Università degli Studi di Cagliari

PHD DEGREE

PhD program in Life Environmental and Drug Sciences

Cycle XXXIII

TITLE OF THE PHD THESIS

Long-term effects of sport on segmental body composition: a study in adult and elderly subjects.

Scientific Disciplinary Sector

BIO/08 ANTROPOLOGIA

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Final exam. Academic Year 2019 – 2020
Thesis defence: April 2021 Session

To my grandparents

Licia and Efisio

Abstract

Research investigating the effects of sport in the elderly has demonstrated that physical exercise contributes to maintain muscle mass and to contrast the increase of fat mass. However, the effects of long-term sport on body composition, in particular on segmental body composition, have been less investigated. Furthermore, the results of the literature are not conclusive about which sport can be more effective on health.

The objective of this doctoral thesis was to study the long-term effects of physical exercise on physiological and psychological well-being in the elderly.

At this purpose, a first section of the thesis concerned methodological aspects related to body composition assessment, including: 1) the comparison of *specific* bioelectrical impedance vector analysis (*specific* BIVA) with dual energy X-ray absorption (DXA) and dilution techniques; 2) the association between *specific* BIVA and self-perceived body image, assessed by silhouettes; 3) the comparison of results from three widely used impedance devices.

Different samples and techniques were used for each objective.

Objective 1) 202 athletes (139 men and 63 women; 20.6 ± 5.1 y of age) were involved for the whole-body composition study, and 50 young active students (25 men and 25 women; 24.3 ± 4.6 y of age) for the study on segmental body composition. DXA was used as the criterion method to assess fat-free mass (FFM), fat-mass (FM), and %FM; deuterium dilution and bromide dilution were used for total body water (TBW) and extracellular water (ECW), respectively. The agreement between techniques was evaluated by Pearson’s correlations, two-way ANOVA, depth-depth analysis, and confidence ellipses.

Objective 2) 632 young adults (238 men and 394 women; 22.8 ± 2.3 y of age) and 162 middle-aged and elderly adults (96 men and 66 women; 61.4 ± 7.6 y of age) were involved. The Williamson’s figure scale was used to evaluate current body size. Statistical analysis included Spearman’s correlation and confidence ellipses.

Objective 3) 31 adults (8 men and 23 women; 39.8 ± 14.2 y of age) were involved. The row data, collected with three devices, were compared with confidence ellipses and regression analysis.

As to the first objective, *specific* BIVA confirmed to be accurate in the assessment of %FM in athletes and sensitive to the ECW/ICW ratio. The segmental approach of *specific* BIVA showed a good agreement with DXA too. The proposed protocol for the electrodes positioning demonstrated to be adequate for segmental body composition.

The analysis of the relationship between silhouettes and body composition (objective 2) showed that young and elderly normal weight individuals of both sexes recognise themselves correctly, and consider their current body image mainly associated with %FM.

The comparison among three bioimpedance devices (objective 3) showed systematic differences in the measure of reactance. The bias was amended by a correction factor, defined for the whole body and each body segment.

In summary, *specific* BIVA demonstrated to be a suitable tool for monitoring whole-body and segmental body composition changes. The comparability of different instruments was allowed by the application of the correction factors.

Following these results, the second section of the thesis investigated total and segmental body composition (arms, legs and trunk), muscle strength, morphological and functional symmetry, the degree of depression and body image perception in middle aged and elderly individuals. A sample of 106 active subjects of both sexes (72 men and 34 women; 60.9 ± 7.5 y of age), involved in three different sports (Tennis, Tai Chi, Running), and a sample of age-matched controls of 105 subjects (49 men, 56 women) involved in normal daily living activities were considered.

The results showed that active individuals had better nutritional status (lower BMI and waist circumference, higher MNA and hand grip strength) with respect to the control sample. They also showed lower values of %FM and higher muscle mass in the whole body, in the arms and, particularly, in the trunk, thus showing a less accentuated loss of muscle mass, as well as a reduced increase and central accumulation of fat mass.

Runners and tennis players showed lower values of %FM and higher values of muscle mass than Tai Chi subjects, both at the total and the segmental level. Tennis players exhibited the highest values of muscle mass in the whole-body, whereas runners registered the highest values in the trunk.

Tai Chi subjects and, especially, runners exhibited less sexual dimorphism in body composition than controls, in the whole body and the limbs.

Active subjects as a whole and in each sport separately were more symmetrical than controls, thus suggesting a role of sport in maintaining balance and preventing falls in old age.

Finally, the active sample exhibited better body image satisfaction and psychological well-being than controls. The only difference among sports was related to the ideal body image, that was slimmer among runners' men.

In summary, the long-term practice of a sport positively influences whole body and segmental body composition, mainly of the arm and the trunk. The effects were more accentuated among runners and tennis players than Tai Chi subjects. Active men and women were less affected by the age-related process of %FM increase, muscle mass and strength reduction, are more symmetrical and hence are further away from the emergence of sarcopenia, sarcopenic obesity, risk of falls and frailty. They were also more satisfied about their body image. All this concurs to maintain health and mental well-being and promotes successful ageing.

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1. GENERAL INTRODUCTION

1.1 BODY COMPOSITION

Body composition is defined as the determination of the main components that constitute the human body.

It is therefore an indicator of nutritional status and is in fact widely used in both clinical and medical-sports fields. Furthermore, it is also very important in the anthropological field, where it is widely used for studies on the variability of human populations and among individuals.

Body composition is based in the interconnection between different areas (Wang et al., 1992): biological factors that may have an influence on body composition (e.g. ageing, training), body composition rules (levels and their organizational rules) and technical measurements.

It can be estimated at whole-body, segmental (arm, leg, trunk), or localised level (specific body region, e.g. muscles of the thigh).

Segmental body composition finds many applications both in the clinical and sports fields. In fact, it can be used to identify certain pathologies, especially those present in the elderly population such as sarcopenia, by estimating the muscle mass of the limbs, or to monitor metabolic and cardiovascular pathologies, by estimating the levels of fat mass in the trunk.

Segmental body composition is sometimes also used to replace total body composition, as body segments such as the arm can be more easily measured in people with major disabilities, or in the elderly.

Segmental body composition is also commonly applied in sport applications, to monitor body asymmetry in order to prevent the risk of injury, and to provide useful information on the training and performance of athletes.

Individuals show a great variability in body composition, largely depending on age, sex, environment and lifestyle.

The variability includes the differences between populations which are mostly due to a greater sharing of genetic heritage and to the similarities in lifestyle given by both culture and environmental context. In fact, each population is characterised by the sharing of habits both regarding the predominance of a certain type of diet and the amount of physical activity, as well as the levels of knowledge in the nutritional and medical fields.

Urbanisation is an important factor to consider among those influencing people's lifestyles. In highly urbanised cities there has been observed a progressive change in lifestyle and diet, which has switched from being traditional and balanced to becoming increasingly international and poorer from a nutritional point of view.

This transition, which takes the name of ‘nutritional transition’ (Popkin et al., 2012), together with the increase in sedentariness has leads to a general increase in overweight and obesity in the developed countries.

In underdeveloped countries, with the increasing number of overweight and obese people still remains a high prevalence of child undernutrition, as a reflects of social inequalities. Such paradoxical where both undernutrition and obesity coexist is named ‘dual burden of malnutrition’ (Doak et al. 2005).

Each stage of life is characterised by different characteristics of body composition with related sex differences.

As far as dimorphism is concerned, the differences are very small during childhood and manifest themselves starting from the reproductive period. In the adult stage, sexual dimorphism is expressed with a greater adipose mass in women, that is mainly located in the peripheral area of the body, and with a greater muscle mass in men. Minor differences are found during old age, due to the hormonal changes that characterise both sexes.

Models of body composition

In the 1992, Wang et al. proposed the five-levels human model for body composition analysis, biological levels of increasing complexity: atomic, molecular, cellular, tissue-system, whole-body.

Atomic level

The sum of all the elements present in the body provides body mass: Total body mass = O + C + H + N + Ca + various elements present in lower concentration (Fe, Mg, Cu, K, etc.). This type of measurement is generally carried out on a corpse or isolated tissue samples. Within this type of measurement, it is in fact possible to determine total potassium, sodium, chlorine, phosphorus, calcium, nitrogen and carbon. The techniques used for dosing these elements are sophisticated, they require complex equipment and extremely specialised skills.

Molecular level

The above-mentioned elements are organised into molecules: body water, lipids, glycids, proteins and minerals (bone and soft tissue minerals). The elements can generally be identified by specific isotopes marking techniques. In addition, by means of special mineral nucleus excitation techniques, it is possible to define the bone and non-bone mineral component.

This level can be detailed into two (2-C) or more compartments models. The 2-C model considers fat mass (FM), that includes subcutaneous and visceral FM, and fat-free mass (FFM), which includes muscle mass, body fluids and internal organs (Behnke et al., 1942). The 3-C add one more compartment, also including the evaluation of total body water (TBW). Hence, body weight is subdivided into TBW, FM and the residual mass, that is composed by mineral and proteins (Siri 1961). The 4-C model is considered the most accurate and a gold standard. It includes the determination of bone mineral (Mo), achieving a more detailed description of body weight, composed by TBW, FM, Mo and proteins (Wang et al., 2002a).

Cellular level

In the cellular level study, the body can be divided into three main body compartments: intracellular fluids, extracellular fluids and extracellular solid component. These three components can be determined using radioactive tracers and dilution techniques.

Tissue level

At these level ten different systems are identified: circulatory, respiratory, nervous, tegumentary, muscular, endocrine, lymphatic, digestive, skeletal and reproductive. From the point of view of body composition this subdivision is simplified by identifying four main components of interest: adipose tissue, muscle tissue, bone tissue and blood.

Techniques such as ultrasound, computer tomography (CT) and magnetic resonance imaging (MRI) can be successfully used to determine these components.

Whole-body level

The whole-body level is a sum of the four previous levels and interest the dimension and considers the silhouette and the physical segments and proportions.

The techniques commonly used at this level include measuring the circumference and length of various body segments, skinfold thickness, body surface, body volume, mass.

1.2 TECHNIQUES FOR THE ASSESSMENT OF BODY COMPOSITION

Nowadays several methods for body composition analysis are available, each with different advantages and disadvantages (Heymsfield et al., 2015). The choice of a technique depends on several factors like: the purpose of the research, the accuracy, the resources, the time available and the sample size.

Body composition assessment techniques can be divided into three main categories that vary in accuracy, precision, invasiveness, and procedural complexity: direct, criterion, and indirect techniques.

Direct techniques

Direct techniques analyse body composition at the atomic, molecular or cellular level. These techniques are very accurate, but complex, expensive and hence difficult to access.

Neutron activation analysis (NAA)

The Neutron activation analysis provides a qualitative and quantitative estimate of the elements present in the human body (e.g. nitrogen, carbon, sodium and calcium) (Knight et al., 1986). The procedure exploits the response of the human body, expressed as reflected radioactivity, to a source of thermal neutrons. The results can be used to estimate body components; for example, the measurement of nitrogen can be is used to estimate the amount of proteins and to predict FFM.

The invasiveness due to radiation and the high costs of this technique make it unsuitable for most research studies.

Isotope dilution

Isotope dilution is based on the principle of volumetric dilution (Schoeller, 1996). This technique allows the estimation of TBW that can be quantified with the formula: TBW = dose / concentration, using deuterium as a tracer. In the case of extracellular water, the tracer is sodium bromide (NaBr) (Van Marken et al., 1996). TBW can be also used to estimate FM, assuming a constant tissues hydration of 0.73%. However, such an assumption does not consider individual variability in hydration, can be acceptable in case of healthy and properly hydrated individuals, but leads to prediction errors when measuring subjects with peculiar body composition characteristics.

Total body counting

Total body counting (Total body potassium, TBK) is used to quantify the gamma rays emitted by the human body for the decay of radioactive potassium (40 K), at the intracellular level (Ellis et al., 1996). This technique was firstly applied to estimate body fat, but more recent studies have proposed using TBK for the estimation of BCM (body cell mass) and total body protein (Wang et al., 2007).

However, the use of total body counting remains limited due to both the very high costs and the complexity of the technique.

Criterion techniques

The criterion techniques, currently considered the reference ones, allow the evaluation of body composition through the analysis of physical parameters (e.g. densitometry), or the measurement of the different components at tissue level. These techniques are accurate, but still quite difficult to use, especially in large scale studies.

Densitometry

Densitometry is based on the 2-C model, distinguishes FM and FFM assuming specific densities of the two components (0.900 g/cm^3 and 1.100 g/ cm^3, respectively).

This method determined the total body density (body mass/volume) (Behnke et al., 1942), and it is based on the principle of Archimedes.

Body volume it has been commonly estimated using the underwater weighting (UWW), with the subjects completely immerged in the water, and using a correction factor to eliminate the residual volume of the lungs.

Recently, has been developed an alternative to UWW, the air-displacement plethysmography (ADP), that is faster and better tolerated by the subjects than UWW. ADP is considered nowadays a valid system for estimating body composition, and particularly suitable for elderly and children.

Dual-energy X-ray absorptiometry (DXA)

The DXA technique gives accurate estimations of body composition both for the total body and regional areas. It is based on " the attenuation of two beams of X-rays of different energy (dual-energy X-ray) penetrating to a depth of about 30 cm; absorption by the patient's body depends on the thickness, density and chemical composition of the tissues (absorptiometry)" Frigerio et al., 2020.

DXA results are processed by highly specialised technicians with specific designed software, different for each DXA machine. The data processing is based on algorithms, tested on physical and biological models.

The technique was initially proposed for the assessment of bone mineral content (BMC). Indeed, one of the main applications of DXA is still the diagnosis of osteoporosis. Successively, it has been increasingly used to estimate body composition. In fact, the output gives information not only on the bone mineral density (BMD) but also is able to quantitatively distinguish Fat Mass (FM) from Fat-Free Mass (FFM) and divide the latter into its bone (BMC) and non-bone (Lean Mass, LM) components (Frigerio et al., 2020).

Compared to other techniques, DXA has the advantage of using a low dosage of radiation, making it a technique not too invasive and quite accessible (Marra et al., 2019), with the inconvenient that it cannot be transported, is quite expensive and complex to be used.

Computed tomography (CT) and Magnetic resonance imaging (MRI)

CT is an X-ray scan technique that permit to reconstruct a detailed cross-sectional image of the body. The images are created from a computer-generated analysis of the attenuation of an X-ray beam as it passes through a body section.

MRI is an imaging technique that uses the absorption and emission of energy in response to magnetic fields produced by a large magnet. MRI produced a detailed three-dimensional image of the internal anatomy the human body.

CT scans and MRI are both excellent imaging techniques that provide an accurate quantitative and qualitative assessment of body composition, including visceral and subcutaneous adipose tissue (Shen et al. 2003)

In term of applications, the differences between the two techniques are that MRI is not suitable in subjects with a very large body, as obese or high-level athletes, while CT can be used in all cases.

Both techniques are invasive and expensive, hence not very easy to use in research, but highly appropriate in clinical diagnostics.

Indirect techniques

The indirect techniques, such as anthropometry and bioelectrical impedance analysis (BIA), are calibrated on criterion techniques, i.e. developed through the comparison with the results obtained with those techniques, used as reference. These procedures are simple and relatively economic, hence have the advantage to be usable in large samples and under a wide set of experimental conditions; however, they are generally less accurate than the others.

Anthropometry

Anthropometry is a branch of anthropology that studies the physical characteristics of the human body with the aim of defining their association with sex, age, nutritional status, physical activity, and population. It is the most diffused method for nutritional status assessment (WHO, 1995) and is also used for body composition analysis (Lohman et al., 1988).

The wide application of anthropometry in epidemiology is due to the advantage of being easily applicable, economical, practical and non-invasive. It also enables the assessment of differences between different body regions, especially the different distribution of adipose tissue. However, the technique has also some disadvantages, such that it is potentially subject to measurement errors. For this reason, the precision and accuracy of the measurement must be guaranteed.

The technical measurement error (TEM) (Ulijaszek and Kerr 1999) is an index that measures the precision of an operator or different operators in repeating the same measurements (intra-evaluator TEM, or inter-evaluator TEM).

The intra-evaluator TEM is calculated using the formula: $TEM = \sqrt{((\Sigma D^2)/2N)}$; where D is the deviation between repeated measurements, and N is the number of individuals. TEM is likely greater with larger measurements, hence it can be standardised in order to be compared: TEM% = (TEM/mean) *100 (Ulijaszek and Kerr 1999).

To improve the precision and accuracy, the operator needs to be adequately trained and internationally recognised assessment standards must be followed (Lohman et al., 1988; WHO, 2008; ISAK, 2019). The anthropometric measurements should be taken in the morning between 8.30 a.m. and 1.30 p.m. to reduce the effect of pressure exerted by gravity on the spine, which causes compression of the intervertebral discs, hence avoiding within day physiological variations. Anthropometric measurements are normally taken on the left side of the body, on the right side if in association with bioimpedance or on both sides if laterality is to be studied.

When an anthropometric measurement is taken, it is necessary to precisely locate the points of detection and ensure that the orientation of the subject and the posture assumed are correct. Lastly, before each detection session, each anthropometric instrument must be calibrated.

The most frequently used anthropometric measurements are height, weight, body circumferences, body lengths and skinfolds.

Height is one of the main indexes of bone size and length and a fundamental parameter for a correct evaluation of nutritional status, especially if combined with body weight (Lohman et al., 1988).

Weight is a measure of the subject's mass, although it is commonly accepted to indicate such variable. It is the most measured anthropometric variable since it measures the total body size and is an index of many metabolic, growth and nutritional disorders (Lohman et al., 1988).

The Body Mass Index (BMI) is an index derived from the measurement of height and weight and is precisely the ratio between weight expressed in kg and height squared expressed in m (kg/m^2). BMI is very simple to measure and is a widely used indicator of malnutrition. Based on its association with morbidity and mortality, the World Health Organisation has defined the cut-off for the classification of adults in underweight, normal weight, overweight and obese (WHO, 2000) (Table 1.1).

Table 1.1 - Adult cut-off values (adapted from WHO 1995, WHO, 2000)

Classification	**BMI (kg/m^2)**
Underweight	< 18.50
Normal weight	18.50 ≥ BMI < 24.99
Pre-obesity	25.00 ≥ BMI < 29.99
Obesity	≥ 30

BMI is positively correlated with FM and FM percentage (%FM) (WHO, 2000), and for this reason it is considered a good proxy for adiposity and overweight-related problems in general population. However, BMI gives only general information and fails to provide a precise indication about the body composition. Furthermore, the relationship between BMI and %FM can change according to the population, sex and lifestyle. Such as in the case of Asian population where the WHO proposed different cut off points (WHO, 2014). For these reasons, the use of BMI is preferable in epidemiological studies rather than individual applications. In fact, at individual level, especially in sport, it can produce wrong diagnosis since high mass values can be due to muscle mass rather than FM.

Furthermore, BMI does not recognise body fat distribution and ‘normal weight obesity’ condition, which is characterised by normal BMI values but high %FM.

In order to provide more information on body composition and fat, the World Health Organisation suggests the association of BMI with waist circumference measurement, which indicates visceral fat. Body circumferences express the transverse dimensions of body segments and are widely used as indices of growth, nutritional status, body composition, and fat distribution (Lohman et al., 1988). The most widely used are: waist circumference (index of visceral fat); three thigh circumferences (proximal, median and distal), indicators of muscle development; calf circumference (index of body composition in adults) (Chumlea et al., 1984; Guo et al., 1987); and arm circumference, an index of the body's energy reserves and protein mass (Lohman et al., 1988).

Lengths are also widely used, given their numerous fields of application. In addition to defining the contribution of each body segment to the total body size, they are useful in the identification of growth disorders (Robinow and Chumlea, 1982; Smith, 1986), ergonomic applications and for example in the design of everyday or special clothing, such as aerospace (Lohman et al., 1988). Both the height and length of a body segment can be measured. Height corresponds to the vertical distance between the subject's supporting surface and a particular skeletal *repere* point, while length is given by the distance between two *repere* points (Lohman et al., 1988).

Plicometry represents a non-invasive and easy-to-use anthropometric technique for the topography of subcutaneous fat. The term *plica* indicates thickness of a fold of skin and subcutaneous fat tissue relative to a specific point on the body (Lohman et al., 1988). It is measured with a calibre (plicometer). These measurements, more than others, are often subject to detection error due to absence of easily detectable skeletal points as well as different compressibility of the skin and adipose tissue, which varies according to numerous variables such as age, body size and degree of hydration (Lohman et al., 1988).

Bioelectrical impedance analysis (BIA)

BIA is a non-invasive, rapid and economic technique that is used to obtain quantitative and qualitative information on body composition.

BIA can be performed in different ways, depending on the type of equipment used. A distinction is made between single-frequency BIA (SF-BIA) and multi-frequency BIA(MF-BIA).

Single-frequency BIA, at 50 kHz is more frequently used for the estimation of FM, FFM and TBW. BIA-SF analysis is performed by applying an alternating current of 500–800 mA at a known frequency of 50 kHz and a low current intensity, generally 400–800 μÅ, which is considered non-invasive and not perceptible by the subject under examination (Kyle et al., 2004a). SF-BIA offers the ability to determine impedance under conditions where alternating current is expected to pass through both extracellular and intracellular fluids. This method is very valid in the healthy population; however, it does not offer the possibility to discriminate between the intracellular and extracellular compartments (Alves et al., 2014).

Multi-frequency BIA, from 5 kHz at 100 kHz, gives more detailed information regarding the distribution of body fluids, particularly the ratio between intra and extra cellular water (ICW/ECW). When MF-BIA is administered at low frequencies (5 and 10 kHz), the electrical current predominantly pass through extracellular fluids that provides an estimate of the ECW. At higher frequencies (50,100 and 250 kHz) it exceeds cell membranes that penetrate intracellular fluids and provides an estimate of the ICW and TBW (Cha et al., 1995).

BIA provides the two impedance components: resistance (R) and reactance (Xc). Resistance corresponds to ability of body tissues to resist the passage of an alternating current and is negatively correlated with TBW and FFM. Reactance, which is only detectable by phase sensitive devices, is related to the capacitance properties of the cell membrane and to variations that can occur, depending on its integrity, function and composition (Baumgartner et al., 1988).

Regarding the total body or whole-body, BIA considers the human body as a cylinder that offers the same resistance in all its parts when current flows (Campaet al., 1985). However, the trunk contributes only 10% to the total resistance and total body is mostly estimated by impedance of the limbs. Therefore, changes that occur at the trunk level are not generally reflected by changes in the whole-body composition (Kyle et el., 2004a).

Segmental BIA refers to the regional body composition of the arms, trunk and legs, considering separately the cylinders of the body (Organ et al., 1994), which furnishes a more accurate analysis of the body composition. In this research thesis, the term ‘segmental’ was preferred to the term ‘regional’ because it is more widely used in literature, and because ‘regional’ may have a wider connotation (e.g. for indicating smaller areas such as the thigh or abdomen).

There are various applications of segmental BIA: in the healthy adult population (Bracco et al., 1996; Ling et al., 2011; Mally and Dittmar, 2012; Organ et al, 1994; Thomas et al., 1998), adolescents (Ohta et al., 2017), athletes (Ishiguro et al., 2006, Stagi et al., 2020a), patients with kidney disease (Nescolarde et al., 2008a; Song et al., 1999; Woodrow et al., 1996).

At present, several approaches to assess segmental BIA are available in the literature, indeed there is a lack of standardisation in the positioning and type of electrodes (Kyle et al. 2004a, De Lorenzo and Andreoli 2003).

To perform segmental BIA, authors have proposed different electrode positions. For instance, in the leg, Chumlea et al. (1990) placed the electrode on the interior line of the thigh, whereas Fuller and Elia (1989) placed the electrode at the level of the iliac crest. According to Mally et al. (2011), the electrode positioning adopted by Fuller and Elia (1989) yielded more accurate results. In the trunk, some authors placed the electrode at the sternal notch level, while others placed it on the anterior part of the shoulder (De Lorenzo and Andreoli, 2003).

In this thesis, a new protocol of electrode positioning was proposed for the segmental assessment of body districts (as described in detail in chapter 3.2). Furthermore, the correction of each segment for cross sectional areas and segment length, adopted in *specific* BIVA, has been tested against DXA (procedures and results detailed in chapter 3.2).

Localised BIA differs from the segmental approach because is focussed on the investigation of body composition variations in highly defined regions, in order to analyse the integrity of muscle structures or fluid accumulation. This technique requires peculiar electrodes positioning that is localised in specific regions of the body and finds interesting applications in patients or athletes who have suffered muscle injuries, such as football players (Nescolarde et al., 2013, 2015, 2017).

Standard measurement conditions of BIA (Heitmann, 1994, Kyle et al., 2004b)

Due to the relevance of BIA in estimating body composition in both research and clinical settings, it is important that bioelectrical impedance measurements are reliable. For this purpose, standardised procedures need to be strictly followed.

For instance, the type of electrodes and their correct positioning is relevant because they can influence the analysis and reproducibility of results (Smye et al., 1993; Nescolarde et al., 2016).

The operator should observe the following rules during the evaluation (Kyle et al., 2004b).

Ideal measurement conditions:

- Measurements should be preferably taken in the morning;
- The subject should be placed in a supine position with arms and legs open (30° and 45° respectively);
- The subject should lie down for 5–10 minutes before taking the measurement;
- The bed in which the measurement is performed should be made with a non-conductive material;
- The device should be calibrated before each measurement;
- The integrity of the electrodes should be checked for each detection;
- The electrodes should be positioned at least 5 cm away from each other;
- No skin lesions should be present in the electrode placement area;
- The temperature of the skin and the environment should be kept constant;
- The conduction area where the electrodes are applied should be clean (if necessary, it is recommended to clean the area with alcohol);
- In the case of a subject with amputations on the right side the detection should be performed on the left side.

Furthermore, *a list of recommendation for the volunteer's should be given before measurement:*

- Do not drink/eat in the previous 8 hours;
- Do not have exercise in the previous 8 hours;
- Empty their bladder;

- Do not take diuretics in the previous seven days;
- Do not put creams or oily substances on the skin.

The analysis is not recommended in the following cases:

- Women in their menstrual period;
- People with metal prostheses or pacemaker implants.

Traditional BIA and BIVA

In the traditional approach, BIA values are examined through regression equations, that are calibrated according to different sample characteristics (age, sex, sporting activity, etc.). The application of predictive equations to different groups can generate errors and it has been suggested that equations specifically appropriate for the sample be used (Kyle et al., 2004b). For this reason, many studies have been directed towards the calibration of regression equations for different kinds of samples (e.g. Koury et al., 2019). However, the proliferation of regression equations has reduced the possibility of comparison between samples. In any case, regression equations can lead to estimation errors in the case of individuals with particular characteristics, who differ from the reference population because they do not consider the variability of some physic or physiological conditions assumed to be constant. For instance, the assumption of body water equal to 73% does not consider the intra-population variability in body hydration (Heitmann, 1994).

In order to overcome the errors associated with the predictive equations, new methods have been proposed, such as the analysis of phase angle (PhA) and classic bioelectrical impedance vector analysis (Classic BIVA) proposed by Piccoli et al. (1994), or its '*specific'* variant known as the specific bioelectrical impedance vector analysis (*specific* BIVA) (Buffa et al., 2013; Marini et al., 2013). These methods are based on direct analysis of resistance and reactance values and allows the evaluation of body composition without the need of predictive equations, thus avoiding a potential source of error and contextually safeguarding the advantages of simplicity, non-invasiveness and cost-effectiveness of BIA.

These techniques have been studied in association with reference methods in order to analyse their performance in evaluating body composition, particularly FM, %FM, FFM, TBW, and ICW/ECW ratio (Buffa et al., 2013; Marini et al., 2013; Marini et al., 2020). Some of these associations have been studied for the first time in this research thesis (Marini et al., 2020; chapter 3.1).

Phase angle [PhA = arctn Xc/R 180/π, degree] is an important body composition indicator that depends on quantity and quality of cells membranes and is related to the distribution of body fluids (Norman et al., 2012). As shown by Gonzalez et al. (2016), the major determinants of PhA variation are age, ICW/ECW ratio, FFM, height, and population. In the present research thesis (chapter 3.1; Marini et al., 2020), the association between PhA and ICW/ECW ratio (detected by dilution techniques) has been verified. In general, based on scientific evidence, a growing body of research is considering PhA as indicative of muscle mass and functional status, a marker of nutritional status, and a prognostic index of morbidity and mortality.

The normal PhA values, observed in healthy individuals, vary between 6° and 7°. Low PhA values (<5°) are indicators of malnutrition and low cellular integrity (Norman et al., 2012, Zanforlini et al. 2019). Higher PhA values are observed for athletes (Marra et al., 2014).

However, analysis of the PhA alone, not considering the information provided by the vector length, can lead to interpretation errors. In fact, groups of individuals characterized by quite similar PhAs, but with different vector lengths, may show different body fluids or %FM (Mereu et al., 2016).

