0.037*R) + (0.096*Xc)-9.734$	0.98	1.1
Quantum II (RJL Systems, Detroit, MI, USA)	n=396 19.2±1.8	$=(0.508*WT)+39.234*(H^2/R)^{\log 10}-48.263$	0.87	2.3
Biodynamics 450 (Shoreline, United States)	Female n=151; 13.0±1.1	$= -2.615+0.603*(age)+0.954*($menarche occurrence$)+0.713*(H^2/R)$	0.84	2.2
	Male n=167; 14.0±0.9	$= -6.340+0.795*(age)+2.071*($skeletal maturity$)+0.744*(H^2/R)$	0.92	2.7
BIA-101, RJL/ Akern Systems, Firenze, Italy	n=142; 22.9±4.9	$= -2.261+(0.327*H^2/R) + (0.525*WT) +(5.462*Sex)$	0.95	2.4

HT: height in cm; WT: body weight in kg; male: 1 and female: 0; skeletal maturity: 0 immature, 1 mature; menarche occurrence: 0 no-occurrence, 1 occurrence

The human body can be understood as five cylinders (two arms, trunk, and two legs), and human height can be considered as the cylinder length. Fat-free mass, composed of intracellular fluids and electrolytes, has higher conductivity than fat mass. Although the body is not a uniform cylinder and its conductivity is not constant, an empirical relationship between the impedance quotient (Length^2/R) and the volume of water can be established (Kushner & Schoeller, 1986). In humans to measure height (H) than conductivity is easier, so the impedance quotient can be described as H^2/R (Figure 1).

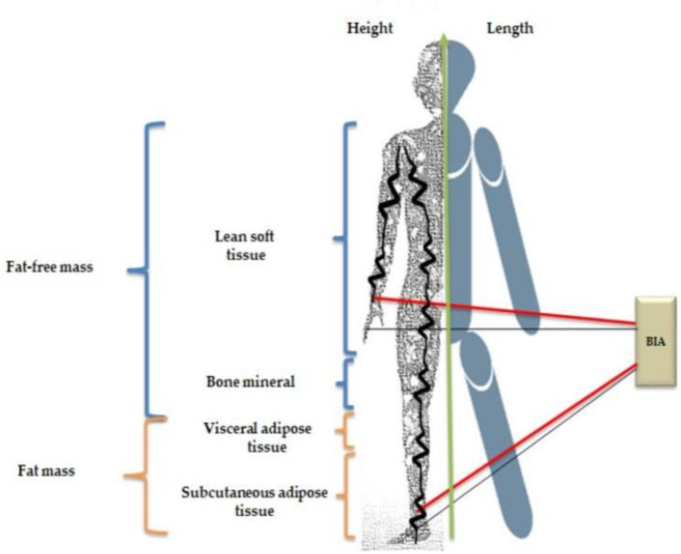

Figure 1. Graphical representation of BIA principles from the cylinder model for the relationship between impedance and geometry. The human body is not a uniform cylinder, and its conductivity is not constant, unlike the cylinder; therefore, it is necessary to apply an empirical relationship that can be established between the impedance quotient (Length^2/R) and the volume of water or H^2/R in predictive equations for the human body composition.

Bioimpedance parameters alone do not support equations for predicting fat-free mass. The inclusion of variables such as H and H^2/R in a regression model increases the participation of the fat-free mass variation in different groups by 11%–53% and 22%–68%, respectively (Diaz et al., 1989).

Basic statistical tests for a predictive equation

Regression models

The regression analysis aims to obtain a mathematical model that predicts the independent variable Y and that best fits the values of the dependent variable(s). If there is an independent variable only, a simple linear regression model is used; if there are other variables and the researcher wants to analyze them simultaneously, the multiple linear regression model is used. Multivariate analysis is more commonly used in body composition protocols. The adjusted values are given by:

$$\hat{y} = \alpha + \beta_1 x_1 + \beta_2 x_{12} + \cdots + \beta_q x_q + \varepsilon$$

However, it is necessary to identify the most relevant variables to the model based on the theoretical assumptions underlying the predictive proposal. With this objective, Sun et al. (2003) suggests the use of multiple linear regression with a stepwise procedure.

Adding more variables to the model does not necessarily mean improving or reducing its quality. Akaike (2011) developed a metric called Akaike information criteria (AIC) that penalizes the addition of terms to a model. In the case of regression, AIC has the following formula:

$$\mathrm{AIC} = \log 2P + n\left(\frac{RSS}{n}\right) \quad \text{where:}$$

P is the number of variables, n is the number of observation units and RSS is the residual sum of squares.

As previously mentioned, when the use of multiple linear regression with stepwise procedure is chosen, the main objective is to find a model that minimizes AIC. In exploratory studies, the use of forward test is suggested, which starts including variables in order of significance. Only variables with $P < 0.05$ will be included in the model. In confirmatory studies, the backward test begins with all variables in the model, and the variables are excluded according to their non-significance. Finally, the stepwise procedure, which is a combination of forward and backward procedures, consists of a procedure that selects variables in a step-by-step manner, adding or removing independent variables one at a time using the variable's statistical significance.

In the regression analysis, it is important to check if there is multicollinearity, that is, if there are moderate to high intercorrelations between independent variables. A sign of multicollinearity is the severely reduced value of the adjusted coefficient of determination (R^2 adjustment).

The development of regression models requires two steps: calibration and validation. Calibration is a process that compares the values of two or more measurement systems. The first is the reference measurement, such as DXA; and the second is the measurement system being calibrated, that is, the prediction equation. Validation refers to the process of adjusting parameters so that they are consistent with reality. The aim is to obtain the highest possible degree of similarity between the observed and predicted values.

The criteria for determining calibration can be: (a) without significant differences between the means of fat-free mass measured by reference measurement (e.g., DXA); (b) hypothesis tests for the regression slope coefficients (β) (paired or unpaired t-test for groups); (c) confidence intervals for the slope coefficients (β); (d) coefficient of determination R^2 (variability ratio between observed values of y, explained by linear regression); (e) adjusted coefficient of determination (also called R^2) (which penalizes the inclusion of little explanatory variables); and (f) standard errors of the estimate SEE.

Concordance tests

Bland-Altman graphic and the limits of agreement

The agreement between measurements performed by different methods but with the same unit of measurement must be correctly performed. The correlation analysis is not suitable for this purpose, as this test assesses the association and not the agreement.

It is important that the measurement of agreement be easy to estimate and interpret. These estimates are significant only if we can assume that the bias and variability are uniform across the measurement range (Bland & Altman, 1999). In this sense, the limits of agreement (LOA) with a probability of 95% certainty can be estimated by the average difference of 1.96 standard deviations of differences, providing a confidence interval within which 95% of differences represent the measurements by both methods. It is important to emphasize that the differences are normally distributed for this purpose; a normality test must be applied before building the graph. If data are nonparametric, it is suggested to do the logarithmic transformation. Agreement limits derived from transformed log data can be returned to the original values to provide limits for the proportion of current measurements (Bland & Altman, 1999).

A standard deviation that leads to wide agreement limits becomes a serious problem. The authors do not recommend excluding outliers from the analyses, but it may be useful to assess their influence on the results. In fact, the decision on what would be an acceptable agreement cannot be made by statistics alone because it is a matter for experts.

Lin correlation coefficient

Lin (1989) criticizes some validation processes. For example, (a) Pearson's correlation coefficient measures a linear relationship but fails to detect any deviation at the 45° line of the correlation graph; (b) the paired t-test fails to detect agreement between data pairs; (c) the least-squares approach fails to detect zero intercept deviation and slope equal to 1, if data are very dispersed and still can reject a highly reproducible process because of a very small residual error; it is also true if the paired t-test is used; and (d) the coefficient of variation and intraclass correlation coefficient allow duplicate readings to be interchangeable, that is, they consider duplicate readings as (random) repetitions rather than two separate readings.

Therefore, Lin (1989) proposed the concordance correlation coefficient, which uses a polarization correction factor that measures how far the best-fit line deviates from the 45° line (precision measurement). This coefficient assesses which pairs fall on the 45° line and expresses the measurements of accuracy (C) and precision

(P). For n sample pairs, it is natural to use homologous samples. For the inference, the author assumes that PC (precision of accuracy) is the correlation coefficient of agreement of the paired samples of a normal bivariate distribution. The proposal for strength-of-agreement criteria for Lin's Concordance Correlation Coefficient, described by Mc Bride et al (2005) (almost perfect> 0.99; substantial> 0.95–0.99; moderate 0.90–0.95; poor <0.90), to classify the results obtained can be used.

Validation tests

Efficiency coefficient

The efficiency coefficient (E) developed by Nash and Sutcliffe (1970) is often used for the evaluation of models. Its value is between - ∞ and 1, and the higher the value, the better the agreement between model and observation. The formula for its calculation is:

$$E = 1 - \frac{\sum_{i=1}^{n}(Oi - Pi)^2}{\sum_{i=1}^{n}(Oi - \bar{O})^2} \quad \text{where:}$$

(Oi) observed data; (Pi) predicted data; ($\bar{O}$) mean observed data; (n) sample size.

The interpretation of the E value can be made as follows: perfect model: E = 1 represents the total agreement between predicted and observed; E = 0 means that the model performs as well as the simple mean of the observed values. Negative values for E indicate that it would be better to take the simple mean than the values predicted by the model.

Root mean square error

The most important performance metric from the data science perspective is the root mean square error (RMSE), which measures the overall accuracy of the model and is a basis for comparison with others. Unlike the previously presented indicators, the RMSE has the same dimension as that of the observed and predicted values. Its value is interpreted as a measurement of the mean deviation between observed and predicted, whose differences are squared, that is, representing a measurement of the variability of the prediction.

$$d = 1 - \frac{\sum_{i=1}^{n}(Oi-Pi)^2}{\sum_{i=1}^{n}(|Pi-\bar{O}|)+(|Oi-\bar{O}|)^2}$$ where:

(O_i) observed data; (P_i) predicted data; $(\bar{O})$ mean observed data; (n) sample size.

Standardization with regard to methodological considerations, such as skeletal or sexual maturity (Koury et al., 2019), lower age range, and distinct equations by sex, help improve fat-free mass values obtained using the BIA equation (Kyle et al., 2004a). In addition, the adoption of other statistical analyses can favor future studies using BIA. For example, machine learning has been used to improve the accuracy of BIA prediction equations for estimating fat-free mass in several populations, such as elderly (Hsieh et al., 2013) and individuals with liver ischemia (Tronstad & Strand-Amundsen, 2019). However, despite ongoing studies, there is still no data on athletes.

Finally, even with the development of an equation with excellent R2 adjustment, robust estimate of agreement, adequate confidence interval, and statistical significance, the results may not be confirmed if applied to other populations, if performed by inexperienced evaluators, or if an inefficient reference instrument is used. Therefore, it is necessary to test different equations to reduce the margin of error in the interpretation of data.

Research Highlights

- If a validation for a specific BIA analyzer, reference method, or population has not been performed yet, it is recommended to develop a cross-validation of the selected equation.
- The main variables considered as bias for developing a new equation are age, sex, maturational status, and anthropometric measurements.
- New statistical analysis, such as machine learning, for predicting athletes' body composition could be incorporated into practice soon.

Phase Angle

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The previous chapters discussed bioelectrical impedance analysis (BIA) technology and how body composition and fluid assessment can be made using predictive equations. This chapter will discuss how the BIA raw measures, resistance and reactance, can be used to estimate phase angle (PhA), an important BIA parameter.

What is phase angle?

The electric current passing through the body will flow through two different pathways: the extracellular pathway and the intracellular pathway. In the extracellular pathway, the current will be conducted through the interstitial fluid and plasma, which will offer a resistance inversely proportional to the fluid and electrolyte content. In the intracellular pathway, the intact cell membranes will act as a capacitive element, storing some energy and delaying the current passage, which then becomes out-of-phase. This delay, or phase shift, is expressed as phase angle, measured directly by a phase-sensitive BIA device (Lukaski et al., 2019). Usually, the phase angle is expressed at a 50 kHz frequency, and it is derived from the relation between the direct measure of resistance (R) and reactance (Xc) (Figure 1). Then phase angle has the advantage of being directly estimated from the raw R and Xc measurements, without the need for weight, height, or any other conversion equation.

Phase angle represents not only cell mass, associated with tissue cellularity and cell size, but also cell membrane integrity and permeability (capacitive function). In health, age and sex are the most significant PhA determinants, but fluid distribution, fat-free mass (as a marker of muscle mass), and BMI can also affect PhA (Figure 2). Subjects with the same PhA could be normal, over or dehydrated and for this reason, a better interpretation of PhA should include the hydration status assessment, usually made by RXc graph, discussed in another chapter.

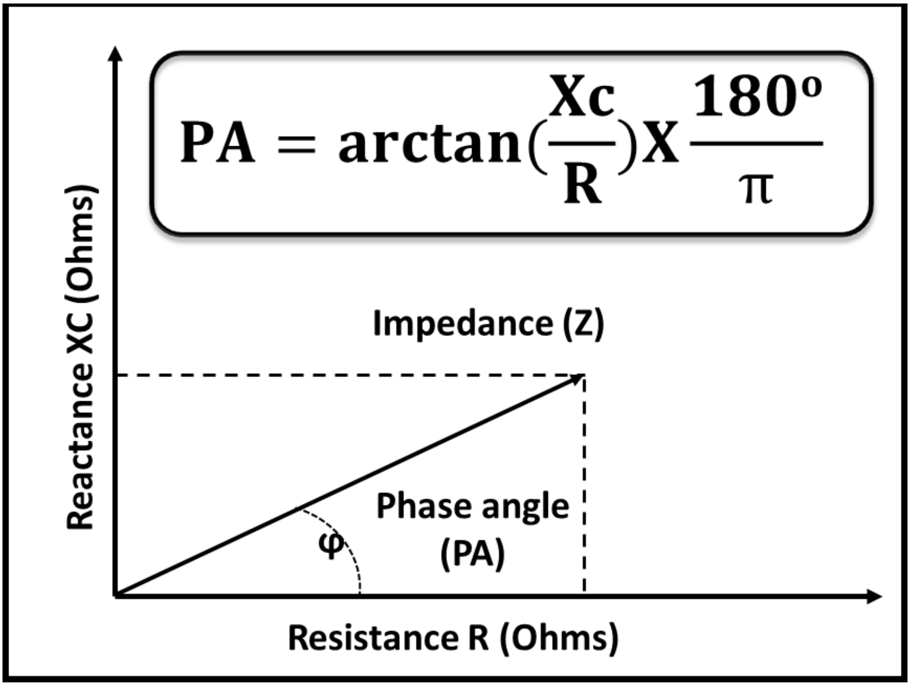

Figure 1. Phase angle graphic representation

Other factors, such as malnutrition, inflammation, or physical activity, appear to be important determinants of PhA values (Figure 2). For this reason, PhA is considered an important prognostic marker in several diseases. More recently, as PhA can be considered a marker of muscle quality and function (Heymsfield et al., 2015b), there is an increasing interest in its role in assessing sarcopenia and as an indicator of muscle quality and performance in athletes.

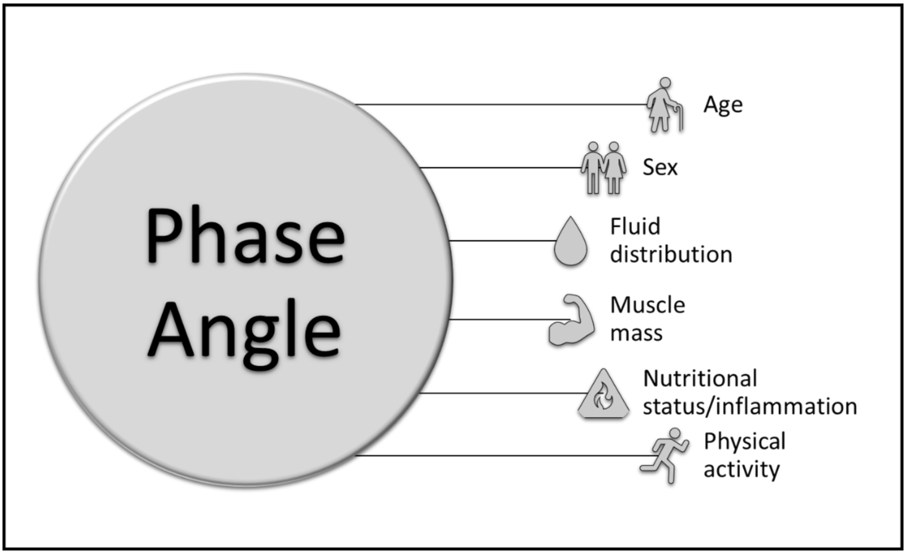

Figure 2. Phase angle determinants

Phase angle in the healthy population

The PhA distribution, according to sex and age, is very similar to that seen with muscle mass. In a database combining four of the largest PhA samples in healthy subjects (n = 282,807), it was shown that men have higher PhA values than women until the 7th decade when the values become similar (Gonzalez et al., 2015). There is a decreasing linear trend with age, being more evident in men after the 3rd decade and after the 4th decade in women. Individuals with African American ethnicity have higher values than all of the other ethnic groups. It was also highlighted that the BIA device is another factor that could influence the PhA values.

A recent meta-analysis combining 46 studies in healthy individuals (n = 249,844) came to the same conclusion (Mattiello et al., 2020). The authors aimed to estimate mean phase angle values in healthy subjects. However, this is an impossible task due to ethnicity differences and the BIA device used for the measurements.

Phase angle cutoff values

Many publications are showing PhA reference values for several different populations. Based on these reference values, a low PhA could be specified using the 5th or 15th percentile or as two standard deviations below the sex- and age-specific mean value.

As PhA varies according to sex, age, ethnicity, and device, this precludes generating a "global" phase angle cutoff or even the comparison of PhA values among the studies from different populations or different devices. One approach to overcome this problem would be transformation of PhA values into Z-scores [standardized phase angle (SPA) = PhA – mean/standard deviation, according to sex and age from the same population]. SPA values below – 1.65 would represent PhA values below the 5th percentile of reference values.

Several studies report disease-specific cutoff values generated from ROC curves predicting higher adverse outcomes (mortality and others) in several clinical conditions. These cutoff values have no external validity, considering they express several factors that could determine PhA variability in that sample: disease inflammation level, mean age of participants, and even sex distribution, beyond the BIA-device specificity. A better approach would be using the lower quartile or quintile of the sample to test any study hypothesis or the population-based cutoff points as defined previously.

Phase angle as a prognostic tool

Besides age and sex, PhA is highly influenced by inflammation, body cell mass, hydration, and nutrition status. For this reason, PhA has been used as a prognostic tool in several clinical situations. Almost 500 references discuss the association between PhA and mortality, and 48 of them were included in a recent systematic review (Garlini et al., 2019). Only six studies could not find a significant association with low PhA values and higher mortality in different clinical conditions as kidney, heart, liver, and pulmonary disease, critical illness, including surgeries and sepsis, several types of cancer, and HIV. The established cutoff points varied from 2.5° to 8°, showing no external validity, only representing the main characteristics (inflammatory level, age, and sex) of the studied sample. A better approach would be the use of cutpoints derived from the reference values for each population (from the mean values, percentiles, or SPA), making possible the comparison among the studies.

Phase angle could be used not only to identify a higher risk for mortality. Low PhA values are also associated with a longer hospital length of stay, higher risk for morbidities after surgery, severity of sepsis, and fibrosis progression in hepatitis C infection (Lukaski et al., 2017).

PhA can also follow-up the patient´s evolution seeing that it is susceptible to changes. Serial measurements could show an improvement or worsening of the clinical situation, according to PhA variability. Especially in the ICU, when clinical status could vary in short periods, PhA could become a useful and straightforward tool for patient monitoring. It can also help follow-up patients with cancer receiving chemotherapy or other continuous therapy when its variation could show the patient´s global answer to the treatment.

Phase angle and malnutrition

Considering PhA as a useful body cell and muscle mass marker, some studies considered PhA as a "nutritional marker." Nonetheless, a systematic review failed to confirm this relationship when Subjective Global Assessment was used to identify malnutrition in different clinical situations (Rinaldi et al., 2019). Another systematic review recently published investigated the relationship of PhA with different nutritional assessment tools or other nutritional objective parameters in patients with cancer (Almeida et al., 2020). Most of the studies found a correlation between PhA and nutritional status or parameters (concurrent validation), showing that PhA could be considered a nutritional marker. Notwithstanding, most of the studies used the ROC curve approach or percentiles of the sample to generate cutoff points, avoiding using in different populations. Future studies using cutpoints generated from the reference population are needed to confirm these findings.