For this reason, the vectorial approach seems to be more efficient, as it considers both influential variables, PhA and vector length. Indeed, BIVA is based on analysis of the impedance vector plotted on a R/Xc graph and characterised by its length and inclination on the Cartesian plane (i.e. by the PhA).

The bioelectrical characteristics can be analysed in relation to the values of the reference population (through tolerance ellipses, Figure 1.1) and for comparison between different groups (confidence ellipses) as proposed by Piccoli et al. (1994).

Three concentric tolerance ellipses are projected on the RXc graph: the internal ellipse represents the variability of 50% of the population, while the intermediate and external ellipses represent the variability of 75% and 95%, respectively. The body composition analysis is based on the comparison of individual or group vectors with reference population ellipses.

The confidence ellipses represent, both graphically and in statistical terms, the area within the average of the reference population that falls with a probability of 95%. They are used to compare different samples and, in association with the Hotelling T^2 test or a MANOVA to provide information on the statistical significance of the difference.

In Classic BIVA R and Xc are standardised for height (R/H, ohm/m; R/H, ohm/m) in order to reduce the effect of the conductor length. Piccoli et al. (1994) observed that individuals that fall towards the upper pole of the ellipse, with a high resistance, are characterised by a tendency of dehydration, while those that fall towards the lower pole, tend to be oedematous (i.e. with a greater accumulation of extracellular liquids); on the right side of the diagram are the vectors of individuals with less cellular mass and in the left one those with greater cellular mass. Furthermore, according to Piccoli et al. (1994), athletes are characterised by vectors that trend to fall in the upper left quadrant, while obese are characterised by those that tend to fall in the lower left quadrant (Figure 1.1).

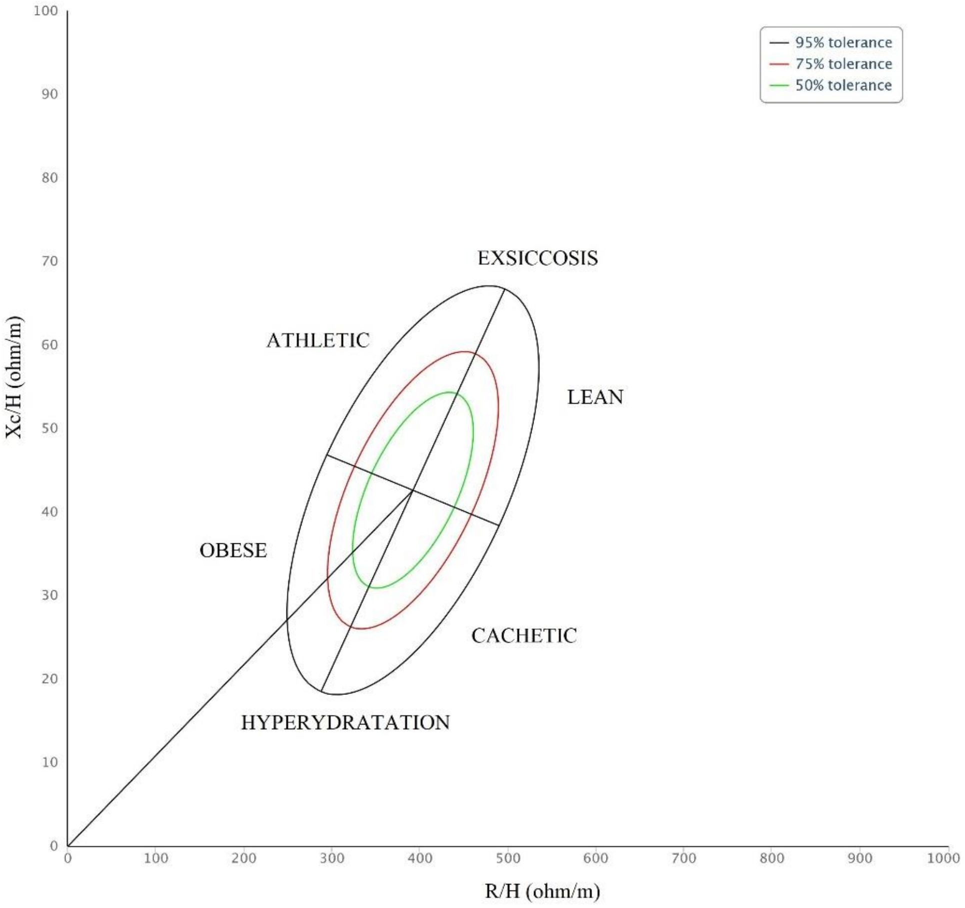


Figure 1.1 - Classic tolerance ellipses interpretation.

Validation studies (Buffa et al., 2013) have shown that the standardisation of R and Xc values only for the length of the conductor is not sufficient to give correct body composition estimation in all the cases. Indeed, in agreement with the Ohm's law, the resistance of a conductor is positively proportional to its length and negatively proportional to its section. Hence, the individuals with large body sections, even if due to the presence of a high percentage of muscle mass and not FM (like athletes), tend to fall in the lower left quadrant, where they could be incorrectly classified as obese (Castizo-Olier et al., 2018). Marini et al. (2013) proposed a variant of the Classic BIVA, *specific* BIVA, in which bioelectrical values (R, Xc; Ω) are multiplied by a correction factor (A/L) in order to minimise the effect of conductor dimensions. In the total body approach, the A value is estimated as: 0.45 arm area + 0.10 trunk area + 0.45 calf area (cm^2); arm, trunk and calf area are calculated as

$C^2/4\pi$, where C (cm) is the circumference of the segment. The length is calculated as L = 1.1H, where H is the height in cm. In the segmental approach, A/L for the arm, leg and trunk is calculated by considering the cross section of the mid arm, calf and wrist (A) and the arm, leg and trunk length (L), respectively. Specific impedance is calculated as $(Rsp^2 + Xcsp^2)^{0.5}$ (Ω cm) and PhA (arctan Xc/R 180/π) is uninfluenced by the correction.

Similarly to Classic BIVA, the specific impedance vector can be analysed by means of tolerance ellipses (Figure 1.2) and confidence ellipses (Figure 1.3). However, in this case, the major axis is referred to the variations of %FM, with increasing values towards the upper pole. The minor axis refers to the variations associated with changes of PhA, which is indicative of body cell mass and muscle mass in particular (left side: more mass; right side: less mass) and to variations of ICW/ECW ratio, with lower values toward the right lower area.

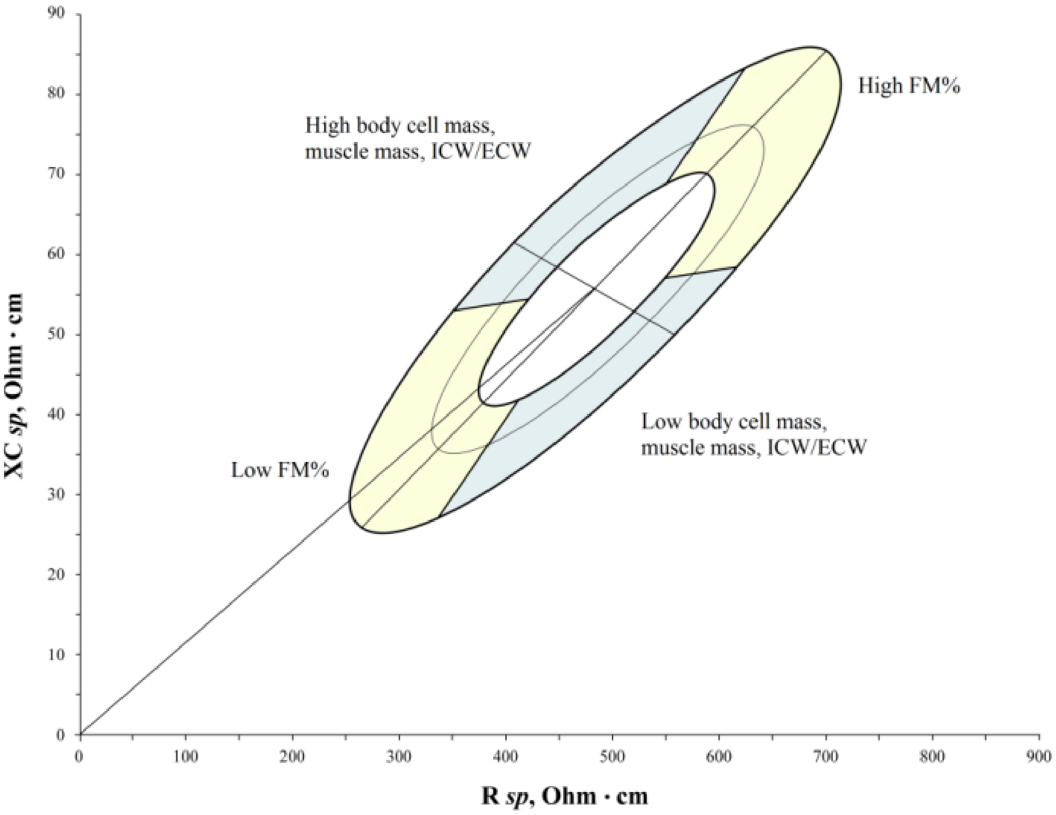


Figure 1.2 - Specific tolerance ellipses interpretation.

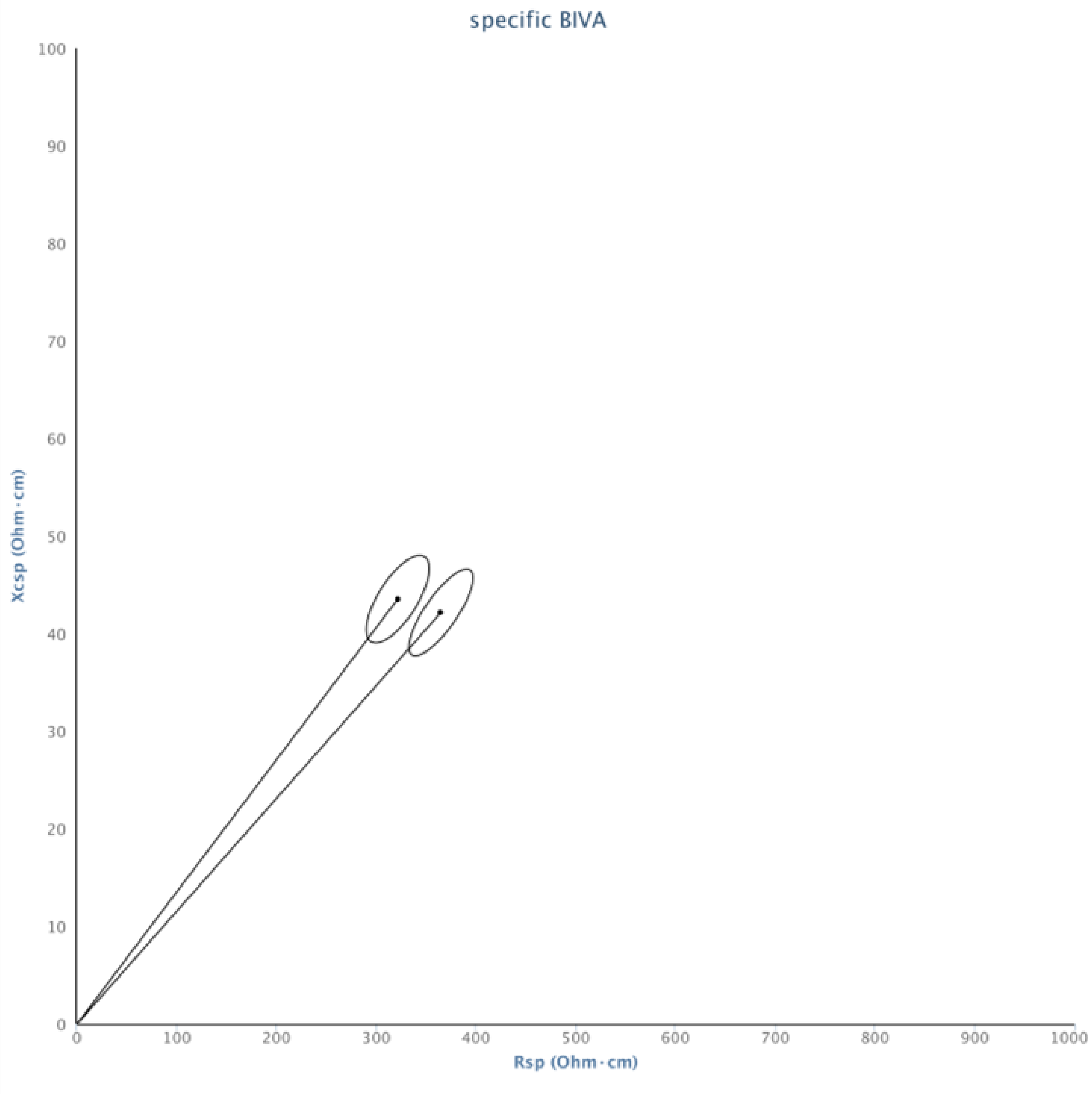


Figure 1.3 **-** Example of confidence ellipses.

1.3 BIOLOGY OF THE AGEING

In the last years, world life expectancy at birth has experimented the fastest increase recorded by World Health Organization since 1960. The global 2020 estimates indicate a life expectancy at birth of 73 years (76 years for women and 71 years for men) (WHO, 2020).

According to the World Population Prospects 2019 report, 703 million people in the world are over 65 years, a number that is expected to grow significantly over the next 30 years. It is estimated that in 2050, 1 in 6 people will be 65 or older.

These data, if analysed in more detail, give information on the different situations in the world, as life expectancy is related to cultural, socio-political and economic issues. Indeed, it varies from 62 years for males and 67 years for females in the African continent to 75 years for males and 81 years for females in Europe, with a gap of 13 years in men and 14 years for women between the two continents. As far as sexual differences are concerned, there is a similar and relatively constant worldwide trend since 2000, women living about 4.4 longer than men.

In Italy, the distribution of the population by gender and age, as of 1 January 2020, shows that a large part of the population is elderly (www.statista.com). In fact, it is estimated that people over 65 years are about 23.1% of the Italian population, that correspond to almost 14 million individuals. Italians are in fact a population that lives a very long time. In 2019, life expectancy at birth is estimated at 83.4 for both sexes, reaching 85.3 years for women and 81 years for men (www.statista.com).

The ageing index, in 2020, showed that in Italy there are 177.9 elderly people for every 100 young people.

In terms of public health, this information is essential to the management of a part of population that needs special cares. The senile population is a fragile population who is more exposed to the risk of chronic diseases, thus requiring special and prolonged care. In addition to the common diseases present in the general population, old age is characterized by a high risk of dementia, increased exposure to diseases, and malnutrition.

At a biological level, ageing is an irreversible physiological process that causes numerous variations in the structure, morphology and functionality of organs and apparatus. The changes involving body composition and phenotype are evident and occur also in the absence of diseases, expressing themselves with different variability and speed in different subjects.

The main physiological changes of body composition that occurs with ageing are:

- increase and redistribution of body fat;
- loss of muscle mass;
- loss of bone mass;
- reduction of total body water.

The increase in body fat is mainly related to the reduction in basal metabolism, which is progressive during adulthood (Buffa et al., 2011). Such increase begins near the age of 25 and ends around the age of 80 for both sexes (Ding et al., 2007), or previously in men (Prentice and Jebb, 2001), with a reversing of the trend, especially related to the loss of subcutaneous fat (Buffa et al., 2011). Furthermore, in advanced age there is a redistribution of body fat, which becomes mainly accumulated in the central area of the body, especially at the visceral level. The accumulation of fat in the trunk has proved to be associated with metabolic disorders at different levels of BMI (Bosy-Westphal et al., 2015), and to contribute to a greater incidence of cardiovascular disease (Neeland 2019, Arsenault et al., 2012; Britton et al., 2013; Torriani et al., 2014). An opposite pattern has been observed for fat accumulation in the lower limbs, where fat mass can provide a protective profile against metabolic diseases (Wu et al., 2010; Zhang et al., 2013).

The loss of FFM, mainly due to the loss of muscle mass, represents one of the main expressions of physiological ageing. The reduction of muscle mass begins early, about the third decade of life (Janssen et al., 2000), and at the age of 50 years a loss of 10% is estimated, while at the age of 80 years the reduction is greater than 50% (Doherty, 2003). The decrease is present in both sexes, but more accentuated among men, and causes a series of negative outcomes such as muscle fatigue, increased risk of falls, functional limitations, and frailty (Andreoli et al., 2009). The association of these changes with a reduced force and possibility of movement, or to disability may lead to sarcopenia (Cruz-Jentoft et al., 2014), that can be also associated to the bone mass loss (osteosarcopenia) (Hirschfeld et al., 2017). The loss of muscle mass in association with the infiltration of fat in the muscle, especially in the limbs, leads to sarcopenic obesity (Polyzos and Margioris 2018), with a major risk of morbidity, reduced mobility and higher risk of falls (Scott et al. 2019).

Sarcopenia is defined as a: "a progressive and generalised skeletal muscle disorder that is associated with increased likelihood of adverse outcomes including falls, fractures, physical disability and mortality" (Cruz-Jentoft et al., 2019). It has been officially recognized as a disease in the September 2016, with the ICD-10-CM code (www.prweb.com-prweb13376057).

In the assessment of sarcopenia, Cruz et al. (2019) recommended the use of hand grip strength or a chair stand measure, using specific cut-off-points for each test (e.g. hand grip strength cut-off: ≤ 27 kg for males and ≤16 kg for females). To confirm the diagnosis, they suggested the use of body composition analysis, in order to detect the low muscle mass, using one of those techniques: DXA, BIA, CT or MRI. Finally, to determine the severity, the guidelines recommended to test the physical performance using the following tests: gait speed, Short Physical Performance Battery (SPPB), Time Up and Go test (TUG) and 400-m walk tests.

The diagnostic approach proposed by Cruz-Jentoft et al. (2019) differs from the previous one (Cruz-Jentoft et al., 2010) because it highlights the importance of muscle strength rather than the amount of muscle mass, which is considered more suitable for predicting adverse outcomes and reserves to reduced functionality only a role for detecting severe conditions.

Sarcopenic obesity (SO) is considered as the coexistence of sarcopenia and obesity (Baumgartner et al., 1998). The pathogenesis of SO is multifactorial: there is an interaction between ageing, sedentary lifestyle and unhealthy eating habits, insulin resistance, inflammation and oxidative stress (Polyzos and Margioris 2018).

People suffering from SO are at risk of impairment of lower limb function, the development of cardiovascular disease, and metabolic disease, including hyperglycemia, hypertension, dyslipidemia, insulin resistance (Choi, 2016).

Body composition is a fundamental tool for the estimation of sarcopenia and SO. The anthropometric measurements and BIA, or the imaging techniques can be used for the differentiation between sarcopenic obesity, sarcopenia, or obesity alone.

SO is in fact characterized by normal or high anthropometric values (weight, BMI, circumferences), hight FM and low muscle mass, whereas sarcopenia alone is characterized by low or normal anthropometric values, low or normal FM, and low muscle mass, and obesity alone by high anthropometric values, high FM and normal or high muscle mass (Polyzos and Margioris 2018).

Sarcopenia and SO represent a serious health public problem because their global prevalence is increasing and are now the major causes of frailty of elderly people.

Frailty is defined as a geriatric syndrome associated with the ageing process that increases biological vulnerability and leads to functional inability. In 2001, Fried et al., provided a phenotypical description of fragility using the following criteria:

- involuntary body weight loss (5 % in one year);
- asthenia (assessed by the hand grip strength test, reduced by 20%);
- fatigue (assessed by a fatigue scale);
- slowness (measured by walking speed);
- low physical activity or impaired mobility (calculated in correlation to activity performed during the week).

The diagnosis of fragility is reached when the patient presents three out of five of these criteria (Berner, 2016).

The incidence of frailty varies by sex, region, country and is strictly dependent on diagnostic criteria used (Ofori-Asenso et al., 2019).

A recent review on the global incidence of frailty found that in a very large sample of older adults from 28 countries, the incidence of frailty and prefrailty was estimated at 43.4 and 150.6 new cases per 1000 persons per year, respectively (Ofori-Asenso et al., 2019), thus demonstrating that the risk to develop frailty is very hight in the elderly population.

1.4 PHYSICAL ACTIVITY AND PSYCHO-PHYSICAL WELLNESS

‘Physical activity’ (PA) includes a series of different meanings, from unstructured and daily conducted activities to structured physical exercise, carried out with a set of pre-established rules, or even to a specific sport (Koeneman et al. 2011).

Physical activity substantially contributes to maintaining psychophysical well-being at all stages of life and particularly in ageing (Koolhaas et al., 2018).

The general benefits in old age are mostly related to the reduction of body weight, the maintenance or improvement of mobility, and the associated reduction in the risk of developing diseases.

Indeed, Ribeiro-Santos et al. (2020) have recently demonstrated that insufficient physical activity is a risk factor to develop sarcopenia and SO in older adults. In fact, physical exercise substantially contributes to slow down or reverse the physiological trend towards sarcopenia (Roubenoff 2000). The reduction of sarcopenia with physical activity was estimated of 55% by Steffl et al. (2017) and of 55% and 29% for sarcopenic obese men and women with sarcopenic obesity, respectively (Son et al. 2019). A recent study (Scott et al., 2019) also confirmed that a vigorous or moderate physical activity is positively correlated with muscle density in ageing people.

Physical activity has also an influence on the mind. In fact, it has been proven that PA has a crucial role in reducing anxiety and depression (Mura et al., 2013), or improving mental health and resilience to psychological distress (Hamer et al., 2009; Da Silva et al., 2012).

Furthermore, recent studies have noted that in late age physical activity also contribute to increase the levels of body satisfaction (Condello et al., 2016).

Due to the scientific evidences, the WHO recommendations (2020) for maintaining physical, psychological, and cognitive well-being in older age suggest including at least 150-300 minutes of moderate-intensity aerobic physical activity or 75-150 minutes of vigorous intensity or a combination of the two during the week. Muscle strengthening training should also be included at least twice a week. Finally, older people should include exercise to improve balance and prevent falls in their weekly routine (3 or more days a week).

2. AIMS OF THE RESEARCH

Research investigating the effects of physical activity (PA) in the elderly has demonstrated that PA contributes to maintain muscle mass and to contrast the increase of fat mass. However, the effects of long-term practice of a sport as in the case of master athletes, or, in a broader sense, of individuals who continue to play a sport regularly after the age of 50 years have not yet been adequately investigated. In addition, the effect of prolonged physical exercise on segmental body composition (Mitchell et al., 2020; Mikkelsen et al., 2013; Moysi et al., 2004a), on morphological and functional symmetry (Ireland et al., 2014; Piasecki et al., 2019), and on body image perception is scanty in the literature. However, such information can be useful for the study of the physiological process of ageing and to suggest possible strategies to slow down its effects.

This thesis aimed to study the long-term effects of different sport disciplines (Tai Chi Chuan, Tennis, and Running) in middle-aged and elderly subjects, analysing the whole and segmental body composition, body symmetry, body satisfaction and mental well-being (Figure 2.1).

At this purpose, a first phase of the research concerned methodological aspects (Figure 2.1) mainly related to the evaluation of body composition by means of specific bioelectrical impedance vector analysis (*specific* BIVA) (Buffa et al 2013, Marini et al 2013). As discussed in the introduction, *specific* BIVA is an economical, non-invasive and easy to use technique, that has shown high sensitivity and specificity in the evaluation of total body %FM, against Dual-energy X-ray absorptiometry (DXA) (Buffa et al., 2013; Marini et al., 2013). *Specific* BIVA demonstrated to be suitable in the monitoring of body composition in epidemiological studies, and has been applied in adult and elderly populations (Buffa et al., 2013; Marini et al., 2013, Saragat et al., 2014), including patients with sarcopenia (Marini et al., 2012), or Alzheimer's disease (Buffa et al., 2014; Mereu et al., 2018), and in samples of athletes (Antoni et al., 2017; Castizo-Olier et al., 2018; Stagi et al., 2020a; Toselli et al., 2020).

However, the performance of *specific* BIVA in relation to segmental body composition, body hydration and body image analysed in this research, had not yet considered. Furthermore, the standardisation of bioimpedance measurements, with different devices, were also investigated, an aspect not widely considered in the literature but that can significantly influence BIA measurements (Deurenberg et al. in 1989; Kyle et al., 2004b).

In detail, the methodological objectives that have been addressed were:

- the association between *specific* BIVA and reference techniques for the assessment of body composition in young athletes, using DXA for %FM and dilution techniques for body hydration analysis;
- the association between segmental *specific* BIVA, using a new defined protocol, and DXA in the assessment of regional body composition (arms, legs and trunk) in a group of active students;
- the analysis of the information given by *specific* BIVA on body composition of different body regions and the whole-body, in a group of active students;
- the association between *specific* BIVA and self-perceived body image in a sample of young and elderly subjects;
- the comparison of three impedance devices, in order to determine the magnitude of the differences and define corrective factors, in a sample of adults.

The results of the methodological study have been used to analyse long-term effects of sports on total and segmental body composition and wellness in a sample of middle-aged and elderly practicing Tai Chi Chuan, Tennis, and Running. In particular, the aim to study were:

- nutritional status, total body composition, and hand grip strength;
- segmental body composition of the arms, legs and trunk;
- body composition asymmetry of the limbs and muscle strength asymmetry of the arms;
- psychological status and body image perception and satisfaction.

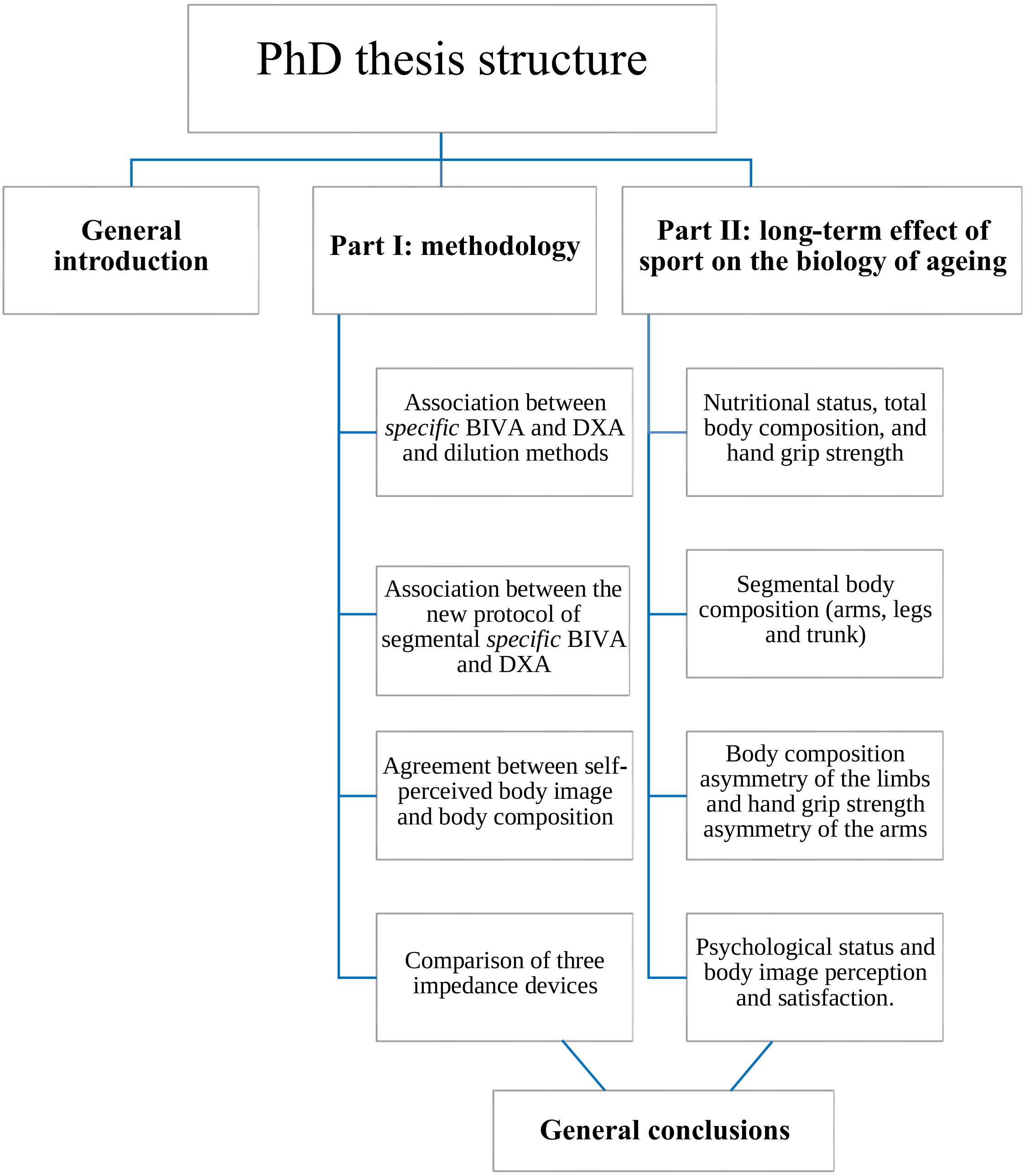


Figure 2.1 - PhD thesis structure.

3. PART I: METHODOLOGICAL STUDIES

3.1 ASSOCIATION OF *SPECIFIC* AND CLASSIC BIVA WITH GOLD STANDARD TECHNIQUES IN THE EVALUATION OF BODY COMPOSITION IN ATHLETES.

INTRODUCTION

The analysis and monitoring of body composition is fundamental in sport, because of its relevance to athletes' health and performance, and to team success. Such analysis can be performed in different contexts and with different approaches, i.e. in cross-sectional studies aimed to characterise sporting group samples, in longitudinal researches finalised to define short-term or long-term changes, or in applications aimed to detect and monitor muscle injuries (Castizo-Olier et al., 2018). Variations of body composition can interest diversely athletes practicing different sport, because of their different exercise type and requirements for body physique and composition. In general, lean mass is considered a predictor of muscular fitness (Henriksson et al., 2016; Köhler et al., 2018). Furthermore, while overhydration is quite uncommon in athletes, physiological dehydration processes can be induced by physical activity, leading to hypotonic, isotonic, or hypertonic dehydration (Oppliger and Bartok 2002).

Several techniques can be used to assess body composition in athletes, among these the quickest and non-invasive ones is Bioelectrical impedance analysis (BIA). As described in the methods of this thesis the analysis of BIA can be performed using raw data, namely phase angles (PhAs), or bioelectrical impedance vectors, i.e. PhA and vector length jointly, as in the bioelectrical impedance vector analysis approaches (BIVA; Piccoli et al., 1994; Buffa et al., 2013; Marini et al., 2013).

PhA, classic and *specific* BIVA have been applied in different groups, particularly obese, athletic subjects, and in the elderly, and in the clinical setting (Kyle et al., 2004a; Barbosa-Silva and Barros 2005; Norman et al., 2012; Buffa et al., 2014a). A growing body of literature on BIVA in sport and exercise research and practice is also noticeable (see the review by Castizo-Olier et al., 2018 and more recently: Campa et al., 2018a; Campa et al., 2018b), and *specific* BIVA has been proposed as a promising approach in this field (Castizo-olier et al., 2018).

Although largely used, reliability studies of PhA, classic or *specific* BIVA in the assessment of body composition (Buffa et al., 2013; Marini et al., 2013; Gonzalez et al., 2016; Wells et al., 2018), or of hydration (Chertow et al., 1995; Lukaski and Piccoli 2012; Gonzalez et al., 2016; Heavens et al., 2016; Wells et al., 2018) through reference techniques are very scarse in the general population and totally lacking in athletes (Castizo-olier et al., 2018).

Therefore the aim of the research was evaluate the accuracy of PhA, classic and *specific* BIVA in body composition assessment of athletes, focusing the analysis on absolute values of body mass (FM, FFM, TBW, ECW, ICW), and on values independent from body dimensions (%FM, ECW/ICW). At this purpose, DXA was used as a reference for FM, FFM and %FM, and dilution techniques for TBW and ECW.

This study was conducted with the researchers from the University of the Lisbon and Bologna.

METHODS

Subjects

This was a cross-sectional, observational study on 202 athletes (139 men and 63 women) over 16 years of age (men: 21.5 ± 5.0; women: 20.7 ± 5.1). The sample included athletes involved in a total of 11 sports (Athletics, Basketball, Handball, Judo, Karate, Pentathlon, Rugby, Soccer, Swimming, Triathlon, Volleyball). The results of a medical screening indicated that all subjects were in good health. The following inclusion criteria were used: 1) 10 or more hours of training per week, 2) negative test outcomes for performance-enhancing drugs, and 3) not taking any medications. Measurements were made by researchers from the laboratory of the University of Lisbon. All subjects and their parents or guardians were informed about the possible risks of the investigation before giving written informed consent to participate. All procedures were approved by the ethics committee of the Faculty of Human Kinetics, Technical University of Lisbon, and were conducted in accordance with the declaration of Helsinki for human studies of the World Medical Association (WHO, 2013). On each subject, all the measurements were obtained in the same morning. Subjects came to the laboratory after an overnight fast (12 h fast), refraining from vigorous exercise at least 15 h, no caffeine and alcohol during the preceding 24 h, and consuming a normal evening meal the night before (Figure 3.1).

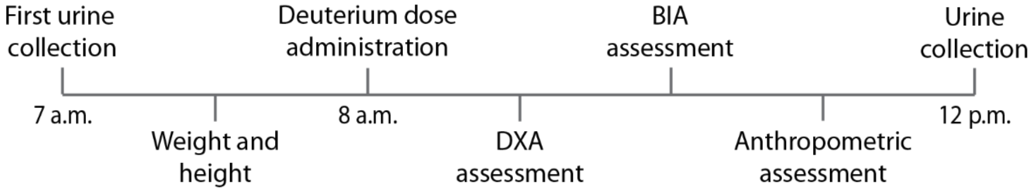


Figure 3.1 - Timeline of stations performed by the athletes involved in the study.

Anthropometry

All anthropometric data were collected by an ISAK accreditation technician according to a standardized protocol (Stewart et al., 2011). Body weight was measured with a scale, without shoes and wearing minimal clothes, to the nearest 0.01 kg; height was measured to the nearest 0.1 cm with a stadiometer (Seca, Hamburg, Germany). Body Mass Index (BMI) was calculated as the ratio of body mass to height squared (kg/m2). Girths were measured by using an anthropometric tape (Lufkin W606PM; Apex Tool Group, Sparks, MD, USA). The intra-observer technical error of measurement (TEM) and the coefficient of variation (CV) were calculated in a subsample of ten subjects (height: TEM=0.06 cm, CV=0.04; weight: TEM=0.04kg, CV=0.07; arm circumference: TEM=0.09 cm, CV=0.3; waist circumference: TEM=0.3 cm, CV=0.4; calf circumference: TEM=0.06 cm, CV=0.2).

Dual-energy X-ray absorptiometry

Athletes underwent a whole-body DXA scan according to the procedures recommended by the manufacturer on a Hologic Explorer-W fan-beam densitometer (Hologic, Waltham, MA, USA). The equipment measures the attenution of X-rayween 70 and 140 kV synchronously with the line frequency for each pixel of the scanned image. According to the protocol described by the manufacturer, a step phantom with six fields of acrylic and aluminum of varying thicknesses and known absorptive properties was scanned to serve as an external standard for the analysis of different tissue components. For athletes who were taller than the scan area, we used a validated procedure that consisted of the sum of a head and a trunk plus limbs scans (Santos et al., 2014). The same technician positioned the participants, performed the scan, and executed the analysis (QDR for Windows software version 12.4; Hologic, Waltham, MA, USA) according to the operator's manual by using the standard analysis protocol. The DXA measurements included whole-body measurements of absolute FM (kg), %FM and FFM (kg).

Body fluids

Following the collection of a baseline urine sample, each participant was given an oral dose of 0.1 g of 99.9% ^2H_2O per kg of body weight (SigmaeAldrich; St. Louis, MO) for the determination of TBW by deuterium dilution using a Hydra stable isotope ratio mass spectrometer (PDZ, Europa Scientific, UK). Subjects were encouraged to void their bladder prior to the 4-h equilibration period and subsequent sample collection, due to inadequate mixing of pre-existing urine in the bladder (Schoeller, 2005). Urine samples were prepared for 1 H/2H analyses using the equilibration technique by Prosser and Scrimgeour (Prosser and Scrimgeour 1995). Our laboratory has reported a CV in ten subjects for TBW of 0.3%. ECW was assessed from a baseline saliva sample using the sodium bromide (NaBr) dilution method after the subject consumed 0.030 g of 99.0% NaBr (SigmaeAldrich; St. Louis, MO) per kg of body weight, diluted in 50 mL of distilled-deionized water. ICW was calculated as the difference between TBW and ECW.

Bioelectrical impedance

The impedance measurements were performed with BIA (BIA 101 Anniversary, Akern, Florence, Italy) using an electric current at a frequency of 50 kHz. Measurements were made on an isolated cot from electrical conductors, the subjects were in the supine position with a leg opening of 45° compared to the median line of the body and the upper limbs, distant 30° from the trunk. After cleaning the skin with alcohol, two electrodes (Biatrodes Akern Srl, Florence, Italy) were placed on the right hand back and two electrodes on the neck of the corresponding foot (Lukaski and Piccoli 2012). Bioelectrical impedance vector analysis was carried out using the classic and *specific* BIVA methods (detailed described in the introduction of the thesis).

The test-retest CV in 10 participants in our laboratory for R and Xc was 0.3% and 0.9%, respectively. Italo Spanish bioelectrical *specific* values (Ibáñez et al., 2015) were used as a reference. Italo Spanish bioelectrical classic values (unpublished data) were: R/H (men: 284.9 ± 33.6; women: 391.2 ± 41.1); Xc/H (men: 38.0 ± 5.0; women: 44.0 ± 5.8).

Statistical Analysis

Descriptive statistics including means ± standard deviations were calculated for all outcome variables. Normality was evaluated using Shapiro-Wilk test. Since the data showed a normal distribution, the association between bioelectrical impedance and body composition values was investigated using Pearson's correlation analysis. Multiple regression analyses were performed to understand the associations between FM, %FM, FFM, TBW, ICW, and ECW and bioelectrical values. Model adjustments included age and sport practised. If more than one variable was a predictor in the model, a variance inflation factor (VIF) for each independent variable was calculated to evaluate multicollinearity, and values below 5 were considered not to have multicollinearity issues. The sample distribution of %FM, TBW and ECW/ICW was divided into quartiles and the bioelectrical values of cases below the first quartile (Q1) were compared with those above the third quartile (Q3) by means of Hotelling's T^2 test.

Data were analysed with IBM SPSS Statistics version 24.0 (IBM, Chicago, IL). Bioelectric variables were calculated using the *specific* BIVA software (www.specificbiva.com). For all tests, statistical significance was set at $p < 0.05$.

RESULTS

Athletes of both sexes showed a condition of normal weight, with low mean values of BMI and low average values of %FM, as expected in a sample of young sportive subjects (Table 3.1).

Table 3.1 - Descriptive statistics of participants characteristics.

	Men (n=142)	Women (n=66)
Variable	Mean ± SD	Mean ± SD
Age (y)	21.5 ± 5.0	20.7 ± 5.1
Height (cm)	183.3 ± 9.1	171.1 ± 8.2
Weight (kg)	77.2 ± 11.4	63.7 ± 8.9
Upper arm crf (cm)	32.3 ± 3.2	28.6 ± 2.6
Waist crf (cm)	81.3 ± 6.4	76.5 ± 5.7
Calf crf (cm)	37.6 ± 2.4	36.1 ± 2.8
BMI (kg/m^2)	22.9 ± 2.6	21.8 ± 2.1
R (ohm)	467.9 ± 51.4	566.1 ± 67.4
Xc (ohm)	63.1 ± 8.0	67.6 ± 10.5
PhA (degrees)	7.7 ± 0.8	6.8 ± 0.8
Rsp (ohm*cm)	324.3 ± 31.2	368.3 ± 46.1
Xcsp (ohm*cm)	43.9 ± 6.2	44.0 ± 7.1
Zsp (ohm*cm)	327.3 ± 31.5	370.9 ± 45.9
R/H (ohm/m)	255.8 ± 30.6	331.5 ± 41.2
Xc/H (ohm/m)	34.6 ± 5.1	39.6 ± 6.4
Z/H (ohm/m)	258.2 ± 30.8	334.3 ± 41.3
FM (kg)	10.8 ± 4.3	15.4 ± 4.4
FM (%)	13.9 ± 3.9	24.1 ± 4.8
FFM (kg)	65.7 ± 8.6	47.9 ± 6.2
TBW (kg)	49.5 ± 7.5	35.8 ± 5.3
ECW (kg)	19.2 ± 3.1	14.6 ± 1.9
ICW (kg)	30.4 ± 5.7	21.2 ± 3.8
ECW/ICW (kg)	0.6 ± 0.1	0.7 ± 0.1

BMI, body mass index; R, resistance; Xc, reactance; PhA, phase angle; Rsp, resistance multiplicated for coefficient; Xcsp, reactance multiplicated for coefficient; Zsp, vector length multiplicated for coefficient; R/H, resistance standardized for height; Xc/H, reactance standardized for height; Z/H, vector length standardized for height; FM, fat mass; FFM, fat free mass; TBW, total body water; ECW, extracellular water; ICW, intracellular water.

Anthropometric and body composition measurements showed significant differences between sexes. Consistently with the known pattern of sexual dimorphism in adults, men showed higher values of all anthropometric measurements, FFM, TBW, ECW, ICW, while women showed higher bioelectrical values (with the only exception of specific reactance), and higher FM, %FM, and ECW/ICW (Table 3.1).

Both men and women showed significantly higher stature ($p<0.001$), significantly larger circumferences ($p<0.001$ for waist and upper arm circumference; $p<0.05$ for calf circumference, only in men), but a similar BMI with respect to the Italo-Spanish reference population (Ibáñez et al., 2015). Classic bioelectrical values (R/H and Xc/H) were significantly lower in Portuguese athletes of both sexes than in the reference population ($p<0.001$), whereas specific values were not significantly different in the two populations, with the exception of Rsp which was higher in the Italo-Spanish group ($p<0.05$). PhA was similar in men and significantly higher in Portuguese females ($p<0.001$).

Table 3.2 shows the correlation matrix between bioelectrical impedance and body composition variables. Following adjustment for covariates, including age and sport practised, bioelectrical values remained significantly associated with body composition variables. In fact, in the multicollinearity diagnosis we found no VIF above 5, which is the rule of thumb used in regression models to assess if the β is affected.

Table 3.2 - Correlation between bioelectrical and body composition variables

Men										
	R	Xc	Z	R/H	Xc/H	Z/H	Rsp	Xcsp	Zsp	Phase
FM	-0.312**	-0.356**	-0.316***	-0.406**	-0.398**	-0.443***	0.602**	0.340**	0.588***	-0.085
%FM	-0.144	-0.228**	-0.147	-0.160	-0.215*	-0.214*	0.589**	0.313**	0.569***	-0.105
FFM	-0.539**	-0.462**	-0.542***	-0.781**	-0.625**	-0.778***	0.173*	0.127	0.204*	0.010
TBW	-0.731**	-0.484**	-0.732***	-0.883**	-0.586**	-0.880***	0.068	0.186*	0.099	0.184*
ECW	-0.484**	-0.565**	-0.490***	-0.701**	-0.694**	-0.702***	-0.028	-0.156	-0.019	-0.165
ICW	-0.705**	-0.339**	-0.703***	-0.792**	-0.405**	-0.783***	0.104	0.326**	0.140	0.327**
ECW/ICW	0.295**	-0.170	0.288**	0.207*	-0.204*	0.187*	-0.122	-0.472**	-0.153	-0.493**
Women										
	R	Xc	Z	R/H	Xc/H	Z/H	Rsp	Xcsp	Zsp	Phase
FM	0.059	-0.128	0.055	-0.126	-0.256*	-0.127	0.734**	0.414**	0.737	-0.232
%FM	0.281*	0.001	0.277*	0.222	-0.033	0.218	0.774**	0.407**	0.773	-0.295*
FFM	-0.475**	-0.333**	-0.475***	-0.734**	-0.525**	-0.731***	0.029	0.055	0.026	0.052
TBW	-0.598**	-0.368**	-0.597***	-0.829**	-0.549**	-0.829***	-0.171	-0.018	-0.156	0.146
ECW	-0.543**	-0.489**	-0.545***	-0.781**	-0.667**	-0.788***	-0.033	0.086	-0.043	0.083
ICW	-0.547**	-0.258	-0.545***	-0.746**	-0.419**	-0.743***	-0.219	0.018	-0.193	0.243
ECW/ICW	0.214	0.127	-0.209	0.244	0.085	0.229	0.256	-0.117	0.215	-0.408**

r values are reported in the table; R, resistance; Xc, reactance; R/H, resistance standardized for height; Xc/H, reactance standardized for height; Rsp, resistance multiplicated for coefficient; Xcsp, reactance multiplicated for coefficient; PhA, phase angle; FM, fat mass; %FM, percentage of fat mass; FFM, fat free mass; TBW, total body water; ECW, extracellular water; ICW, intracellular water; Z, vector length; Zsp, vector length multiplicated for coefficient; Z/H, vector length standardized for height.

In classic BIVA, the correlation between TBW, ECW, ICW and R/H, Xc/H, Z/H was highly and negatively significant in both sexes (Table 3.2, Figure 3.2a), and the mean vectors of groups with lower and higher amounts of body water (below Q1 vs. above Q3 of the TBW) were significantly different (Figure 3.3a,g). The association between FFM or FM and R/H, Xc/H, Z/H was negative in both sexes (Table 3.2), while the correlation with %FM was inconsistent in the two sexes (Z/H negatively correlated in men and positively in women) and reached the significance level only in men (Table 3.2, Figure 3.2c). To be noted that the classic mean vectors of one or both opposite quartiles were located in the left lower region of the tolerance ellipses, towards the region of obesity (Figure 3.3c, i).

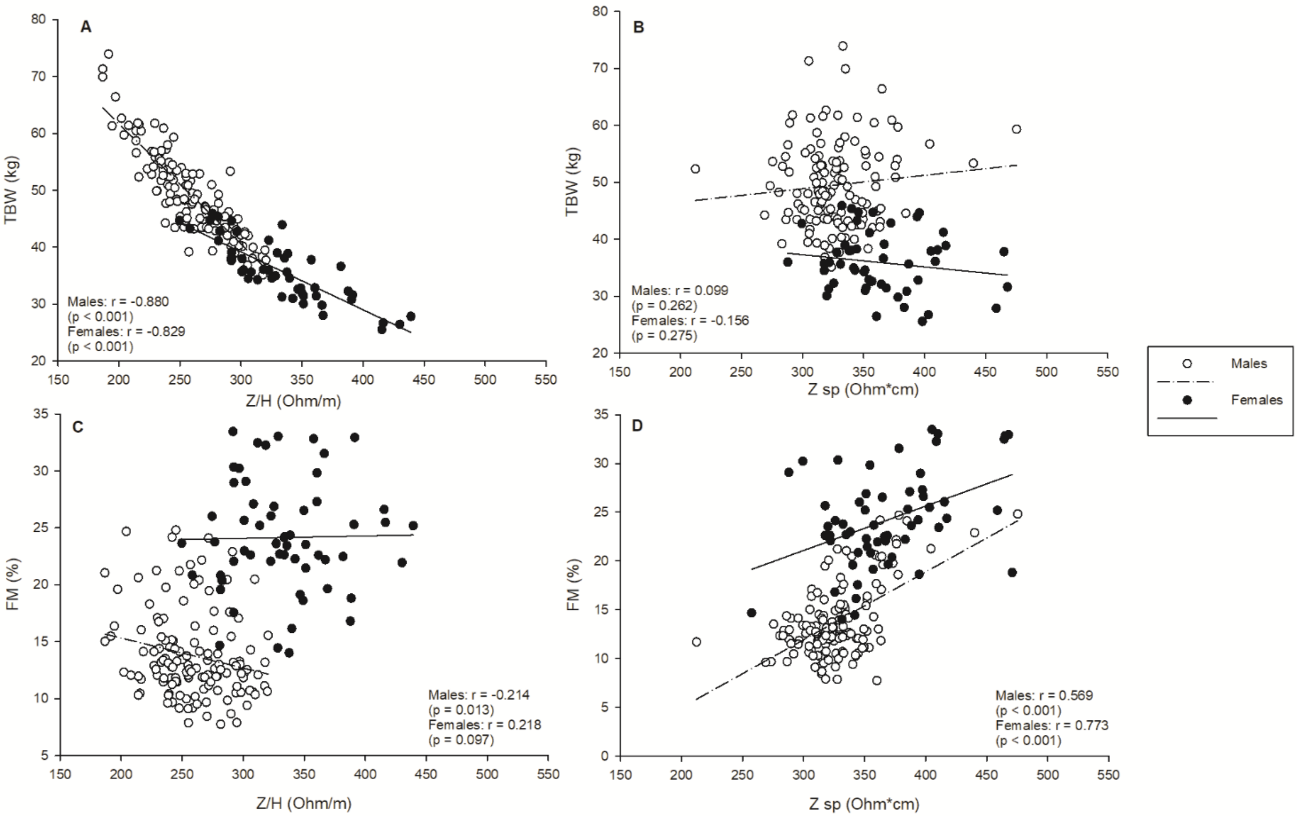


Figure 3.2 - Correlation between classic or specific impedance vectors with total body water or fat-mass% in men and women. a: Z/H vs. TBW; b: Zsp vs. TBW; c: Z/H vs. %FM; d: Zsp vs %FM. Z: impedance; H: height; sp: specific; TBW: total body water; %FM: percentage of fat mass.

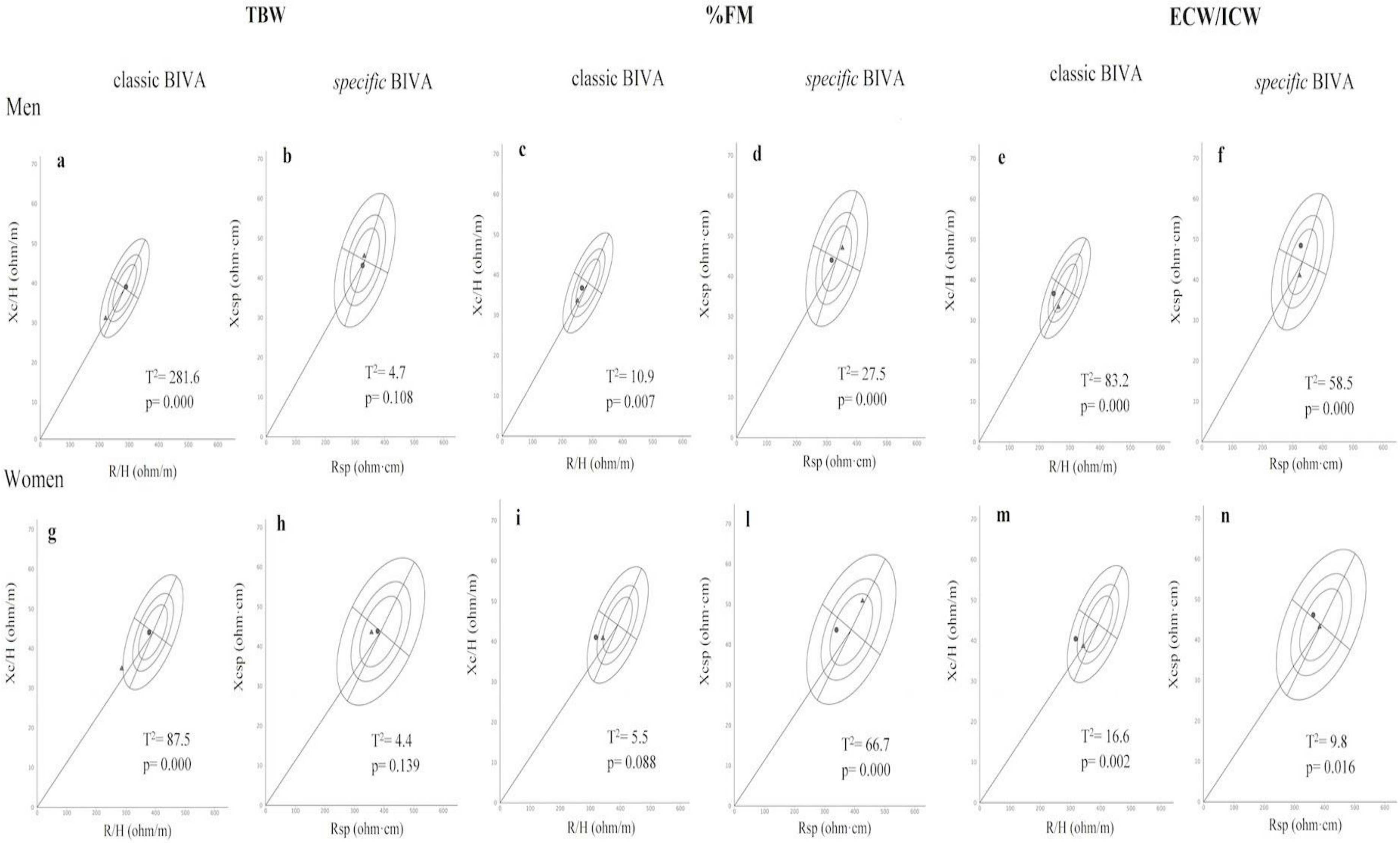

TBW
%FM
ECW/ICW
classic BIVA
specific BIVA
classic BIVA
specific BIVA
classic BIVA
specific BIVA
Men
a
T^2= 281.6
p= 0.000
b
T^2= 4.7
p= 0.108
c
T^2= 10.9
p= 0.007
d
T^2= 27.5
p= 0.000
e
T^2= 83.2
p= 0.000
f
T^2= 58.5
p= 0.000
Women
g
T^2= 87.5
p= 0.000
h
T^2= 4.4
p= 0.139
i
T^2= 5.5
p= 0.088
l
T^2= 66.7
p= 0.000
m
T^2= 16.6
p= 0.002
n
T^2= 9.8
p= 0.016
Xc/H (ohm/m)
R/H (ohm/m)
Xcsp (ohm·cm)
Rsp (ohm·cm)
0
10
20
30
40
50
60
70
100
200
300
400
500
600

Figure 3.3 - Classic and *specific* mean vectors of quartiles (below Q1 vs. above Q3) with different total body water, fat-mass%, and extracellular/intracellular water ratio in men and women.
Circles: below Q1; triangles: above Q3; a: classic BIVA and TBW (men); b: *specific* BIVA and TBW (men); c: classic BIVA and %FM (men); d: *specific* BIVA and %FM (men); e: classic BIVA and ECW/ICW (men); f: *specific* BIVA and ECW/ICW (men); g: classic BIVA and TBW (women); h: *specific* BIVA and TBW (women); i: classic BIVA and %FM (women); l: *specific* BIVA and %FM (women); m: classic BIVA and ECW/ICW (women); n: *specific* BIVA and ECW/ICW (women); TBW: total body water; %FM: percentage of fat mass; ECW/ICW: extracellular/intracellular water ratio.

In *specific* BIVA, the correlation between FM or %FM and bioelectrical values (Rsp, Xcsp, Zsp) was positive and highly significant in both sexes (Table 3.2, Figure 3.2d), while the association with FFM rarely reached the significance level. The mean vectors of groups with different percentages of body fat (below Q1 vs. above Q3 of the %FM) were significantly separated (Figure 3.3d, l). The mean vectors of opposite quartiles were located within the 50% tolerance ellipses and the group with higher %FM (above Q3%FM) toward the pole of higher %FM, as expected. The association of *specific* bioelectrical values with TBW, ICW or ECW, instead, was not significant, with the only exception of the positive correlation between Xcsp and ICW and TBW in men (Table 3.2, Figure 3.2b, Figure 3.3b, h).

PhA, and hence both classic and *specific* BIVA, detected ECW/ICW differences in both sexes, with lower PhA values in subjects with higher ECW/ICW ratio (Table 3.2, Figure 3.3e, f, m, n, Figure 3.4). It was also positively associated with ICW and TBW in men and negatively associated with %FM in women (Table 3.2).

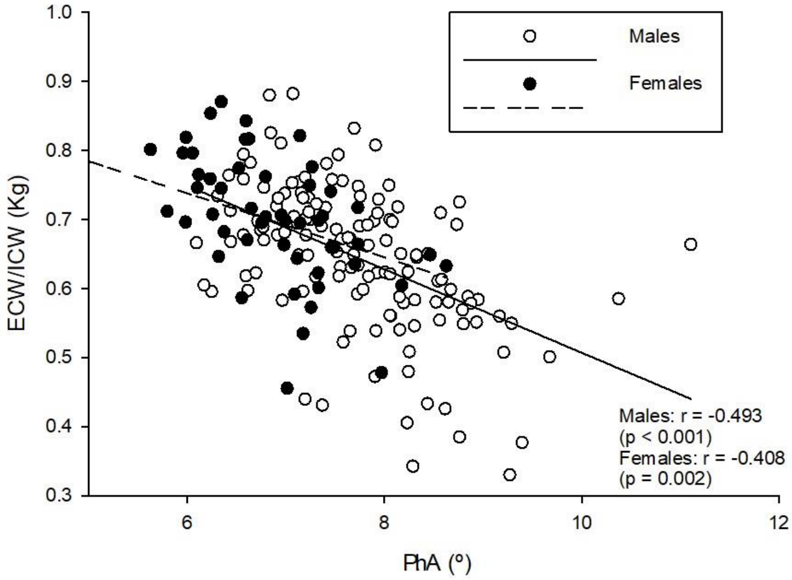


Figure 3.4 - Correlation between phase angle and extracellular/intracellular water ratio in men and women.