Although the actual possibility of using PhA to diagnose malnutrition is limited, PhA seems to be a potential tool to monitor nutritional intervention´s effectiveness. It is an open research area where more studies are needed.

Phase angle and sarcopenia

Besides its correlation with muscle quantity, PhA also seems to be a useful marker of muscle quality and functional capacity (Bourgeois et al., 2019 & Tomeleri et al., 2019). In virtue of these characteristics, there is a growing interest in the PhA usefulness as a marker of sarcopenia. There is evidence suggesting that PhA is lower in individuals diagnosed with sarcopenia by different definitions, and a higher sarcopenia prevalence is found among older subjects with lower PhA values (Di Vicenzo et al., 2020a).

Some studies have also showed that PhA could be sensitive to changes after interventions addressing sarcopenia prevention or attenuation. Phase angle values increase after a 12-week progressive physical activity program or resistance programs and nutritional supplement, and its increase is associated with gains in strength and muscle mass (Lukaski et al., 2017). These results highlight the importance of PhA as a sensitive and useful tool to identify sarcopenia and follow-up the response after nutritional and exercise interventions.

Phase angle and physical activity

Physical activity has been shown to have a positive effect on PhA: PhA is higher in active individuals and also significantly increases in active subjects in longitudinal studies when compared to controls (Mundstock et al., 2019). The intracellular water and body cell mass increase together with the strengthening of the cellular membrane could be some of the possible explanations for this positive effect.

The PhA differences between athletes and controls seem to vary according to the sport/physical activity modality, according to a systematic review and meta-analysis published by Di Vincenzo et al. (2019). Phase angle seems to be higher than controls in professional bodybuilders males, ballet dancer females, cyclists, and marathon runners. Nonetheless, PhA values from female rhythmic gymnasts are not different from the age and sex reference values. Competition seems to have a negative impact in PhA on children, as shown by a study where the competitive group had lower values, even after a one-year follow-up. No conclusions could be drawn concerning PhA differences among different sports or performance levels.

Conclusions

Phase angle, a simple parameter from BIA, appears to be a promising tool as a marker of muscle quality and quantity. Future studies may show its usefulness to follow up responses after nutritional, clinical, and exercise interventions.

Research Highlights

- Phase angle is obtained directly from the relationship between the BIA raw measures (resistance and reactance), exempting the use of any prediction equation.
- Phase angle reflects body cell mass and hydration status and can be affected by nutritional status, inflammation, or physical activity.
- Phase angle varies according to sex and age: men show higher values than women and decrease with age after the 3rd decade (men) or the 4th decade (women).
- Phase angle can be considered a marker for muscle quantity and quality.
- Besides its usefulness as a prognostic marker in several clinical situations, phase angle can help assess and monitor malnutrition and sarcopenia.

Bioelectrical impedance vector analysis (BIVA)

In memory of Professor Antonio Piccoli (1949-2020)

Doctor in Medicine, 1975 and Doctor in Statistical Sciences, 1983

Lexa Nescolarde Selva, PhD

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In 1994, four professors of the Division of Nephrology of the University of Padova under the direction of Professor Antonio Piccoli published the method who had revolutionised the standard analysis of body fluid variation (Piccoli et al., 1994). The BIVA method is based on the multivariate Hotelling's T^2 test (Hotelling, 1931) and in contrast to other bioimpedance methods this approach does not yield any absolute estimate of ECW, ICW or TBW, makes no assumptions about body geometry, hydration state, or the electrical model of cell membranes and is unaffected by regression adjustments.

Electrode placement for BIVA method

The electrode placements used for BIVA is the standard tetra-polar non-invasive configuration known as *whole-body* (also called "right-side"): two electrodes (an injector I, and a sensor V) are dorsally placed on the right hand in the third metacarpus-phalangeal articulation and in the carpus, respectively, 5 cm apart. The pair on the foot is located in the third metatarsus-phalangeal and in the articulation, 6 cm apart. In his configuration, the total impedance of a subject with normal hydration is determined by 90% of the upper and lower limbs and by 10% of the impedance of the trunk (Lukaski, 1996). The electrode usually choose for non-invase BIA measurements is Ag/AgCl electrodes.

BIVA pattern, RXc-graph

The BIVAguide described by Piccoli and Pastori (2002) is the main source of this summary and where the technique is fully explained:

http://www.renalgate.it/formule_calcolatori/BIVAguide.pdf

Before to introduce the summary of BIVA is important to refresh that the binomial form of the complex biompedance is: Z=R-jXc. From this equation we can obtain the module of impedance $|Z| = \sqrt{R^2 + X_C^2}$ which is the lengthy of the complex vector and the trigonometric ratio between reactance (Xc) and resistance (R) known as phase-angle [arctg (Xc/R) · (180°/π)] expressed in radian degrees.

In the classic BIVA approach, the complex bioimpedance is represented in the RXc-graph (Figure 1) following a bivariate distribution forming elliptical probability regions. The two components of the complex bioimpedance (Resistance R: real part of the complex number and reactance Xc: imaginary part of the complex number) are normalized by the height of the subject (R/H and Xc/H). It is worth noticing that Z/H is normalized by standing stature height in meter and not by the real length of the conductor currently assessed between wrist and ankle in a recumbent position.

The bivariate reference intervals of the healthy population are represented by three tolerance ellipses of 50%, 75% and 95% of individual points in the RXc graph (Piccoli et al., 1994; Piccoli and Pastori, 2002).

Tolerance ellipses for an individual vector and clinical interpretation: By plotting an individual impedance vector (Figure 2) on the RXc-graph allows an evaluation of soft tissues through patterns based on percentiles of their electrical properties without prior knowledge of body weight.

Case A. Vector migration parallel to the major axis of tolerance ellipses indicate progressive changes in tissue hydration.

In renal failure, the inferior pole of the 75% tolerance ellipse (vectors falling out of the 75% tolerance ellipse) is considered the threshold for oedema (Piccoli et al., 1996a; Piccoli et al., 1998, Nescolarde et al., 2004), with 100% sensitivity and 92% specificity (Piccoli et al., 1998).

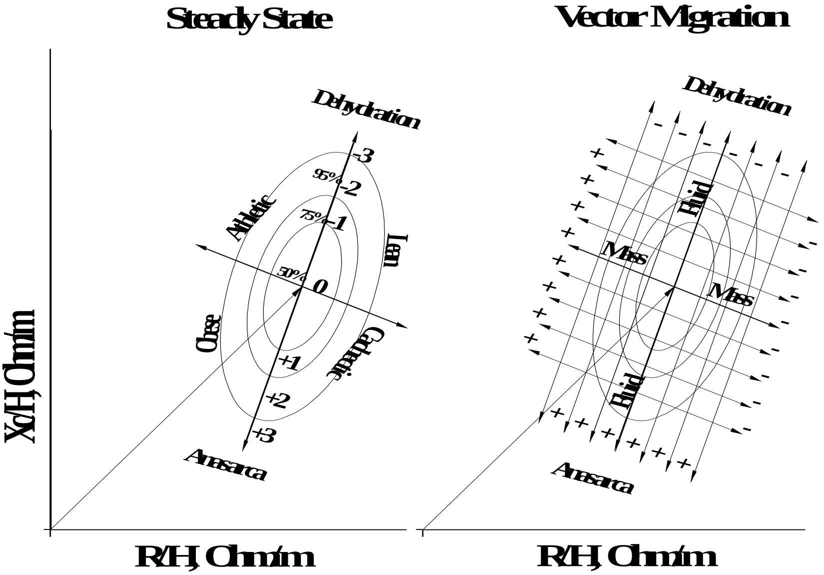

Figure 1. BIVA PATTERN, RXc-graph (Reproduced with the author's permission from - Piccoli A., Pastori G. BIVA software. 2002. University of Padova).

Case B. Vectors falling (Figure 2A, steady state) or migrating (Figure 2B, dynamic state) parallel to the minor axis indicate more (left) or less (right) cell mass, respectively, contained in soft tissues.

Case C. Different trajectories indicate combined changes in both hydration and tissue mass.

Confidence ellipses for mean vector: The means of R/H and Xc/H calculated in a sample can be plotted as a mean impedance vector with its 95% confidence ellipse, thus defining the position and variability of the corresponding population.

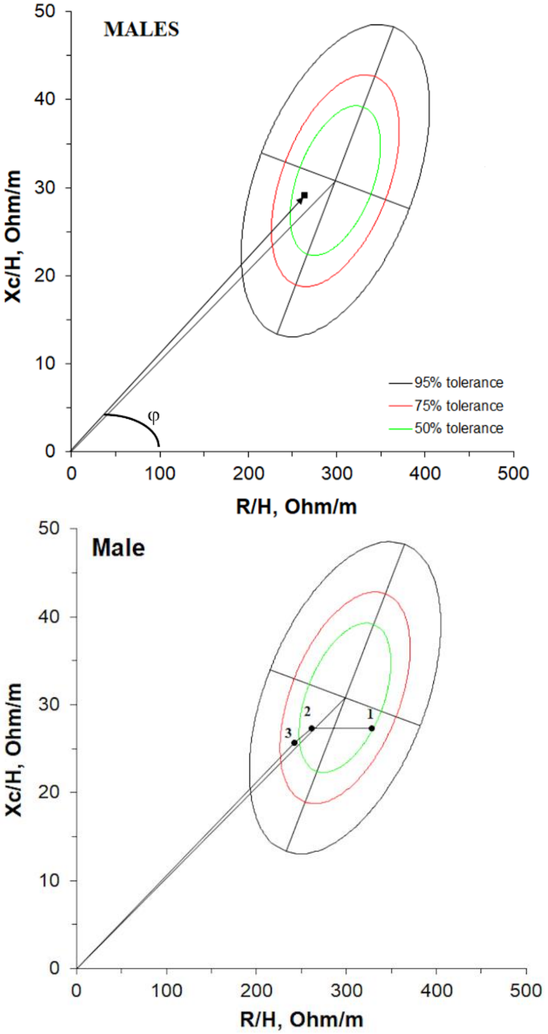

Figure 2. Individual bioimpedance vector on the RXc-graph (A) and trajectory of three individual vector (B)

Comparison of mean vectors through confidence ellipses and statistical interpretation: Confidence intervals help us to compare vector position of different populations and to interpret the statistical significance of the tests, such the generalized Mahalanobis distance (D) widely used in multivariate statistical tests.

In unbalanced groups, if the 95% confidence intervals of the means for each group do not overlap, the difference among groups is not statistically significant ($P < 0.05$). However, the reverse is not necessarily true. Figure 3 shows an example when the statistical significance it not clear.

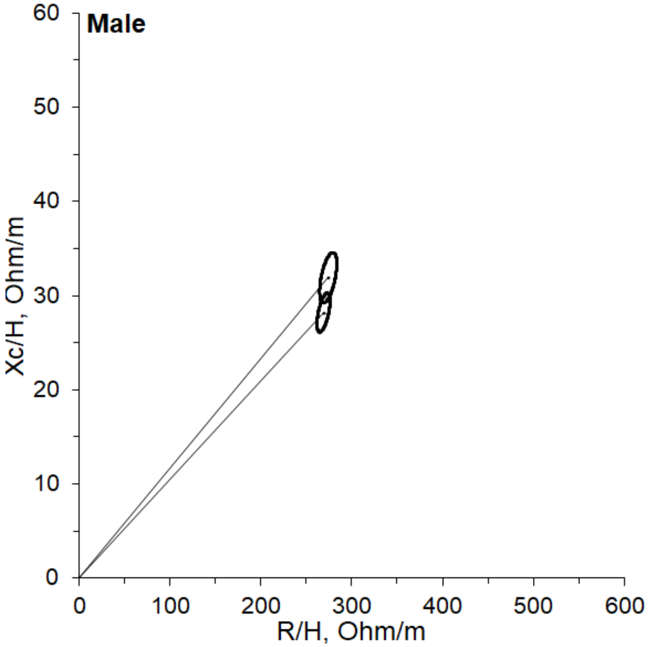

Figure 3. Confidence ellipses (two unbalanced groups)

For this reason, it is recommendable to compare two sample means *based on their difference*, both in statistical tests and in graphical procedures (Figure 4). When the 95% confidence ellipse of the mean vector difference does not cover the null vector (0,0), the statistical comparison will be significant ($P < 0.05$). Viceversa, if the ellipse covers the zero point, the difference between groups will result not statistically significant. In balanced groups with the same variance, the graphical criterion is equivalent to the statistical test.

With software, through *BIVAconfidence,* it is possible to perform the two-sample and the paired one-sample (i.e., repeated measures on the same subjects) Hotelling's T^2 test.

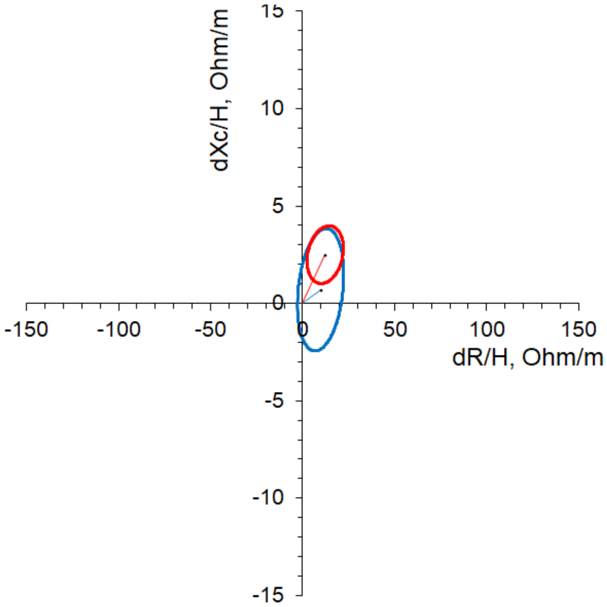

Figure 4. Paired graph for one-sample.

As summary, the RXc-graph method allows: 1) to evaluate an individual subject, plotting the point vector on the reference bivariate tolerance ellipses; 2) to evaluate the successive measurements of the impedance vector in an individual patient; 3) to evaluate groups of subjects using the bivariate 95% confidence ellipses of the mean vectors.

The main advantage of the BIVA method is that works without making any assumption about constant soft tissue hydration, body composition and independently of the body weight. Other advantages of the method are its simplicity and objectivity.

Since the BIVA method was proposed by a group of researchers led by Prof. MD Antonio Piccoli (Piccoli et al., 1994) to the present, it has been validated in numerous clinical research (https://www.ncbi.nlm.nih.gov/pubmed/?term=BIVA) and referenced more than 600 times (https://scholar.google.com/). Among the most relevant works are: hemodialysis (Piccoli, 1998), peritoneal dialysis (Piccoli, 2004), obesity (Piccoli et al., 1998), heart failure (Piccoli et al., 2012), anorexia nervosa (Piccoli et al 2005), pediatrics (De Palo et al., 2000), healthy adults (Ward et al., 2001; Piccoli et al., 2002b), geriatrics (Piccoli et al., 1995), lung cancer (Toso et al., 2000) and in athletes (Micheli et al., 2014) among others.

Effect of intrinsic impedance of Ag/AgCl electrodes on bioimpedance vector displacement using BIVA

As a final remark, it is important to highlight the effect of the intrinsic impedance of the electrodes that are commonly used in non-invasive measurements of electrical bioimpedance at 50 kHz. In Nescolarde et al. (2016) nine different electrodes were studied and the impedance vector was finally plotted on a sample of healthy population with the highest and lowest intrinsic impedance electrodes, finding different displacement within the RXc-graph. Therefore, it is important to previously verify the intrinsic impedance of the electrode that will be used for the analysis with the BIVA method.

Research Highlights

- Body composition analysis from direct R and Xc measures with phase-sensitive bioimpedance analyzer.
- Without any assumption of hydration state, model and without regression equation.
- BIVA has been the first method under which, following the mathematical-statistical basis of multivariate analysis, it has been possible to obtain the R-Xc pattern of healthy reference population. Tolerance ellipses have been obtained from several populations (taking into account sex, age class, and ethnicity).
- High correlation with clinical state of patients with renal failure, heart failure, cachexia, anorexia and critical ill patients, among others.

Specific bioelectrical impedance vector analysis (*specific* BIVA)

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The validity of conventional single-frequency bioelectrical impedance analysis (BIA) in assessing body composition by means of predictive equations is influenced by sex, age, ethnicity, level of fatness, sexual maturity and health status. To overcome possible estimation errors associated with equations use, alternative approaches have been proposed, based on the analysis of raw bioelectrical data (resistance, R, and reactance, Xc), or derived measures (phase angle [PhA = arctn Xc/R · 180/π] and vector length [$(R^2 + Xc^2)^{0.5}$]).

Phase angle has been already discussed in the dedicated chapter by Gonzalez and Heymsfield. It reflects the quantity and quality of cells' membranes, and the distribution of body fluids; is considered a marker for muscle mass and functionality, and a prognostic indicator of nutritional status and morbidity. However, bioelectrical impedance vector analysis (BIVA), that is the simultaneous analysis of phase angle and vector lenght, allows a more detailed evaluation of body composition. Indeed, groups characterized by similar phase angles, but different vector lengths, show different amounts of body fluids or fat mass percentage (%FM) (Piccoli et al., 1994; Mereu et al., 2016).

The classic BIVA approach, designed by Piccoli et al. (1994), is based on the semi-quantitative analysis of the bioelectrical vectors defined by resistance and reactance normalised by height (H). As described in the chapter by Nescolarde, classic BIVA is an original, simple and performing method to monitor nutritional and hydration status, that has been used by a wide body of scientific literature.

However, as firstly pointed out by Ward and Heitmann (2000), classic BIVA results may be affected by the "geometric effect" of transversal areas of the body. In fact, in agreement with the Ohm's law, R is directly proportional to the conductor's length (L) and inversely proportional to its cross-section area (A), besides depending on the intrinsic property of the conductor, that is resistivity (or *specific* resistance) ($R = \varrho L/A$). The standardisation by height alone used in classic BIVA does not completely eliminate the volume effect. In fact, when compared with reference techniques, the classic BIVA approach proposed by Piccoli et al. (1994) showed to be highly sensitive to absolute measures, such as TBW (Marini et al., 2020b), but much less to the relative content of body compartments, such as %FM, and to FM (Buffa et al., 2013; Marini et al., 2013; Marini et al., 2020b; Wells et al., 2021). The short vectors characterising the obesity region within classic BIVA tolerance ellipses are linked to the large body circumferences of individuals with overweight or obesity. However, athletes too can show large body circumferences, due to high FFM, and could be wrongly classified.

In order to reduce the influence of body size and shape, and to increase the sensitivity of *specific* bioelectrical values to tissues properties, a methodological variant, named *specific* BIVA, has been proposed (Buffa et al., 2013; Marini et al., 2013). *Specific* BIVA uses the same empirical approach of classic BIVA, but, following the Ohm's law, differs in that bioelectrical values are normalised by conductor length and cross-sectional areas, besides than by length alone, thus obtaining resistivity: $\varrho = R A/L$. This is not completely new in the literature, as the use of resistivity as an indicator of body fatness had been already suggested by other authors (e.g., Chumlea et al., 1988; Biggs et al., 2001). The novelty of *specific* BIVA is that it coniugates the information given by resistivity with the vectorial approach proposed by Piccoli et al. (1994).

According to the *specific* BIVA bioelectrical model, the conductive volume of the human body consists of three serial elements, corresponding to the leg (l), trunk (t) and arm (a) segments. The total conductor length is calculated as body height (H, cm) multiplied for a factor 1.1 (ratio between the sum of arm length and acromial height, and stature).

Segment cross-sectional areas (A, cm^2) are geometrically calculated, as follows:

$A_l = C_l^2/4\pi$, where C_l is the calf circumference;

$A_t = C_t^2/4\pi$, where C_t is the waist circumference;

$A_a = C_a^2/4\pi$, where C_a is the arm circumference.

Assuming that the arms account for 45%, the legs for 45%, and the trunk for 10% of the whole body resistance (NIH, 1996), average *specific* resistance (ϱ, ohm · cm) of the body can be estimated by the formula: $\varrho = (R/1.1\ H)\ (0.45\ A_l + 0.10\ A_t + 0.45\ A_a)$.
The same procedure is applied for the estimation of *specific* reactance, whereas phase angle is unaffected by the correction.

As in the classic BIVA procedure, impedivity vectors can be analysed by means of tolerance ellipses, probability graphs showing the 50%, 75%, and 95% percentiles of the *specific* bioelectrical values of the reference population (figure 1). However, diversely from classic BIVA, the major axis of *specific* tolerance ellipses refers to variations of the relative quantity of fat mass (%FM), with higher values falling towards the upper right pole. Indeed, this pattern is consistent with theoretical expectations based on human body electrophysiology, predicting a lower conductivity of fat tissues. The information given by the minor axis is similar to classic BIVA: the left upper area of the ellipses is indicative of higher values of body cell mass, skeletal muscle mass in particular, and of intracellular/extracellular water ratio (ICW/ECW). Such similarity between classic and *specific* BIVA is to be expected, considering that the minor axis is mainly determined by variations of the phase angle, which is equal in the two approaches.

As in the classic BIVA procedure, the mean vector of a group can be graphically represented by the confidence ellipse, corresponding to the area around the sample mean within which the "true mean" is expected to lie with a probability of 95%. Inter-group comparisons can be performed by means of Hotelling's T^2 test or MANOVA.

Population reference values

Currently, *specific* bioelectrical reference values are available for U.S.

American adults (21 to 49 y) (Buffa et al., 2013), healthy elderly Italian population (65 to 100 y) (Saragat et al., 2014), and Italo-Spanish young adults (18 to 30 y) (Ibáñez et al., 2015), élite soccer players (only males, 10-15 y) (Toselli et al., 2020).

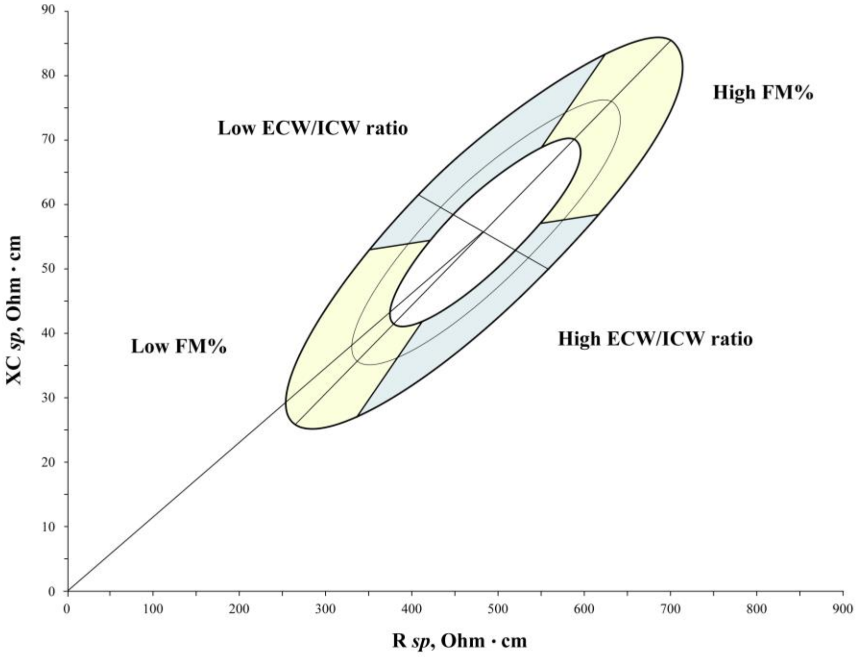

Figure 1. *Specific* BIVA tolerance ellipses

Validation and methodological studies

Specific BIVA has been validated against dual x-ray absorptiometry (DXA) in a cross-sectional multiethnic sample of 1590 U.S. adults from the NHANES 2003–2004 survey (836 men and 754 women) (Buffa et al., 2013). The sensitivity and specificity were evaluated using receiver operating characteristic curves, and compared to classic BIVA. *Specific* BIVA demonstrated significantly higher accuracy than classic BIVA in assessing %FM, whereas the two procedures were similarly accurate for the evaluation of ICW/ECW, mainly due to the effect of phase angle, which is the same in the two approaches. A reanalysis of the same sample highlighted the different information provided by body mass index (BMI) and *specific* BIVA in the measure of relative body fat (Buffa et al., 2017), showing that groups of individuals with similar BMI exhibited different %FM, as detected by *specific* BIVA and confirmed by DXA.

The efficacy of *specific* BIVA for assessing %FM or FM compared to DXA has been verified in a sample of 207 Italian elderly individuals (75 men and 132 women) (Marini et al., 2013), in a sample of 202 young Portuguese athletes (139 men and 63 women) (Marini et al., 2020b), in a sample of 50 young Spanish athletes (25 men and 25 women) (Stagi et al., 2021), and in a sample 281 European children and adolescents (Wells et al., 2021). The Italian elderly survey showed that *specific* BIVA (unlike classic BIVA) was able to distinguish individuals with different %FM (Marini et al., 2013). The analysis of Spanish athletes demonstrated the agreement of *specific* BIVA and DXA in evaluating %FM at the segmental level (arms, trunk, and legs) (Stagi et al., 2021). Furthermore, the segmental analysis showed that the whole body represents a synthesis of the different bioelectrical properties characterising the trunk, arms and legs, thus confirming the efficacy of the analytical approach used in *specific* BIVA to balance the effect of body size. The research on Portuguese athletes included the analysis of body fluids, assessed using dilution techniques, confirming that classic and *specific* BIVA similarly evaluate ICW/ECW (mainly due to the effect of the same phase angle), whereas classic BIVA only (unlike *specific* BIVA) is able to detect the quantity of total body water (TBW) (Marini et al., 2020b). The low accuracy of *specific* BIVA in detecting TBW is not surprising. In fact, the correction by lenght and cross-sectional areas is properly intended to eliminate the volume effect, and hence the correlation between *specific* biolectrical variables and absolute quantities of body compartments, such as TBW, is not expected (unless absolute values are correlated with relative quantities, as for example in the case of FM and %FM).

Applicative studies

Applicative researches implementing *specific* BIVA as body composition assessment technique have concerned geriatric and gerontology settings, and, more recently, sports science.

In a sample of 207 free-living elderly individuals aged 65 to 93 years, Marini et al. (2012) investigated the suitability of *specific* BIVA in assessing sarcopenia. The technique recognized significant differences between sarcopenic and nonsarcopenic individuals, with the sarcopenic groups showing a lower *specific* reactance and phase angle. The technique also was able to discriminate between sarcopenia and sarcopenic obesity, with bioelectrical values of sarcopenic elders clustering toward the right lower area of the tolerance ellipses and those of sarcopenic obese ones toward the right upper (higher values of *specific* resistance).

Buffa et al. (2014a) analysed body composition in relation to nutritional, cognitive and functional status in a sample of seventy patients with mild-moderate stages of Alzheimer's disease (AD). In comparison with a reference group, patients with AD showed similar scores of mini nutritional assessment, but peculiar bioelectrical characteristics (lower phase angles and longer vectors), indicative of a reduction of lean tissue mass and an increase of FM%. The worsening of functional and cognitive status was accompanied by an accentuated lean mass reduction and FM% increase, respectively. Similar results were obtained by Mereu et al. (2018) in a larger sample of 127 patients with AD, where the same body composition peculiarities were detected considering only the right arm.

In a cross-sectional study, including fifty-four patients with dementia in moderately severe to very severe stages, classic and *specific* BIVA variations, in relation to psycho-functional and nutritional indicators, were studied by Camina Martin et al. (2014). The findings indicated that *specific* BIVA was more efficient than classic BIVA in identifying bioelectrical changes associated with psycho-functional and nutritional status. Similarly, Bonaccorsi et al. (2016) showed the efficacy of *specific* BIVA in the assessment of nutritional status in a sample of 321 Italian nonagenarians.

The efficacy of *specific* BIVA in body composition assessment within exercise and sport contexts has been recognised in the systematic review by Castizo-Olier et al. (2018a) (up to July 2017) and in subsequent research. The review included a study on cavers experiencing a 10 hour subterranean exploration, where a reduction of extracellular water, probably due to hypo-osmolal dehydration related to the prolonged underground exercise, was observed (Antoni et al., 2017). Another reviewed paper was aimed to study the effects of a 6-month resistance training program on leg strength in a sample of twenty elderly women (Fukuda et al., 2016). The authors detected a higher leg strength and *specific* reactance after the training program, suggesting improved cellular integrity and cellular health. More recently, *specific* BIVA has been applied to study different samples of athletes. Toselli et al. (2020) analysed body composition in a sample of 178 male élite youth soccer players, where they did not found %FM differences related to maturity status. Lastly, as discussed in a dedicated chapter of this book, Stagi and colleagues studied the long-term effects of physical activity on body composition, in middle-aged and elderly subjects.

Research perspectives

Specific BIVA represents a valid tool for estimating body composition, furnishing reliable information on %FM, muscle mass, and ICW/ECW. Its accuracy, quickness and lack of invasiveness provides an interesting alternative to standard techniques, which can be prohibitively complicate, expensive, or invasive in several contexts, such as clinical routine or sports science.

In particular, segmental *specific* BIVA allows quantifying body components in distinct body regions (arms, legs and trunk), adding selective information about the risk of some diseases (e.g. type 2 diabetes, or sarcopenia), the effect of medicaments (e.g. in lymphedema), the effects of training, and making possible the study of body asymmetry.

The *specific* BIVA software

Calculations and analysis of *specific* BIVA may be performed using the software available at: http://www.specificbiva.com/. The software includes three main sections: *specific BIVA calculus*, *specific BIVA tolerance*, *specific BIVA confidence*.

Specific BIVA calculus: In this section, by clicking on *new case* individual values of resistance and reactance, anthropometric measures and other information related to a subject can be inserted. *Specific* bioelectrical values and body mass indexes are automatically calculated. The *add to subjects* function allows the selection of a set of subjects to be moved into the *subjects* page of the *specific BIVA tolerance* section for further analysis.

Specific BIVA tolerance: This section comprises three sub-sections: reference population, subjects, and point graph.

Reference population: here the populations used as a reference for the analysis are managed.

The software includes the specific bioelectrical reference values for U.S. American adults, healthy elderly Italians, Italo-Spanish young adults. New reference values can be added by mean of the *new reference population* function.

Subjects: here subjects retrieved from the *specific BIVA calculus* section can be selected for their graphical representation within the tolerance ellipses.

Point graph: here the tolerance ellipses and the subjects' specific impedance vectors are plotted.

An appropriate population has to be chosen from the list *reference population* before groups of cases can be plotted with the corresponding tolerance ellipses. The graphs can be modified (title, subtitle, legends, axes range) and saved as png, jpeg, pdf, or svg files.

Specific BIVA confidence: This section comprises two sub-sections: *groups* and *mean graph* (Figure 2).

Groups: it allows the addition of data of new groups. The *draw* option can be used to select the groups to be analysed.

Mean graph: here mean values and confidence ellipses of selected groups are plotted in the Cartesian plane defined by R_{sp} and Xc_{sp}. The graphs can be modified (title, subtitle, legends, axes range) and saved as png, jpeg, pdf, or svg files.

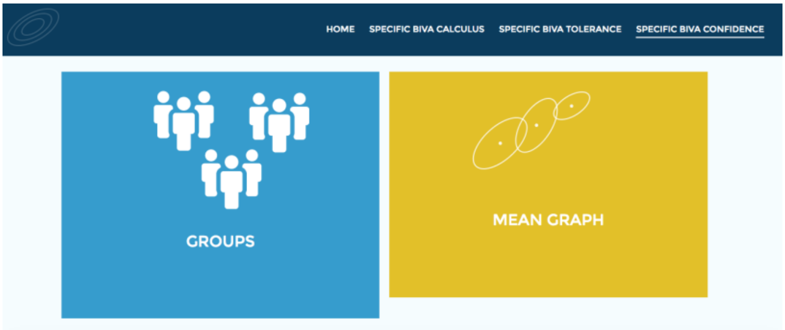

Figure 2. The *specific BIVA confidence* page

Research Highlights

- *Specific* BIVA follows the same procedure proposed by Piccoli et al. (1994) for classic BIVA, differing in that *specific* bioelectrical values are normalized by length and cross-sectional areas of the body, besides than by height alone, in order to overcome the "geometric effect" of the human conductor.
- Validation studies showed that *specific* BIVA is significantly more accurate than classic BIVA in estimating %FM (both at the whole body and segmental level), provides information on ICW/ECW similar to classic BIVA, and performs worse than classic BIVA in detecting TBW.
- Calculations and analysis of *specific* BIVA may be performed using the software available at: http://www.specificbiva.com/, which also includes *specific* bioelectrical reference values for U.S. American adults, healthy elderly Italians, and Italo-Spanish young adults.
- Applicative researches implementing *specific* BIVA concerned geriatric and gerontology setting, and, more recently, sports science.
- An emerging field of research is related to segmental *specific* BIVA, with promising applications in clinical practice and sports science.

Part III – Bioelectrical impedance analysis: applications in sports science

Body composition in sports practice

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In various sports, body composition is considered a determinant of athletic performance (Hogstrom et al., 2012; Silva, 2019; Thomas et al., 2016). For instance, in sports where the body mass must be repeatedly lifted against gravity (e.g., running or jumping), or where a lean appearance is required for aesthetic reasons, a physique, e.g., one characterized by low adipose tissue content, is seen as an important component of bodily fitness and success (Fogelholm, 1994). Obviously, sport performance cannot be adequately predicted solely based on body composition. A multitude of factors determine athletic performance, with some apparently having an even greater influence on success than body composition. Of these, next to genetic endowments, adequate training and recovery strategies seem to be of high importance. Physical performance and success is also clearly dependent on the athlete's health and ability to avoid injuries. Body composition may influence those parameters; for example, adequate muscle mass may help to prevent sport injuries, while poor weight management in weight-sensitive sports may lead to health issues (Sardinha and Santos, 2017; Silva, 2019). Figure 1 provides an overview of the relationships among body composition, training, health and sports performance.

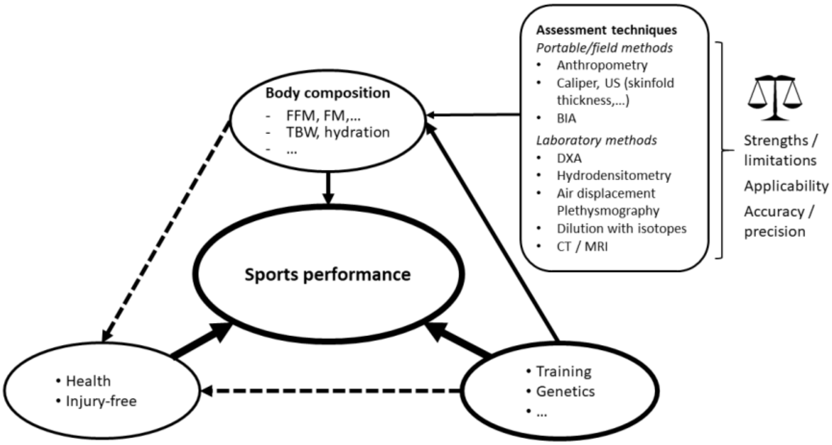

Figure 1. Relationships among body composition, training, health and performance, and an overview of body composition assessment techniques

Body composition and sports performance

Sports practice and more general physical activity can change the physical appearance of the body. The type of training and activity involved for a given sport discipline clearly influences this process. In sports involving strength and speed/power, athletes will aim to increase fat free mass (FFM) and reduce fat mass (FM) by applying specific training and nutritional strategies (e.g., muscle hypertrophy training and high protein intake). Here, a distinction must be made between athletes aiming solely to increase muscle mass and those trying to optimize the power to weight ratio (Fukuda et al., 2017; Silva, 2019; Thomas et al., 2016).

On the other hand, endurance athletes may seek to optimize body mass and body composition (Moon and Kendall, 2017; Silva, 2019). It is important to mention here that, in endurance sports, optimized body composition may depend largely on the type of endurance sport. Whereas rowers may profit from high muscle mass and bone mineral density, which necessarily increase body weight, long distance runners may profit most from low body mass and body fat (Moon and Kendall, 2017).

In general, a rigid notion of the optimal body composition should not be applied to every sport (Thomas et al., 2016). Strategies to achieve the best possible physique for each individual athlete and sport discipline form part of a comprehensive approach to maximize the performance of the athlete.

Besides an adequate physique, body water content and hydration status may exert some influence on sport performance. For example, overhydration or excessive fluid intake may increase body weight and thus lead to a weight penalty during weight-bearing activities; moreover, they may pose a health risk (e.g., hyponatremia) (Gatterer et al., 2011; Sawka et al., 2007). On the other hand, dehydration may undermine aerobic exercise performance by increasing physiological strain and perceived effort, especially in warm-to-hot conditions (Sawka et al., 2007). Therefore, for optimal sport performance, especially in endurance sports, particular attention should be paid to proper hydration and body water content.

Body composition in sports practice: the validity of assessment techniques and the body compartments of interest

Because of the importance of an appropriate body composition for athletic performance, determination and monitoring thereof is of great importance. However, this is also a challenge, as a multitude of methods exist, none of which is currently free from error (Ackland et al., 2012; Armstrong, 2007; Gatterer et al., 2017; Sardinha and Santos, 2017). Therefore, coaches, athletic trainers, nutritionists and all staff working with athletes and physically active people, who want to determine and monitor body composition, should be aware of the advantages and disadvantages of each method (Figure 1) so that they can select the right one for a specific purpose (Sardinha and Santos, 2017). To understand the advantages and disadvantages of the different approaches to measuring body composition, the terms precision and accuracy, which along with some other terms describe the validity of a measurement method, have to be invoked (Gatterer et al., 2017). Accuracy can indicate the extent of the agreement between two different assessment methods, and how close a measured value is to the true value (Gatterer et al., 2017; Sardinha and Santos, 2017). On the other hand, precision refers to the extent of agreement among repeated measurements obtained using the same method, that is, how consistent the measurements are under the same circumstances (Gatterer et al., 2017; Sardinha and Santos, 2017). As well as being accurate and precise, to be practicable, a method must also be easy to use and time-efficient.

As mentioned earlier, selection of the right method also depends on the purpose of measuring components of body composition. In many sports, excessive FM is considered detrimental to performance, whereas a high proportion of FFM –skeletal muscle mass is sometimes referred to as FFM, even though FFM additionally includes bone, organs, and connective tissue (Dengel et al., 2017) - is seen as beneficial. Hence, determining FM and FFM, and monitoring changes therein, can be viewed as an important goal in exercise science. Knowing the body mass and either the FM or FFM is sufficient, as the unknown mass can be calculated according to the formula FM + FFM = body mass.

Nearly all body composition methods allow estimation of these components of body composition, mainly by using equations with certain assumptions. For example, there are equations allowing the estimation of FM from the body mass index or waist circumference. However, since the latter cannot be regarded as accurate, its applicability to the athletic population is highly questionable; thus, these methods will not be further discussed here.

The most accurate procedures to estimate FM and FFM are multicomponent approaches and models. Multicomponent models combine two or more measurement methods and show an accuracy and precision to within 1% to 2% (Gatterer et al., 2017). However, these procedures are time-consuming and costly, and so cannot easily be applied on a regular basis; thus, they are ultimately considered impractical. In this context, a distinction can be made between laboratory-based and portable methods; the latter include skinfold caliper and bioelectrical impedance analysis, and can be considered practicable given that they are easy to use. If measurements are performed by adequately trained personnel (especially in the case of skinfold measurements), precision and accuracy is usually reasonably high, with the latter being within 3.5 to 5% (Ackland et al., 2012; Gatterer et al., 2017). Ultrasound (US) measurements of skinfold thickness have also been introduced recently (Muller et al., 2020). At present, no equations are available to estimate FM and FFM from US measurements, although due to the high accuracy and precision of subcutaneous adipose tissue measurements, detection of changes in FM to within 0.2 kg are expected (Muller et al., 2020).

Laboratory-based procedures including underwater weighing, air displacement plethysmography, dilution with isotopes, dual X-ray absorptiometry, computer tomography and magnetic resonance imaging (Figure 1) are more time-consuming and costly, but may also be slightly more accurate. These measurement methods are accurate to within ~2-4%, and also show high precision (Fosbøl and Zerahn, 2015; Gatterer et al., 2017). It is important to remember that no current single or combined method can be considered definitive for accurate measurement of FM and FFM, and that all methods rely on assumptions and/or calculations that have been validated against reference methods that are themselves not error-free. Therefore, independent of the measurement method, for accurate estimation of mass it is crucial to select the right equation, which must be population-specific and based on appropriate assumptions. Moreover, even though the various methods may have low mean biases (i.e., mean error at the group level), the wide limits of agreement (i.e., error at the individual level) may limit their utility for a single athlete when the aim is to accurately establish FM and FFM (Gatterer et al., 2017). The situation is different if the aim is to determine changes in body composition over time. Any method showing a high degree of precision may enable changes in body composition over time to be satisfactorily determined. Therefore, even simple methods can be valuable for monitoring purposes, provided that high precision can be guaranteed.