DISCUSSION

The present study, for the first time, analysed the association of PhA, classic and *specific* BIVA with DXA and dilution techniques, for body composition assessment in athletes. Data showed that classic BIVA correctly detect differences of TBW, but was weak in the assessment of %FM. On the contrary, *specific* BIVA detected changes of %FM, but not those of TBW. The relation with FM and FFM was different in classic and *specific* BIVA: classic bioelectrical values were negatively related to body compartments (particularly to FFM), while specific bioelectrical values showed a positive correlation (particularly with FM). Also, the relation with water compartments was different: R/H and Xc/H were negatively related to ICW and ECW, while in *specific* BIVA only Xcsp was positively related to ICW and only in men. PhA, which is the same in classic and *specific* BIVA, was sensitive to ECW/ICW ratio and ICW. These results were unaffected by age and sports practised. Although the sexual dimorphism, the association between bioelectrical and body composition variables was quite similar in the two sexes. The only exception was the stronger relation between classic values with %FM or

FM in men (with an opposite direction in the two sexes in the case of FM%), and the stronger relation between specific values with FM observed in women.

Previous reliability studies on body composition assessment in the general population, using DXA as a reference, have shown quite similar results. Indeed, *specific* BIVA has demonstrated to evaluate FM, FFM, and %FM accurately in US adults (Buffa et al., 2013) and in Italian elderly (Marini et al. 2013). Further, both specific vector length and phase angle have shown to be able to detect skeletal muscle mass differences (Buffa et al., 2014). The same studies have also shown that classic BIVA can recognize different quantities of absolute mass, but does not perform accurately in evaluating %FM and in the recognition of the obesity and athletic regions within the RXc graph (Buffa et al. 2013; Marini et al. 2013), as in the present research. Furthermore, Wells et al. (Wells et al. 2018) recently tested classic BIVA in a sample of healthy children against the criterion 4-component model and recognized inconsistencies in body composition outcomes, particularly for FFM. Accordingly, the recent review on the applications of BIVA in sport sciences (Castizo-olier et al., 2018) has shown that the majority of the studies using classic BIVA did not observe bioelectrical vectors falling in the region of the tolerance ellipses expected for athletes. As suggested by Castizo-Olier et al. (2018), this could indicate the need of reference values for each population or sport. However, as discussed with more detail elsewhere (Buffa et al., 2014b; Castizo-olier et al., 2018), these unexpected results of classic BIVA could be due to the solely effect of body geometry cross-sectional areas in particular on bioelectrical parameters. In fact, according to the Ohm’s law, resistance is directly proportional to the conductor’s length and inversely proportional to its cross-section. Indeed, our sample of athletes, characterized by shorter classic vectors (significantly lower values of R/H and Xc/H) with respect to the reference sample of Italo-Spanish young adults (Ibáñez et al., 2015) is also characterized by significantly higher circumferences. The correction for cross-sections applied in *specific* BIVA reduces the differences related to body size and shape, increasing the sensitivity of bioelectrical values to tissues' properties and body composition, such as %FM. In fact, the vectors of Portuguese athletes are located toward the obesity region of classic tolerance ellipses of the Italian-Spanish young adults

(Ibáñez et al., 2015), while they are centrally located within the specific tolerance ellipses of same reference population.

Classic BIVA is commonly used to monitor hydration changes, with fluid overload indicated by shorter vectors, i.e. falling towards the lower pole of the classic tolerance ellipses. The technique has been clinically validated for the evaluation of TBW (Piccoli et al., 1996; Bronhara et al., 2012; Lukaski and Piccoli 2012; Piccoli, 2014) and used for detecting body fluids changes in athletes (Gatterer et al., 2014). Further, Wells et al. (Wells et al., 2018) showed that, BIVA outcomes behaved as expected on the basis of theoretical assumptions in the case of FFM hydration, using the 4-component model as a reference. The vector migration has also shown to be consistent with fluid loss determined using dilution techniques (Lukaski and Piccoli 2012; Heavens et al., 2016). However, Heavens et al. (Heavens et al., 2016) noticed that the area of normal hydration on the tolerance ellipses is wider than expected on the basis of dilution techniques.

The classic vector length, mainly determined by R/H values, can be also considered indicative of extracellular water (negative relation), being ECW strongly correlated with TBW (Pierson et al., 1991), while Xc/H, which is related to body cell mass, should be positively associated with ICW (Piccoli et al., 1994). Instead, we have observed a negative relation between Xc/H and ICW. However, it should be noted that ICW, as well as ECW, is also positively correlated with TBW. Further, nor R/H or Xc/H are expected to give information on fluid distribution between compartments and tissue hydration, especially if considered separately. Fluid distribution is more related to the ECW/ICW ratio, which is not dependent on body dimensions (and hence on absolute values of ICW, ECW, TBW), and mainly detected by PhA. In fact, PhA has demonstrated to be related to water distribution between the extra- and intra-cellular spaces using dilution as reference technique: the higher PhA, the greater proportion of ICW compared to ECW, i.e. the lower ECW/ICW ratio (or ECW/TBW) (Chertow et al., 1995; Gonzalez et al., 2016). PhA is identical in classic and *specific* BIVA and, accordingly, the two techniques have demonstrated a similar accuracy in detecting

ECW/ICW in US adults, based on the comparison with bioelectrical impedance spectroscopy (Buffa et al., 2013).

Body composition and body fluids monitoring is a relevant topic in sports. In fact, an elevated body FM can negatively affect the quality of movement and performance in athletes (Lovell et al., 2015; Campa et al., 2018b), while hypo-hydration and fluid accumulation may compromise physical and cognitive performance, and eventually health (Maughan and Shirreffs 2010a); especially in certain sports (Maughan and Shirreffs 2010b; Reljic et al., 2016). Futhermore, ICW variations are related to changes in performance (Silva et al., 2010; Silva et al., 2011; Silva et al., 2014). However, it should be stressed that different physiological adaptations and dehydration processes, diversely affecting the extra cellular and intracellular spaces, can be induced by physical exercise and their relations with bioelectrical changes should be better explored (Cheuvront et al., 2013). Moreover, as also suggested by Wells et al. (Wells et al., 2018), further work is needed to improve the understanding of PhA meaning at the physiological level.

This research has the main point of strength of being the first study performed in athletes analysing the association of PhA, classic and *specific* BIVA with DXA and dilution techniques in the assessment of body composition and body fluids.

Despite the encouraging results obtained in this study, some limitations are present and should be considered. In fact, our results are applicable to BIA equipment using the 50 kHz frequency and to a similar population. Indeed, even if multifrequency equipments are widely used with acceptable accuracy at the group level to assess and track FFM (Matias et al., 2012; Matias et al., 2013; Matias et al., 2016), BIVA was originally developed and proposed using single-frequency devices. Moreover, a recently published research (Silva et al., 2018) showed that BIS values at 50 kHz are not directly comparable to those obtained by single-frequency devices. Thus, further analysis using multifrequency equipments are required and could give useful information. Additional studies should focus on health and disease populations, different age groups, ethnicity, and body regions to better define the suitability of BIVA approaches for body composition assessment.

In conclusion the present study shows that *specific* BIVA is more accurate than classic BIVA in the %FM assessment in athletes, whereas the classic method is able to analyse body fluids with a higher accuracy. PhA (and hence both classic and *specific* BIVA) was sensitive to ECW/ICW ratio. Physicians and sports coaches should consider using both BIVA approaches (classic and *specific*) to obtain reliable body composition evaluations in athletes. More research is needed to analyse the sensitivity of BIVA to each type of dehydration and to body water compartments. Further, validation studies are also necessary with regard to the variations of body composition and hydration that occur during the competitive season and in pre- to post-exercise.

3.2 ASSOCIATION OF *SPECIFIC* BIVA AND DXA IN THE EVALUATION OF SEGMENTAL BODY COMPOSITION

INTRODUCTION

Although body composition is mostly applied to the whole-body, the definition of variations at a segmental level, i.e. in different body segments (limbs, trunk), is growing in interest. Most studies have been directed at using segmental bioelectrical impedance measurements to predict whole-body composition (Ward, 2012). However, segmental body composition is also useful to provide selective information about the risk of some diseases (e.g. trunk adiposity for type 2 diabetes; Roh et al., 2020), in diagnostic investigation (e.g. limb FFM in sarcopenia; Guglielmi et al., 2016), for analysing the effect of medicaments (e.g. arm hydration in lymphedema; Ward et al., 2011), in the evaluation of training effects (Tinsley et al., 2019), and for studying body asymmetry (e.g. in athletes; Czeck et al., 2019; Milsom et al., 2014). Furthermore, in some experimental conditions, such as in the elderly where total body measurements may not be convenient, the information on limbs can be used as an alternative to total body composition (Mereu et al., 2018; Biggs et al., 2001). Indeed, the association between the whole-body and segmental approaches has been observed in various experimental contexts (Shafer et al., 2009; Kim and Kim, 2013; Nescolarde et al., 2008b).

Specific BIVA has been applied in several contexts (Buffa et al., 2014; Castizo-Olier et al., 2018; Stagi et al., 2020a), while the segmental approach has been introduced more recently (Mereu et al., 2018; Stagi et al., 2020b). As previously discussed, in the whole-body approach the bioelectrical values have shown to be associated to the %FM and ECW/ICW. However, at the present day, no studies have evaluated the performance of *specific* BIVA at the segmental level.

The present research aims to analyse the relationship between *specific* BIVA and DXA, used as the reference technique, for segmental body composition, and to analyse comparatively the information retrieved from different body segments and the whole-body.

This study was conducted with the researchers from the University of Barcelona.

METHODS

Study partecipants

Fifty active students (25 women, 25 men) from the National Institute of Physical Education of Catalonia (INEFC) volunteered for this research. Sample size was determined by fixing the type I error at 5% while minimizing the type II error at less than 5% and by using standard formulas for comparing independent normal populations. The average age of the volunteers was 24.37 (± 4.79) years for men and 24.32 (± 4.43) years for women. The sample includes students involved in different sports: swimming, football, running, tennis, cycling, padel, badminton, skiing, dancing, water polo, basketball, climbing, taekwondo, rugby, gymnastics, callisthenics and weightlifting.

Before the measurements, each participant was informed about the aims of the project and the type of measurements. The following exclusion criteria were adopted: electronic medical implants such as a pacemaker, diuretic therapy, pregnancy, alcohol or drug abuse, a physical disability that might interfere with body composition measurement and the use of contraceptives. Each participant provided his or her consent before the examination. The experimental protocols were approved by the Ethics Committee for Clinical Research of the Catalan Sports Council (24/CEICGC/2020).

Protocol

Subjects were asked to come to the laboratory after at least three hours of fasting and no previous exercise. For the evaluation, volunteers were asked to wear light, casual clothing, and remove all metal jewellery. The experimental protocol was performed following a precise order of measurement steps. First, anthropometrical measurements were recorded. Then, the densitometric analysis was conducted. Finally, the total and segmental bioimpedance analysis was performed. The data registration procedure was done between 9:00 and 14:00.

Anthropometric measurements were obtained by an ISAK-certified technician following an international standardised protocol (Esparza-Ros et al., 2019). Body mass was measured with a scale (Seca 700®, Seca Corp©, Hamburg, DE) to the nearest 0.01 kg and height was measured to the nearest 0.1 cm with a stadiometer (Holtain stadiometer®, Holtain Limited®, Crymych, UK).

Circumferences of the relaxed right arm, right calf and waist were taken. Also, lengths were measured for the right arm, right leg and trunk. Arm length was measured as the distance between the acromion and the stylion, leg length as the distance between the trochanter and the malleolus and trunk length as the distance between injector electrodes.

The technical error of intra-observer measurement (TEM) and TEM% were calculated in a sample of ten subjects (height: TEM=0.04 cm, TEM%=0.02; weight: TEM=0.04 kg, TEM%=0.07; arm circumference: TEM=0.16 cm, TEM%=0.22; waist circumference: TEM=0.14 cm, TEM%=0.53; calf circumference: TEM=0.10 cm, TEM%=0.29).

DXA analysis was performed using a whole-body DXA scan (Lunar Prodigy Advance model with an enCORE v18 software platform, from GE Medical Systems Madison, Wisconsin, USA). The scanning method involves a narrow fan beam (4.5° angle) with an intelligent fan and MVIR. X-ray characteristics include a constant potential source at 76 kV, K-edge filter at efficient dose, tube current: 0.15 to 3.00 mA. DXA quality control calibration procedures were performed using dedicated circuit (120 VAC 50–60 Hz 20 A or 230–240 VAC 50–60 Hz 10 A; ± 10%). Ambient requirements were a temperature between 18°C–27°C and humidity between 20%–80%. A specialised technician positioned the subjects in a supine position within the edges outlined on the scan table. Each full-body scan took about seven minutes. DXA measurements included whole-body and segmental measurements of %FM, FM (kg) and FFM (kg).

FFM indexes were calculated for total and body segments using the formula: FFMItotalbody=FFM/height^2 (kg/m^2) or FFMIsegmental=FFM/segment length^2 (kg/m^2).

A total and segmental bioelectrical impedance analysis was performed on the right side of the body, using a single-frequency phase-sensitive impedance device (BIA 101 Anniversary Sport Edition, Akern, Firenze, Italy; 50 kHz and 400 μA). The BIA device and cables were checked for each session with a test circuit. Subjects were measured lying on a non-conductive bed. The positioning of the electrodes (BIATRODES, Akern, Firenze, Italy) for the entire body followed the standard hand-to-foot position (NIH 1996) (Figure 3.5). For segmental body composition, to ease the procedure and to optimise the representation of different body segments, an ad hoc protocol was defined (Figure 3.6). As suggested by Chumlea et al. (1988), on the arm, a pair of electrodes were placed on the shoulder and the hand. On the leg, the procedure suggested by Fuller and Elia (1989) was used, the electrodes were placed at the level of the iliac crest and the foot, which was considered to be less affected by measurement error. On the trunk, the same pair of electrodes that were placed on the shoulder and the iliac crest were used. The difference between the sum of raw bioelectrical values measured in the arm, the trunk and the leg, and the value of the total body was below the threshold of biological significance ($R=2.7$; $Xc =-0.5$).

Specific BIVA was applied for the estimation of body composition (Buffa et al., 2013; Marini et al., 2013). The resistance (R) and reactance (Xc) values were adjusted for a correction factor (A/L). For the whole-body, A was estimated as 0.45 arm area + 0.10 trunk area + 0.45 calf area (cm^2); the arm, trunk and calf areas were calculated as $C^2 4\pi$, where C (cm) is the circumference of each segment. The length was calculated as $L = 1.1H$, where H is the height in cm. The correction factors for the arm, the leg and the trunk were calculated using the cross-sections (A) and the length (L) of the arm, the calf and the trunk, respectively.

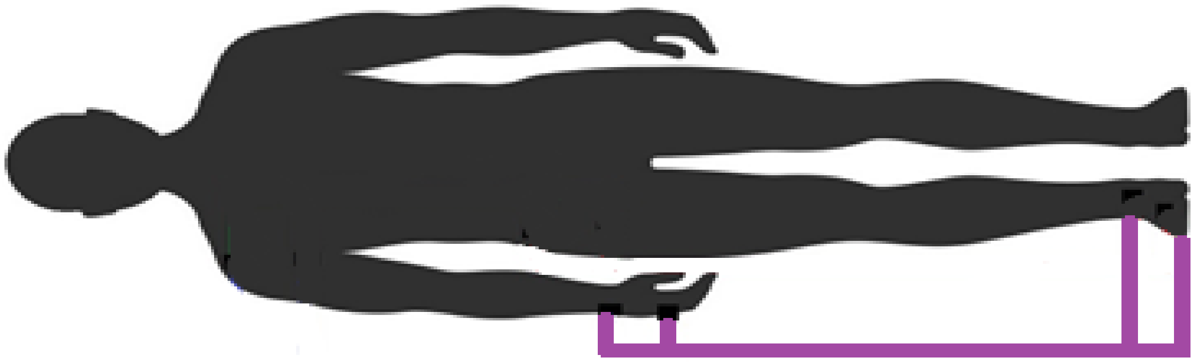


Figure 3.5 - Whole-body BIA electrodes positioning.

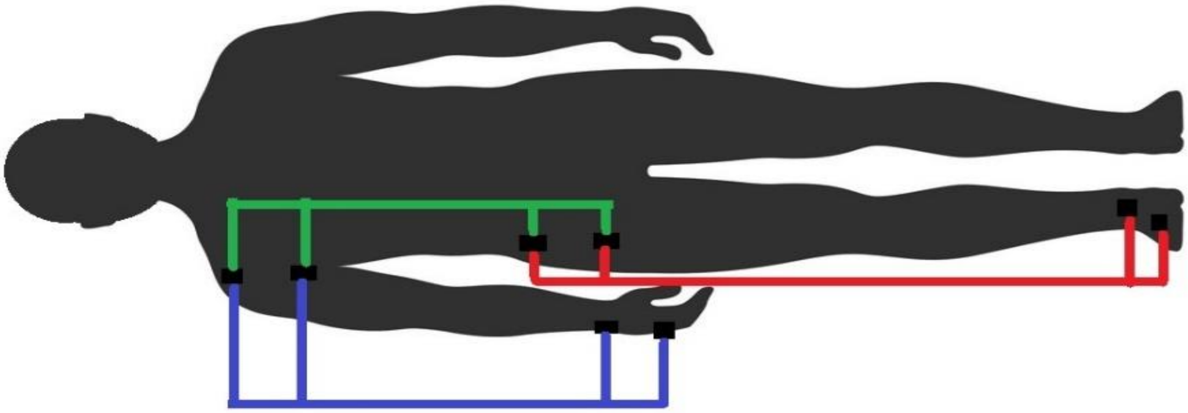


Figure 3.6 - Segmental BIA electrodes positioning. Arm: blue; Trunk: green; Leg: red.

Statistical analysis

Descriptive analyses of the total and segmental bioelectrical and DXA variables were performed. The distribution of bioelectrical specific vectors was evaluated with tolerance ellipses representing the Italo-Spanish reference population.

According to Shapiro-Wilk, all the variables were normally distributed. The comparison between sexes was made using the Student's t-test.

Pearson's correlation was used to estimate the correlation between specific bioelectrical variables

(Rsp, Xcsp, Zsp), PhA and %FM, FM, FFM, and FFMI for total and segmental body composition. The general agreement between *specific* BIVA and DXA was evaluated with a depth-depth analysis (Mosler, 2012; Ochoa and Cascos, 2019). The depth statistics measures the compatibility of a single multivariate observation with the rest of the sample. The more the depth, the less different is the sample. In particular, we considered the measures of %FM and FFMI obtained in each subject with DXA and compared them with the measures for Zsp and phase measured with BIVA. The two sets of measures lead to two corresponding unknown multivariate distributions and, thus, to two sets of depth measures. In this case, we used the so-called Zonoid depth, which is suitable for small sample sizes that provide low information regarding the two unknown multivariate distributions (Ochoa and Cascos, 2019). The two sets of depths from the two techniques were compared using ANOVA. If the subjects measured with *specific* BIVA and DXA received similar depths, the two techniques provided similar information on their body composition.

Statistical analyses were performed using the free software R (http://www.R-project.org) with the MASS library and *specific* BIVA (www.specificbiva.com). The analysis was performed in collaboration with a statistician from the University of Madrid.

RESULTS

The sample of young students practising physical exercise showed that both sexes had normal weight, as indicated by their BMI, and low %FM values (Table 3.3). Considering the whole-body, the majority of specific vectors among men (84%) and women (92%) fell on the left side of tolerance ellipses, indicating high values of cell mass, muscle mass in particular, and ICW/ECW (Figure 3.7).

A normal pattern of sexual dimorphism was detected in the total body and different body segments. Compared with women, men showed higher anthropometric values, FFM, FFMI, and PhA, and lower values of %FM, and, in most cases, of Rsp and Zsp (Table 3.3).

Table 3.3 - Subject characteristics, including the bioelectrical variables of total and segmental *specific* BIVA and the comparison between the sexes.

	Men (n= 25)		Women (n= 25)		t-test
TOTAL	Mean	s.d.	Mean	s.d.	*p*
Weight (kg)	72.4	7.9	57.1	7.6	<0.001
Height (cm)	175.7	7.0	163.0	7.4	<0.001
BMI (kg/m^2)	23.5	2.4	21.5	2.0	<0.001
FFM (kg)	60.4	7.4	43.1	6.6	<0.001
FM (kg)	12.0	3.6	14.0	3.4	0.046
%FM	16.5	4.2	24.5	5.5	<0.001
FFMI (kg/m^2)	19.6	2.2	16.2	1.6	<0.001
Rtot (ohm)	460.9	55.7	559.5	58.7	<0.001
Xctot (ohm)	65.9	7.3	69.1	5.5	0.091
Rsptot (ohm·cm)	306.5	19.6	324.6	30.3	0.017
Xcsptot (ohm·cm)	44.2	4.0	40.3	4.9	0.004
Zsptot (ohm·cm)	309.7	19.7	327.1	30.5	0.022
PhA (°)	8.2	0.7	7.1	0.6	<0.001
ARM					
Arm C. (cm)	30.9	3.1	26.9	2.2	<0.001
FFM (kg)	3.8	0.7	2.2	0.5	<0.001
FM (kg)	0.6	0.8	0.8	0.2	0.003
%FM	14.0	4.1	26.4	7.4	<0.001
FFMI (kg/m^2)	11.0	2.3	7.7	1.1	<0.001
R (ohm)	195.6	34.7	257.6	34.8	<0.001
Xc (ohm)	25.7	3.6	29.0	3.0	0.001
Rsp (ohm·cm)	247.2	29.0	274.4	39.1	0.007
Xcsp (ohm·cm)	32.8	4.8	31.1	5.1	0.241
Zsp (ohm·cm)	249.4	29.2	276.2	39.3	0.009
PhA (°)	7.6	0.8	6.5	0.8	<0.001
LEG					
Calf C. (cm)	36.7	1.9	34.3	1.9	<0.001
FFM (kg)	10.3	1.5	7.2	1.1	<0.001
FM (kg)	2.4	0.7	3.0	0.7	0.002
%FM	18.1	4.7	29.5	5.1	<0.001
FFMI (kg/m^2)	14.2	1.7	11.4	1.3	<0.001
R (ohm)	223.7	23.6	250.9	24.3	<0.001
Xc (ohm)	33.6	4.2	33.6	3.2	0.976
Rsp (ohm·cm)	280.2	13.6	293.7	23.5	0.017
Xcsp (ohm·cm)	42.1	3.9	39.4	4.4	0.024
Zsp (ohm·cm)	283.4	13.8	296.4	23.7	0.022
PhA (°)	8.6	0.7	7.6	0.6	<0.001
TRUNK					
Waist C. (cm)	77.3	4.8	67.4	3.9	<0.001
FFM (kg)	28.5	3.3	20.9	3.4	<0.001
FM (kg)	5.2	2.1	5.6	1.9	0.453
% FM	15.2	4.2	21.2	7.0	0.001
FFMI (kg/m^2)	70.6	11.5	58.5	7.9	<0.001
R (ohm)	39.0	5.5	48.1	6.7	<0.001
Xc (ohm)	7.3	0.8	7.0	0.9	0.250
Rsp (ohm·cm)	287.0	41.4	290.7	39.9	0.754
Xcsp (ohm·cm)	54.0	7.9	42.1	5.1	<0.001
Zsp (ohm·cm)	292.1	28.8	293.8	38.9	0.888
PhA (°)	10.7	1.2	8.3	1.1	<0.001

BMI, body mass index; R, resistance; Xc, reactance; PhA, phase angle; Rsp, resistance multiplicated for coefficient; Xcsp, reactance multiplicated for coefficient; Zsp, vector length multiplicated for coefficient; FFM, fat free; FM, fat mass; mass; %FM, fat mass percentage; FFMI, fat free mass index .

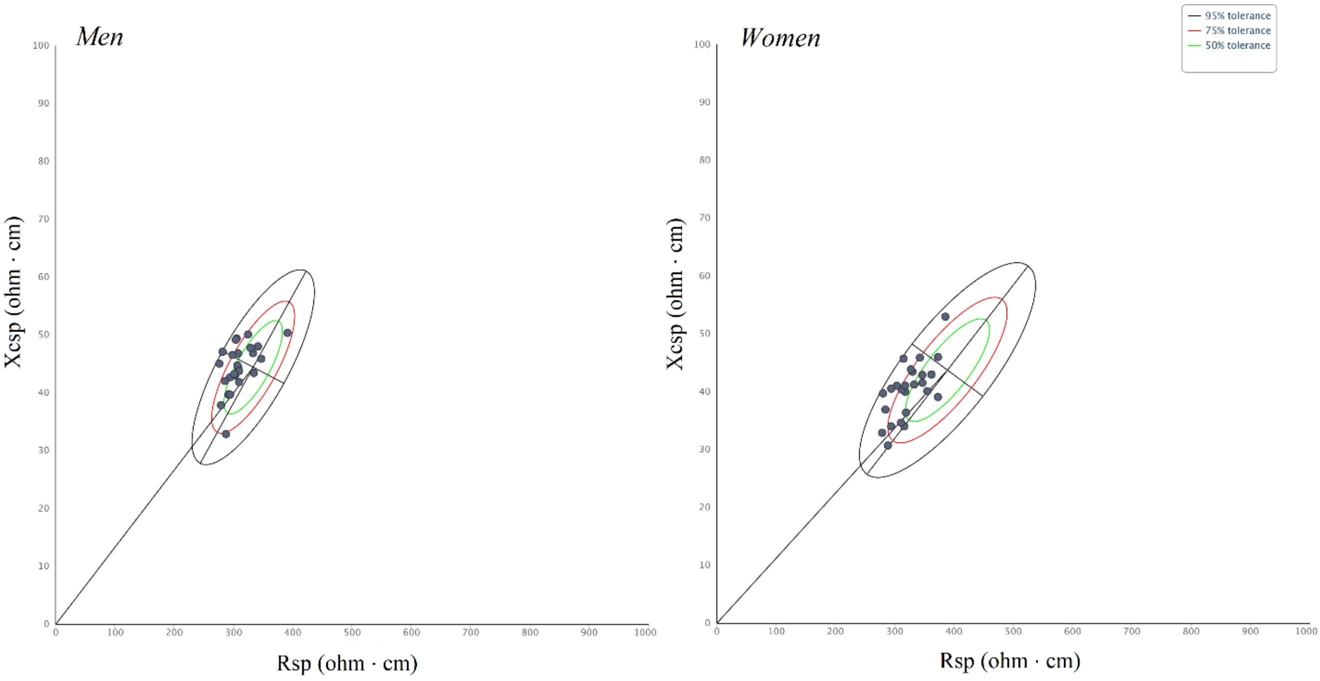


Figure 3.7 - Distribution of bioelectrical vectors of subjects on the sex-specific bivariate tolerance ellipses.

The bivariate depth-depth analysis showed good agreement between the results of DXA on FFMI and %FM and those of *specific* BIVA based on the PhA and vector length (F=14.89, p<0.001). The relationship was similar in men and women, as the effect of sex was not significant (F=0.27, p=0.84), in different body segments (F=0.77, p=0.51), without interactions (sex * body segment, F=1.39, p=0.25).

In both sexes, in the total and the segmental approach, vector length was positively correlated with %FM (Table 3.4, Figure 3.8), and in some cases with FM (Table 3.4). Among women, a negative correlation between the vector length and FFMI was also detected at the trunk level (Table 3.4, Figure 3.9).

PA was positively correlated with total body FFMI in both sexes and all segments, with the only exception of the trunk among women (Table 3.4, Figure 3.9). It was also negatively associated with %FM in the total body in men, and in the arm and trunk in both sexes (Table 3.4, Figure 3.8).

Table 3.4 - Total and segmental correlations between bioelectrical and body composition variables.

	Pearson's correlations							
	Men (n=25)				Women (n=25)			
TOTAL	*%FM*	*FM*	*FFM*	*FFMI*	*%FM*	*FM*	*FFM*	*FFMI*
Rsp	0.755**	0.788**	-0.077	-0.206	0.782**	0.765**	-0.240	-0.096
Xcsp	0.231	0.376	0.313	0.282	0.331	0.528**	0.218	0.503*
Zsp	0.751**	0.787**	-0.070	-0.197	0.778**	0.765**	-0.233	-0.084
Phase	-0.447*	-0.315	0.420*	0.528**	-0.350	-0.077	0.532**	0.769**
ARM	*%FM*	*FM*	*FFM*	*FFMI*	*%FM*	*FM*	*FFM*	*FFMI*
Rsp	0.486*	0.089	0.134	0.292	0.754**	0.734**	-0.281	-0.147
Xcsp	-0.067	0.127	0.493*	0.692**	0.304	0.537**	0.250	0.399**
Zsp	0.478*	0.090	0.143	0.303	0.750**	0.734**	-0.274	-0.140
Phase	-0.591**	0.030	0.526**	0.639**	-0.453*	-0.109	0.680**	0.719**
LEG	*%FM*	*FM*	*FFM*	*FFMI*	*%FM*	*FM*	*FFM*	*FFMI*
Rsp	0.611**	0.695**	0.018	0.029	0.589**	0.441*	-0.193	0.146
Xcsp	-0.032	0.093	0.275	0.365	0.319	0.372	0.086	0.403*
Zsp	0.596**	0.684**	0.029	0.044	0.587**	0.443*	-0.188	0.153
Phase	-0.386	-0.287	0.310	0.409*	-0.143	0.062	0.292	0.404*
TRUNK	*%FM*	*FM*	*FFM*	*FFMI*	*%FM*	*FM*	*FFM*	*FFMI*
Rsp	0.626**	0.686**	0.047	0.070	0.832**	0.796**	-0.452*	-0.440*
Xcsp	0.008	0.152	0.494*	0.475*	0.077	0.209	0.221	-0.087
Zsp	0.612**	0.676**	0.063	0.085	0.826**	0.794**	-0.443*	-0.438*
Phase	-0.669**	-0.554**	0.547**	0.502*	-0.731**	-0.583**	0.628**	0.339

Rsp, resistance multiplicated for coefficient; Xcsp, reactance multiplicated for coefficient; Zsp, vector length multiplicated for coefficient; FFM, fat free; FM, fat mass; mass; %FM, fat mass percentage; FFMI, fat free mass index.