Along with the assessment and monitoring of FM and FFM, cognizance of the body water content and hydration status of athletes is a further important goal for some athletic coaches. As mentioned earlier, body water content and hydration status may exert some influence on sport performance. Similar to the estimation of FM and FFM, different methods are available for assessing body water content for different body compartments (Armstrong, 2007). These include the isotope dilution method (described in detail in the chapter entitled "Assessing fat and fat-free mass: two-, three-, and four-compartment models at the molecular level"), the use of various tracers for body compartment analyses (i.e., intra- and extracellular water content) and bioelectrical impedance analysis (Maughan and

Shirreffs, 2017). In the field of sport science, monitoring hydration seems to be of even greater importance than estimating total body water. Hydration status is not the same as total body water, as it also reflects the distribution of water and the balance of electrolytes (Maughan and Shirreffs, 2017). Assessment and monitoring of hydration status is a controversial topic, and can be achieved using various methods. Laboratory tests focus on the analysis of serum parameters (e.g., osmolality and sodium concentration), hematocrit or urine osmolality (Maughan and Shirreffs, 2017). Monitoring changes in body mass, and food and fluid intake, constitute additional methods that can be informative regarding hydration status (Maughan and Shirreffs, 2017). It is important to mention that none of those methods can be considered as the "gold standard"; in fact, no "gold standard" method exists (Armstrong, 2007; Maughan and Shirreffs, 2017). Recently, bioelectrical impedance vector analysis (BIVA) was introduced in the field of sport science, and appears to be a valuable method for assessing hydration status. The advantages and disadvantages/limitations of this method are addressed in other chapters of this book; thus, it will not be discussed here.

Summary

In summary, body composition can be considered as an important determinant of athletic performance and success, which make its assessment and monitoring important for sport scientists and people working with athletes. There are various methods for estimating body composition, all of which have advantages and disadvantages/limitations. Different methods and models may be more convenient for certain situation; thus, selecting the appropriate method with regard to cost, time, validity and applicability is important (Gatterer et al., 2017). Care should be taken to preserve the long-term performance and health of the athlete, by carefully monitoring body composition, understanding the optimal physique for a given athlete, and avoiding potentially harmful practices that may lead to excessively rapid and/or extensive changes in body composition (Thomas et al., 2016)

Research Highlights

- Monitoring and assessing body composition is important in the field of sport science, as an adequate physique is a determinant of the athletic performance, success and health of athletes.

- There are a variety of methods for determining body composition; selecting the right one for a given situation is a challenge that every athletic coach must face. Knowledge of the different methods, including their strengths and limitations, is important to ensure the right method is chosen.

- Care should be taken to preserve long-term performance and health by carefully monitoring body composition, understanding the optimal physique for a given athlete, and avoiding potential harmful practices.

Differences in whole-body bioimpedance parameters among athletes of different sports and age

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Bioelectrical differences in athletes practicing different sports

Bioelectrical impedance analysis (BIA) according to its traditional and vectorial approach (bioelectrical impedance vector analysis = BIVA) has become one of the most used methods to evaluate body composition in sports today. Piccoli et al. (2007) were pioneers in considering the bioelectrical characteristics of a group of athletes compared to the normal population and it was immediately apparent that these subjects, in particular body builders, had very different bioelectric proprieties, including a much higher phase angle (PhA). Subsequently, in 2014, Koury et al. (2014) also confirmed the same theory, shown in chronological order by the findings of Micheli et al. (2014), Campa and Toselli (2018) and Giorgi et al. (2018), which analyzed élite soccer players, volleyball players, and cyclists, respectively. As mentioned above, the first raw bioelectrical variables to be particularly different from the general population was the bioelectrical PhA. However, while this aspect was immediately clear, which also differed among athletes of different competitive levels, some doubts instead arose regarding the ability of PhA to discriminate athletes of different sports (Di Vincenzo et al., 2019). In fact, in some groups of athletes such as élite soccer and volleyball players, even if characterized by a widely different morphology, PhA was similar. It wasn’t long before this concept was clarified and understood by the scientific community, thanks to the studies by Marini et al. (2020b) and Campa et al. (2020) who showed how PhA faithfully represented the ratio of intra (ICW) and extra cellular (ECW) fluids in athletes. In particular, these articles demonstrated how PhA reflects the ICW/ECW ratio, evaluated with dilution techniques, not only in a cross-sectional design, but also its changes over time. In this regard, BIVA demonstrates how its application exceeds the limits linked to an evaluation of the PhA alone. Figure 1 demonstrates how volleyball players and soccer players differ in mean vector length while showing a similar PhA. This means that with the same ICW/ECW ratio and in a condition of normohydration, the total body water (TBW) content can be different. In fact, TBW also varies according to body weight and in this case, as volleyball players were heavier than soccer players, they showed a shorter vector length. Similarly, in the comparison among athletes, bodybuilders show a shorter and left-shifted vector, while cyclists find their position in the upper portion and within the 50th percentile of the reference ellipses of the general population (Piccoli et al., 1995).

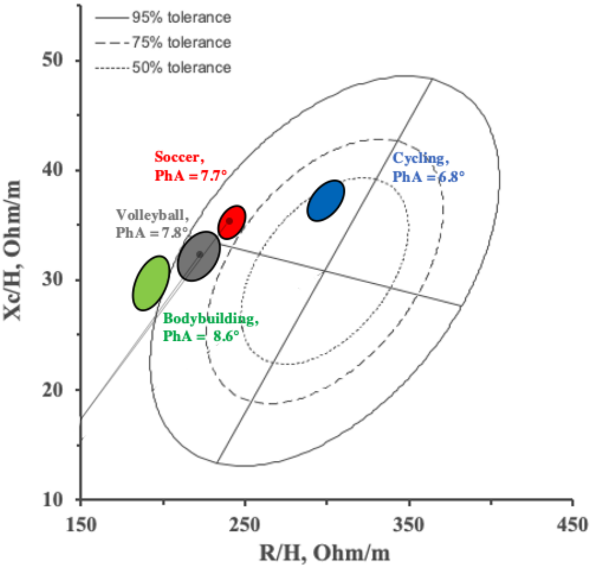

Figure 1. Bioimpedance mean vectors position of soccer players (Micheli et al., 2014), volleyball players (Campa and Toselli, 2019), bodybuilders (Piccoli et al., 2007), cyclists (Giorgi et al., 2018) on the reference ellipses of the general population (Piccoli et al., 1995). Vector length are shown for volleyball and soccer players to highlight the ability of classic BIVA to overcome the only phase angle (PhA) evaluation. Adapted from Campa et al. (2019b).

Body fluids content remains as one of the most important factors in determining the vector position in classic BIVA. On the contrary, by adjusting the bioimpedance parameters according to the *specific* BIVA approach, the vector length will no longer be representative of TBW, but of the fat mass percentage (%FM), where a longer vector will represent greater values than a shorter vector (Marini et al., 2020). Classic and *specific* BIVA agree on lateral vector shifts, which represent changes in the PhA and therefore in the ICW/ECW ratio for both methods. Among the major causes of fluid shifts between the compartments, there are the effects of physical exercise. In fact, increases in PhA and lateral displacements in the R-Xc graph can be measured after a resistance training program or as a result of proper training strategies in team sports (Reis et al., 2020). Underlying these changes are changes in body composition such as those that occur in athletes' morphology. In fact, with the same TBW it can be observed that the athletes who are positioned furthest to the left in the R-Xc graph are those with a high mesomorphic component (Figure 2). In this regard, Campa et al. (2020) showed PhA to be positively associated with the musculoskeletal component of the somatotype, the mesomorphic one, and negatively correlated with ectomorphy, a dominant component in athletes who tend to be thin and long-limbed. Although presenting a variability between roles, soccer and volleyball players show on average an ectomorphic-mesomorph somatotype and in the light of what has already been said, this could explain the similarities in the PhA measured among these two sports. If, on the other hand, bodybuilders and cyclists are taken into consideration, the bioelectrical differences can also be representative of their morphological differences. In fact, athletes engaged in strength sports are characterized by a dominant mesomorphic component, while better endurance performances can be found in ectomorphic athletes, therefore with reduced body dimensions and slenderer. Indeed, body builders and cyclists show not only a different vector length, but also a different PhA and therefore a laterally opposite position.

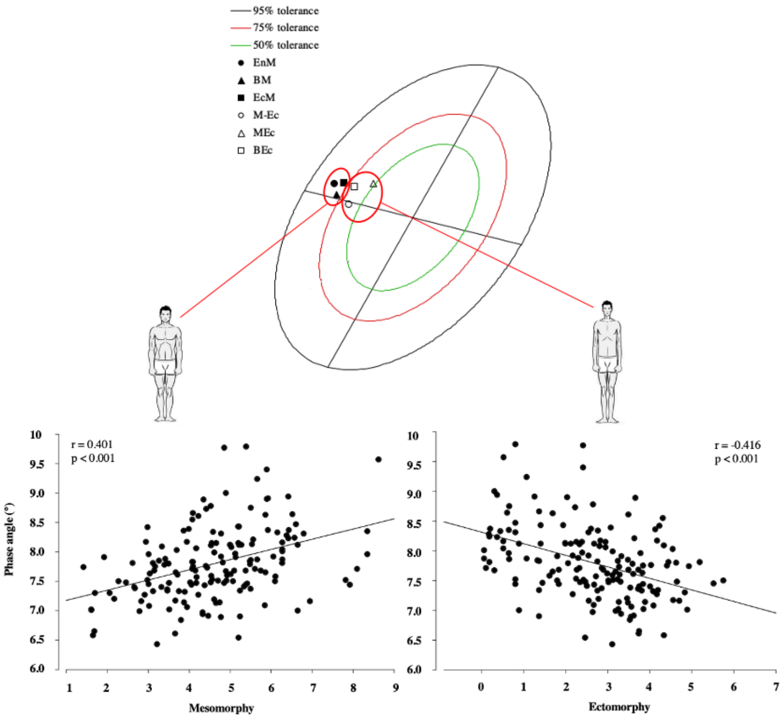

Figure 2. Mean bioimpedance vector of different somatotype categories in athletes with similar total body content; on the bottom, the associations of phase angle with mesomorphy and ectomorphy are shown. EnM = Endomorphic Mesomorph, BM = Balanced Mesomorph, EcM = Ectomorphic Mesomorph, M-Ec = Mesomorph Ectomorph, MEc = Mesomorphic Ectomorph, BEc = Balanced Ectomorph. Adapted from Campa et al. (2020).

Above all, what is apparent is that the athletic population presents clear bioelectrical differences compared to the general population (Campa et al. 2019b) (Figure 3). For this reason, in recent years numerous authors have proposed new reference ellipses for assessing body composition through BIVA in athletes. These references are now known for soccer players, volleyball players, cyclists, and swimmers according to the classic method and for young élite soccer players according to the classic and *specific* approaches (Toselli et al., 2020).

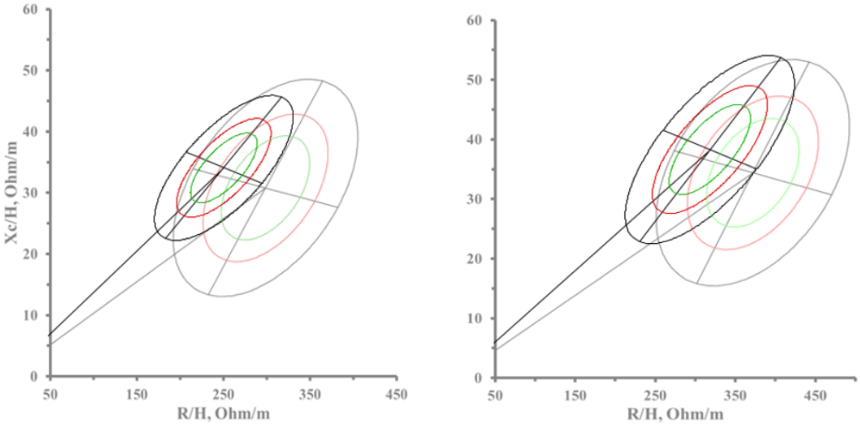

Figure 3. New classic bioelectric reference ellipses for athletes overlapped on those of the general population (Piccoli et al. 1995) for males on the left and female on the right. Adapted from Campa et al. (2019b).

Differences and bioelectrical changes over the competitive season

The raw bioimpedance parameters (resistance, reactance, and PhA) also vary in the individual athlete throughout the competitive season (Silva et al., 2020; Reis et al., 2020; Campa et al., 2020). The changes in body composition are the basis of the changes recorded in the bioelectric data as a consequence of the different training load and nutritional strategies adopted during the competitive period. In any case, while increases in PhA almost always identify beneficial effects of the training periodization, its reductions may represent conditions of fatigue (Nabuco et al., 2019) and cell damage (Nescolarde et al., 2013). For this reason, given that the needs in sport are more specific than what one might have when measuring the general population, the bioelectric references should also consider additional aspects. For example, a new strategy could be to propose bioelectrical references for the different competitive phases in each individual sport. In this regard, Bongiovanni et al. (2020) have proposed new classic BIVA references to evaluate élite soccer players at the start of the season period, where an optimal body composition as well as high physical performance are required. Therefore, technicians using the BIVA references proposed in the literature should carefully consider the period in which the athletes included in the research were measured.

It is preferable to use tolerance ellipses proposed by studies in which bioimpedance measurements are collected over long and non-specific periods only for the general classification of the athlete's body composition characteristics. This is because, while a certain vector position within the reference ellipses may be appropriate in a particular phase of the season, the same position may no longer be informative at another time and therefore war against an optimal interpretation of the data. For example, in a condition of high training load, typical of a pre-season phase, a functional overreaching situation can occur and therefore a PhA reduction is not necessarily concerning (Reis et al., 2020; Mascherini et al., 2014).

Maturity related differences among adolescent athletes

Finally, differences in bioelectrical parameters occur in young athletes as a result of the somatic maturation. If athletes are grouped based on their skeletal maturity, a higher PhA is measured in athletes in a more advanced maturity status (Koury et al., 2019). In these athletes, the difference in PhA is mainly generated by a lower bioelectric resistance, a parameter inversely correlated with the body fluid content. Fat-free mass is also higher in early maturing athletes and, as mentioned in the previous chapters, this molecular compartment is mainly formed by water. In fact, by using BIVA, and an approach not limited to bicompartmental interpretation, a shorter vector length is shown in these athletes, as a consequence of a greater total body water (TBW) or lower FM%, according to the classic and *specific* approach, respectively (Campa et al., 2019b; Toselli et al., 2020). It is then possible to identify specific transition breakpoints in which the bioelectrical parameters show an increase, a decrease or a plateau (Figure 4). Particularly, in young élite soccer players, it has been seen that PhA begins to increase rapidly beginning at 2 years prior to the peak high velocity (PHV) and continues to do so for the 4 years following this phase (Campa et al., 2019c). Otherwise, the vector length shows a sharp decrease up to 1 year after the PHV, identifiable with the maturity offset, and then reaches a plateau. However, in these athletes the age at PHV can be lower than that measured in the general population, where somatic maturity is reached on average, at the age of 14 for males and 12 for females. This is a particularly common scenario in élite teams as often there is a tendency to select athletes with greater body dimensions and better physical performance, typical in more mature athletes.

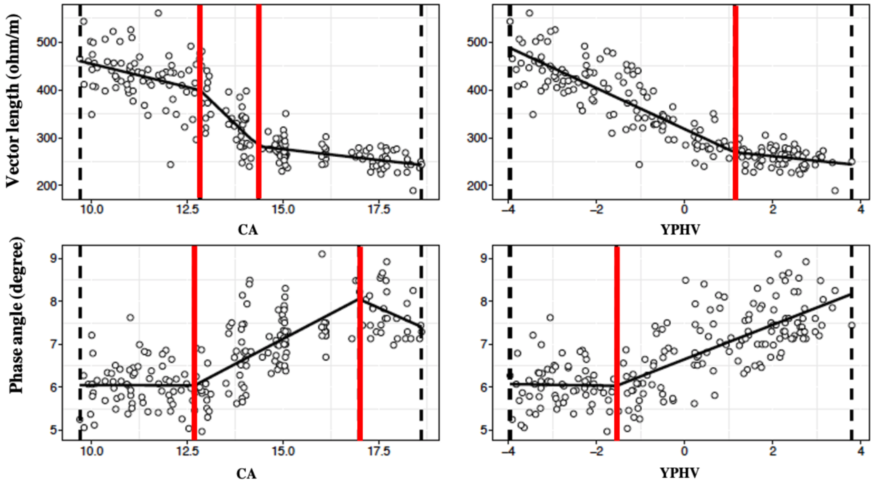

Figure 4. Bioimpedance values development according to the player chronological age (years) (CA) and somatic maturity status (age from peak height velocity = YPHV). Transition points are highlighted with red lines. Adapted from Campa et al. (2019c).

Furthermore, young athletes in many competitive sports are divided according to chronological age. This may mean that in a group of athletes belonging to the same category, regardless of their chronological age, there may be athletes with a different maturation status. In particular, it may happen that an early-maturing athlete finds himself competing against a late-maturing subject and therefore with completely different body composition features. If we consider the young players and the body composition variables investigated by classic BIVA, these reach the 50th ellipse percentile proposed for adult soccer players (Micheli et al., 2014) at an average chronological age of 19 years, while already at 15 they are positioned interior of the tolerance ellipses (Campa et al., 2019c) (Figure 5). This process in which the bioelectric vector moves towards the center of the reference ellipses is achieved with a vector shortening and a progressive increase of PhA over the years. However, as mentioned above, it is possible for an adolescent athlete, whose group of peers on average has bioelectric and body composition characteristics still very far from those foreseen by the soccer player profile, shows already body dimensions similar to that of an adult. This may be the case of a young athlete who at 14 years has already reached the maturity offset for several years (Figure 5). In this specific population the application of BIVA therefore appears extremely useful for the identification of sports talent, as well as for the purposes related to body composition monitoring for which it is normally used.

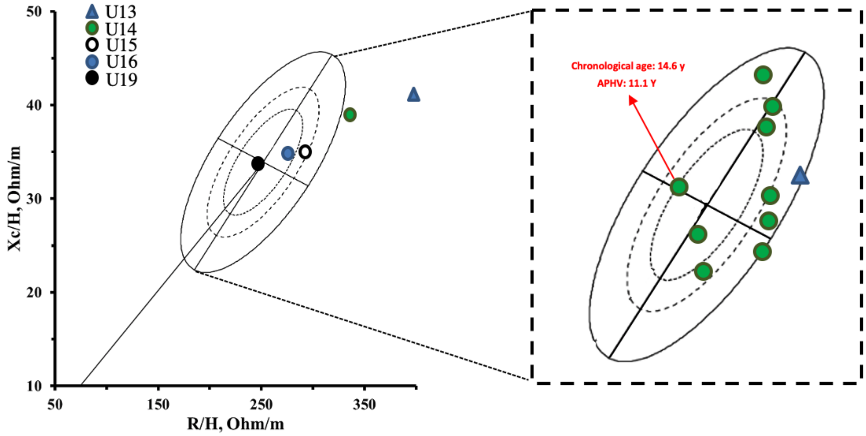

Figure 5. Mean impedance vectors for each age category (on the left) and single vectors (only for U13 and U14 players entered in the tolerance ellipses) plotted on the adult soccer players tolerance ellipses (on the right) (Micheli et al., 2014). Adapted from Campa et al. (2019c).

The BIVA vector, whose position is due to the phase angle and vector length values, provides additional information with respect to the interpretation of the phase angle alone.

Research Highlights

- BIVA discriminates athletes practicing different sports, as a consequence of sports-specific body composition features.
- Early maturing athletes present higher phase angle and shorter vector length than those categorized as maturing late or on time considering the stage of skeletal and somatic maturity.

Bioelectrical Impedance Vector Analysis of regional body composition among athletes practicing different sports

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The term “regional body composition” refers to the study and analysis of body compartments in different body portions, such as the trunk, the limbs or even specific muscles. In literature, there is no consensus on standardized protocols regarding the nomenclature of different techniques, and the position of the electrodes used to investigate bioelectric properties of body regions varies according to different researches. In this chapter, in order to clarify the topic of discussion, we used the term “regional” referring generally to the analysis of body portions, while “segmental” defines the most common BIA method to analyse body segments, which involves different protocols. The designation “localized BIVA”, instead, applies to the analysis of the impedance of specific muscles (Nescolarde et al., 2011).

Regional body composition offers a deeper insight into what it is possible to achieve using total body impedance and allows a better understanding of body composition compartments of just a single body region. It analyses compartments among each body segment, distinguishing the impedance values of arms, trunk and legs. This technique has been applied in different contexts, such as clinical field, nutrition, ageing and sport. The evaluation of regional body composition may be useful to assess appendicular lean body mass, for example of lower limbs, to estimate muscle size and its relationships with movement and performance efficiency, or for fluid accumulation detection and the shift in their balance (ECW/ICW), due for instance to metabolic complications or injuries.