** The correlation is significant at 0.01 level.

* The correlation is significant at 0.05 level.

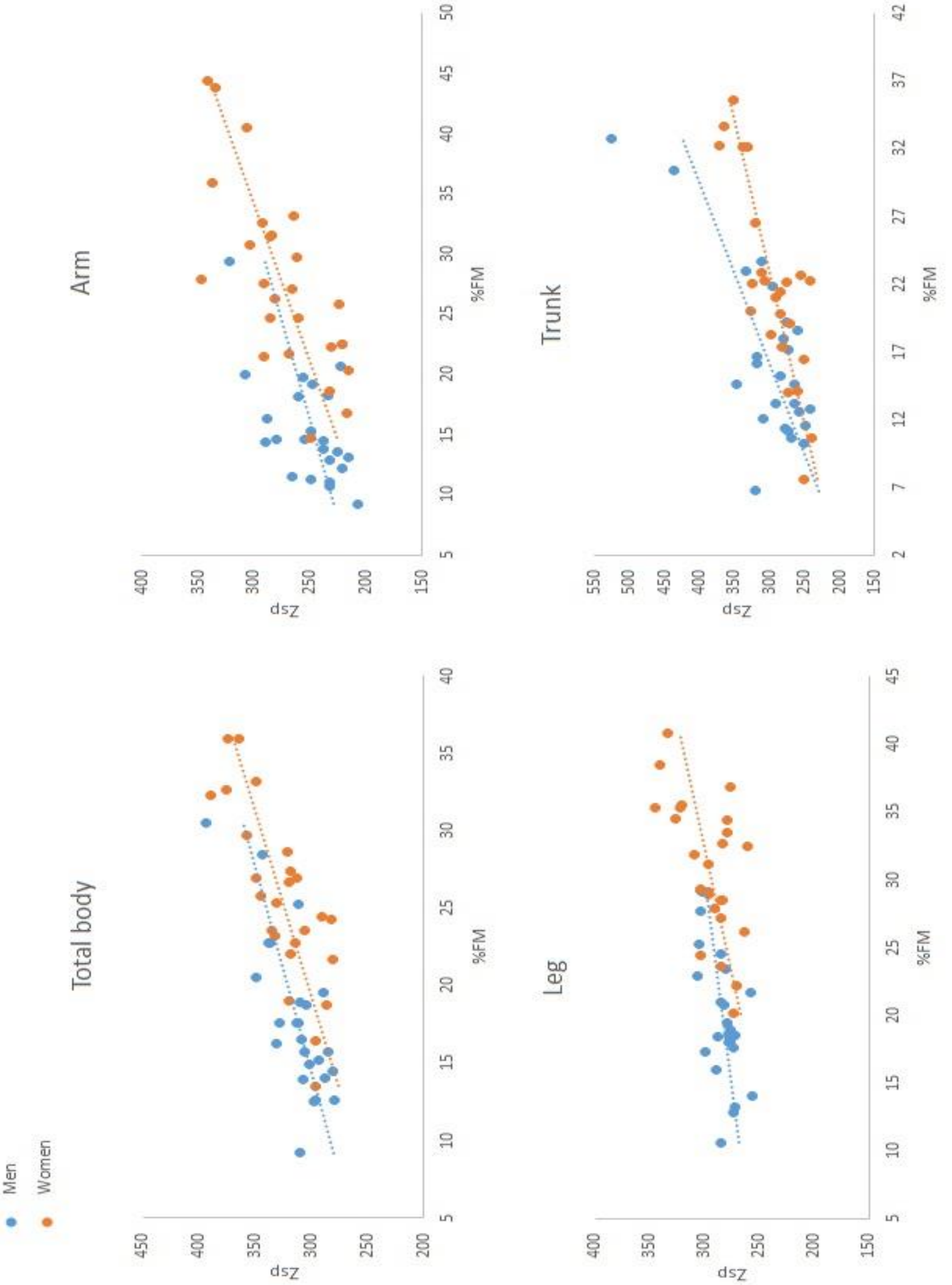


Figure 3.8 - Correlation between specific impedance vectors and %FM of the whole-body and body segments.

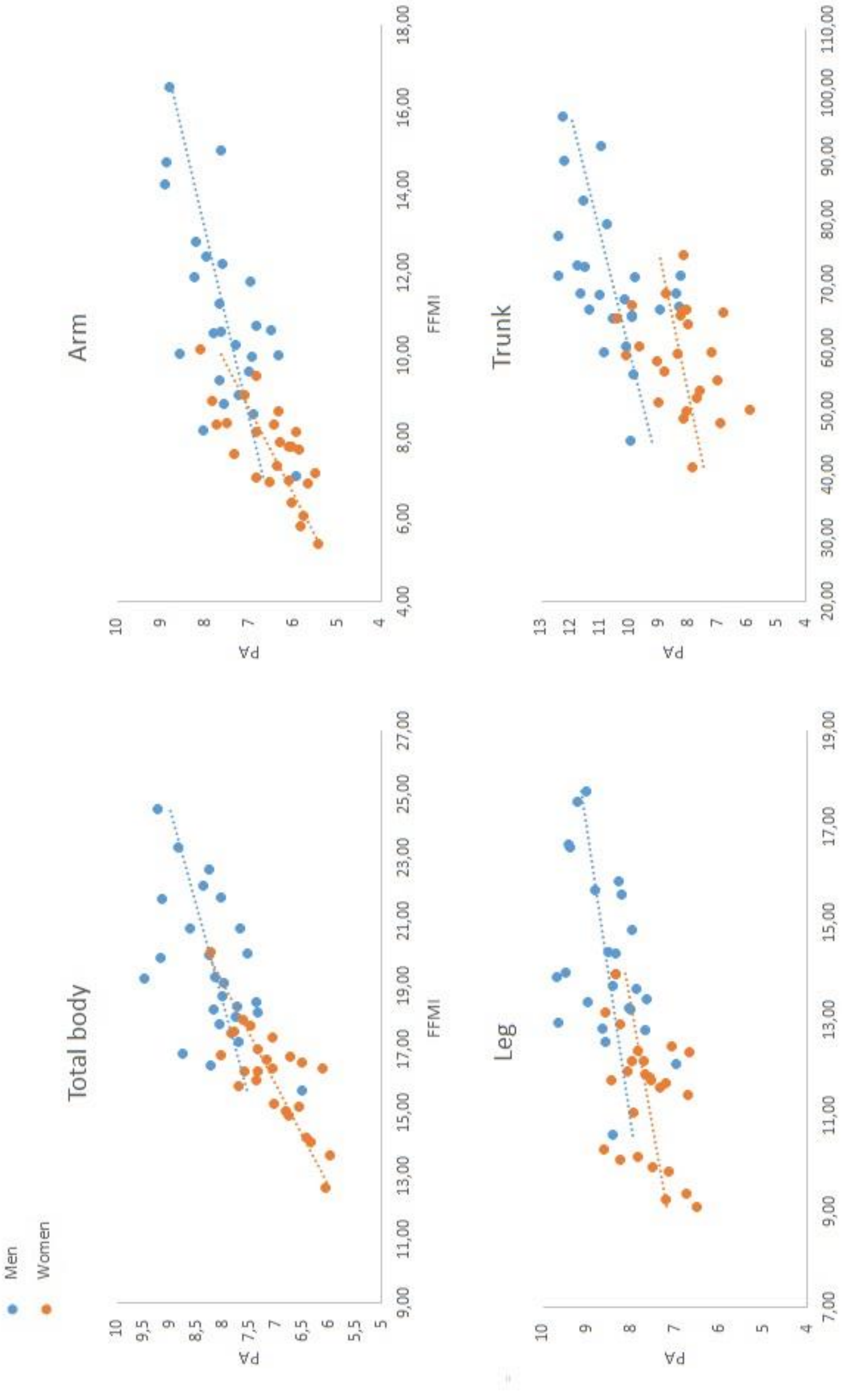


Figure 3.9 - Correlation between phase angle and FFMI of the whole-body and body segments.

DISCUSSION

This research showed that body composition evaluation performed with *specific* BIVA agrees well with that of DXA. The analysis showed a similar association in both sexes, in the total body and the trunk, arms and legs. These results are consistent with previous research on total body composition, where *specific* BIVA was compared with DXA in different samples (Buffa et al., 2013; Marini et al., 2013; Marini et al., 2020). However, this research study is the first to demonstrate that such an association has also been detected at the segmental level.

BIVA is based on the joint analysis of variables that are correlated (R and Xc, or PhA and vector length) and provides information on variables also related to each other, such as those describing body composition (e.g. FM and FFM). Thus, the bivariate statistical approach used in this study to analyse the performance of BIVA regarding DXA is very appropriate. The analysis of body composition based on single variables may not be fully informative, and it may furnish the wrong information. As shown by Mereu et al. (2016), for example, individuals with the same PhA but different specific vector length, can be characterised by %FM differences as high as 60%.

However, it is undeniable that the vector components are influenced differently by different body compartments. The specific vector length is strongly and positively related to %FM, as clearly indicated by the results of this research (whole-body and all body segments) and those of previous studies (whole-body: Biggs et al., 2001; Buffa et al., 2013; Marini et al., 2013; Marini et al., 2020; body districts: Fuller et al., 2002). PhA shows a positive association with FFMI (this research, except the trunk among women), with skeletal muscle mass index (Marini et al., 2012) or with FFM (this research; Maddocks et al., 2015; Gonzalez et al., 2016). Other relationships are less clear and consistent among studies. PhA shows a tendency to be negatively related with %FM (whole- body, arm and trunk in men, arm and trunk in women: this research; men only: Buffa et al., 2013; women only: Marini et al., 2020; men: Tanaka et al., 2018), or with FM (trunk: this research; Gonzalez et al., 2016). In contrast, the association between vector length and FFMI or FFM is rarely significant and inconsistent among studies (negative, only in the trunk among women: this research; positive, in the legs and arms among men: Fuller et al., 2002; positive, whole-body among men: Marini et al., 2020).

When the body composition of different body segments was considered comparatively, the trunk's higher FFM content with respect to the limbs was detected by both *specific* BIVA and DXA, consistently with the results of other studies in athletes (Buehring et al., 2014) and the general population (Bracco et al., 1996). However, the Zsp values of the trunk were indicative of %FM values tendentially higher than expected on the basis of DXA, whereas recent research based on traditional BIA have shown an underestimation of FM at the trunk level (Wingo et al., 2018; Majeed et al., 2019). Also, the literature and our results on raw R and Xc data show that the Z of the trunk accounts for only about 10% of the total impedance, whereas the trunk represents 45% of body mass (Fuller et al., 2002). This difference has been attributed to the composition and shape of this segment (Biggs et al., 2001; Fuller and Elia, 1989; Fuller et al., 2002; Kyle et al., 2004). The trunk includes internal organs, visceral and subcutaneous fat with variable density and distribution, and empty spaces, such as the air volume included in the lungs (that overemphasises the trunk volume). Furthermore, the trunk is characterised by a wider cross-sectional area concerning the limbs, whereas the length is similar. Hence, based on Ohm's law, the current passage in the trunk is easier, and the resistance is consequently lower.

The volume effect problem is overwhelmed by the *specific* BIVA approach, where the bioelectrical values are adjusted by A/L, i.e. by an estimate of body cross-sectional areas and length.

This study also showed that the information provided by *specific* BIVA for the body segments is also aligned with the results of the whole-body approach, confirming the correctness of the analytical procedure. The confidence ellipses of the whole-body are located in an intermediate position with respect to those of different body segments (Figure 3.10).

This study has some limitations. In fact, the lack of individuals with different expressions of body composition, particularly overweight individuals, reduces the potential generalisation of the results, that should be verified in different samples. Moreover, it was not possible to carry out the analysis on body water, for which the classic BIVA would have been appropriate because there are no reference methods to estimate body fluids at the segmental level.

However, this study has the strength of being the first research to analyse the relationship between *specific* BIVA and DXA and demonstrate that the consistency between two approaches is appreciable the sexes and different segments. Furthermore, a new protocol regarding electrode position was used in this study, selecting and integrating previous research contributions. This method has proven to be adequate, as the sum of raw bioelectrical values at the segmental level corresponded to those of the whole-body. Hence, the criticism highlighted by Ward (2012) about the imprecision in locating electrodes in the segmental approach does not apply to our case. Furthermore, the adequacy of the analytical approach used in *specific* BIVA for the whole-body, that weights the contribution of different body segments differently, was confirmed to be correct.

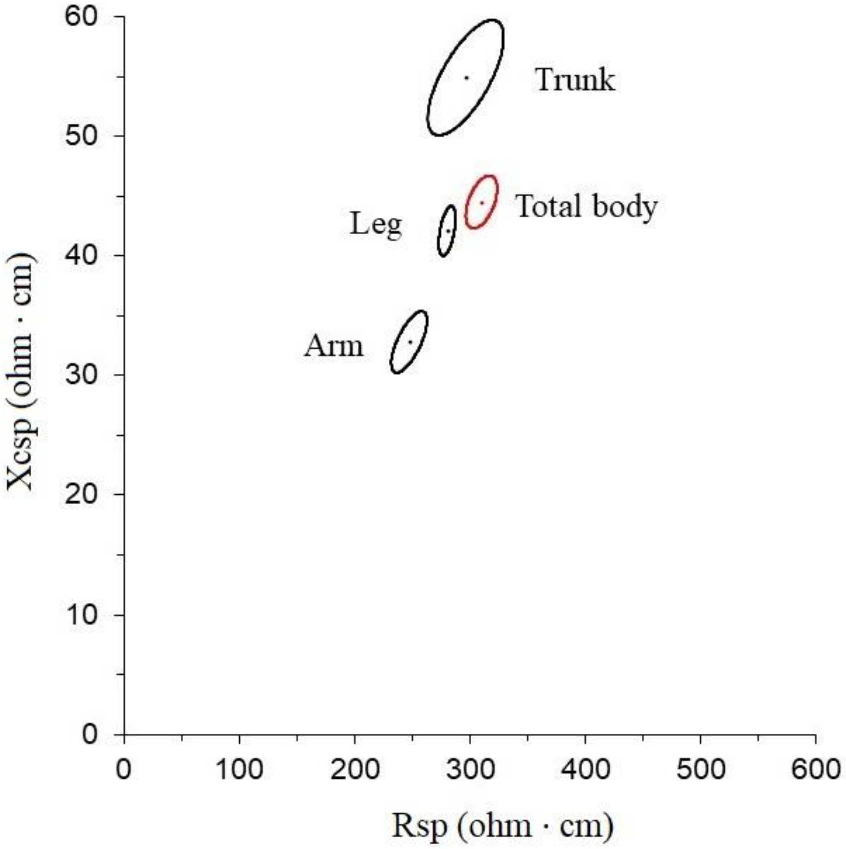


Figure 3.10 - Confidence ellipses representing the whole-body and body segments.

In conclusion *Specific* BIVA has shown to be associated with DXA in both sexes and the whole-body and all body segments. The indices %FM and FFMI obtained with DXA were correlated to vector length and phase angle in each segment, respectively.

From a methodological point of view, the new protocol proposed for segmental analysis proved to be effective. The comparative analysis of different body segments indirectly confirmed that *specific* BIVA effectively balance the effect of body size in both whole and segmental approaches. *Specific* BIVA represents a promising technique for monitoring segmental body composition changes in sport science and clinical applications.

3.3 AGREEMENT BETWEEN SELF-PERCEIVED BODY IMAGE AND BODY COMPOSITION

INTRODUCTION

Body image is a multidimensional concept that includes subjective beliefs and feelings about physical appearance (Grogan, 2008). It is influenced by factors such as sex, age, ethnicity, personality, family, media and nutritional status (Grogan, 2008). Studies of body image are based on different methods, such as interviews, questionnaires, and silhouette collections. Silhouettes generally include a range of body figures that represent increments of weight.

Nowadays only a few studies have examined the association between actual body image assessed by silhouettes and body composition estimated with an accurate technique (Zaccagni et al., 2020). Some authors have analysed the relationship between silhouette collections and the %FM calculated using anthropometry (e.g. Costa et al., 2016; Greeff, 2016), or to the body mass index (BMI) (e.g. (Stunkard, 1983; Williamson et al., 2000). Other studies investigated different methods for assessing body image (mainly questionnaires) in relation to body composition using anthropometry (Altintaş et al., 2014; Brodie and Slade, 1988; Buscemi et al., 2018), bioimpedance (Duncan and Schofield, 2011) or dual-energy X-ray absorptiometry (Streeter et al., 2012). The research has been mainly focused on young individuals, and few studies on the elderly have been based on BMI (Bricio-Barrios et al., 2020; Evans et al., 2015; Knight et al., 2009; Pinto et al., 2017; Runfola et al., 2013; Sánchez et al., 2015; Schuler et al., 2004).

Considering the gaps in the literature, this study focused on the association between Williamson's silhouettes and body composition estimated by specific bioelectrical impedance vector analysis (*specific* BIVA; (Buffa et al., 2013; Marini et al., 2013) in a sample of young and older subjects of both sexes. *Specific* BIVA has been considered adequate as it has been validated against dual-energy X-ray absorptiometry, showing high sensitivity and specificity in the evaluation of %FM (Buffa et al., 2013).

This study was conducted with the researchers from the University of the Basque Country (Bilbao).

METHODS

Subjects

This cross-sectional sample included 632 young adults (238 men and 394 women; age, 23.1 ± 2.36 and 22.5 ± 2.25 years, respectively), from the Basque Country (Spain), sampled by researchers from the University of Bilbao, and 162 middle-aged and elderly adults (96 men and 66 women; age, 61.43 ± 7.72 and 61.40 ± 7.51 years, respectively) from Sardinia (Italy). The participants were informed about the study design and signed consent before the examination. The experimental protocols were approved by the Ethics Committee for Human Research (CEISH) of the UPV/EHU and by the Independent Ethical Committee of the A.O.U. of Cagliari.

Anthropometric and bioelectrical measurements

Anthropometric measurements (height, cm; weight, kg; waist arm and calf circumference, cm) were taken following standards procedures (Lohman et al., 1988). BMI was calculated.

Bioimpedance measurements were obtained using a single-frequency phase-sensitive 50 kHz device (BIA 101 Anniversary, Akern, Florence, Italy). For each session the BIA device was checked with a calibrated circuit (R = 380 Ω, Xc = 47 Ω; ±2% error). Subjects were asked not to drink or eat, and to void their bladder before the evaluations.

For total BIA, two pairs of electrodes were placed following the standard position hand-to-foot (NIH 1996). *Specific* BIVA (Buffa et al., 2013; Marini et al., 2013) was applied to evaluate body composition.

Body image

BIA-O scale (Williamson et al., 2000) is a validated collection of silhouettes to measure body image, which includes many figures (eighteen silhouettes for each sex) representing body size individuals from very thin to very obese (https://www.nature.com/articles/0801363/figures/1). This method was enhanced from a previous body image assessment, which included nine silhouettes only for women (BIA) (Williamson et al., 1989). The new scale developed in 2000 also incorporates silhouettes for men and covers different degrees of obesity; it has been widely used not only in research on obesity, but also in studies of populations in which overweight and obesity are not very common (e.g., Muñoz-Cachón et al., 2009).

The BIA-O figure scale (Williamson et al. 2000) was used to evaluate the current body size (CBS) perception. Eighteen silhouettes of each sex, ranging from very thin (number 1) to very obese (number 18), were presented to participants and they were asked to choose the one that best represented their actual shape.

Silhouettes chosen by fewer than 5 subjects (silhouettes number 1, 12-18, plus 10-11 among older women) were not included in the analysis of the confidence ellipses, which excluded 18 young adults and 6 older subjects. The silhouettes were categorised into in five main groups (I= silhouettes 2-3; II=4-5; III=6-7; IV=8-9; V=10-11) to increase the number of cases per group after checking the absence of significant differences within groups.

Statistical analyses

The Mann-Whitney U-test was employed to evaluate sex differences in the chosen CBS. The associations between CBS and anthropometrical or bioelectrical measurements were investigated using Spearman's correlation analysis. The relationships between the silhouettes and body composition were studied using confidence ellipses and Hotelling's T^2. The differences between the sexes and among groups of silhouettes were analysed separately by two-way analysis of variance in the sample of young adults and older adults. Statistical analyses were performed using the SPSS programme (SPSS Inc., Chicago, IL, USA) and *specific* BIVA software (www.specificbiva.com).

RESULTS

The sample showed normal nutritional status in mean with a tendency for overweight in the older group, as indicated by the BMI (young men: 23.60 ± 2.58; women: 22.33 ± 2.82; older adult men: 25.19 ± 2.92; women: 24.30 ± 4.20 kg/m^2). The mean waist circumference values were below the thresholds for abdominal obesity (young men: 79.28 ± 6.74, women: 69.61 ± 2.58; older adult men: 88.36 ± 9.30, women: 77.26 ± 9.0 cm). A few participants (14 young and 8 older adults) were obese

(BMI > 30 kg/m^2). Accordingly, very few individuals' chose large silhouettes numbered 12 or more. The entire sample showed a normal pattern of sexual dimorphism, with men characterised by higher height, weight, BMI, PhA, and lower Rsp and Zsp compared to women (Table 3.5).

Table 3.5 - Descriptive statistics and two-way ANOVA of bioelectrical and anthropometrical values by groups of CBS silhouettes.

	Men (N= 231)					Women (N= 383)							
Young adults	I (N=34)	II (N=67)	III (N=79)	IV (N=42)	V (N=9)	I (N=72)	II (N=170)	III (N=72)	IV (N=56)	V (N=13)	**ANOVA**		
	Mean s.d.	Mean s.d.	Mean s.d.	Mean s.d.	Mean s.d.	Mean s.d.	Mean s.d.	Mean s.d.	Mean s.d.	Mean s.d.	F_{sex}	F_{group}	$F_{sex \cdot group}$
Height (cm)	175.0 (7.24)	175.6 (6.12)	175.3 (5.78)	177.9 (5.90)	176.7 (4.28)	162.2 (5.07)	161.7 (5.41)	162.0 (6.31)	163.7 (6.36)	161.4 (5.60)	<0.001	0.163	0.884
Weight (kg)	65.9 (8.01)	68.4 (6.86)	73.6 (7.28)	82.5 (9.90)	85.3 (7.03)	52.6 (5.11)	56.9 (5.61)	60.3 (8.02)	67.1 (8.76)	72.9 (11.56)	<0.001	<0.001	0.313
BMI (kg/m^2)	21.5 (1.50)	22.2 (1.69)	23.9 (1.74)	26.3 (2.45)	27.3 (2.61)	20.0 (1.42)	21.8 (1.89)	22.9 (2.26)	25.1 (2.89)	27.9 (3.68)	0.002	<0.001	0.084
Rsp (ohm·cm)	317.0 (32.48)	311.5 (24.35)	331.4 (38.93)	352.7 (40.26)	380.1 (55.21)	346.9 (35.20)	367.5 (36.71)	382.3 (50.14)	424.1 (52.04)	445.4 (50.87)	<0.001	<0.001	0.009
Xcsp (ohm·cm)	42.9 (6.15)	43.1 (4.90)	46.0 (6.70)	49.2 (5.46)	49.8 (9.30)	40.1 (6.27)	42.9 (5.58)	44.9 (6.97)	50.4 (7.02)	52.8 (8.07)	0.978	<0.001	0.126
Zsp (ohm·cm)	319.9 (32.77)	314.5 (24.53)	334.6 (39.25)	356.1 (40.52)	383.4 (55.72)	349.2 (35.52)	370.0 (36.88)	384.9 (50.44)	427.1 (52.31)	448.6 (51.03)	<0.001	<0.001	0.009
PhA (°)	7.7 (0.79)	7.9 (0.70)	7.9 (0.75)	7.8 (0.48)	7.6 (0.84)	6.6 (0.67)	6.7 (0.67)	6.7 (0.63)	6.8 (0.64)	6.8 (0.89)	<0.001	0.166	0.503
	Men (N= 95)					Women (N= 61)							
Middle-aged and elderly	I (N=10)	II (N=21)	III (N=38)	IV (N=19)	V (N=7)	I (N=14)	II (N=17)	III (N=18=)	IV (N=12)				
	Mean s.d.	Mean s.d.	Mean s.d.	Mean s.d.	Mean s.d.	Mean s.d.	Mean s.d.	Mean s.d.	Mean s.d.		F_{sex}	F_{group}	$F_{sex \cdot group}$
Height (cm)	168.2 (6.43)	170.0 (7.50)	170.06 (6.95)	168.7 (6.30)	169.0 (4.65)	154.9 (8.01)	156.7 (6.88)	155.5 (7.00)	154.2 (5.14)		0.901	0.098	0.883
Weight (kg)	64.6 (8.59)	66.7 (6.95)	72.07 (8.46)	80.0 (9.03)	80.1 (6.65)	50.6 (3.94)	55.2 (6.45)	60.2 (8.42)	66.9 (10.08)		<0.001	0.775	0.989
BMI (kg/m^2)	22.8 (2.01)	23.1 (2.46)	24.89 (2.23)	28.1 (2.00)	28.0 (1.57)	21.3 (2.91)	22.5 (2.34)	24.9 (3.36)	28.1 (3.96)		<0.001	<0.001	0.853
Rsp (ohm·cm)	330.5 (64.07)	328.1 (34.03)	338.74 (34.25)	377.9 (64.15)	376.2 (66.72)	343.8 (57.18)	362.6 (40.68)	390.6 (77.88)	426.0 (54.53)		<0.001	<0.001	0.676
Xcsp (ohm·cm)	41.9 (7.38)	41.9 (6.27)	42.34 (7.07)	50.9 (10.44)	43.3 (7.99)	38.3 (7.03)	41.7 (8.57)	44.1 (7.43)	43.5 (7.38)		0.468	0.011	0.091
Zsp (ohm·cm)	333.2 (63.91)	330.8 (34.35)	341.46 (34.09)	381.5 (63.53)	378.8 (66.54)	346.0 (57.28)	365.1 (40.68)	393.2 (77.66)	428.2 (54.83)		<0.001	<0.001	0.674
PhA (°)	7.3 (1.44)	7.3 (0.72)	7.2 (1.39)	7.9 (2.32)	6.7 (1.43)	6.4 (1.01)	6.6 (1.41)	6.6 (1.54)	5.8 (0.63)		0.001	0.978	0.193

Groups: I= silhouettes 2-3; II= silhouettes 4-5; III= silhouettes 6-7; IV= silhouettes 8-9; V= silhouettes 10-11.
BMI, body mass index; Rsp, resistance; Xcsp, reactance; Zsp, vector length; PhA, phase angle.