In clinical and ageing settings, this method has been applied on a sample of elderly population, divided into healthy subjects and Alzheimer's Disease (AD) patients (Mereu et al., 2018). Results showed how subjects affected by AD were characterized by a higher percentage of %FM (longer *specific* BIVA vectors) and lower lean mass (lower phase angle) compared with the control group. Among the results, authors highlighted how in studies regarding elderly population with limited mobility, regional body composition assessment, applied also to upper limbs, can be particularly useful for the screening of sarcopenia and sarcopenic obesity, which are common conditions affecting elderly subjects. This was the first study to use regional *specific* BIVA and to show its efficacy in discriminating body composition compartments of the arm and it can be used together with whole-body composition analysis.

In sport, regional body composition can be related either to performance levels or to physical activity, and applications concern both competitive and non-competitive sports (Castizo-Olier et al., 2018a; López-Fernández et al., 2020). It has been applied as an evaluation method: to monitor body composition conditions in post-injury/trauma recovery phases (structural and functional), to analyse training effectiveness and even for talent identification. It can also be used in assessments in order to investigate, for example, the role of physical activity in several elderly people samples characterized by various conditions, such as sarcopenia or obesity (Stagi et al., 2020).

Regional raw data can be analysed as previously demonstrated for whole-body impedance, employing different methodological approaches. It has been verified, in fact, that concepts shown in the present volume (Chapter II) are also applicable to a single body region: classic BIVA values, calibrated for example on a single limb's length (Nescolarde et al., 2008), are sensitive to hydration and TBW changes (Marini et al., 2020b), while *specific* BIVA values, adjusted for length and transversal area of the same limb, are sensitive to %FM changes (Mereu et al., 2018; Stagi et al., 2020). The same approach can be followed for the phase angle, which is considered as a proxy of muscle mass, and the balance of ECW/ICW (Stagi et al., 2020), related to a single body portion.

Regional body composition has been proposed as a valuable tool aimed at estimating the total one, starting from all the values of body segments (Stahn et al., 2012).

Applications of regional body composition in sport

The analysis of regional body compartments is particularly useful in élite athletes, whose training is based on strengthening the lower limbs, as in the case of soccer players (Nescolarde et al., 2011) and cyclists (Marra et al., 2016), as opposed to upper limb-based sports such as volleyball (Di Vincenzo et al., 2020b) and water polo (Marra et al., 2021), or others more based on a particular body hemisoma, i.e. tennis (Stagi and Marini, 2019).

Competition. Athletes are often exposed to high stress and fatigue on body composition (fluids and fat free mass in particular), especially during competitions. A fast and non-invasive evaluation close to the time of performance (both before and after) can easily provide valuable information concerning the optimization of hydration, possibly related to the level of performance. In a study conducted by monitoring élite cyclists throughout the three weeks of Giro d'Italia, Marra et al. (2016) demonstrated that during competition total body phase angle significantly decreased, however they found significant differences differentiating between the values of arms and legs: only the legs' phase angle showed reduction as well as ICW compartment (the leg is the most stressed body segment in this case), whereas the arms' phase angle did not change during the competition.

Sport and ageing process. Stagi et al. (2020) demonstrated that people who practice a particular discipline or sport for a long period of time showed a better body composition status compared with a sample of sedentary people. This was the first study to apply *specific* BIVA to the investigation of regional body composition in the field of sport. Moreover, Stagi and Marini (2019) showed how asymmetry due to %FM in both arms and legs in middle aged and older athletes of Tai Chi Chuan, athletics and tennis, can be notably connected to a specific focused training.

Symmetry and training effects. Localized BIVA, which involves the bioelectrical impedance analysis of single muscles, has been applied to study muscle symmetry in élite athletes (e.g. soccer and basketball players) (Nescolarde et al., 2011). The authors suggested that well-trained muscles of lower limbs seem to be symmetrical between right and left side, even though the training protocol is also determinant. Maintaining symmetry effects due to an optimal training in élite athletes was also demonstrated by López-Fernández et al. (2020) in a sample of male futsal élite players. Authors highlighted how sub-élite athletes, instead, presented asymmetry in lower limbs.

Muscle injury and recovery. Starting with the muscle symmetry situation in optimal conditions of élite athletes, the discovery of asymmetry through bioelectrical impedance can serve as an indicator to detect changes in muscular structure and hydration, as occurs in particular circumstances such as muscle injury and recovery (Nescolarde, Chapter III). For example, Nescolarde et al. (2011), performed localized BIVA on lower-limbs muscle groups in a sample of professional soccer athletes. Athletes in ideal conditions showed limb symmetry, while athletes affected by muscle strain tended to hyperhydration of the injured limb, which presented lower values of R/H and Xc/H. The return to normal conditions could thus be monitored through these values so professionals and researchers can better examine the recovery process and the return to play. Lower limb localized BIVA values in soccer players are also indicated as markers of the injury extent (Nescolarde et al., 2013).

In a current study on élite kayakers (national level) (research in progress by the same authors of this Book Chapter: MM and AM, in collaboration with the medical and training team), the use of regional BIVA analysis according to Stagi et al. (2020) brought out asymmetry values linkable to injury outcomes. The Authors analysed a female élite kayaker who showed upper limb asymmetry BIVA values after (1) surgery due to a dislocation that affected the right shoulder, (2) a consequent inflammation event (Adhesive Capsulitis), and (3) shoulder retraction procedure. At measurement, performed 4 months after trauma, the right arm resulted with limited movements and muscle deficits: bioelectric values showed higher raw R and Xc compared with the left arm. Although *specific* BIVA values (sensitive for %FM) remained similar between the two hemisoma, classic BIVA values highlighted less hydration and TBW in the injured arm as opposed to the healthy one (higher values of R/H and Xc/H). The explanation could be that the right arm and shoulder could perform very limited movements for a rather long extent of time, so muscles lost training and thus optimal level of hydration, albeit with no increase in FM% compared with the healthy arm. Instead, the phase angle decrease was very low.

Comparison among athletes practicing different disciplines

Few studies investigated the training effects and differences in terms of body composition through regional BIVA among groups practicing different sports (Stagi, in this Chapter III). Regarding élite athletes, Donatucci et al. (2018), investigated segmental body composition of élite canoe, kayak and baseball athletes. No differences in phase angle has been detected in those three sports, while canoers and kayakers (highest values of R and Xc) showed a higher development of muscle mass in the trunk. On the other hand, baseball players showed a greater muscle development in both lower and upper limbs at the expense of the trunk. The identification of specific body composition features in regard to a particular discipline can help, with many other markers such as BMI, anthropometry and skinfold thickness, to better understand whether the athlete is training in line with the specific body composition compartments of the population. This concept can be considered in monitoring young athletes' growth as well (Campa et al., 2019c).

Therefore, it is more frequent to find comparative studies conducted among athletes practicing different sports through conventional BIA, in order to analyse bioelectrical values (R, Xc, Z and phase angle) in distinct body regions, trying to find expected differences. Marra et al. (2021), in a study conducted on élite cycling, water polo and ballet athletes, demonstrated that the identification of segmental phase angle among athletes practicing different sports may be useful to detect body composition differences and changes due to specifics training. Their results showed that the lower limbs phase angles were higher in all three groups than in the control (non-competitive sample), while similar results for the upper limb phase angles could be found only in water polo players and ballet dancers, but not in cyclists, probably due to the relatively less development of the arms during training and competitions (Marra et al., 2021). Among the three groups, the study did not display significant differences in terms of phase angle, even adjusting for FFM. In addition, whole body and limb phase angles were slightly higher in water polo players. Results highlighted how they also presented more FM than cyclists and ballet dancers, possibly because they tend to retain slightly more fat to assist with floating during training and matches. Ballet dancers had less FM, probably due to a reduced dietary energy/high-demanding performance. This study showed that segmental phase angle, and thus evaluation of regional body composition among athletes of different sports, can be employed by researchers and specialist in assessing variations in physiological variables (e.g. response to intervention), to monitor athlete's body composition close to a competition, to assess intra and inter differences among athletes and slight variations due to training in specific body segments. In a study conducted on volleyball players, Di Vincenzo et al. (2020b) underlined how total body and lower limb Xc and phase angle were significantly higher in the athletes as compared to the control group composed of healthy non-athletes, just as expected. Moreover, they found, for the first time in the context of volleyball, that Hand Grip Strength (HGS) and upper limb phase angle were strongly correlated in the athletes' sample. The authors anticipate major attention to develop studies regarding the correlation between phase angle and HGS, as the latter seems to be related to sprint acceleration, jumping ability and motor coordination, and consequently to be a potential proxy of the volleyball players' performance.

Regional BIVA among athletes practicing different sports: future perspectives

Building specific ellipses in terms of different sports could potentially improve the interpretation of both total body (Castizo-Olier et al., 2018a) and regional analysis. A first approach in this direction has been proposed by Stagi et al. (2020), and we applied part of the protocol to an ongoing study conducted on professional kayakers and soccer players (in-progress research by the same authors of this Book Chapter: MM and AM). We could perform regional BIVA and subsequently observe how notable differences between body regions were among the two distinct groups. To date, data has been collected on a numerically limited sample, but results of the preliminary analysis seemed to highlight differences worth exploring and studying, opening future perspectives with the objective of building regional reference ellipses for different disciplines. Below, we describe two individual cases to show data which could lead researchers to invest resources in the investigation of specific regional body composition features of each sport, as was already done for total-body composition (Campa, Chapter III).

We started from the best élite athletes of two sport groups, kayakers and Italian First Team soccer players, to study intra and inter sport variability: we report the comparison between two males (1 for each sport) of the same age (26 years old) who showed similar BMI (kayaker: 24,8; soccer player: 23,5), both categorized as normal weight, to compare their total and regional body composition. Classic BIVA values indicated a higher hydration and TBW in the soccer player, while the kayaker had greater values of %FM as highlighted by higher *specific* BIVA values. Phase angles resulted similar and were both high (more than 7°). Regarding upper limb differences, we found higher %FM (*specific* BIVA) in the kayaker on both right and left arm, while hydration and TBW (classic BIVA) on upper limbs values seemed to be quite similar, as also found for the phase angles. Lower limbs analysis showed how the soccer player was characterized by greater levels of hydration and TBW (lower values of classic BIVA).

Starting with similar BMI, the kayaker seemed to have greater total-body values of %FM, while the soccer player body composition was characterized by more TBW and better hydration levels. Considering only the total body impedance, we couldn't completely understand how exactly body compartments differed in terms of body regions and so how FM and TBW were differently localized. Indeed, with classic and *specific* regional values, we found that the greater amount of %FM in the kayaker was more localized in the upper limbs, while the soccer player showed better hydrated lower limbs, as expected, probably due to the intense training focused on these areas. Analysing individual cases, though, we could assume that the explanation of the found differences could be subjective and also due to genetic features of both the athletes. Additionally, analysis of about 20 kayakers and 15 soccer players has confirmed these outcomes, in particular for lower limbs, although the study is still in progress. Future studies and collecting a more considerable amount of data could allow to compare classic and *specific* regional values and, as a consequence, better characterize upper and lower limbs body composition for each sport, comparing intra and inter individual differences.

Research Highlights

- Regional body composition analysis in term of bioelectrical impedance represents a useful method of evaluation in different fields such as sport, and not only in clinics, ageing and nutrition
- In sport, the analysis of regional body composition can be applied to the contexts of performance, training effects, ageing processes, as well as in the recovery phase following an injury
- Literature regarding regional body composition assessment among different sports is still limited, and measurements are usually taken with different protocols
- Generally, athletes practicing different kinds of sports showed different bioelectrical values, either in total body or in regional assessments
- Regional body composition offers a deeper insight into what we have already learnt by studying total-body composition. Therefore, future researches are needed for the purpose of building ellipses for every discipline we could base useful interventions on, for sports and physical activity

Body composition changes in relation to physical exercise

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In the area of physical exercise and sport field is important to monitor body composition longitudinal changes induced by practice. This lies in the fact that the physical stress imposed during trainings and competitions may lead to body composition alterations, which can be detrimental to athletes. Furthermore, body composition has been suggested to discriminate athletes of different performance levels and has been shown to influence physical performance and sport success.

On the other hand, monitoring the hydration status in exercise and sport is important because dehydration is recognised to impair sport performance, as well as increasing the injury risk. Therefore, monitoring body fluid variations may help to adequately prescribe fluid intake and thus limit deleterious effects.

As reviewed in Castizo-Olier et al. (2018b), "classic" BIVA has been used to monitor body composition (i.e. hydration status and body cell mass – BCM) longitudinal changes induced by exercise in athletes and active individuals. To date, different studies aimed at analysing longitudinal vector variations with protocols ranging from short- to long-term changes. In this chapter we will review the current findings and the next challenges to overcome will be indicated.

Short-term vector changes

Studies analysing short-term vector changes (<24 hours after the first measurement) are those which currently face more methodological issues, since their validity can be easily compromised, mostly because of the factors that may affect the accuracy of the pre to-post BIA measurements despite any attempts to control them (Koulmann et al., 2000; Castizo-Olier et al., 2018b).

The concerns to be considered in any study design focused on pre- to post-exercise bioelectrical changes have been previously reviewed by Castizo-Olier et al. (2018b). These include: controlling skin conditions (skin electrolyte accumulation produced by physical exercise) and temperature (affected by the variations in cutaneous blood flow); checking pre-exercise euhydration status of individuals; informing participants not to perform strenuous physical exercise 48 hours before assessments; avoiding/controlling (in laboratory studies) or registering (in field studies) consumption of food or beverage before, during and after exercise; replicating electrodes placement and body position during BIA measurements; performing a 10-min period of body fluid stabilisation; taking into account possible biases caused by biological intra-day variations and pre- to post-exercise environmental conditions. All these considerations will dramatically determine any approach that aims to provide rigorous, valid and reliable information on the quality of the bioelectrical signal in the assessment of short-term vector changes. justifications. For example, different exercises induce different types and degrees of dehydration in athletes. The most common is hypertonic dehydration (i.e., primarily a loss of water) induced by a prolonged and relatively strenuous exercise in which heavy sweating occurs. But in competitive sports, especially in aesthetic and weight-categories disciplines, it is crucial to discern if dehydration is mainly based on a loss of electrolytes (hypotonic) or on a balanced loss of electrolytes and water (isotonic). To our knowledge, there are currently no studies that have analysed the sensitivity of BIVA according to different types of acute dehydration (short-term changes) and how the vector is pointed with respect to tolerance ellipses. Therefore, vector analysis could be the key for serial measurements of hydration status in athletes.

Currently, there are still very few studies analysing the short-term vector changes. These studies are characterised by two different methodological approaches: a) pre-post experimental studies performed in laboratory conditions or with highly controlled protocols (Gatterer et al., 2014; Campa et al., 2019d; Campa et al., 2020); b) field studies with athletes on real sporting scenarios (Antoni et al., 2017; Carrasco-Marginet et al., 2017; Castizo-Olier et al., 2018a; Nescolarde et al., 2020a). These differences between research designs are critical when assessing the effective applicability of BIVA in the short-term vector changes since both athletes and technical staff demand support tools that can provide them with real-time information that is easy to record and interpret during training and competition.

Practically all of these current BIA short-term studies (independently of their research design) indicate an increase in resistance (R) and reactance (Xc), as well as a significant vector migration, especially after performing sufficiently prolonged and intense physical exercise. An example of this can be observed in Figure 1, which shows the main pre-post exercise results in synchronised swimming (Carrasco-Marginet et al., 2017) and ultra-endurance triathlon (Castizo-Olier et al., 2018a). Since R is the pure opposition of the conductor to the flow of current, the significantly increase of this parameter experienced by athletes post-exercise would indicate a decrease in body fluids, which is supported by the decrease in body mass (BM). However, characterising Xc behaviour in the short-term changes induced by different types of physical exercise is more complex. Basically, Xc could vary its response as a function of two different but related adaptations induced at the cellular level by physical exercise: 1) the fluids shift between intracellular and extracellular compartments; 2) the modifications in cell size that would directly affect its dielectric mass, that is, cell membrane and tissue interfaces. Regarding fluids shift, Gatterer et al. (2014) observed that significant increases in plasma osmolality were negatively correlated to significant increases in Xc/H just after 60 minutes of an intense dehydration session. As Xc/H reflects intracellular fluid content, authors suggest that this relationship indicates that lower intracellular fluid losses mean greater plasma osmolality increases. The contributions of Campa et al. (2020) are also interesting. In their study analysing a cycling exercise at 65% of VO_{2max} for 60 minutes followed by a time-to-trial

(TT) at 95% of VO_{2max}, they noted that, although the bioimpedance vectors lengthened as a result of the loss of body fluid, the slope and phase angle did not change, which would imply that the ICW/ECW ratio remained unchanged. On the other hand, it is well known that Xc maintains a relationship with cell membrane capacitance, which is affected by the size, thickness, composition and distance between cell membranes. Physical exercise generates acute processes which modify the characteristics of muscle cells. When cell membrane becomes thinner, the cell swells and capacitance increases, and the opposite happens as the cell shrinks, thus affecting Xc (Castizo-Olier et al., 2018b). Nescolarde et al. (2020a) reported in a novel way the relationship between kidney function and the kinetics of the bioimpedance vector components immediately after and 48 hours post-running a marathon. Serum and urine biomarkers and Xc values just after the race suggested an impairment in the cell structure integrity. According to the authors, this impairment was due to muscle activation during race, which affected its length and tension. Consequently, this induced a major delay between injection of current and voltage and, as consequence, a greater Xc post-race.

Only the study developed by Campa et al. (2019d), which focused on demonstrating the effectiveness of a 10-min cold shower (22°C) to stabilise the impedance parameters, showed a significant decrease in R an Xc immediately after 30 minutes of high intensity running. These results, as the authors clarify, could be produced by the increase in conductivity due to the body/skin temperature and blood flow, and by a decrease in weight that, although statistically significant, was probably due to the loss of fluid due to sweat. It was concluded that a 0.2°C increase in skin temperature and a decrease of less than 0.5% in body weight loss would likely have a minimal effect on whole-body BIA measurements immediately after exercise.

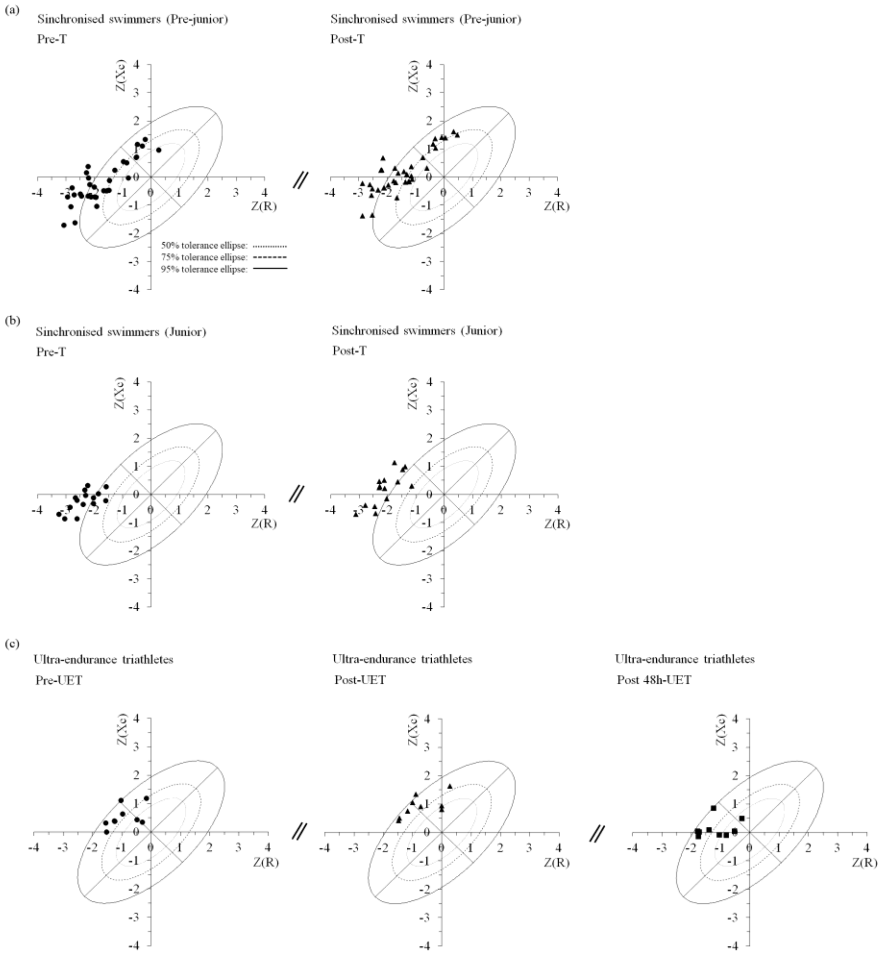

Figure 1. BIVA patterns before and after training/competition. Individual impedance score vectors of the (a) pre-junior and (b) junior synchronised swimmers (Carrasco-Marginet et al., 2017), and (c) ultra-endurance triathletes (Castizo-Olier et al., 2018a), plotted on the 50%, 75%, and 95% tolerance ellipses of the corresponding healthy reference population (De Palo et al., 2000; Piccoli et al., 1995), are displayed for pre-, post-training/competition and post 48 hours-competition. Z(R), resistance Z score; Z(Xc), reactance Z score; Pre-T, pre-training; Post-T, post-training; Pre-UET, pre-triathlon race measurements; Post-UET, post-triathlon race measurements; Post 48h-UET, 48 hours post-triathlon race measurements.