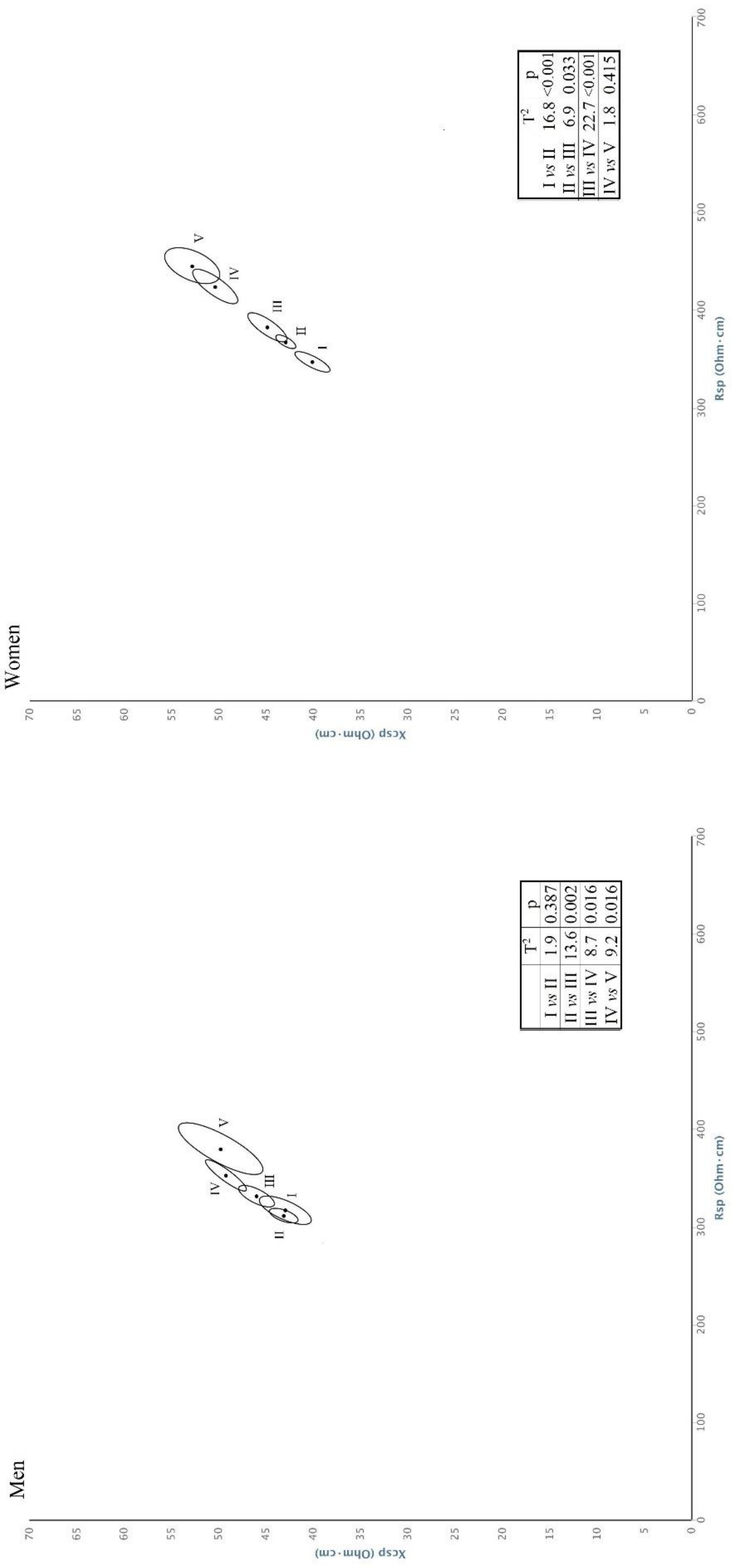
Sexual differences were also observed in the CBS choices by the young adults (Mann-Whitney U-test; $p < 0.001$). Men tended to choose larger silhouettes than women; the most chosen silhouettes were number 6 by the men (18.2%) and number 4 by the women (22.7%). In the older group, sex differences in the CBS choice were not significant (Mann-Whitney U-test; $p = 0.070$), with both sexes choosing silhouette number most frequently 7 (21.9% in men; 16.7% in women).

In both sexes, individuals choosing different groups of silhouettes had similar mean height and PhA values, while weight, BMI, Rsp, Xcsp and Zsp were significantly different, with greater values in those choosing bigger silhouettes (Table 3.5). In fact, weight, BMI and the bioelectrical variables, with the only exception of PhA, were positively correlated with the silhouettes (Table 3.6). The pattern was similar between the sexes, but more regular in women (Figure 3.11). Men choosing larger silhouettes (group V) tended toward a declining phase, which was significant among younger subjects ($p < 0.05$).

Table 3.6 - Correlation between bioelectrical variables and CBS in the two groups and sexes.

	Young adults		**Middle-aged and elderly**	
	Men	Women	Men	Women
Height (cm)	0.081	0.054	-0.040	-0.040
Weight (kg)	0.589**	0.544**	0.610**	0.682**
BMI (kg/m^2)	0.687**	0.618**	0.723**	0.642**
Rsp (ohm·cm)	0.378**	0.482**	0.374**	0.409**
Xcsp (ohm·cm)	0.352**	0.444**	0.267**	0.313*
Zsp (ohm·cm)	0.379**	0.483**	0.375**	0.405**
PhA (°)	0.032	0.073	-0.006	-0.080

r values are reported in the table: Rsp, resistance multiplicated for coefficient; Xcsp, reactance multiplicated for coefficient; Zsp, vector length multiplicated for coefficient; PhA, phase angle.
** The correlation is significant at 0.01 level.
* The correlation is significant at 0.05 level.



Men

	T^2	p
I *vs* II	1.9	0.387
II *vs* III	13.6	0.002
III *vs* IV	8.7	0.016
IV *vs* V	9.2	0.016

Women

	T^2	p
I *vs* II	16.8	<0.001
II *vs* III	6.9	0.033
III *vs* IV	22.7	<0.001
IV *vs* V	1.8	0.415

Figure 3.11 - Confidence ellipses representing body composition in the groups of current body size silhouettes (I=silhouettes 2-3; II=silhouettes 4-5; III=silhouettes 6-7; IV=silhouettes 8-9; V=silhouettes 10-11) in young men and women. The older adults showed a similar trend.

DISCUSSION

In the analysed sample, CBS, estimated by Williamson's silhouettes, was associated with variations in body size and body composition in both age classes and sexes, but particularly in women. In fact, CBS was positively correlated with weight and BMI, and with bioelectrical vector length, while it was unrelated to PhA and height. According to *specific* BIVA (Buffa et al., 2013; Marini et al., 2013), the observed associations indicate that individuals choosing bigger silhouettes are characterised by higher %FM values (longer vectors), but similar muscle mass (similar PhAs).

Previous studies, using different populations and figure collections established a robust relationship between BMI and silhouette collections (e.g. Bulik et al., 2001; Mũoz-Cachón et al., 2009). As *specific* BIVA recognises differences in body composition that are not detected by the BMI (Buffa et al., 2017; Marini et al., 2020), this study allowed us to clarify that the association is due to the FM component of the body, and not to the muscular component. A similar result was reported by Zaccagni et al. (*2020*) in a sample of Italian students of both sexes, where self-perceived body image was consistent with FM measured using conventional bioimpedance, although with a weakening of the relationship in underweight and overweight individuals.

In the present study, we also observed that the association was similar between the sexes, although it was more regular among women. Men who chose the bigger silhouettes of group V, especially the youngest men, despite a similar BMI to women (27.3 vs. 27.9 kg/m2), were characterised by a decrease of their PhAs. The different trend between the fattest men and women can be interpreted in view of their body composition differences, with overweight men characterized by a reduction of muscle mass not appreciable among women.

We observed similar relationships between body composition and CBS among young and older adults. This result is insightful as it suggests a possible application of self-perceived body image among elderly people, who are prone to the risk of malnutrition (Morley, 2012) and where other techniques may be more difficult to use. Other studies on body satisfaction detected no significant differences between younger people and older adults (Evans et al., 2015), and observed that ideal body image remains quite unchanged across the lifespan, particularly in women (Pruis and Janowsky 2010; Runfola et al., 2013; Schuler et al., 2004). However, some authors have observed that elderly people, especially the heaviest and fattest women, tend to underestimate their actual weight (Bricio-Barrios et al., 2020; Knight et al., 2009; Pinto et al., 2017; Sánchez et al., 2015).

The main strength of the present study was that it represents one of the few attempts to analyse the association between CBS, which is a broad construct that includes perceptual, attitudinal, behavioral, and cognitive dimensions (Roy et al., 2012), and body composition evaluated using an accurate technique. In particular, to our knowledge, this is the first study applying *specific* BIVA, a validated technique for the evaluation of percent FM, also sensitive to ICW/ECW.

Additionally, the sample included both sexes and two different age groups, thus allowing an analysis of the relationships from a large perspective.

However, this research has some limitations. The sample was mainly composed of normal weight individuals, with very few cases choosing large silhouettes. Hence, we were unable to analyse if the relationship remained similar at the extreme of body composition variability.

In conclusion the present study demonstrated that Williamson’s silhouettes used to assess the CBS are strongly related to body fat in both sexes and until an older age. The whole sample composed of mostly normal weight individuals, including both sexes and classes of age, showed a coherent perception of their current body size.

3.4 COMPARISON OF BIOIMPEDANCE DEVICES: CORRECTION OF BIAS DIFFERENCES

INTRODUCTION

Considering the relevance of BIA in science and medicine, a bioelectric impedance measurement is expected to be reliable. However, there are likely to be many factors that could affect its reliability. As mentioned in the general introduction, the application of BIA requires standardisation, and to do this it is necessary to know all the factors that may influence both accuracy and precision.

The factors influencing the measurement of BIA can be classified in:

- ambient temperature, which in general does not affect resistance and reactance measurements if maintained in a range between 24° and 34° C;
- operator, which is associated with inter-variability (in the case of one operator) and intra-variability (when more than one operator is involved in the same study);
- subject to be measured, to which several factors interfere with the measurement, such as the position assumed by the subject, skin temperature, skin preparation, food and drink intake, the practice of intense physical exercise and also the period of the menstrual cycle (detailed in the general introduction of the thesis);
- instrumentation, which includes all the components used in the measurements: characteristics and position of the electrodes, characteristics of the connection cables and the device, which can give rise to both intra-instrumental and inter-instrumental variability. One important factor that must be checked is the calibration of the device. The operator must check the device before each measurement session, through repeated measurements of circuits with known values of resistance and reactance.

Another important aspect to be considered is that devices produced by different manufacturers, as well as different models produced by the same manufacturer, can provide different impedance measurements for the same subject. Deurenberg et al. in 1989 stated that differences in the voltage administered may be at the origin of these discrepancies.

Several studies have investigated different BIA devices in order to determine inter-instrumental variability. Most of them referred to the comparison between multifrequency and monofrequency BIA (Gutin et al. 1996; Yamada et al. 2013; Raimann et al. 2014; Ellegard et al. 2018; Silva et al. 2019; Tinsley et al. 2020). Less frequently, single-frequency BIA devices have been compared (Graves et al. 1996; Deurenberg et al. 1989; Smye et al. 1993; Bosy-Whestphal et al. 2006; Bellopede et al. 2011).

In this study different single frequency bioimpedance devices have been compared in order to identify the agreement between the devices and to standardize the measurements by means of correction factors, in case of systematic differences.

METHODS

Subjects

The sample examined was composed of 31 adult volunteers, including 8 men and 23 women (Table 3.7), 39.8 ± 14.2 years of age, mainly recruited at the University of Cagliari.

Table 3.7 - Descriptive statistics of the sample

	Men (8)				**Women (23)**			
	Mean	**DS**	**Min**	**Max**	**Mean**	**DS**	**Min**	**Max**
Age (years)	41.1	14.6	22.3	62.8	39.4	14.3	23.2	71.1
Weight (kg)	76.5	14.3	56	105.5	57.1	13.5	33	89
Height (cm)	175.8	6.1	168.2	188.2	155.7	7.1	142	165.8
Wrist (cm)	85.9	8.3	71	101	73.5	10.9	56.3	95
Arm (cm)	30.9	2.5	26.7	35	27.9	5.3	19.5	39.5
Calf (cm)	35.9	2.6	32	40	33.8	3.9	27.3	41

The survey campaign lasted two months, from March to May 2019; the measurements were carried out at the Laboratory of Body Composition and Anthropometry of the Section of Neuroscience and Anthropology, Department of Life Sciences and Environment of the University of Cagliari. Before each session the volunteers were informed of the purpose of the research and signed the informed consent before undergoing the examination.

Protocol

Anthropometric measurements of weight, height and body circumferences (arm, waist and calf) were taken to describe the sample.

In order to carry out the comparative analysis among instruments, at the beginning of each measurement day, the impedance devices were tested to check the calibration. A variability range of ±10 ohms for R and ±5 ohms for Xc was considered acceptable. Participants were asked to strictly follow the recommendation for BIA measurement described in the general introduction of this thesis. During the whole examination, they were asked to stay as still as possible. The total and segmental BIA were performed on the right hemisoma. The measurements were taken on each volunteer and on each body segment using the three different impedance devices (AKERN 1; AKERN N.E. 2; BIA ANALYZER), and repeating the sequence twice, in the same order.

For total BIA, two pairs of electrodes were placed following the standard position hand-to-foot (NIH 1996) and for segmental BIA the standard protocol proposed in this thesis (chapter 3.2) was applied. In order to reduce disturbance effects in the results, measurements on each individual site were made using the same electrodes.

Bioimpedance devices

Three single-frequency (50 kHz) BIA devices were used, named according to the following list:

AKERN 1 – AKERN BIA 101

The instrument is equipped with a sensor that shows on the display (Figure 3.12) the resistance and reactance values obtained from the injection of a sinusoidal alternating current maintained at a constant intensity of 800 µA at a frequency of 50 kHz. The applied voltage is 11.1 V, powered by a 400 µA rechargeable battery. It has a weight of 1.1 kg, length=25 cm, width=16 cm and height=11 cm.

AKERN N.E. 2 – AKERN BIA 101 NEW EDITION

As in the previous version, the sensor shows directly on the display (in this case with blue ‘low power’ LED segments) the resistance and reactance values obtained by injecting a sinusoidal alternating current maintained at a constant intensity of 400 µA at a frequency of 50 kHz. The applied voltage is case 11.1 V also in this and the instrument has the same size as the AKERN 1 version. The instrument communicates with the user through a series of LEDs and internal self-control circuits, signalling that the quality of the electrodes or skin impedance are within the limits of measurability (Figure 3.13). It is equipped with a rechargeable lithium-ion battery powered at 1000-2600 mAh, for an operating autonomy of up to 10 hours in continuous use and a reduced recharge cycle; a sensor checks the reliability of the analysis carried out and in case of errors or artefacts in the measurement warns with appropriate alarms the nature of the problem; the patient cable has Amphenol connector and a native USB communication port.

In both instruments the reading range is between 0 and 999 Ω for the resistance and between 0 and 200 Ω for the reactance, with resolution ± 1 Ω. Furthermore, both include the possibility of analysing bioelectric values for quantitative estimates of body composition through a software supplied, which is installed on the PC (equipped with Microsoft operating systems). These elaborations depend on predictive equations and have not been taken into account in this research.

Figure 3.12 - Akern BIA 101

Figure 3.13 - Akern BIA 101 New Edition

BIA ANALYZER^{TM}

It is a battery-operated impedance analyser (Figure 3.14) designed to meet the professional, commercial and practical needs of users of the instrument.

Compared to the AKERN impedance devices described above, it has a reduced size (11.5x7.3x2.4 cm). It shows on the display the measurements of resistance (R), reactance (X), impedance (Z) and phase angle (PA). The sinusoidal alternating current is maintained at a constant intensity of 425 ± 25 μA at a frequency of 50 kHz.

Figure 3.14 - BIA Analyzer ^{TM}

Statistical analysis

Descriptive statistics of R, Xc, Z and PhA for each device were performed using the SPSS software. The bivariate comparisons among devices were conducted for the whole-body and each segment using confidence ellipses and Hotelling’s T^2.

The regression analysis, performed with R software, was applied to estimate the relationships between variables and to define a possible systematic error, considering the effects of individuals (sex and age) as stable. This analysis was performed in collaboration with a statistician from the University of Madrid.

RESULTS

At both the whole-body and segmental level, the results showed that measurements repeated with the same instrument on the same subject gave deviations statistically significant, but within the range of variation allowed for the instrument, and hence biologically tolerable. For example, AKERN 1, AKERN N.E. 2, BIA ANALYZER showed in the whole-body a variation lower than 5 ohm for R, and than 1 ohm for Xc.

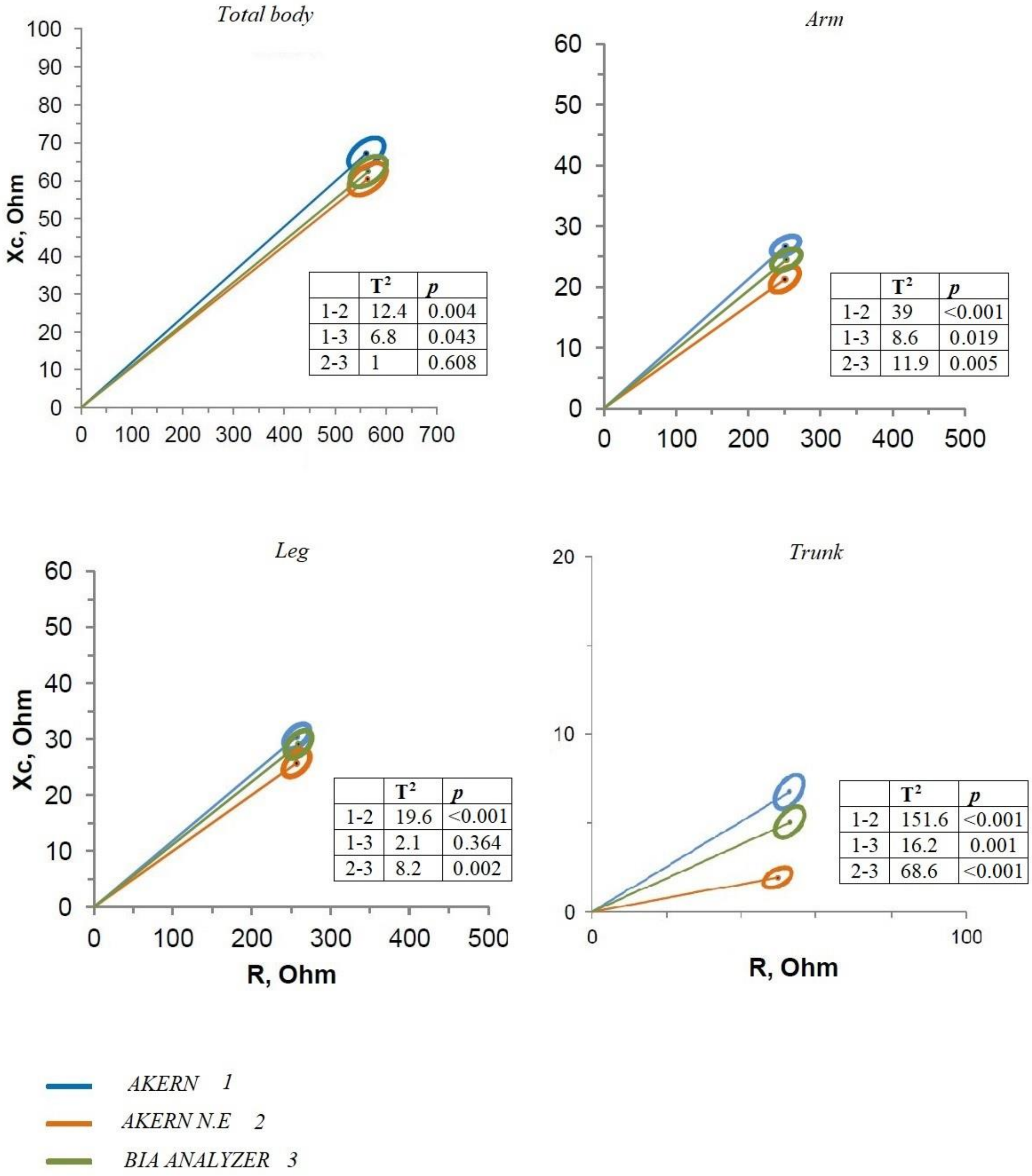
The data collected with each instrument, for each district (whole-body, arm, leg, trunk) and for each bioelectric variable are indicated in Table 3.8.

The comparison of the three devices by means of confidence ellipses and Hotelling's T^2 test showed significant differences in the whole body and all segments, except for the comparison between AKERN 1 and BIA ANALYZER in the whole-body, and between AKERN N.E 2 and BIA ANALYZER in the leg (Figure 3.15). The AKERN N.E. was the most differing device.

Table 3.8 - Descriptive statistics of bioelectric measurements

		Total body		**Arm**		**Leg**		**Trunk**	
		Mean	**DS**	**Mean**	**DS**	**Mean**	**DS**	**Mean**	**DS**
AKERN	R (Ω)	561.3	74.3	251.3	41.3	257.3	34.5	52.8	8.1
	Xc (Ω)	67.2	8.8	26.7	3.4	30.5	4.7	6.8	2
	Z (Ω)	565.3	74.6	252.7	41.3	259.1	34.7	53.2	8.1
	PhA (°)	6.9	0.7	6.8	0.8	6.8	0.8	7.4	2.2
AKERN N.E.	R (Ω)	563.9	76	250.4	42.2	257	36	49.7	8
	Xc (Ω)	60.4	9	21.3	4.4	25.7	5.1	1.9	1.2
	Z (Ω)	567.2	76.3	251.3	42.3	258.3	36.9	49.8	8
	PhA (°)	6.1	0.7	4.9	0.8	5.7	0.8	2.2	1.4
BIA ANALYZER	R (Ω)	564.9	76.1	252.5	42.9	259.5	35.6	52.9	8.2
	Xc (Ω)	62.4	8.5	24.5	3.8	29.1	5.1	5.1	1.9
	Z (Ω)	568.3	76.2	253.7	42.9	261.2	35.7	53.2	8.2
	PhA (°)	6.3	0.7	5.6	0.8	6.4	0.8	5.6	2.1

R: resistance; Xc: reactance; Z: impedance vector; PhA: phase angle



Total body

	T^2	*p*
1-2	12.4	0.004
1-3	6.8	0.043
2-3	1	0.608

Arm

	T^2	*p*
1-2	39	<0.001
1-3	8.6	0.019
2-3	11.9	0.005

Leg

	T^2	*p*
1-2	19.6	<0.001
1-3	2.1	0.364
2-3	8.2	0.002

Trunk

	T^2	*p*
1-2	151.6	<0.001
1-3	16.2	0.001
2-3	68.6	<0.001

Figure 3.15 - Confidence ellipses of the comparison between the three devices analysed.

In the comparison among devices by means of multivariate regression analysis, the AKERN 1 device was used as the reference. The values in tables 3.9-3.12 represent the differences with BIA

ANALYZER and AKERN N.E. 2. The sign of the coefficients represents the direction of the difference, being negative in case of their underestimation.

The differences were statistically significant for reactance and resistance, phase angle and impedance vector, with the BIA ANALYZER and AKERN N.E. 2 devices underestimating Xc and PhA in the whole-body and all body districts, and overestimating R and Z in most cases. However, the differences of resistance were not biologically influent, as they were always well below the acceptable range of 10 ohm (Table 3.9-3.12). On the contrary, the differences of reactance were relevant also from a biological point of view, being around 5 ohm, or higher, i.e. very closed to or outside the acceptable range of variation (Table 3.9-3.12).

Table 3.9 - Multivariate analysis report. Comparison of instruments on total body.

TOTAL BODY					
	Estimate Std.	**Error**	**T value**	***p***	
Xc					
AKERN N.E.	-6.129	0.2942	-20.833	< 2-16	<0.001
BIA ANALYZER	-4.1129	0.2942	-13.980	< 2-16	<0.001
R					
AKERN N.E.	3.1452	0.5523	5.695	6.19^{-8}	<0.001
BIA ANALYZER	3.9839	0.5523	7.214	2.41^{-11}	<0.001
PhA					
AKERN N.E.	-0.65532	0.02702	-24.254	< 2-16	<0.001
BIA ANALYZER	-0.44968	0.02702	-16.643	< 2-16	<0.001
Z					
AKERN N.E.	2.4306	0.5590	4.438	2.51^{-5}	<0.001
BIA ANALYZER	3.4871	0.5590	6.238	4.19^{-9}	<0.001

Table 3.10 - Multivariate analysis report. Comparison of instruments on arm.

ARM					
	Estimate Std.	**Error**	**T value**	***p***	
Xc					
AKERN N.E.	-4.9677	0.2397	-20.725	< 2-16	<0.001
BIA ANALYZER	-1.6935	0.2397	-7.064	5.42^{-11}	<0.001
R					
AKERN N.E.	-0.6452	0.3571	-1.807	0.072187	0.1
BIA ANALYZER	1.4516	0.3571	4.066	6.74^{-5}	<0.001
PhA					
AKERN N.E.	-1.15339	0.05666	-20.357	< 2-16	<0.001
BIA ANALYZER	-0.42597	0.05666	-7.518	8.11^{-12}	<0.001
Z					
AKERN N.E.	-1.0935	0.3621	-3.020	0.002836	0.01
BIA ANALYZER	1.2774	0.3621	3.528	0.000513	<0.001

Table 3.11 - Multivariate analysis report. Comparison of instruments on leg.

LEG					
	Estimate Std.	**Error**	**T value**	***p***	
Xc					
AKERN N.E.	-4.4839	0.2156	-20.796	< 2-16	<0.001
BIA ANALYZER	-0.9355	0.2156	-4.339	2.21^{-5}	<0.001
R					
AKERN N.E.	0.3548	0.3271	1.085	0.279	N.S.
BIA ANALYZER	2.8710	0.3271	8.778	5.47^{-16}	<0.001
PhA					
AKERN N.E.	-1.00710	0.04842	-20.800	< 2-16	<0.001
BIA ANALYZER	-0.28113	0.04842	-5.806	2.30^{-8}	<0.001
Z					
AKERN N.E.	-0.1339	0.3271	-0.409	0.682794	N.S.
BIA ANALYZER	2.7371	0.3271	8.367	7.79^{-15}	<0.001

Table 3.12 - Multivariate analysis report. Comparison of instruments on trunk.

TRUNK					
	Estimate Std.	**Error**	**T value**	***p***	
Xc					
AKERN N.E.	-4.677	1.262^{-1}	-37.067	< 2-16	<0.001
BIA ANALYZER	1.532	1.262^{-1}	-12.143	< 2-16	<0.001
R					
AKERN N.E.	-2.83871	0.18954	-14.977	< 2-16	<0.001
BIA ANALYZER	0.48387	0.18954	2.553	0.011385	0.05
PhA					
AKERN N.E.	-5.02177	0.12971	-38.715	< 2-16	<0.001
BIA ANALYZER	-1.69355	0.12971	-13.056	< 2-16	<0.001
Z					
AKERN N.E.	-3.2306	0.2067	-15.628	< 2-16	<0.001
BIA ANALYZER	0.3177	0.2067	1.537	0.102697	N.S.

The coefficients obtained with regression analysis represent the appliable correction factors for the observed systematic differences between pairs of instruments. For example, in order to compare the reactance values obtained with different AKERN instruments for the total BIA, a factor of + 6.129 ohms have been added to the reactance measured with AKERN N.E. 2 device and of less than + 5 ohms for the other body segments. Even not significantly different among devices, for internal consistency of the procedure, the resistance too should be corrected for the relative coefficients.

DISCUSSION

The analysis of the results highlighted the risk of using different bioimpedance devices in the assessment of body composition. The comparison among the three analysed devices showed statistically significant differences in the values of R and Xc, also reflected in the magnitudes of PhA and impedance vector. In the case of R or Z, the differences were not relevant at biological level, whereas the reactance and the derived magnitude of the PhA showed to be particularly sensitive to the instrument. Such differences cannot be attributed to physiological variability.

Research focussed on both validation and comparison of bioimpedance devices are present in the literature, but comparisons between different monofrequency devices are quite rare.

In the comparison of monofrequency and multifrequency bioimpedance, together with DXA and plicometry on a sample of children between 9 and 11 years of age, the methods provided non-overlapping results (Gutin et al., 1996). A study conducted on subjects suffering neuromuscular pathologies compared a multifrequency bioimpedance and two instruments for spectroscopic impedance analysis, with DXA as the reference (Ellegard et al., 2018), underlining differences in PhAs values. In the evaluation of the musculoskeletal system in the elderly, the Single Frequency BIA (SF-BIA) showed to underestimate Z values compared to the Multiple Frequency BIA (MF-BIA) and Bioelectrical Impedance Spectroscopy (BIS) (Yamada et al., 2013).

A recent study (Tinsley et al., 2020) highlighted differences between measurements taken with MF-BIA (Seca mBCA 515/514) and BIS (ImpediMed SFB7), with MF-BIA recorded R values 9-14% higher than BIS, although strong correlations ($r \geq 0.96$) were observed at all frequencies. Also, BIS produced higher Xc and PhA values at lower frequencies, while MF-BIA produced higher values at higher frequencies, with values correlated each other ($r \geq 0.92$) were also observed up to frequencies of ~ 150 kHz.

Raimann et al. (2014) analysed the results produced in the measurements of 35 patients undergoing haemodialysis using a 50 kHz monofrequency bioimpedance analyser (Hydra 4200 Bioimpedance Analyzer) and multifrequency BIS. The study highlights the correlation between the two methods, but with a systematic error and a lower level of accuracy for SF-BIA than for MF-BIS. Silva et al. (2019) found a discrepancy between resistance, reactance, PhA, and impedance vector measurements obtained with single-frequency and multi-frequency impedance measurements using the BIA-101 RJL/Akern Systems device at 240 µA at 50 kHz and the Xitron4200 device at frequencies between 5 and 1000 kHz. The tests were conducted on a sample of 126 adults including 101 (men and women) nationally competitive athletes and 25 very active men. The resistance and reactance measurements obtained with BIA 101 with respect the precision tolerance indicated in the verification circuit (10 Ω for R and 5 Ω for Xc, as in this study). The authors found differences, with Xitron overestimating R of 9.91 Ω in mean (representing an average difference of 2.0%) and underestimating Xc of ~1 Ω (representing an average difference of 1.5%). These values are relatively close to the device limits, indicating that instruments should not be used interchangeably.

There are few studies addressing the comparison between single frequency bioimpedance devices, which is the main issue of this research.