Finally, no correlation was found between body mass and bioimpedance vector kinetics in some of the pre-post short-term field studies (Carrasco-Marginet et al., 2017; Castizo-Olier et al., 2018a), probably by exercise-related factors, such as sweat rate, respiratory water loss and oxidative water production that may lead to BM loss without an effective net negative fluid balance (Gatterer et al., 2014).

BIVA's research focused on short-term vector changes is a topic of great interest as it poses a challenge for researchers, not only because of the variety and complexity of the different types of exercise but also because of the focus on the design of the studies. Although laboratory research allows controlling the quality of data recording, it is necessary to continue making progress in the preparation of field studies with athletes on a real sporting scenario.

Medium-term vector changes

Investigations assessing medium-term vector changes (<7 days after the first measurement) have fewer limitations than the short-term vector changes analysis, since they compare two basal measurements, and no exercise is performed immediately before the second measurement. However, the methodological weaknesses of these protocols rely in the control of possible external factors between every each measurement, which could affect the intra-subject bioelectrical signal.

Castizo-Olier et al. (2018a) evaluated bioelectrical changes 48 hours after performing an ultra-endurance triathlon (UET) race. Regarding the findings, individual vectors’ migration along the major axis was observed (Figure 1c). This was due to significant decreases in R and Xc from the end of UET, indicating fluid restoration at 48 hours post-UET, while BM values were still significantly lower than at baseline. This highlights the potential advantage of BIVA in providing additional information about hydration changes in comparison with BM alone. These results are also consistent with those obtained by Nescolarde et al. (2020a) after 48 hours post-marathon, where significant decreases in R/H and PhA were observed in relation to the values recorded immediately after the race. The authors concluded that BIVA along with serum biomarkers could be used to follow up the kidney function in runners.

Again, the Xc medium-term changes induced by physical exercise requires special attention. While R is directly affected by the opposition to the flow of an alternating current through intra- and extra-cellular ionic solutions and it is related with the hydration status of soft tissues, Xc implies the capacitive component of tissue interfaces and cell membranes. Consequently, it is associated with the integrity of the soft tissue structures. Based on the scientific evidence elucidated by the 48 hours post-exercise results after the completion of an UET (Castizo-Olier et al., 2018a) and a marathon (Nescolarde et al., 2020a), although it should be considered that both studies were performed with non-professional athletes, it seems conclusive that Xc is sensitive to muscle adaptations (i.e. cell structure integrity) induced by both races. Thus, those runners who registered higher values of creatinine 48 hours post-marathon reported lower values of Xc/H (P <0.01), probably due to the expansion of extracellular water (fluid overload) and inflammation. In addition, Xc correlated with C-reactive protein -a marker of kidney function that indicates physical stress- 48 hours after the race, showing a relation with the inflammatory processes of tissue disruption induced by the marathon race (Nescolarde et al., 2020a).

All in all, several biological mechanisms have a multifactorial influence on the progressive restoration of fluids (related to R values), balance of the ratio ECW/ICW (related to PhA values) and cell integrity of soft tissues (related to Xc values) during the days after performing physical exercise, compromising their homeostasis (Castizo-Olier et al., 2018b): transient expansion of plasma volume due to a higher activity of aldosterone and antidiuretic hormone; protein catabolism with consequent fluid shifts (hypoproteinemic oedema); increased plasma protein concentration – especially albumin – inducing an increase in plasma oncotic pressure; or impairment of the kidney function due to the rhabdomyolysis. To date and to our knowledge, only two studies have analysed, under real circumstances of sport competition, the medium-term changes of the biompedance vector. BIVA research on medium-term vector changes induced by physical exercise needs to continue exploring the possible relationship between these biological mechanisms and the subsequent kinetics of the bioimpedance components (R, Xc, PhA). Isolating or controlling the external variables between the different BIA measurements that could interfere with the intra-subject biological response (measured at 24h - 48h - 72h... post-exercise) should be one of the greatest challenges to overcome in future longitudinal studies focused on medium-term vector changes.

Long-term vector changes

Longitudinal studies investigating long-term vector changes (≥7 days after the first measurement) have some protocol-specific advantages in comparison with investigations focused on short- or mid-term vector changes, mainly because the quality of the bioelectrical signal can be assessed independently from the acute adaptations related to exercise.

There are multiple follow-up studies performed with athletes that have analysed long-term whole-body vector changes, usually throughout a sports season in soccer (Gatterer et al., 2011; Bonuccelli et al., 2012; Mascherini et al., 2014), boxing (Reljic et al., 2013), judo (Silva et al., 2020), youth gymnastics and swimming (Meleleo et al., 2017), handball, basketball, swimming, volleyball and triathlon (Campa et al., 2019a). Stage competitions/events in cycling (Pollastri et al., 2016a; 2016b) and in climbing at high altitude (Piccoli et al., 1996b) have also been studied.

Regarding the studies performed in soccer, Gatterer et al. (2011), in their study assessing body composition using "classic" BIVA in the 2008 European Football Championship, found a significant lengthening of the vector within a period between 1 and 2 weeks. They attributed it to changes in BCM and ECW after the first match with respect to baseline values, indicating body fluid loss. After the second match, only the athletes who played more showed a significant lengthening of the vector possibly due to a decrease in ECW. Therefore, they concluded that changes in body composition were mainly due to changes in ECW. On the other hand, Bonuccelli et al. (2012) and Mascherini et al. (2014) assessed football teams across one sport season and also reported significant shortening of the vectors in the pre-season associated with an improvement in endurance performance, possibly due to plasma volume expansion and enhanced glycogen storage (Bonuccelli et al., 2012; Mascherini et al., 2014). A significant lengthening of the vector in the mid-season compared to pre-season results was also observed. This could indicate a reduced body fluid volume (i.e., decreased plasma or interstitial volume) despite an increased intracellular fluid associated with an increase in BCM, and consequently in PhA. However, while Mascherini et al. (2014) reported a significant shortening of the vector at the end of the season compared to the mid-season, Bonuccelli et al. (2012) observed a significant water content decrease. Sports calendars could have conditioned different (or even opposite) training strategies that would explain these results.

Two studies emphasised the relationship between relatively rapid loss of BM and the subsequent lengthening of the bioimpedance vector in climbers (Piccoli et al., 1996; Reljic et al., 2013) and boxers (Reljic et al., 2013), which were identified by different hydration markers. In relation to the climbers, after descent to sea level, the impedance vector underwent a significant shortening and returned close to baseline values. Lastly, significant relationships were found between changes in bioelectrical variables of climbers (R/H and Xc/H) and changes in the following hydration biomarkers along measurements performed at altitude and at sea level: BM, urine volume, plasma osmolality, serum sodium, potassium, chlorine and glucose, and urine osmolar excretion.

Besides boxing, there is another study analysing combat sports that reported interesting results, in this case applied to a group of élite judokas (Silva et al., 2020). The authors assessed their body composition from the beginning of the sport season (September) to December and they concluded that judokas who reported an increase in R experienced a reduction in TBW mainly from ECW, while counterparts who reported an increase in PhA showed an expansion of ICW and, as a result, a decrease in the ratio ECW/ICW.

Campa et al. (2019a) analysed several sports and concluded that long-term vector changes reflected the loss or gain of fluids during a sport season. Peripheral vectors that lied on the left (> PhA) or the right side (< PhA) of the minor axis of the tolerance ellipses indicated more or less soft tissue, respectively. Furthermore, authors noted that PhA was inversely related to the fluid distribution evaluated from the ECW/ICW ratio, as reflected by their results.

On the other hand, Pollastri et al. (2016a; 2016b) found a significant shortening of the vector along three weeks of multistage road bicycle race, indicating fluid gain during the tour and they attributed these results to muscle oedema, haemodilution, released water from muscle glycogen oxidation, and excess fluid intake. Although the vector shortening was not related to power output or rating of perceived exertion, it was negatively associated with performance during the last stages, being suggested that increases in plasma volume and improved thermoregulatory capacity could explain these outputs.

Meleleo et al. (2017) evaluated the body composition in participants of swimming and gymnastics along one year. The baseline measurement (T0) was performed at a period preceding races and sporting events, just as the third measurement (T2) one year later. The second measurement (T1) was executed six months after T0 in a period characterised by a softer daily training. They found a significant increase in Xc from T0 to T1, along with increased PhA and ICW (derived from ECW:TBW ratio). The authors hypothesised that this was due to an improvement in the muscular trophism with higher levels of intracellular proteins and glycogen and to a lower stress from training program. After one-year follow-up, no significant differences were found in R, Xc and PhA.

Finally, most of the studies that monitor athletes through BIVA during a sport season do not include quantitative variables of training load (internal or external) and/or competitive performance parameters depending on the period the season. Beyond the research for possible relationships between the long-term vector changes of with certain physiological variables, future studies should provide this information of special interest to athletes and coaches. We should note that the final results will not only depend on the type of sport or the characteristics of each athlete but also by the different training systems and by the planning strategies applied.

Research Highlights

- Research on the short-, medium-, and long-term vector changes induced by physical exercise is still an emerging area with great potential.
- Despite the need of further research, BIVA seems to be an adequate technique to detect certain biological changes induced by physical exercise at the short-, medium and long-term.
- While the arguments about the behaviour of R and PhA regarding certain biological adaptations induced by physical exercise seem to be increasingly resolved, the Xc behaviour in relation to physical exercise still needs further specific research.
- Short- and medium-term vector changes studies are scarce due to the difficulty of applying these protocols, which are affected by the acute effects of the physical exercise performed. Laboratory and field studies should be promoted to achieve a balance between scientific rigor and the necessary applicability in real sports scenarios.
- Beyond the research for possible relationships between the long-term vector changes and certain physiological variables, it is necessary to include quantitative variables of training load (internal or external) and/or competitive performance parameters.

Localized bioimpedance (L-BIA) in professional football players

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The electrode placements used in clinical applications are: whole-body (or right-side), as the standard bioimpedance configuration; segmental BIA and localized bioimpedance (LBIA) measurements. All of them, using tetra-polar configuration's; two electrodes for injecting current (I), and two electrodes for sensing voltage (V).

The difference of segmental BIA respect to localized BIA (L-BIA) is that in segmental BIA the injector (I) electrodes are placed in standard position for "whole-body", while the detector (V) electrodes are positioned indistinctly on arms, legs and body's trunk according to several modalities. In L-BIA both the pair of injecting and sensing electrodes are positioned in the area of interest, such as quadriceps, hamstring, calf, etc.

The diagnosis and follow-up of muscle injury until the return-to-play (RTP) is carried out mainly by magnetic resonance imaging (MRI) and the grade of the injury is based fundamentally using the British Athletics Muscle Injury Classification (BAMIC) according to the site and the severity of the injury (Pollock et al., 2014). Starting from localized impedance myography (Rutkove, 2009) and wound assessments by localized bioimpedance (Lukaski and Moore, 2012) as references, the use of tetra-polar L-BIA arises for the muscle assessment at 50 kHz (Nescolarde et al., 2013) with phase-sensitive bioimpedance analyzer using Ag/AgCl electrodes.

In professional football players the L-BIA measurements has as objective to identified connective tissue disruption through changes in reactance (Xc) and fluid distribution through changes in resistance (R). The L-BIA measured are taken 24 hours after injury and previously diagnosed by MRI exam. Percentage difference of R, Xc and phase angle (PhA) of the injured side are calculated considering contralateral non-injured side as the reference value.

The sensing pair of electrodes (V) are placed 5 cm proximally and 5 cm distally from the center of the injury (located by ultrasound) or 10 cm proximally and 10 cm distally depending on the anatomical depth (Sanchez et al., 2016; Rutkove et al., 2017) and the electrodes for injecting the current (I) close to the sensing electrodes. In adductors, a transverse position of the electrodes can be used, with two electrodes for detecting voltage (V) 5 cm to the center of the injury (Nescolarde et al., 2015).

Nescolarde et al. (2015) extended the first L-BIA study (Nescolarde et al., 2013) over a larger sample and have found most significant change is evidenced by Xc, 24-h after injury, showing a pattern in line with the severity of the injury, while variations in R are not as indicatives. There is evidence that part of the RTP is conditioned by the severity of the injury, and this severity is associated with muscle gap (quantifiable retraction between fibers). Actually, Futbol Club Barcelona Medical Department working in the validation of muscle injury classification, in which muscle gap plays an important role (Valle et al., 2019), with the inconvenience that it is not always possible to be identified by MRI.

A third work (Nescolarde et al., 2017) related L-BIA with RTP and muscle gap, regardless of the anatomical location of the muscle injury. On grouping data according to the muscle gap (by MRI exam) there are significant differences in R between grade 1 and grade 2f (myotendinous or myofascial muscle injury with feather-like appearance), as well as between grade 2f and grade 2g (myotendinous or myofascial muscle injury with feather and gap). As conclusion, a significant decrease in Xc and PhA was detected between each grade of muscle injury with decreasing severity. In addition, the severity of muscle gap adversely affected the return-to-play.

Finally, the last study (Nescolarde et al., 2020b) is aimed to differentiate the muscle injury, by L-BIA, according to anatomical location between tendinous, myotendinous junction (MTJ), myofascial junction (MFJ) injuries and the severity of MTJ injuries graded from 1 to 3. In addition to find the relationship between days to RTP and L-BIA. The muscle injuries were previously disgnosed following BAMIC (Pollock et al., 2014) and the histoarchitectural approach to skeletal muscle injury described by Balius et al. (2020). By L-BIA was found that muscle injury with a greater percentage of change in Xc (-24 to -43%) has a long RTP (38 to 66 days).

In conclusion, L-BIA could help in the diagnosis of muscle injury and contribute to give a forecast by the RTP. However, a limitation is that L-BIA method is insensitive to the detection and monitoring of tendon injuries.

Research Highlights

- Muscle assessment and quantifications of muscle injury by localized bioimpedance (L-BIA).
- Muscle injury classification by quantifications of muscle "gap" and anatomical location of muscle injury.
- Relationship between percentage decrease of reactance 24 h after injury and time to return-to-play (RTP).

Effects of resistance training programs on body composition in older adults

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The increase of the elderly population worldwide is providing a demographic transition process, in both developed or developing countries, that also represents an essential epidemiological transition. This implies changes in morbidity patterns and in leading causes of death. The main factors increasing the proportion of diseases in older adults, especially chronic non-communicable diseases, are cardiovascular diseases (30.3%), malignant neoplasms (15.1%), chronic respiratory diseases (9.5%), neurological and mental disorders (6.6%) (Prince et al., 2015). In this context, understanding the demographic and epidemiological transition is essential to explain the process of population aging and its economic and social impact on the health system.

Within the biological processes studied in aging, those related to morphological adaptations are of particular interest to gerontology professionals, considering the various intervention alternatives to mitigate the harmful effects of aging on the body composition components. Of the studied components, special emphasis is placed on the fat, lean and bone components.

Body composition and aging

An increase in body fat with a decrease in lean mass and bone mineral content has been revealed during the aging process. Such changes are accentuated by adipose tissue distribution specificity, with a more significant accumulation of intramuscular, visceral, and liver fat, with less representation of subcutaneous fat for total body fat (Reinders et al., 2017). Also, the reduction in skeletal muscle mass from the third decade of life, with greater accentuation at older ages, contributes to a pro-inflammatory process responsible for several chronic diseases.

In older adults, obesity and reduced skeletal muscle mass are associated with a severe functional decline. When the reduction in muscle mass is related to the decrease of muscular strength and mobility, the phenomenon is called sarcopenia, which is also related to the worsens of functional status and a higher decline in functional fitness in a period over five years (Ponti et al., 2020). When obesity and sarcopenia are presented concomitantly (sarcopenic obesity), the risks for multiple health outcomes are even more remarkable.

To better monitor these dramatic changes in body composition throughout aging and to avoid possible abnormal conditions in the distribution of body components, detailed and specific assessment of body composition has long been proposed for older people with the objective, especially, of preparing strategies and intervention alternatives to reduce the risks for age-related diseases.

Resistance training for older adults

Among the various intervention strategies, resistance training (RT) is a widely accepted proposal, with sufficient evidence on its benefits for the health of older adults, especially to mitigate or even reverse the harmful effects of aging, especially sarcopenia and obesity.

The National Strength and Conditioning Association presents RT programs as a non-pharmacological strategy to neutralize age-related changes in contractile function, atrophy, and aging morphology of skeletal muscle (Fragala et al., 2019). In addition, RT can promote increases in muscular strength, power, and neuromuscular function, as well as reduction of body fat (Fragala et al., 2019). Therefore, RT is beneficial for treating and preventing sarcopenia and maintaining functionality in older adults, increasing muscular strength, and improving walking speed. Given the benefits on mobility, physical functioning, and performance in performing activities of daily living, the participation of older adults in RT programs favor the preservation of functional independence, resistance to injuries and catastrophic events, such as falls, in addition to improving psychosocial well-being (Fragala et al., 2019).

Body composition assessment

Several methods of assessing body composition have been used to assess older adults at a specific moment and monitor changes in body components in intervention processes. Among the most used and verified methods in the literature, bioelectrical impedance analysis (BIA), dual-energy X-ray absorptiometry (DXA), densitometry (underwater weighing), hydrometry (deuterium dilution), and Echo-MRI can be mentioned as those widely applied. Each method has its positive and negative points, which vary between the cost of acquisition and operation, the complexity in the operationalization of the equipment, the invasiveness, the duration of the evaluation, the portability, the validity, among other factors. Among the most used methods in the clinical and scientific environment, BIA stands out for its low cost (compared to other evaluation methods with more advanced technologies), for its portability, operation facilities, low invasiveness, and validity accepted for the evaluation of different body components, especially for the assessment of muscle mass, because of its good validity for measuring total body water (TBW) and fat-free mass (FFM). It is noteworthy that in aging changes in fat and lean components are partly influenced by essential changes in the hydric component, with reductions in TBW and the intracellular water-to-extracellular water (ICW/ECW) ratio.

As is already known, conventional simple frequency BIA assessment techniques provide an estimate of TBW and FFM using regression models that consider impedance, resistance, and other variables, which can lead to considerable bias, mainly if used in groups of people with different characteristics from the sample used to validate the model. These errors can be enhanced if the group comprises of older adults, who have even more significant differences in body hydration. Therefore, the analysis of the raw parameters of BIA, such as resistance (R) and reactance (Xc), in addition to the phase angle (PhA) and bioimpedance vector analysis (BIVA), has been suggested to mitigate these errors (Piccoli et al., 1994).

BIA in older adults

As is already known, the PhA has been suggested as a biomarker of muscle quality, muscular strength, and functionality. Recent studies indicate that the PhA can be influenced by the level of physical activity (Mundstock et al., 2019) and is higher in athletes than non-athletes (Di Vicenzo et al., 2019). The PhA is usually higher in males than in females at all life stages due to the amount of muscle mass and it increases progressively with age until approximately the fourth decade of life, and then decreases with aging, with greater reductions after the seventh/eighth decades of life phase angle (Mattiello et al., 2020).

Along with the PhA, BIVA allows an assessment of body cell mass and TBW with valuable information in the clinical area, as also an indication of nutritional status and frailty. With the normalization of the conductors' lengths by body segment measurements (arm, waist, and calf circumference), for the vector analysis using the *specific* BIVA, more accurate values of R, Xc, and consequently, the analysis of the vectors and the cellular integrity started to be presented (Marini et al., 2013). The use of classic BIVA has been shown to have a better application for assessing nutritional status and body hydration level (Piccoli et al., 2014). Instead, the *specific* BIVA shows a better potential to distinguish lean and fat body components (Buffa et al., 2014b), and hence, to better classify older people under sarcopenia conditions and/or sarcopenic obesity.

In monitoring RT programs, the use of BIA and the BIVA proposal (classic or *specific*) to evaluate older adults in various clinical conditions, in addition to the best cost-benefit and practicality, can provide answers related not only to body composition (muscle, fat and hydration components), as well as cellular integrity analysis. Thus, BIVA's relationship with morphological and functional indicators can be considered an alternative methodology for approximating the health conditions of the elderly population, especially concerning functional capacity and frailty.

The use of the BIA methodology to assess body composition, especially for the analysis of fat and lean body mass, using predictive equations, with variables such as impedance, R, Xc, age, sex, among others, has been widely presented in the literature for monitoring RT programs in the elderly. However, despite advances in the number of studies that indicate that BIA parameters (R, Xc and PhA) have clinical relevance and are related to physiological and functional biomarkers, there is still a lack of information regarding the effects of RT on these parameters, especially in older adults.

In this regard, we will present the main studies using the new proposals of BIA and BIVA in RT programs in the elderly.

Resistance training programs and BIA raw parameters

The search for the articles for this session was carried out in various databases based on the use of the criteria: older adults, aged 60 or over, involved in RT programs or similar equivalent to a randomized clinical trial with group control, and with assessments of BIA parameters R, Xc, PhA and/or BIVA (*specific* or classic). Seven studies were found, of which six with the analysis of raw BIA variables, and only one investigation with BIVA compared the classic model with the *specific* one. All the studies, interestingly, were carried out with older women. There seems to be a particular focus on women when analyzing the deleterious effects of aging on body composition and the benefits of intervention programs, especially physical exercise, possibly due to the significant hormonal and metabolic changes generated during and after menopause and its consequences.

The six studies that analyzed the results of RT programs on BIA variables presented positive responses, in general, with an increase in the PhA and Xc and reduced R values (Tables 1 and 2). Such changes in BIA values throughout RT programs can be explained by physiological mechanisms associated with training principles, especially overload.

Table 1. General characteristics of the studies with BIA variables, resistance training and elderly people.

First Author, Year	Sample size	Age, yrs (Mean±SD)	Intervention	RT Protocol Sets x Reps	Main results
Ribeiro et al., 2017	76	68.5±5.7	RT with different loads	8 exercises 3x8-12 3x12-10-8	> PhA (GCC = +2.9%; GCP = +4.1%) < R (GCC = -3.7%; GCP = -2.6%) > Xc (GCC = +3.4%; GCP = +7.4%)
Souza et al., 2017	41	67.2±4.5	RT	8 exercises 3x10-15	> PhA = +6.5% < R = -3.2%
Campa et al., 2018	30	66.1±4.7	TRX	6 exercises 4x12	> PhA = +7.2% < R = -3.2% > Xc = +7.1%
Cunha et al., 2018	62	68.6±5.0	RT with different volumes	8 exercises 1x10-15 3x10-15	> PhA (G1S = +4.8%; G3S = +7.5%) < R (G1S = -4.9%; G3S = -5.3%) > Xc (G1S = +6.1%; G3S = +6.8%)
Ribeiro et al., 2020	33	68.1±5.7	RT	8 exercises 3x10-12	> PhA = +3.4% < R = -4.6%
Tomeleri et al., 2018	51	70.6±5.1	RT	8 exercises 3x10-15	> PhA = +7.4%

Note. SD = standard deviation; RT = Resistance training; TRX = suspension training; GCC = Group with constant load; GCP = Group with pyramidal load; G1S = Group with single set per exercise; G3S = Group with three sets per exercise; PhA = Phase Angle; Xc = reactance; R = Resistance; BIA = Bioelectrical impedance; > = higher; < = lower.

During a training session, the progressive overload mechanically stimulates events such as gene transcription, translation, and modification of the post-translation process until the integration of events, which stimulate the synthesis of proteins and tissue adaptations. In addition to mechanical tension, metabolic mechanisms and muscle damage at the cellular level promote localized adaptations that stimulate hypertrophy, often resulting in edema (Wackerhage et al., 2019).

Table 2. Changes in the values of BIA markers from studies with Elderly in Resistance Training Programs

Authors, Year	**Group (n)**	**PhA – Pre**	**PhA – Post**	**R – Pre**	**R - Post**
Souza et al., 2017	GT (19)	5.53 ± 0.53	5.89 ± 0.63*	591.2 ± 75.0	572.1 ± 69.2*
	GC (22)	5.62 ± 0.55	5.49 ± 0.60	585.2 ± 74.2	590.3 ± 74.3
Ribeiro et al., 2017	GCC (25)	5.60 ± 0.49	5.76 ± 0.59*§	586.5 ± 65.2	564.9 ± 74.2*§
	GCP (26)	5.41 ± 0.65	5.63 ± 0.61*§	574.7 ± 59.5	559.8 ± 60.4*§
	GC (26)	5.56 ± 0.50	5.48 ± 0.46	580.5 ± 80.9	587.0 ± 79.2
Campa et al., 2018	GTS (15)	5.6 ± 0.4	5.9 ± 0.5*	555.2 ± 46.9	540.2 ± 49.2*
	GC (15)	5.6 ± 0.4	5.5 ± 0.5	536.2 ± 46.7	540.7 ± 46.2
Cunha et al., 2018	G1S (20)	5.87 ± 0.59	6.12 ± 0.49*§	552.65 ± 51.77	528.10 ± 45.60*§
	G3S (20)	5.50 ± 0.58	5.90 ± 0.61*§	603.54 ± 59.89	570.93 ± 58.57*§
	GC (22)	5.67 ± 0.60	5.36 ± 0.51*	589.06 ± 74.34	594.61 ± 71.12
Ribeiro et al., 2020	GT (18)	5.59 ± 0.51	5.78 ± 0.63*§	570.5 ± 43.7	544.4 ± 51.6*§
	GC (15)	5.52 ± 0.47	5.44 ± 0.44	558.8 ± 69.9	571.5 ± 70.8
Tomeleri et al., 2018	GT (24)	5.4 ± 0.6	5.8 ± 0.7*§	560.3 ± 56.1	547.1 ± 56.7*§
	GC (22)	5.6 ± 0.5	5.4 ± 0.5*	579.8 ± 71.5	584.1 ± 70.3

GT = training group; GC = Control group; GCC = Group with constant load; GCP = Group with pyramidal load; GTS = Training group in suspension; G1S = Group with a series per exercise; G3S = Group with three sets per exercise; PhA = Phase Angle, expressed in degrees; R = Resistance, expressed in ohms per meter; Xc = Reactance, expressed in ohms per meter.

Considering the biophysical principles of BIA, with the human body being a circuit formed by resistors, represented by body fluids and their electrolytes, and cell membranes and interfaces with tissues acting as capacitors, changes in the volume and composition of body fluids, in the amount of electrolytes and/or in the cell structure, associated with RT, may influence the responses in R, Xc and, consequently, in the PhA. .

Table 2.
(continues)

Authors, Year	**Group (n)**	**Xc – Pre**	**Xc - Post**
Souza et al., 2017	GT (19)	57.2 ± 8.2	58.8 ± 9.5
	GC (22)	57.6 ± 9.5	56.5 ± 8.3
Ribeiro et al., 2017	GCC (25)	55.6 ± 6.2	57.5 ± 7.6*§
	GCP (26)	53.9 ± 7.7	57.9 ± 8.4*§
	GC (26)	55.7 ± 9.1	54.6 ± 8.0
Campa et al., 2018	GTS (15)	53.6 ± 4.1	57.1 ± 4.5*
	GC (15)	52.3 ± 7.9	51.2 ± 7.2
Cunha et al., 2018	G1S (20)	56.32 ± 5.32	59.49 ± 4.51*§
	G3S (20)	58.07 ± 7.59	61.88 ± 8.45*§
	GC (22)	58.67 ± 10.10	55.89 ± 8.61
Ribeiro et al., 2018	GT (18)	54.1 ± 5.2	55.9 ± 5.9
	GC (15)	53.4 ± 8.7	52.5 ± 8.9
Tomeleri et al., 2018	GT (24)	53.3 ± 7.9	55.9 ± 8.7*
	GC (22)	57.0 ± 9.8	55.4 ± 8.3

All values are express in mean ± SD. *$p < 0.05$ vs. pre-training; §$p < 0.05$ vs. group control.

Once R depends directly on tissue hydration and is inversely associated with fluid content and muscle tissue is composed of ~80% of water and electrolytes, it can be considered an excellent electrical conductor. The cell membrane (with lipidic material), body fat, and bones, instead, are composed of anhydrous materials with reduced conductivity. Based on these assumptions, most studies have presented plausible answers to RT. Five out of the six studies presented body fat reduction between - 1.3% to 7% (Campa et al., 2018; Ribeiro et al., 2020; Souza et al., 2017; Tomeleri et al., 2018). Other five studies showed increases in ICW, ranging from + 3.1% to + 12.4% (Cunha et al., 2018; Ribeiro et al., 2017; Ribeiro et al., 2020; Souza et al., 2017; Tomeleri et al., 2018), and four studies showed increases in muscle tissue, ranging from 1.3% to 6.6% (Ribeiro et al., 2017; Ribeiro et al., 2020; Souza et al., 2017; Tomeleri et al., 2018). All of these responses to RT meet the reductions seen in variable R, which can lead to the hypothesis that reduction in favor of RT groups can increase intracellular hydration and muscle tissue, reduce body fat, or a combination of these changes.

It is worth noting that, except for the study by Ribeiro et al. (2020), which lasted eight weeks, all other investigations had an intervention lasting 12 weeks. The results with the parameter Xc show significant increases in favor of the training groups. As mentioned previously, the RT program promoted a rise in ICW, which is positively related to increases in Xc. During a training session, there are some acute adaptations on muscle fibers, mainly due to the accumulation of metabolites in the muscle induced by exercise, depending on volume or intensity. This acute adaptation is called cellular swelling, and it acts as a regulator of cell function by stimulating the accumulation of proteins and strengthening structures, especially the cell membrane (Wackerhage et al., 2019), which work as capacitors, as presented previously. When analyzing the chronic effect from several accumulated RT sessions, the response in Xc values (higher values) was plausible.

Finally, the proper combination of changes in parameters R and Xc resulted in increases in the variable PhA of ~0.4° in favor of the training groups. Due to the positive effects of the overcompensation that occurs after RT sessions, with the damaged muscle fibers repaired, the PhA is expected to be higher (Lukaski et al., 2019), supporting previous studies which found a positive relationship of PhA and physical activity, especially when compared to their non-active and/or non-athlete peers (Di Vincenzo et al., 2019; Mundstock et al., 2019).

Resistance training programs and BIVA

The study of Fukuda et al. (2016) was the only one that analyzed the effects of an RT program on classic or *specific* BIVA. Initially, it was found in the study that the patterns in the classic and *specific* BIVA charts were different after the six-month training period (Figure 1). For the classic BIVA, the main changes were observed only after six months. On the other hand, for the *specific* BIVA, the changes occurred after between 3 and 6 months. Therefore, the results suggest that the analysis of classic and *specific* BIVA can provide conflicting values after an RT program.

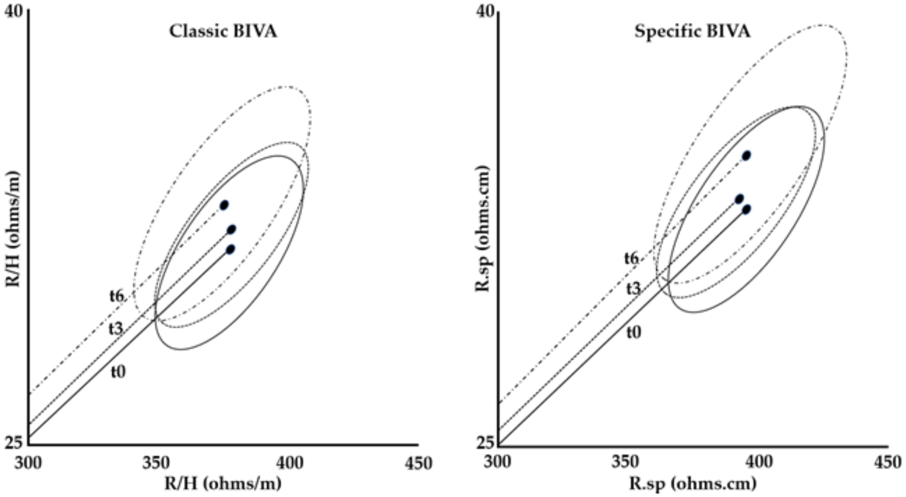

Figure 1. Classic and *specific* BIVA confidence ellipses at baseline (t0), after 3 months (t3), and after 6 months (t6) of resistance training in elderly women (Adapted from Fukuda et al., 2016).

As seen in the previous six studies, decreases in R values throughout the RT program for classic and *specific* analyzes (for *specific* resistance from three months onwards) may reflect improvements in body composition (Buffa et al., 2014b), depending on the reduction of fat mass, or the increase in lean mass/cellular hydration. For Xc, the expected increase occurred only when the analysis was based on the *specific* BIVA. As previously mentioned, these changes are related to cell adaptation. This qualitative distinction in the contributions of *specific* resistance and *specific* reactance by a *specific* BIVA as of the three months of RT is consistent with the literature concerning strength training adaptations.

Conclusions

The RT programs have shown positive effects on the body composition in older adults, already well demonstrated by several studies with different assessment methodologies. With the new proposals for evaluation by BIA, with the raw parameters R, Xc PhA, and BIVA, either in the classic or the *specific* model, although few studies have been carried out yet, scientific evidence points to a good validity of BIA and BIVA for the evaluation of distinct morphological and functional components in older adults.

This study area appears to be prominent. There are still gaps to be studied, such as the effect of training on older men and investigations with BIVA. Moreover, it is not yet verified in the literature studies on localized BIA in older adults involved in RT programs.

Research Highlights

- Elderly people benefit from resistance training programs, with positive effects especially on lean body mass and body fat.
- The effects of resistance training programs on the body composition of older adults are subject to evaluation using the raw BIA variables.
- Throughout a resistance training program, an increase in the variables Xc and phase angle, and a reduction in the variable R is expected in older adults, due to the increase in intracellular and body hydration and lean tissue and the reduction of body fat, in addition to the increased in cell function and integrity.
- Further studies with BIA and especially BIVA are needed with the older people and resistance training, in view of the incipience of the topic in the literature.

Long-term effects of physical exercise on whole and regional body composition

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The objective of reducing and delaying the process of ageing and its consequences is crucial to scientific research, as well as a matter of public health concern. The World Health Organization defines wellness as 'a state of complete physical, mental, and social well-being, and not merely the absence of disease or infirmity'. Research can provide very useful information to define intervention and prevention strategies for maintaining wellness in old age.

The ageing process is characterised by a progressive change in body mass and composition, even in the absence of disease. These changes affect the whole body and different body segments and mainly concern the increase in fat mass (FM) that tends to accumulate at the visceral level and the decrease in muscle mass, which is more pronounced in men and mainly affects the limbs.

The ageing process exposes the elderly population to the risk of malnutrition, sarcopenia and frailty, especially when the physiological trend is combined with other factors, such as depression or other psychological disorders, physical inactivity and poor dietary habits. In particular, it is related to muscle fatigue, functional limitations and increased risk of falls that significantly accelerate the functional decline and increase the risk of morbidity. For this purpose, an aspect that could interfere with the above scenario, particularly in relation to the risk of falls, is body symmetry. Body composition symmetry has been widely studied in sports science, in relation to performance and injury prevention (Hinton et al., 2017). In the elderly population, previous studies have found an association between falls and body asymmetry (Chon et al., 2018; McGrath et al., 2020). However, research mainly referred to strength and functional asymmetry, while the literature investigating the role of body composition asymmetry is still limited.

The quality of life in the elderly population, in terms of both physical and psychological well-being, is strongly related to lifestyle, with a significant role played by physical activity (PA). Studies on the influence of PA on the elderly population have shown an effect on body composition. Both interventional studies and studies that focussed on the effects of a sport have demonstrated that PA is negatively associated with body fat and positively with muscle mass, even being able to slow down or reverse the physiological trend towards diseases such as sarcopenia. However, the study of the long-term effects of PA on regional body composition, morphological symmetry and body image satisfaction is limited. Moreover, the comparative analysis of the effect of different sports disciplines has been poorly explored.

Whole body composition

Of the limited studies investigating the long-term effect of sports on whole body composition, a few studies were conducted in tennis and running practitioners. A study on veteran tennis players found that the practice of tennis until old age was effective in reducing FM (Moysi et al., 2004a), but the effects on muscle mass were less investigated.

Studies in old runners have shown that regular practice of resistance exercise, even in the old age, demonstrated effectiveness in reducing body mass index (BMI) and body fat and in improving lean mass (Mikkelsen et al., 2013; Mitchell et al., 2020).

A recent PhD thesis (Stagi, 2021) was carried out in a sample of 106 middle-aged and elderly participants (72 men, 34 women; 61.0 ± 7.44 years) involved in three sports commonly practised until old age (tennis, Tai Chi and running), and 105 (49 men, 56 women) age-matched controls selected for being sedentary but normally active in daily living activities. Specific bioelectrical impedance vector analysis was applied to analyse whole and regional body composition. The results were in line with the literature, showing that active participants had lower body weight, BMI and values of all body circumferences (waist, mid-arm and calf) and higher mini nutritional assessment scores. Active individuals also exhibited lower FM percentage (%FM) and higher muscle mass than controls, as shown by the lower vector lengths and the higher phase angles (Figure 1).

These results were similar in both sexes (Figure 1). All sports were effective in reducing %FM, although running and tennis demonstrated a more pronounced effect in improving muscle mass than Tai Chi.

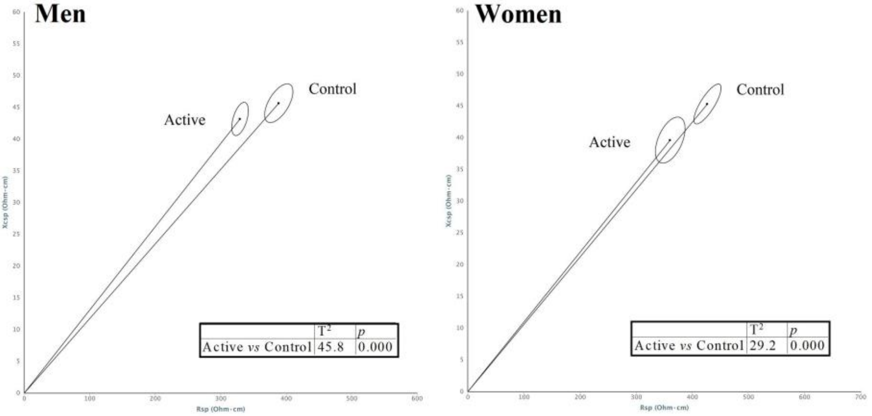

Figure 1. Confidence ellipses of whole body composition of active and control sample.

Regional body composition

The literature on regional body composition in middle-aged adults and older individuals who regularly engage in sports is scarce, but the interest on this topic has been growing in recent years (Piasecki et al., 2019; Mitchell et al., 2020). Studies on regularly active senior subjects were conducted in a sample of subjects engaged in rowing, tennis and running. Studies on male rowers have shown that athletes have higher muscle mass in all body segments (arm, leg and trunk) (Sanada et al., 2009) or in the trunk and leg only (Asaka et al., 2010) than age-matched untrained controls. Among veteran tennis players, research on regional body composition is mainly concerned on bone mass (Moysi et al., 2004a) or asymmetry between dominant and non-dominant arms (Piasecki et al., 2019; Ireland et al., 2014), while very few studies have compared elderly tennis players with sedentary subjects or with players of other sports. Moysi et al. (2004b) stated that regular practice of tennis can maintain or increase leg muscle mass in middle-aged men, while Moysi et al. (2004a) found no differences in body lean mass between tennis and postmenopausal women, but higher fat values in controls, especially at the trunk level.

A study of old runners detected low levels of regional body fat (Piasecki et al., 2019; Mitchell et al., 2020) and greater lean mass in the leg (Piasecki et al., 2019) and trunk (Mitchell et al., 2020). The results of Stagi (2021) partially confirmed results of previous studies, highlighting the differences with the controls and among sports players. Essentially, all analysed sports players showed a lower %FM in the arm and trunk and a higher level of muscle mass, especially in the trunk, than the control subjects of the two sexes. The comparison among sports showed that runners and tennis players exhibited lower %FM and higher muscle mass values than Tai Chi practitioners in both the arm and trunk, and runners had the highest muscle mass values in the trunk. In the legs, differences were not significant in all cases.

Body composition asymmetry

Body composition asymmetry in long-term active older people has been investigated in tennis players only, and these studies have shown conflicting results. Piasecki et al. (2019) detected symmetrical muscle size in the arms of older male tennis players, whereas Ireland et al. (2014) observed greater lean mass in the dominant arm. A study investigating body composition asymmetry in the general population and the whole life cycle showed that the dominant legs and arms were characterised by higher lean mass values, while the differences in the FM were less accentuated (Hinton et al., 2017).