Graves et al. (1989) compared R values measured in two different laboratories (University of Florida and U.S. Department of Agriculture, San Francisco) in a sample of 146 healthy adults with three instruments in each laboratory: Valhalla Scientific 1990, BIA-101 and Medi-Fitness 1000 in Florida, Bioelectrical Sciences 2002, Valhalla Scientific 1990, BIA-101 in San Francisco. The raw R values showed high correlation on repeated tests for each instrument ($r \geq 0.99$ and $P \leq 0.01$) and also between Valhalla and Akern instruments ($r \geq 0.99$ and $P \leq 0.01$), but not between these two and Bioelectrical Sciences 2002 ($r=0.59$ and $r=0.61$ respectively). The results obtained leads to the conclusion that several bioimpedance analysers, even if calibrated on a common resistor, can produce significantly different R values (Graves et al., 1989).

Deurenberg and colleagues (1989) compared three 50 kHz single frequency devices of the same manufacturer (BIA-101 RJL System). The devices were tested on a sample of 8 individuals and on 7 different ceramic resistors; statistically significant differences were found in one of the three instruments, but they were very small and within the limit values indicated by the technical specifications of the instruments.

Smye et al. (1993) conducted a study in a sample of 21 subjects on four single frequency BIA devices: Holtain, RJL, Bodystat and EZComp. Significant differences (about 6%) were found between the Holtain instrument and the other devices (Bodystat and RJL), that gave higher values then the Holtain and very small differences among them (on average 0.6%). The differences detected with the Holtain instrument were hypothetically attributed to its inadequacy in maintaining a constant current intensity (800 µA) for contact resistances > 200 Ω: as the contact resistance increases, the intensity decreases of current and voltage and this is interpreted as a reduction in whole-body resistance.

Bosy-Whestphal et al. (2006) affirmed that the values of resistance and reactance obtained with two single frequency BIA instruments (Xitron 4000B and DataInput BIA 2000S), although significantly different, contributed to small differences in PhA values.

More recently, in a study conducted on a sample of 133 patients undergoing nutritional status assessment, resistance and reactance measured with two monofrequency BIA instruments, AKERN and Cube, with AKERN as reference, were almost overlapping, although the Cube overestimated the PA in the group of obese patients (Bellopede et al., 2011).

The results obtained in these previous studies are in line with those of this research.

In fact, the authors generally showed that instruments are not interchangeable in the same study and may lead to non-comparable results. Instead, the values detected by different instruments should not compromise the results from a biological point of view but allow a reliable diagnosis. The error could question the meaning of the results obtained in the applied. The reliability of the measured resistance and reactance values also determines the reliability of the derived quantities and the position of the impedance vector in the RXc graph (Oh et al., 2019), from which interpretations on the body composition and health status of the individual are derived.

In conclusion the present study has shown that the measurements of the resistance and reactance variables, carried out with three monofrequency bioimpedance devices, even maintaining constant measurement conditions (ambient temperature, subject position, electrode position) with the only replacement of the patient cable, present systematic differences, biologically relevant in the case of reactance and derived phase angle. Hence, the measures obtained with the three devices are not directly comparable. However, the correction factors proposed in this study can be used to compare data collected with the devices applied in this research.

4. PART II: ANALYSIS OF LONG-TERM EFFECTS OF SPORT ON TOTAL AND SEGMENTAL BODY COMPOSITION AND WELLNESS

4.1 INTRODUCTION

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.”

As mentioned in the general introduction, the aging process is characterised by a progressive change in body mass and composition, even in the absence of disease. These changes affect both the whole-body and the different body segments, and mainly concern the increase in fat mass, that tends to accumulate at the visceral level, and the decrease in muscle mass, more pronounced in men and mostly affecting the limbs.

The process of ageing exposes the elderly population to the risk of malnutrition, sarcopenia and fragility, 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 (Andreoli et al, 2009). At this purpose, an aspect that could interfere with the above depicted scenario, particularly in relation to the risk of falls, is body symmetry. Previous studies found an association between falls in the elderly and body asymmetry (Skelton et al., 2002; Chon et al., 2018; McGrath et al., 2020a). However, the research is mainly referred to strength and functional asymmetry, while the literature investigating the role of body composition asymmetry is still scanty (Lee et al., 2019). However, body composition asymmetry has been widely studied in sport science, particularly in relation to performance and injury prevention (Hinton et al., 2017).

The objective of reducing and delaying the process of ageing with its consequences is central to the scientific research, as well as a matter of public health concern. The quality of life in the elderly, is strongly related to lifestyle, where a determinant role is played by physical activity. Studies on the effect of physical activity in the elderly, both interventional studies and studies investigating the effects of a sport, demonstrated that it is able to slow down or reverse the physiological trend towards diseases such as sarcopenia (Roubenoff 2000) and that it is positively correlated with muscle mass density in aging people (Scott et al., 2019). However, the study of the effect of prolonged physical activity, especially at the segmental level, is very scanty in the literature. Also, the comparative analysis of the effect of different sports disciplines has been poorly studied (Yu et al., 2007; Latorre-Román et al., 2015; Kelly and Gilman 2017). The most studied sports are those that can be generally practised until old age, such as Tennis, Running and Tai Chi Chuan (Marks et al., 2006; Wang et al., 2002b; Kelly and Gilman 2017).

Tai Chi Chuan (TCC) is an ancient Chinese martial art. The practice consists of the repetition of a sequence of slow and harmonious movements, focused on balance and based on respiration techniques. This discipline is particularly adequate for elderly people, who find difficult to perform rapid movements, and has been recommended to improve quality of life (Kelly and Gilman 2017). In fact, TCC practice may help prevent falls (Wong et al. 2009), improve coordination and balance (Wong et al. 2009), retard bone loss (Qin et al. 2005), maintain cardiorespiratory fitness and flexibility (Lan et al. 1996). It also contributes to promote social and psychological health (Chan et al. 2017), and cognitive function (Lim et al. 2019).

Tennis is one of the most popular sports in the world (Chandler et al. 2000); it requires flexibility, agility, cardiorespiratory ability, speed, strength and muscular endurance (Chandler et al. 2000; Groppel and Roetert 1992; Konig et al., 2001; Juzwiak et al, 2008) and is considered a low risk sport, with a minimal risk of serious injury (Changstrom et al., 2016). It has also the advantage that can be practised at any age and at any level (Pluim et al., 2018): children can start at the age of 4 years, using balls and rackets appropriate to their age and playing on courts suited to their characteristics, adults can begin at any age and practice it throughout all their lives. It is in fact considered a ‘lifetime sport’ (Marks et al., 2006) so that there are international competitions involving categories of ‘over 80’ and even ‘over 85’.

Running is a very popular aerobic exercise too, one of the most practised among the world (Pedisic et al., 2020), and one of the most studied (Oja et al., 2015). The health benefits of running include improvements in aerobic fitness, cardiovascular function and postural balance, with limited effects on adiposity, in both women and men (Oja et al., 2015).

Considering the benefits of physical activity and particularly of the sports described above, this research aims to study the long-term effects of these sport disciplines in middle-aged and elderly subjects. In particular, the effects on whole-body and segmental body composition, morphological and functional symmetry between limbs and hand grip strength, nutritional status, depression symptoms and body image satisfaction will be considered. The results will be described and discussed separately using the following scheme of topics: total body composition; segmental body composition; body asymmetry; body image perception and psychological well-being. In all cases, the analysis will firstly consider the whole group of active people and then each sport singularly.

This type of research could provide useful information for the study of physiological ageing and possible strategies to slow down its effects.

4.2 METHODS

Subjects

The whole sample of active middle-aged and elderly subjects was composed in total of 106 subjects (72 men and 34 women) aged 60.9 ± 7.5 years.

The sample of Tai Chi Chuan included 34 volunteers (14 men and 20 women) aged 62.8 ± 7.4 years, recruited from the A.S.D. Tai Chi Chuan school of Cagliari and La Porta d'Oriente of Quartu S.Elena (Italy). At the time of the measurement, the subjects had already been practicing Tai Chi Chuan for an average of six years and were training four times a week or more.

The sample of tennis players was composed of 35 (31 men and 4 women) aged 63 ± 8.5 years in mean and recruited from the Tennis Club of Selargius and Associazione Dopolavoro Ferroviario of

Cagliari. Subjects had already been practicing Tennis for an average of twenty-two years and training three days a week.

The sample of runners was constituted of 37 (27 men and 10 women) aged 57.5 ± 5.4 in mean and recruited from the Atletica Fossano 75 and the Polisportiva of Isili. Subjects had already been practicing running for an average of seventeen years and training three days a week.

The control group was composed of 105 volunteers (49 men, 56 women) aged 62.8 ± 6.4 years, and living in the same geographical area, selected for not practicing a regular physical exercise, but normally active in everyday activities (in some cases including manual labour).

The exclusion criteria of this research were physical handicaps, pathologies that might influence the measurements (e.g., significant cardiovascular or pulmonary disease, endocrine or renal diseases, cancer or sever inflammatory conditions), metallic prostheses, pacemakers, or limb amputations.

The research was approved by the Independent Ethical Committee of the A.O.U. of Cagliari (PG/2017/1700). Each participant was informed about the purposes and methods of the research and signed the informed consent to participate.

Detection Board

For this study a card was filled for each volunteer. Data collection includes:

- personal data of the subject;
- information regarding the sports activity (years of training, frequency of training);
- results of anthropometric and impedance measurements.

Anthropometric measurements

During the measurements the subject was asked to remain barefoot and, where possible, in underwear.

Height and weight

The height was measured with a portable anthropometer; with the subject’s feet placed on a flat surface perpendicular to the vertical rod, the head positioned on the Frankfurt horizontal plane, the arms along the sides of the body with the palms of the hands facing the thighs; the heels together and resting against the vertical rod and with the toes open at approximately 60° (Lohman et al., 1988).

The weight was measured with the balance (Seca).

Circumferences

The measurement of body circumferences was carried out using a flexible tape. Waist, calf and arm circumference were taken. The circumferences of the calf and arm were measured in both sides of the body.

Waist circumference

The waist circumference was measured at the level of the narrowest part of the abdomen, ensuring that the tape measure was parallel to the floor (Lohman et al., 1988).

Calf circumference

The circumference of the calf was measured with the subject standing and with feet at about 20 cm in order to ensure an equitable distribution of body weight. The tape has been moved at calf level until the maximum circumference has been identified (Lohman et al., 1988).

Arm circumference

The circumference of the arm was measured at the midpoint of the arm, which was localised, before measurement, by flexing the subject's elbow at 90° and measuring the distance between the lateral edge of the acromion and the olecranon (Lohman et al., 1988).

Lengths

The length of the arms, trunk and trochanter-malleolus were measured.

Arm length

The length of the arm was measured using a flexible tape, with the subject in an upright position, measuring the distance between the acromion and the stylion (Peebles and Norris, 1998). The measurement was made on both upper limbs.

Length of the trunk

The length of the trunk was determined as the distance between the injector electrodes used for impedance analysis, measured with the subject in a supine position.

Length of trochanter - malleolus

The length between the trochanter and the malleolus was measured with the subject standing upright, placing the tape measure at the height of the trochanter and sliding it vertically to the malleolus.

Hand strength

The measurement of hand grip strength is a widely used technique in the evaluation of muscle strength and is considered an important marker of health, especially in ageing (Dodds et al., 2014).

The hand grip strength was taken using a hydraulic dynamometer (Hidraulic Hand Dynamometer of the Sahean Corporation, MSD buba Belgium).

The subject was asked, with his elbow flexed at 90° (Hillman et al., 2005), to hold the instrument and squeeze it with the greatest possible force.

The measurement was carried out three times for both right and left hands, alternately, in order to guarantee a few seconds of recovery. In this study the maximum value obtained from the three repetitions was considered.

Bioelectrical measurements

Bioelectric impedance measurements were carried out using BIA 101 single-frequency devices: Akern BIA 101 and Akern BIA 101 New Edition.

Before each session the device was checked with a calibrated circuit whose impedance values are known: R= 80 Ω, Xc=47 Ω (± 2% error). Measurements were taken in the morning and volunteers were asked not to drink, eat, to empty their bladders, wear light clothing and remove any metal objects before the examination.

In the present study, the whole-body and the segmental *specific* BIVA (see chapter 3.2; Stagi et al., 2021) were applied.

Questionnaires on nutritional and psychological status

Geriatric Depression Scale (GDS)

The Geriatric Depression Scale (GDS), in its short form (https://dementiapathways.ie/_filecache/0c8/57e/37-gds.pdf) (Sheikh and Yesavage 1986), is a validated scale commonly used for the assessment of depressive symptoms in older people.

GDS is in the form of a self-assessment questionnaire filled directly by the subject.

The questionnaire, which can be completed in less than 5 minutes, consists of 15 questions arranged in one page, easy to understand and with a binary answer (yes/no). Of the 15 questions, 10 indicate the presence of depression symptoms when the answer is yes, while 5 (1, 5, 7, 11, 13) are associated with depression symptoms when the answer is negative.

Mini Nutritional Assesment

The Mini Nutritional Assessment (MNA®) (https://www.mna-elderly.com/mna_forms.html), is a simple and widely used tool in clinical practice to assess the nutritional status of elderly people (Guigoz et al., 1994) ensuring high sensitivity, specificity and reliability.

The questionnaire was administered only to volunteers aged 60 years and over.

The test provides a maximum of 30 points: with 24 to 30 points total is considered a normal nutritional status, from 17 to 23.5 points there is a risk of malnutrition, and with a score of less than 17 points there is malnutrition (Guigoz et al., 1994). In this case, it is essential to deepen the assessment and determine the severity.

Body image test

A silhouette scale BIA-O developed by Williamson et al. (2000), was used to assess body image perception.

To each subject were shown 18 cards (representing silhouette of different body size and specific for sex). Each volunteer was asked to indicate the silhouette that corresponded to their current size and, if different, the silhouette corresponding to their ideal body image. In this way, the following variables were obtained: Current Body Size (CBS); Ideal Body Size (IBS) and Dissatisfaction score (D). Dissatisfaction score is the result of the difference between CBS and IBS.

The CBS assessed by this silhouette scale was previously tested with *specific* BIVA (see chapter 3.3; Stagi et al., 2020b), demonstrating its validity of application in a sample of normal weight individuals of both sexes and two age groups (young and old adults).

Statistical analysis

The specific bioelectric values of total and segmental body composition were calculated using the *Specific* BIVA software (www.specificbiva.com).

Bioelectrical values obtained with different devices were made comparable by using a correction factor. Raw data obtained with the Akern BIA 101 New Edition were corrected using the following values: whole-body: R=-3.15, Xc=+6.13; arm: R =+0.65, Xc=+4.97; leg: R=-0.35, Xc=+4.48; trunk: R=+2.84, Xc=+4.68.

Descriptive statistics were calculated for all variables in total and segmental approach.

The anthropometric and bioelectric variables and the results of questionnaire (i.e. GDS and MNA) of active subjects were compared with those of the control sample through the Two-way ANOVA analysis, considering the effect of sex and exercise. One-way ANOVA was applied to assess differences between sport and control, Tukey's b test was used to perform the post hoc analysis. The differences between the averages of the impedance values were evaluated through confidence ellipses and Hotelling T^2 test for all groups and for total and segmental body composition.

The differences between limbs (dominant *vs* non-dominant arm; right *vs* left leg) were graphically represented in the paired data graphs and tested by the Hotelling T^2. For the hand grip strength, a paired data t-test was applied to test differences between dominant and non-dominant arm.

Pearson’s correlation was used to test the association between hand grip strength and body composition of the arm.

Mann-Whitney's U-test was used to assessed differences among groups (sexes, active, control, TCC, Tennis, Running) for body image variables (actual, ideal and dissatisfaction score). Spearman’s correlation was used to test the association between GDS and dissatisfaction score.

4.3 TOTAL BODY COMPOSITION

4.3.1 RESULTS

Whole active sample

The sample of active and control subjects were characterised by similar age but different anthropometric and bioelectric measurements.

Active subjects were characterised by normal weight, while the control subjects showed mean values of overweight, as indicated by BMI values (Table 4.1).

As to sexual dimorphism, in both active and control group, women showed lower BMI, body circumferences, and PhA values, and higher Rsp and Zsp values, compared to men (Table 4.1, Figure 4.1). Women also showed lower values of hand grip strength than men in both groups (Table 4.1). MNA (tested in a subset of aged ≥60) was not significantly different between sexes (Table 4.1).

With respect to the control group, active subjects showed lower body weight, lower BMI and lower values of all body circumferences (waist, mid-arm and calf), lower values of Rsp and Zsp and higher values of PhA (Table 4.1, Figure 4.2). These results are similar in both sexes with the exception of arm circumference difference, which was significant in women only (Table 4.1). The strength of the hand was similar in active and control (Table 4.1).

Active older subjects of both sexes showed a better nutritional status, as indicated by MNA (Table 4.1).

Table 4.1. - Descriptive statistics and comparisons between the sample of active older and the control sample.

	Control				**Active**				**Two-way ANOVA**		
	Men (49)		Women (56)		Men (72)		Women (34)				
	Mean	DS	Mean	DS	Mean	DS	Mean	DS	p_{sex}	p_{sport}	$p_{sex \cdot sport}$
Age (y)	62.8	6.57	62.8	6.35	61.1	7.56	61.1	7.29	0.968	0.079	0.961
Height (cm)	166.1	6.18	153.1	6.69	170.4	6.85	156.4	6.25	<0.001	<0.001	0.581
BMI (m/kg^2)	28.1	4.08	27.3	4.71	24.5	2.73	22.0	2.76	0.001	<0.001	0.135
Weight (kg)	77.8	11.78	64.0	11.46	71.1	9.36	53.8	7.58	<0.001	<0.001	0.241
Waist (cm)	97.4	11.27	85.2	12.07	86.4	8.17	72.7	6.89	<0.001	<0.001	0.625
Arm (cm)	30.3	3.11	29.0	3.24	29.4	2.40	26.3	2.49	<0.001	<0.001	0.038
Calf. (cm)	36.8	3.32	34.9	3.70	35.7	2.22	33.1	2.24	<0.001	<0.001	0.455
Rsp (ohm·cm)	388.4	59.20	426.6	71.41	329.2	42.65	352.3	56.82	<0.001	<0.001	0.362
Xcsp (ohm·cm)	45.6	8.36	45.3	9.25	43.2	8.83	40.1	7.75	0.175	0.002	0.281
Zsp (ohm·cm)	391.1	59.40	429.0	71.79	332.2	42.37	354.7	56.64	<0.001	<0.001	0.354
PhA (°)	6.7	1.01	6.1	0.78	7.6	1.86	6.6	1.52	<0.001	0.001	0.477
H.G. (kg)	38.0	9.40	23.6	5.41	40.6	7.73	25.4	5.17	<0.001	0.070	0.739
MNA	25.7	2.75	25.5	2.25	26.9	2.08	26.6	1.97	0.686	0.021	0.965

BMI: body mass index; Rsp: specific resistance; Xcsp: specific reactance; Zsp: specific impedance; PhA: phase angle; H.G.: hand grip strength; MNA: mini nutritional assessment.

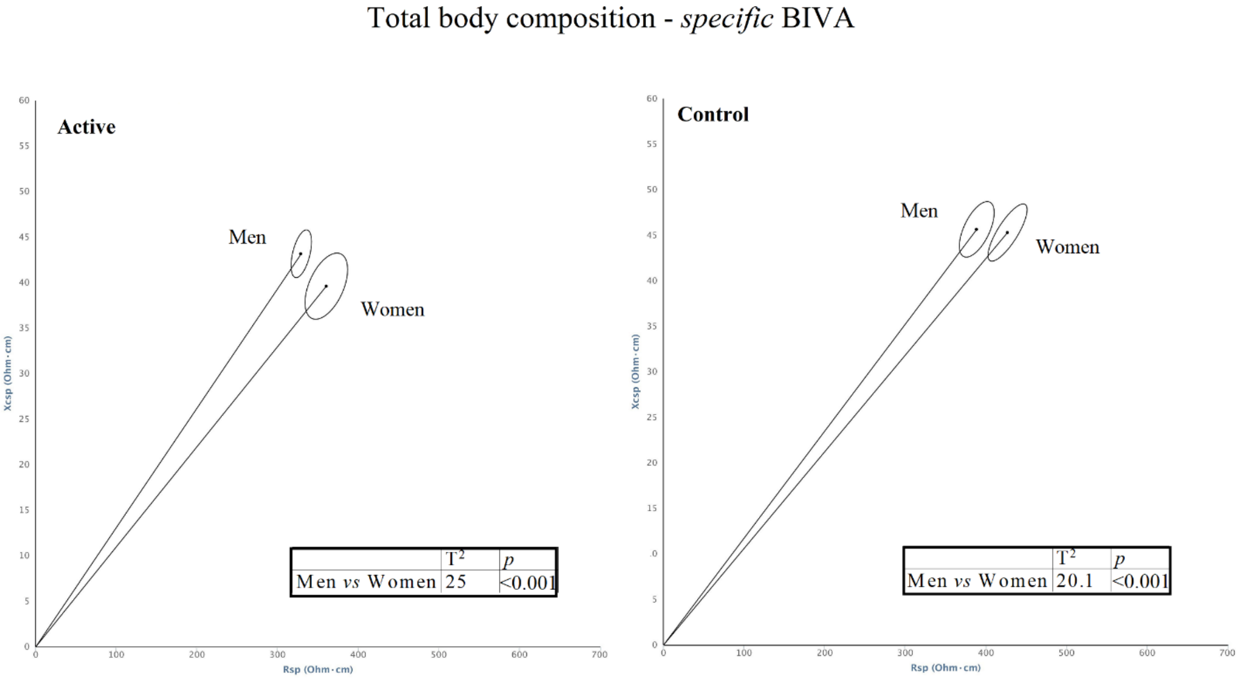


Figure 4.1 - Total body confidence ellipses of active and control samples. Comparison between sexes.

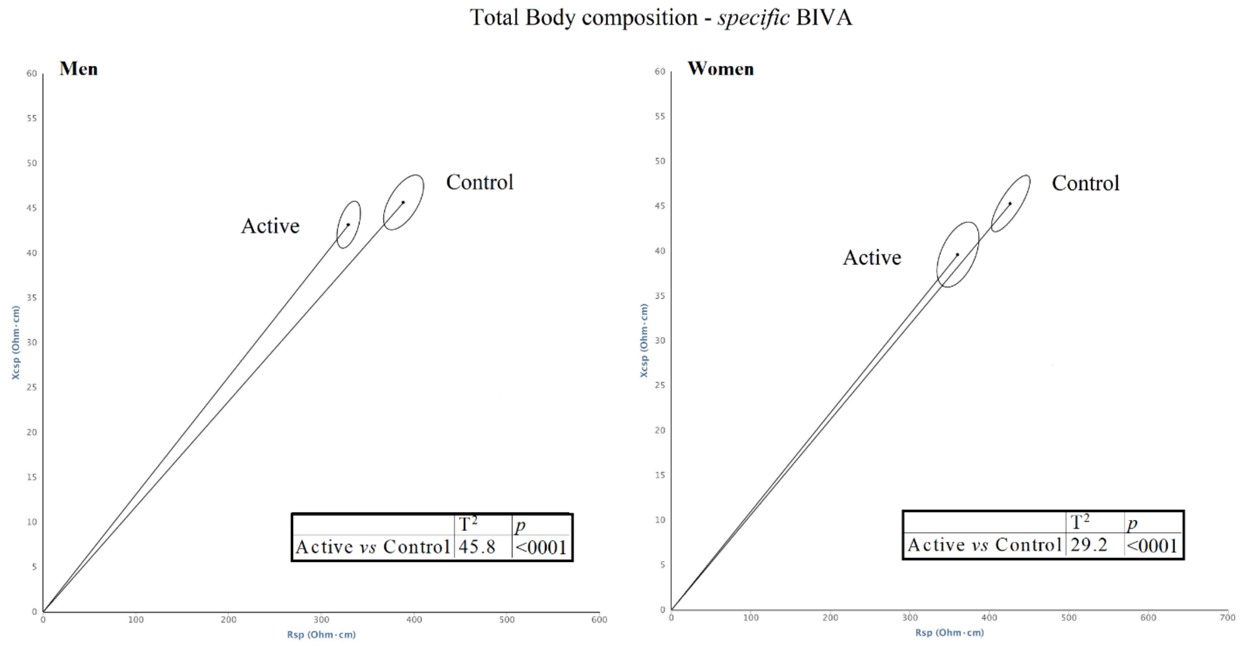


Figure 4.2 - Confidence ellipses of active and control sample.

Analysis by sport

The sample of active men, where three disciplines have been analysed (tennis, TCC, running) showed in each sport results consistent with those obtained from the joint sample. In fact, in all sports with respect to the controls, men showed lower values of waist circumference, BMI, Rsp and Zsp and different confidence ellipses (Table 4.2, Figure 4.3). However, the bivariate approach showed less differences between TCC and control groups (Figure 4.3).

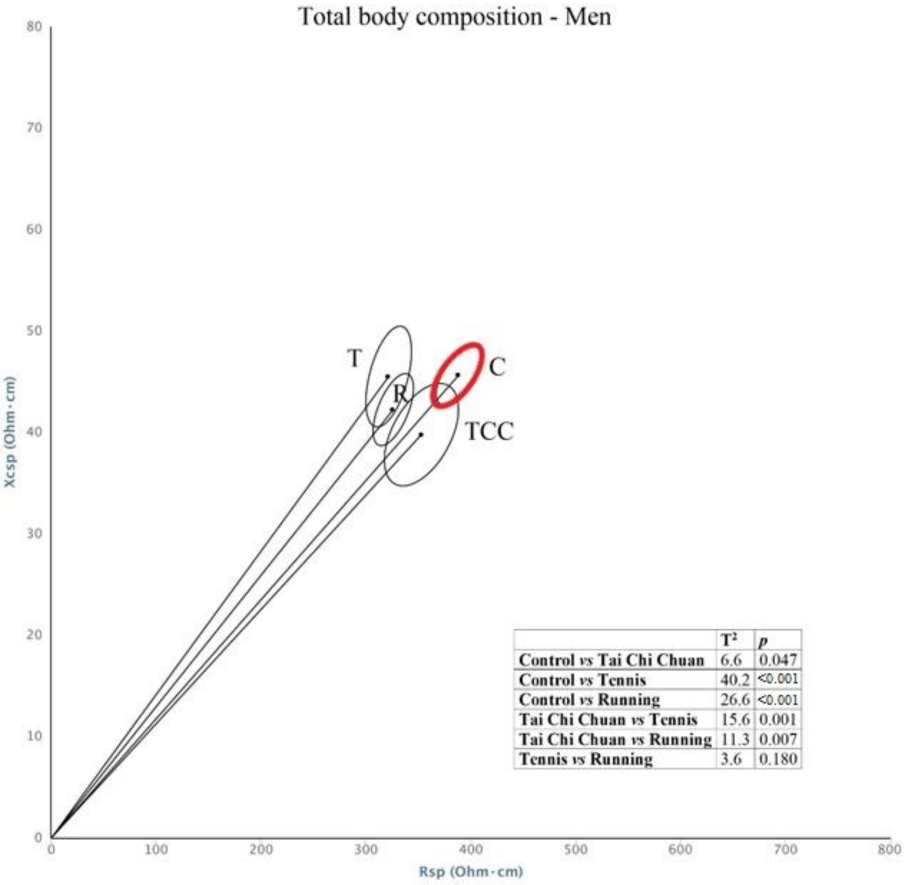
Differences were also observed among sports. The runners showed a slightly lower age (near 4 years in mean). Tennis players showed the highest values of BMI, circumferences (arm and waist), and PhA, while runners the lowest values of waist circumference and BMI (Table 4.2). Rsp and Zsp were similar in three disciplines. Considering bioelectrical variables under a bivariate approach, the differences between runners and tennis players appeared of low degree, while those of the two disciplines with respect TCC were significant (Figure 4.3).

No differences were found among groups for the hand grip strength and MNA (Table 4.2).

Table 4.2 - Descriptive statistics and comparisons between sports in men.