Stagi (2021) showed that active subjects engaged in different sports, tennis players included, have symmetrical lower and upper limbs, while the control group has asymmetrical limbs, because of the higher muscle mass in the dominant arm and the higher %FM in the left leg.

Sexual dimorphism

The literature on sexual dimorphism in sports shows conflicting results. In young adult élite athletes engaged in football and running, few sex differences were found in the whole body composition. Pate et al. (1985) affirmed that athletes with similar performance also exhibited similar body composition. By contrast, Lewis et al. (1986) and Buffa et al. (2001) detected greater sex differences in body composition among young individuals practising different physical exercises compared with people having sedentary lifestyle, due to a weaker effect observed among women. Stagi (2021) showed that middle-aged and elderly Tai Chi practitioners and runners exhibit less sexual body composition differences than the control group. This pattern was observed at the whole body and segmental levels, with greater sex differences at the trunk level.

The selective pressure of long-term sports practice or of high-level competitions could be the cause of the observed lower degree of sex differences. Body composition could be related to the performance needs of a particular sport, which is comparable between sexes, whereas among generally active practitioners, the pressure toward a physique would have less influence.

Psychological well-being

The few studies in aged individuals engaged in sports have shown that PA has an effect on body image satisfaction and mental wellness. Rica et al. (2018) observed that older women who undergo resistance training are much more satisfied of their appearance than the control sample, and Condello et al. (2016) stated that PA at old age is important not only for the management of body weight but also for its positive influence on body satisfaction and mental health.

Stagi (2021) observed similar results in long-term active subjects. Active individuals, independently of the sports practised, are generally satisfied with their body image (50% of men and women were totally satisfied of their appearance). In addition, they did not demonstrate depression symptoms and had a better mental health condition than controls based on the geriatric depression scale questionnaire.

In summary, the long-term practice of sports demonstrated positive influence on body composition, body composition symmetry and psychological well-being. Active individuals were characterised by a better nutritional status and a lower %FM at the whole body and regional levels, particularly the trunk, thus with a less accentuated age-related trend toward the central accumulation of fat mass. As regards muscle mass, athletes more effectively maintain muscle mass both at the whole body and regional levels, especially at the trunk. Among sports, analysis highlighted differences according to the type of sports practised. In all body segments, runners, tennis and Tai Chi practitioners were characterised by lower %FM than controls. Higher regional muscle mass was also observed in tennis, running and rowing athletes than in controls.

Long-term sports practice was also associated with reduced sex differences in body composition, with Tai Chi practitioners and runners showing less sexual dimorphism in the whole body and limbs than controls, whereas sex differences persist in the trunk.

As regards body composition symmetry, the active samples in three sports disciplines have shown limb symmetry in body composition, indicating a possible positive effect of sports on maintaining body balance and consequently on providing better mobility until old age.

Finally, the active sample showed an improvement in body image satisfaction and psychological well-being.

In conclusion, all these results confirmed the positive influence of PA on health. Regular PA contributes to maintaining mental and physical well-being, thus promoting successful ageing.

Research Highlights

- Long-term athletes showed lower %FM and higher muscle mass at the whole body and regional levels than controls.
- Among the considered disciplines, Tai Chi was less effective in maintaining muscle mass.
- Long-term sports practice appears to reduce sex differences in body composition.
- Subjects who regularly engaged a sport showed limb symmetry.
- Body satisfaction and mental state appears to be positively influenced by regular physical exercise, independent of the types of sports.

Applications of bioelectrical impedance vector analysis in physical activity and sports science

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Conventional bioelectrical impedance analysis (BIA) is a non-invasive technique widely used in body composition assessment, nutritional status and hydration status, all considered areas of interest to monitor general health and well-being, but also training and performance levels. However, its accuracy is compromised because of its reliance on regression equations, mostly derived from non-athletic or sport-specific populations. Furthermore, the standard errors of the best BIA regression equations were estimated to be ~3–8% for total body water (TBW) and ~3–6% for fat-free mass (FFM), both considered too large to be used in the clinical setting (Kyle et al., 2004a). In the physical exercise and sport practice, this is especially relevant. For example, dehydration rates lower than these standard errors which may affect negatively the sport performance could be not adequately detected. On the other hand, conventional BIA relies on assumptions such as constant tissue isotropy or constant tissue hydration, conditions that are not frequently met.

Alternative techniques such as the bioelectrical impedance vector analysis (BIVA) emerged to overcome the above-mentioned BIA limitations, founding their main strength on the use of raw impedance variables.

Due to its strengths, there has been a rapid growth of interest in the application of BIVA in sport and exercise research in the recent years. Despite the fact that the current literature in this field is still scarce and very heterogeneous, the increase in the number of publications is justified in order to investigate the applicability of the method for assessments in real time and in a precise, accurate, reliable, non-invasive, portable, inexpensive, safe and simple way.

In order to compile the current knowledge on the topic, this chapter shows the applications of BIVA in the exercise and sport field. More information and references can be found in a systematic review of the literature (Castizo-Olier et al., 2018a).

BIVA applications in the exercise and sport field

In the physical exercise and sports area, BIVA has been used in three different ways: 1) to characterise the vectors and the body composition (i.e., hydration status and body cell mass –BCM) of athletes and active individuals/groups; 2) to monitor the bioelectrical and body composition changes induced by a particular sport or physical exercise; 3) to determine the degree of sensitivity of the localised bioimpedance vector analysis for the identification and follow-up of muscle injuries and as indicator of the training workload adaptation.

In the following lines a brief introduction will be made on each of these areas or applications.

Whole-body vector characterisation

This application consists in performing a cross-sectional comparison of the whole-body vector of athletes and active individuals/groups with their reference populations and also with other sport/active groups.

Vectors shifted to the left with greater phase angle (PhA) have been found in both young and adult athletes compared to the corresponding reference populations (Figures 1 and 2), indicating increased BCM and fluid content. This might reflect a better cell functioning and suggests that the differences are due to sport-specific adaptations (Micheli et al., 2014). Athletes generally possess increased soft tissue mass and differing fluid content compared to the sedentary population. Since total body fluid is affected by factors such as training, trained athletes have a greater amount of body fluid and different fluid distribution between the intracellular and extracellular compartments. This could be because of their larger muscle mass, increased plasma volume and muscle glycogen reserves, which could increase water transport into the muscle and fluid-regulating hormone adaptations (i.e., aldosterone). In relation with this, a negative correlation between the extracellular (ECW) to total body water (TBW) ratio and PhA has been found in athletes (Carrasco-Marginet et al., 2017), indicating a differing fluid distribution (i.e. increased intracellular water –ICW- content), likely due to the hypertrophy of muscle fibres.

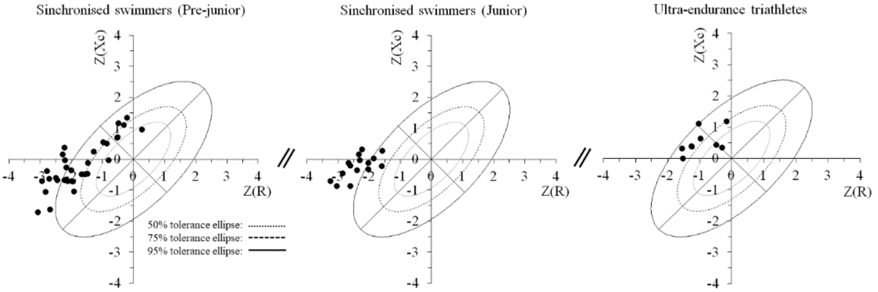

Figure 1. BIVA score graph. Individual vector score values of synchronised swimmers (Carrasco-Marginet et al., 2017) and ultra-endurance triathletes (Castizo-Olier et al., 2018b) are plotted on the 50%, 75%, and 95% tolerance ellipses of the corresponding reference populations. Z(R), resistance Z score; Z(Xc), reactance Z score.

When sport samples where compared, vectors shifted to the left have been reported with increasing age (age ranged from 12 to 44 years old) and performance level in sport samples (Figure 2). The differences could be the result of vector displacement due to the increase in metabolic tissues because of the biological maturation, to the specific training process or a combination of both. It remains to be clarified the influence of performing bioelectrical comparisons in young populations according to the biological or the chronological age. Besides, athletes who had better performance in an ultra-endurance event (and that, presumably, had higher performance levels) were plotted to the left before the start, due to lower height-adjusted resistance -R/H- values (Castizo-Olier et al., 2018b). Accordingly, athletes who were better hydrated before the competition (e.g., with lower R/H values), registered better competitive performance, a lower subjective perception of effort, and less changes in post-competition hydration status. This particularly relevant finding highlights the need of further research regarding this matter, since the application of a non-invasive technique could help to discriminate between performance levels of athletes according to the position of their vectors.

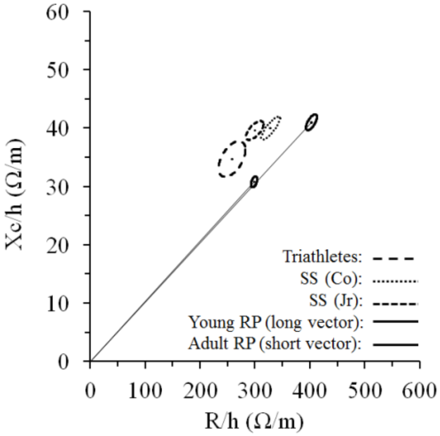

Figure 2. RXc mean graph. The 95% confidence ellipses for the mean impedance vectors of young synchronised swimmers (Carrasco-Marginet et al., 2017), adult ultra-endurance triathletes (Castizo-Olier et al., 2018b), the healthy young female reference population (solid line ellipse with long vector) (De Palo et al., 2000) and the healthy adult male reference population (solid line ellipse with short vector) (Piccoli et al., 1995) are shown. R/H, height-adjusted resistance; Xc/H, height-adjusted reactance; Ω, ohms; m, metres; C_o, pre-junior; J_r, junior; SS, synchronised swimmers; RP, reference population.

It has also been observed that the distance between the confidence ellipses of adolescent and adult athletes is lower than the distance between the ellipses of their respective reference populations. A possible explanation is that the intense training reduced the differences between young and adult individuals (Koury et al., 2014), although this is still to be elucidated.

Regarding the vector position on the RXc graph, the trend is to be outside the 50% tolerance ellipse of the respective reference population in both young and adult athletes (Figure 1). Furthermore, many vectors are plotted outside the 95% tolerance ellipse. This could reflect a specific body composition (characterised by greater soft tissue mass and different fluid content) and suggests that specific tolerance ellipses are needed for sport populations. The relationship between the new specific tolerance ellipses and the hydration status, body composition and sport performance level should be analysed, in order to represent significant hydration changes (that compromise health or performance) or target zones of impedance vectors for athletes.

With regard to the hydration assessment, it should be noted that fluid overload (overhydration) is not common in healthy athletes. Therefore, the analysis of the hydration status should be related to euhydration and physiological dehydration processes. In this way, regarding the identification of dehydration with single measurements according to the tolerance ellipses of the reference population, the limits for “normal hydration” (individuals positioned within the 50% tolerance ellipses, according to the literature) should be reviewed, since subjects experiencing high levels of fluid loss can still be identified as euhydrated. Accordingly, most of the studies applying “classic” BIVA in sport and exercise identify the athletes outside the 50% tolerance ellipse. This is probably due to a range of “normal hydration” comprised by the ellipses wider than a hydration status considered as “dehydration” through other methodologies (Heavens et al., 2016). Therefore, the current classic BIVA point graph should be further investigated in order to detect euhydration and dehydration status in individual athletes with single measurements.

With regard to the body composition assessment and in accordance with “classic BIVA”, athletes have been identified in the upper left quadrant of the reference population and obese individuals in the lower left quadrant. This would generally imply greater R/H and height-adjusted reactance (Xc/H) values of the athletes. Nevertheless, according to the electro-physical assumptions, FFM is characterised by a greater conductivity in comparison with the poorly hydrated adipose tissue, not justifying the relative shortness of vectors of obese individuals with respect to the athletes, unless contemplating their generally greater fat mass (FM), fluid overload and body size. Furthermore, the vector position of athletes regarding the tolerance ellipses of the general reference population is controversial. As mentioned by Buffa et al. (2014), athletic individuals are not always plotted in the "athlete" quadrant of the reference population and their vectors often overlap the "obesity" area. Comparable vector position of athletes and obese individuals would imply similar values of R/H and Xc/H. The already mentioned factors (FM and fluid overload) could compensate the bioelectrical values between both individuals, making difficult to detect these differences by "classic" BIVA (e.g., discriminating the distribution of fluids between compartments, or those athletes with higher ICW content). Moreover, "classic BIVA" would be characterised by a limited sensitivity in assessing the features of body composition due to the no consideration of the effect of cross-sectional areas of the body. "*Specific*" BIVA, a method which performs a correction of whole-body bioelectrical values for body geometry, emerges as the key to overcome this limitation. Although the inclusion of anthropometric measurements can make these plots more sample-specific and perhaps less generalisable than "classic" BIVA, this adaptation may be an advance when comparing athletes with different body composition (in terms of FM and FFM). Therefore, it should be further investigated in the sports field.

Finally, regarding the bioelectrical parameters that determine the vector position, the interpretation is also controversial, and more research is needed to clarify this matter. When athletes present a vector shifted to the left with greater PhA in comparison to the reference population, due to a decrease in R/H with no differences in Xc/H (Carrasco-Marginet et al., 2017), it has been suggested that it reflects different ICW content (Micheli et al., 2014). Moreover, when the vector shifted to the left (with greater PhA of athletes compared to the reference population) is due to a decreased R/H and an increased Xc/H (Castizo-Olier et al., 2018a), the following explanation have been suggested: the decreased R/H is probably due, among other factors, to a greater muscle mass, muscle glycogen reserves and plasma volume, and the increased Xc/H may be due to an increase in the size and number of muscle cells (hypertrophy and hyperplasia, respectively), although the last one is still a controversial topic. Xc/H is not only conditioned by the cell size, but also by the thickness and composition of the cell membrane and also by the distance between them. In this way, lower Xc/H values have been documented in bodybuilders (the best model of extreme muscle hypertrophy) compared to healthy active people and with no differences with the healthy reference population (Piccoli et al., 2007). On the other hand, vectors shifted to the left with lower PhA have been reported in competitive children in comparison with healthy control groups due to significantly lower Xc/H values in absence of differences in R/H (Meleleo et al., 2017). It was suggested that it could be due to an increase in the size of the limbs or to a greater 'sufferance' in cell membranes maybe due to bad response to the workloads (over-training). Therefore, the interpretation of these parameters (R/H, and especially Xc/H) in these cases remains unresolved.

Monitoring whole-body vector changes evoked by training/competition

Whole-body BIVA (classic and *specific*) has been applied to analyse short-term (<24 hours after the first measurement), medium-term (<7 days after the first measurement) and long-term vector changes (≥7 days after the first measurement).

The importance of assessing the body composition and hydration status of athletes lies in the fact that the physical stress imposed during trainings and competitions may lead to alterations, which can impair sport performance and increase the injury risk. Therefore, monitoring theses variations may help to adequately prescribe food/fluid intake and thus limit deleterious effects. Furthermore, body composition has been suggested to discriminate athletes of different performance levels and has been shown to influence physical performance and sport success.

This chapter will not delve into this application of BIVA, since a specific chapter entitled "Body Composition Changes in relation to Physical Exercise" has been dedicated to it. However, we must emphasise one key point: although both classic and *specific* BIVA have been demonstrated as complementary tools when characterising and detecting vector changes, studies investigating these bioelectrical adaptations induced by different protocols of physical exercise or sport are currently still very scarce, especially those related to short-term changes. This could be because its validity can be easily compromised by multiple factors that are difficult to control and that directly affect the precision of the BIA measurements (Castizo-Olier et al., 2018a).

Overall, the relationship between the bioimpedance signal and acute or chronic physiological adaptations induced by physical exercise remains largely unresolved, especially in how the structure and function of the cell are altered and how these affect the behaviour of R, and in particular Xc.

Localised vector as indicator of injury and training workload

The use of bioimpedance as an indicator of muscle status has its origin in the interest of medical sciences in some neuromuscular diseases (Sanchez and Rutkove, 2017). The basic principles of the so-called electrical impedance myography (EIM) are based on the idea that conductivity and permittivity in diseased muscles are altered and these alterations can cause changes in their generated voltage (Foster and Schwan, 1989).

In the field of sports sciences, there are two research areas for which muscle-localised vector has been used: as indicator for diagnosis and follow-up of muscle injuries (Nescolarde et al., 2011; 2013), and most recently as indicator of muscle adaptation to the short- (Li et al., 2016; Freeborn et al., 2019), medium- (Freeborn et al., 2020), or long-term (Mascherini et al., 2015) training load.

Regarding injuries, the application of BIVA consists in identifying (through single cross-sectional protocols) bioelectrical patterns of change depending on the injury type and grade, and also in assessing (through longitudinal protocols) bioimpedance vector sensitivity to monitor injuries and their recovery. Decreases in localised R and Xc were observed in the injured muscles due to the oedema and to the disruption of the muscle structure, respectively. Additionally, the more severe the injury was, the more R and Xc were decreased. On the other hand, a bioelectrical symmetry between muscular groups in lower-limbs was found in soccer players, circumstance positively interpreted by the authors since this could imply a lower risk of injuries induced by imbalances between limbs (Nescolarde et al., 2011; 2013). Therefore, localised bioimpedance vector analysis appears as a method that could help to assess soft tissue injury and to monitor the injury recovery process.

On the other hand, acute and chronic physical exercise evokes some morphological and physiological adaptations within the muscle and these changes in muscle properties could be quantified by the muscle-localised bioimpedance. A relationship seems to be established between the type of muscle activation and the consequently kinetics of R, Xc and PhA. Whereas after maximum voluntary isometric contraction (MVC) impedance values increase significantly, the opposite seems to occur in a series of dynamic isotonic muscle contractions in the case of R. In this way, while the isometric contraction would generate a vasoconstriction and limit blood flow (increasing impedance), dynamic contractions would do the opposite, generating a greater localised fluid volume by muscle swelling (decreasing impedance). Again, the behaviour of Xc and PhA remains unclear, given the complexity of monitoring the physical and chemical changes that occur at the cellular level (Li et al., 2016; Freeborn et al., 2019; Freeborn et al., 2020).

Future perspectives

BIVA in physical activity and sports science is an emerging area of research with great potential. However, some issues should be deeper investigated. The most three relevant are listed below:

Firstly, further research is needed on new classic and *specific* BIVA tolerance ellipses with new reference data of different athletes' profiles. Although progress is being made, there is an evident lack of research carried out with professional or élite athletes stratified by sport, sex, age, and race.

Secondly, further studies should help to clarify the role of Xc (in relation to BCM and PhA) in the short-, medium-, and long-term changes induced at cellular level, depending on the type of exercise performed.

Thirdly, muscle-localised vector analysis must continue to advance beyond the diagnosis and follow-up of injuries. Recent studies using this technique as indicator of training load adaptation seem encouraging and open new challenges for scientists, coaches, and athletes. However, the standardisation of protocols (e.g., electrode placement and position) and the generation of new reference values that allow discriminating between normal or deficient vector outcomes, are the main challenges to overcome in this research area.

Research Highlights

- The bioimpedance vector analysis is a technique that has a great potential in the exercise and sport field, especially for the identification and follow-up of injuries and as indicator of muscle adaption to training load.
- Classic and *specific* BIVA represent different and complementary perspectives of analysis to describe body composition characteristics in athletes.
- In athletes aged from 12 to 44 years old, BIVA has shown a vector shifted to the left with increasing the age and performance level.
- The possibility of generating new BIVA research applied to sport is very wide. In addition, this could have a broad social impact due to the clear transfer in the optimisation of training and competition of athletes.

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List of most used acronyms

BIA: bioimpedance analysis

BIVA: bioelectrical impedance vector analysis

ECW: intra-cellular water

ECF: extra-cellular fluid (water plus electrolytes)

ICW: intra-cellular water

ICF: intra-cellular fluid (water plus electrolytes)

TBW: total body water

FM: fat mass (also referred to as body fat, BF)

FFM: fat-free mass (excludes fat mass, and includes bone mineral content)

LSTM: lean soft tissue mass (FFM - bone mineral content)

STM: soft tissue mass (LSTM + FM)

R: resistance

Rsp: *specific* resistance, or resistivity (ϱ, rho)

Xc: reactance

Xcsp: *specific* reactance

PhA: phase angle

SPA: standardised phase angle

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