Men	**Control** (49)		**TCC (14)**		**Tennis (31)**		**Running (27)**		One-way ANOVA
	Mean	DS	Mean	DS	Mean	DS	Mean	DS	
Age (y)	62.9	6.57	63.4	7.92	63.0	8.48	57.7	4.78	*p= 0.008 (TR*; TnR*; CR*)*
Height (cm)	166.1	6.18	172.2	5.13	168.6	7.56	171.6	6.49	*p= 0.001 (CR*; CT*)*
BMI (m/kg^2)	28.2	4.08	23.7	2.51	26.0	2.60	23.1	2.13	*p< 0.001 (TTn*; TnR*; CR*; CTn*; CT*)*
Weight (kg)	77.8	11.78	70.1	7.22	74.0	10.38	68.3	8.35	*p= 0.001 (CR*; CT*)*
Waist (cm)	97.4	11.27	87.2	8.97	89.7	7.20	82.1	7.05	*p< 0.001 (TnR*; CR*; CTn*; CT*)*
Arm (cm)	30.3	3.11	28.6	2.15	30.2	2.35	28.8	2.39	*p= 0.036*
Calf (cm)	36.8	3.32	35.2	2.01	36.3	2.31	35.4	2.1	*p= 0.075*
Rsp (ohm·cm)	388.4	59.20	352.7	45.58	321.5	46.12	325.8	32.63	*p< 0.001 (CR*; CTn*; CT*)*
Xcsp (ohm·cm)	45.6	8.36	39.8	6.51	45.5	10.54	42.2	6.98	*p= 0.069*
Zsp (ohm·cm)	391.1	59.40	355.0	45.63	324.9	45.57	328.6	32.89	*p< 0.001 (CR*; CTn*; CT*)*
PhA (°)	6.7	1.01	6.5	1.03	8.2	2.41	7.4	1.00	*p< 0.001 (CTn*; TTn*)*
H.G. (kg)	38.0	9.40	39.4	8.67	40.8	8.19	40.9	6.90	*p= 0.525*
MNA	25.7	2.75	27.4	0.99	26.7	2.0	26.6	1.93	*p= 0.311*

BMI: body mass index; Rsp: specific resistance; Xcsp: specific reactance; Zsp: specific impedance; PhA: phase angle; H.G.: hand grip strength. CT: comparison between Control and TCC; CTn: comparison between Control and Tennis; CR comparison between Control and Running; TTn: comparison between TCC and Tennis; TR: comparison between TCC and Running; TnR: comparison between Tennis and Running. Tukey's b test was used for post hoc comparisons. The accepted level of statistical significance was $p < 0.05$ (*).



	T^2	*p*
Control *vs* Tai Chi Chuan	6.6	0.047
Control *vs* Tennis	40.2	<0.001
Control *vs* Running	26.6	<0.001
Tai Chi Chuan *vs* Tennis	15.6	0.001
Tai Chi Chuan *vs* Running	11.3	0.007
Tennis *vs* Running	3.6	0.180

Figure 4.3 - Confidence ellipses of all groups of men. C: Control; TCC: Tai Chi Chuan: T: Tennis; R: running.

In women, where two disciplines were considered (running and TCC), active subjects showed lower weight, BMI, waist and arm circumferences, Rsp and Zsp (near to significant values in the case of TCC) with respect to the controls, while PhA and hand grip strength were higher in runners (Table 4.3). Confidence ellipses showed significant differences between both running and TCC samples and the control group (Figure 4.4). The runners showed a slightly lower age (near 4 years in mean).

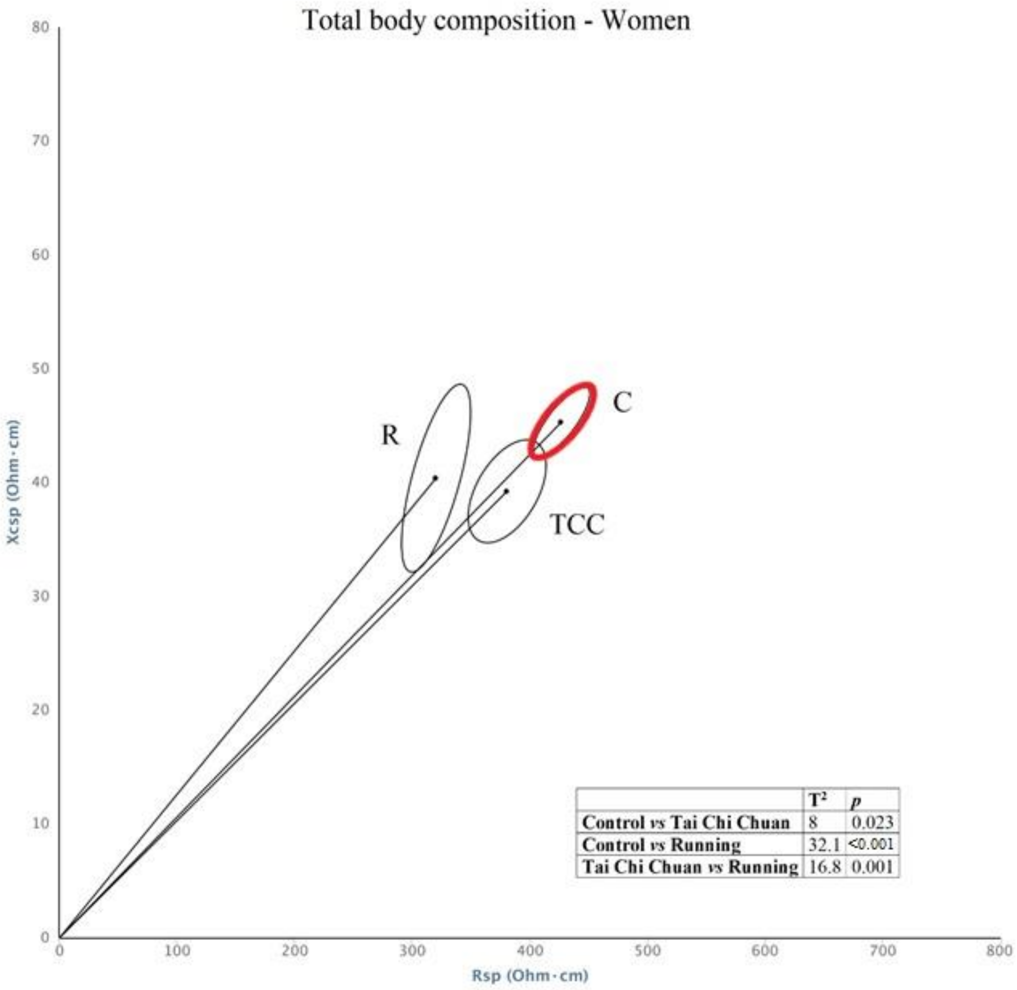
No significant anthropometrical differences were found between sports, while bioelectrical values were different, with runners showing lower Rsp and Zsp, and higher PhA than TCC (Table 4.3). Confidence ellipses confirmed bioelectrical differences (Figure 4.4).

No differences were found among groups for MNA (Table 4.3).

Table 4.3 - Descriptive statistics and comparisons between sports in women.

Women							
	Control (56)		**TCC (20)**		**Running (10)**		One-way ANOVA
	Mean	DS	Mean	DS	Mean	DS	
Age (y)	62.8	6.35	62.5	7.14	57.1	7.20	*p= 0.050 (CR*; TR*)*
Height (cm)	153.2	6.69	155.5	6.70	158.9	4.89	*p= 0.030 (CR*)*
BMI (m/kg^2)	27.3	4.71	22.4	2.98	20.7	1.93	*p< 0.001 (CR*; CT*)*
Weight (kg)	64.0	11.46	54.1	7.37	52.5	6.51	*p< 0.001 (CR*; CT*)*
Waist (cm)	85.2	12.07	74.0	6.93	70.4	4.87	*p< 0.001 (CR*; CT*)*
Arm (cm)	29.0	3.24	26.0	2.21	26.3	2.68	*p< 0.001 (CR*; CT*)*
Calf (cm)	34.9	3.70	33.0	2.38	32.8	1.81	*p= 0.039*
Rsp (ohm·cm)	426.6	71.41	380.4	54.16	320.0	29.33	*p< 0.001 (CR*; TR*)*
Xcsp (ohm·cm)	45.3	9.25	39.2	7.38	40.4	8.24	*p= 0.019*
Zsp (ohm·cm)	429.0	71.79	382.5	54.28	322.6	29.85	*p< 0.001 (CR*; TR*)*
PhA (°)	6.1	0.78	5.9	0.95	7.2	1.08	*p= 0.001 (CR*; TR*)*
H.G. (kg)	23.6	5.41	23.9	4.71	28.0	4.81	*p= 0.059 (CR*; TR*)*
MNA	25.5	2.25	26.6	2.24	28.0	1.00	*p= 0.150*

BMI: body mass index; Rsp: specific resistance; Xcsp: specific reactance; Zsp: specific impedance; PhA: phase angle; H.G.: hand grip strength. CT: comparison between Control and TCC; CR comparison between Control and Running; TR: comparison between TCC and Running. Tukey's b test was used for post hoc comparisons. The accepted level of statistical significance was $p < 0.05$ (*).



	T^2	*p*
Control *vs* Tai Chi Chuan	8	0.023
Control *vs* Running	32.1	<0.001
Tai Chi Chuan *vs* Running	16.8	0.001

Figure 4.4 - Confidence ellipses of all groups of women. C: Control; TCC: Tai Chi Chuan: R: running.

Sexual dimorphism

The difference between active and controls was more accentuated among women than men in both Rsp and PhA (women: 25% lower Rsp and 18% higher PhA than controls; men: 16% lower Rsp and 10% higher PhA than controls). The comparison between men and women practicing TCC or Running showed significant differences in all anthropometric variables ($p<0.05$), while in bioelectrical variables no significant differences were detected. The similarity between sexes were also confirmed in the bivariate approach (Figure 4.5).

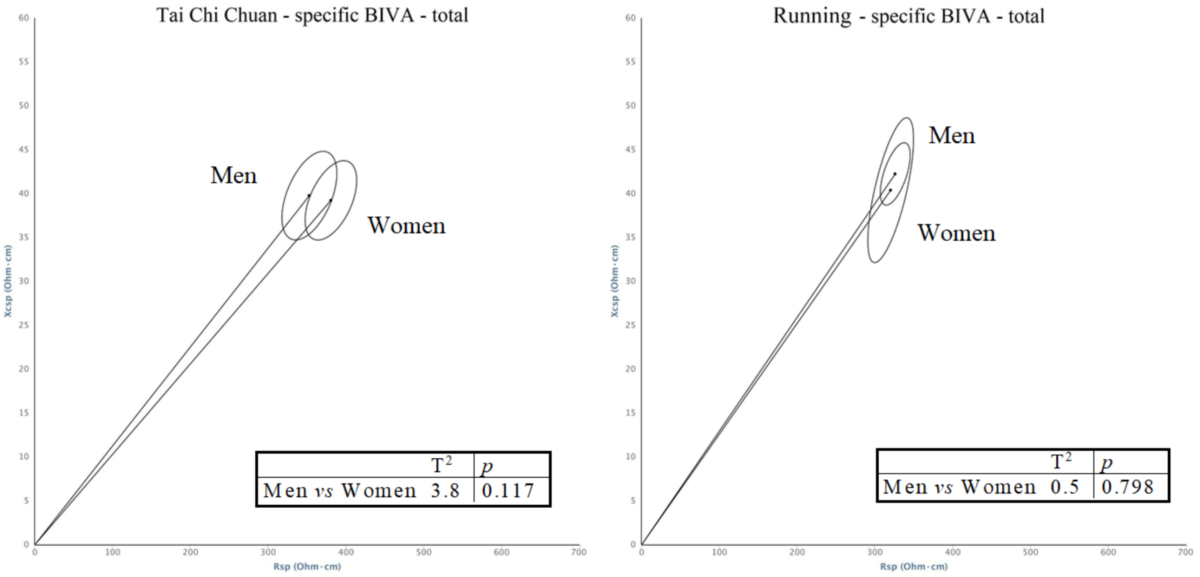


Figure 4.5 - Total body confidence ellipses. Comparison between sexes.

4.3.2 DISCUSSION

The analysis of the results of this research showed that in both sexes and all the disciplines the long-term practice of sport has an effect in the reduction of body mass and body fat (active subjects have lower BMI and waist circumferences, and shorter vectors than controls, i.e. lower %FM) and in maintaining skeletal muscle mass (tendentially higher PhAs in active subjects than controls, i.e. body cell mass, muscle mass in particular, and ICW/ECW). Active elderly also showed a positive effect on the nutritional status, as indicated by their MNA, where higher values were registered when compared to the control sample.

The hand grip strength values were also higher in the sample of active subjects of the two sexes, while they resulted to be above the threshold values for sarcopenia (i.e. higher than 16 kg in women and than 27 in men; Dodds et al., 20014) in all cases.

Consistently with the results of this study the literature suggests the positive effect of physical exercise in the reduction of body fat (Fien et al., 2017; Kelly and Gilman 2017) and its role on the improvement and maintenance of skeletal muscle mass (Fien et al., 2017, Ribeiro et al. 2017).

The literature investigating the effects of physical exercise in the aged population is essentially divided into two types of studies: on the one hand, there are studies that aim to analyse the effects of specific physical exercise programs (Treuth at al., 1994; Walts et al., 2008; Barone et al., 2009 Ribeiro et al. 2017), which have a defined duration that varies from a few weeks to some months, on the other hand studies that investigate the benefits of a specific physical exercise or sport practised for a long period, until late in life (Marks et al., 2006; Piasecki et al., 2019). The intervention studies have shown the effect in the reduction of body fat (Treuth at al., 1994; Walts et al., 2008; Barone et al., 2009), in increase FFM (Treuth et al., 1994; Walts et al., 2008) or PhA (Ribeiro et al., 2017). However, the studies - as the present research - on long-term effects on physical activity analyse the phenomenon from a different perspective and can show the slow down of the ageing process. Among these last studies, the literature considering different sports in order to get a clearer picture of their peculiar effects is limited, even the matter has received a growing interest in recent times.

In the comparison between sexes, active subjects showed similar degrees of sexual differences than the controls. However, this result is probably influenced by the presence of men practicing tennis. Indeed, when sports are considered separately, Tennis was associated with higher values of BMI and muscle mass. Without tennis men, sexual dimorphism among TCC and Running groups is in both cases lower than the control. Such low degree of sexual differences in body composition is a very original results, considering both the age of the sample and the level of sport practised. The literature on sexual dimorphism in sport shows conflicting results. In line with the observations made in this research, young adult elite athletes, practicing football (Mascherini et al., 2018) and running (Pate et al., 1985), show few sex differences in total body composition. In particular, Pate et al. 1985 affirmed that athletes with similar performance also showed similar body composition. In contrast, Lewis et al (1986) and Buffa et al. (2001) detected greater sex differences in body composition among individuals practicing different kinds of physical exercise with respect to sedentary people, due to a weaker effect among women. The difference in the results could be due to the different kind of the samples, with athletes characterised by body composition characteristics typed on the activities requested by the specific sport, similar between sexes, whereas among active practitioners the selective pression toward a particular physique would be less influent.

Considering body composition in each sports and sex separately, Tennis and Running men exhibited more similar values (despite the slightly lower age of runners), while TCC men differed from both due to the higher values of %FM and lower muscle mass (lower PhAs). Similar results were found in running women (Tennis women were not represented in the sample), where also the hand grip strength was higher than in TCC.

Indeed, with respect to tennis and running, TCC is a less conventional discipline. The effects on body composition have been poorly studied and the literature show a still unclear pattern. In line with the results obtained in this research, previous studies observed a lower %FM among TCC practitioners (Lan et al., 1996; Barbat-Artigas et al., 2011; Hsu et al., 2015; Hui et al., 2016). However, other studies failed to detect differences in the comparison with different sport and control (Yu et al., 2007, Lai et al., 2017). As concerns muscle mass our results suggest a weak effect of TCC in improving muscle mass as indicated by the similar hand grip strength and similar or even lower PhA of TCC practitioners with respect to the control group and other sports. Similar results were found by Yu et al. (2007) and Lai et al. (2017), and Kelly and Gilman (2017), that recently affirmed that TCC was not as effective at building muscle compared to other types of physical exercise. Only a few studies showed an improvement of skeletal muscle mass (Barbat-Artigas et al., 2011; Hsu et al., 2015) and muscle strength (Barbat-Artigas et al., 2011) that was not observed in this research.

The effect of tennis on body composition has been more deeply investigated in the literature than that of TCC. In this case the long-term effects of the discipline have been also considered, similarly to the present research. Previous studies on veteran tennis players listing all the benefits of practicing Tennis stated that tennis practice until old age, in line with the results of this research, is effective in reducing FM (Moysi et al., 2004a; Marks et al., 2006) and increasing muscle strength, and particularly hand grip strength (Vodak et al., 1980; Swank et al., 1998; Marks et al., 2006). According to our knowledge, the effects of tennis on muscle mass in veterans have been less investigated. In this research, Tennis demonstrated to be effective in improving muscle mass also in advanced age, as detailed in the chapter on segmental body composition.

Studies in aged runners are more represented in the literature (Mikkelsen et al., 2013; Couppé et al., 2014; Simoes et al. 2017; Piasecki et al. 2019; Mitchell et al., 2020). Previous studies in aged runners showed similar results of those obtained in this research. Indeed, the regular practice of this resistance exercise, even in the old age, has demonstrated to be effective to reduce BMI and (Simoes et al. 2017; Walsh et al., 2018) body fat (Piasecki et al. 2019; Mitchell et al., 2020), and to improve lean mass (Mikkelsen et al., 2013; Couppé et al., 2014; Mitchell et al., 2020).

4.4 SEGMENTAL BODY COMPOSITION

4.4.1 RESULTS

Whole active sample

The results of segmental body composition approach showed differences between sexes and between active and control samples.

Women showed lower PhA than men in all body districts, while the values of Rsp and Zsp were higher in the arms and the legs, but not in the trunk (Table 4.4). In the bivariate approach, confidence ellipses showed significant differences in body composition between sexes in all body districts (Figure 4.6).

Active subjects of both sexes showed lower Rsp and Zsp values than controls in the arm and the trunk, but not the legs, and higher PhA values in the trunk (Table 4.4). The univariate results were confirmed by the bivariate approach, with significant bioelectrical differences for the arm and the trunk, but not the leg (Figure 4.7).

Table 4.4 - Descriptive statistics and comparisons between the sample of active older and the control sample in segmental body composition.

RIGHT SIDE	**Control**				**Active**				**Two-way ANOVA**		
	Men (27)		Women (32)		Men (67)		Women (30)				
ARM	Mean	DS	Mean	DS	Mean	DS	Mean	DS	p_{sex}	p_{sport}	$p_{sex \cdot sport}$
Rsp (ohm·cm)	266.9	44.67	330.0	61.89	231.5	29.69	273.3	48.60	<0.001	<0.001	0.163
Xcsp (ohm·cm)	31.8	7.65	31.2	7.99	25.8	4.78	25.3	5.87	0.596	<0.001	0.983
Zsp (ohm·cm)	268.8	45.03	331.6	62.08	233.0	29.84	274.5	48.73	<0.001	<0.001	0.168
PhA (°)	6.8	1.12	5.4	1.05	6.4	0.92	5.3	0.97	<0.001	0.140	0.408
LEG											
Rsp (ohm·cm)	255.2	44.42	297.9	58.52	250.3	23.49	280.1	31.12	<0.001	0.084	0.323
Xcsp (ohm·cm)	31.3	9.63	31.7	9.92	29.5	5.87	30.9	7.55	0.503	0.346	0.721
Zsp (ohm·cm)	257.2	45.00	299.7	58.94	252.1	23.80	282.0	30.70	<0.001	0.085	0.338
PhA (°)	6.9	1.40	6.0	1.33	6.7	1.00	6.4	1.99	0.011	0.763	0.212
TRUNK											
Rsp (ohm·cm)	520.0	196.78	506.7	159.34	393.6	104.70	443.7	90.99	0.420	<0.001	0.166
Xcsp (ohm·cm)	63.8	25.08	49.0	22.57	66.4	21.99	54.2	16.65	<0.001	0.292	0.723
Zsp (ohm·cm)	524.0	197.97	509.2	160.37	399.5	105.66	447.2	91.29	0.474	<0.001	0.175
PhA (°)	7.1	1.42	5.4	1.42	9.7	2.4	7.1	1.90	<0.001	<0.001	0.145

Rsp: specific resistance; Xcsp: specific reactance; Zsp: specific impedance; PhA: phase angle.

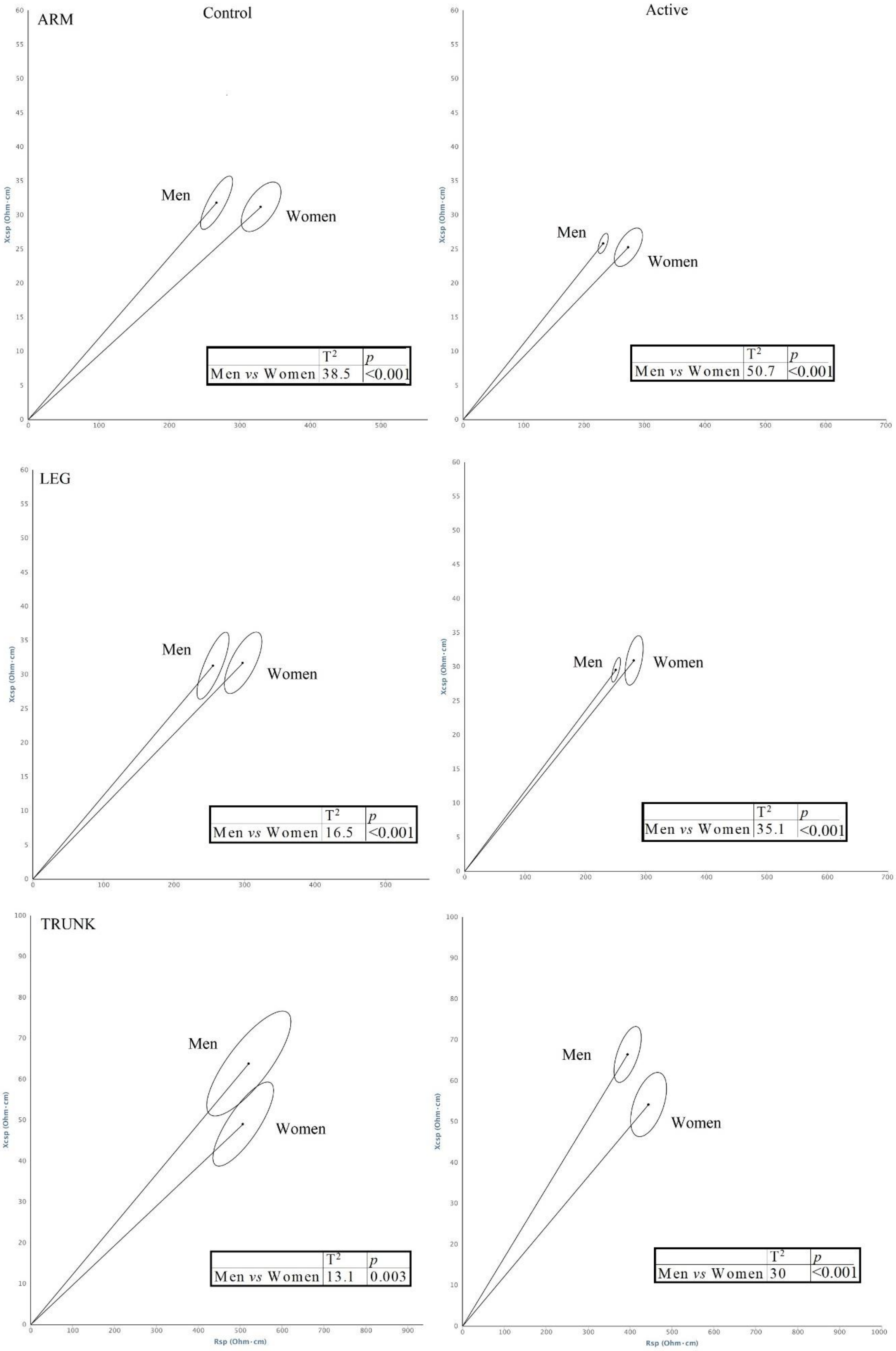


Figure 4.6 - Confidence ellipses of segmental body composition between the two sexes. Sexual differences in active and control subjects.

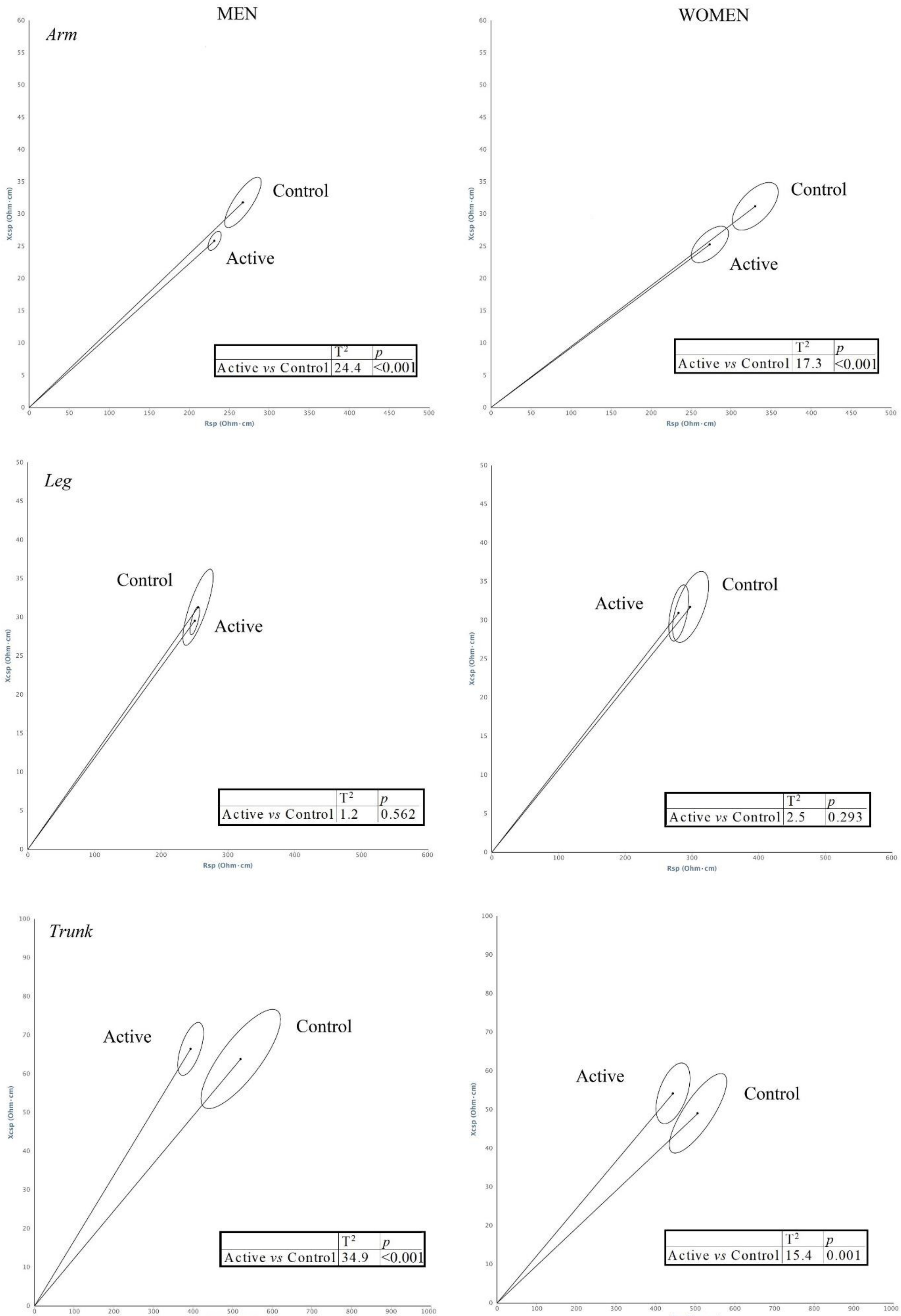


Figure 4.7 - Confidence ellipses of segmental body composition between active and control subjects.

Analysis by sport

In all disciplines, and particularly in Tennis and Running, both sexes showed lower values of Rsp and Zsp and higher values of PhA than controls in the arms and the trunk (Table 4.5, 4.6).

Comparing the sports and considering sexes separately, Tennis and Running men showed lower values of Rsp and Zsp than TCC subjects in the arms and the trunk, while in the legs the differences were not significant (Table 4.5, Figure 4.9). TCC men also registered the lowest values of PhA in all districts and runners the higher values of PhA in the trunk (Table 4.5, Figures 4.8-4.10). The bivariate approach confirmed the previous results, with Tennis and Running men showing similar body composition in the limbs, but differences in the trunk (Figure 4.10), while TCC subjects showed significant differences from the other athletes, with the exception of the leg, whose composition was similar to Tennis men (Figures 4.8-4.10).

Women showed results similar to men, with no differences for Rsp and Zsp between runners and TCC subjects in the arms, the legs and the trunk, while PhA was significantly higher among runners in the arm and the trunk (Table 4.6). Confidence ellipses showed significant differences in all body districts, mainly due to the longer vectors in the arm and the higher PhA in the trunk (Figures 4.11-4.13).

Sexual dimorphism

The difference between active and controls was more accentuated among women than men in the arm and leg (women : 24-10% lower Rsp and 10-18% higher PhA than controls; men: 14-4% lower Rsp and 4%-3% higher PhA than controls) and in men in the trunk (women: 8% lower Rsp and 65% higher PhA than controls; men: 27% lower Rsp and 57% higher PhA than controls).

Vector length and phase angle of Running and TCC men and women were similar, although confidence ellipses reached the significance level in TCC and in the trunk of runners (Figure 4.14).

Table 4.5 - Descriptive statistics and comparison of segmental body composition among sports and control in men.

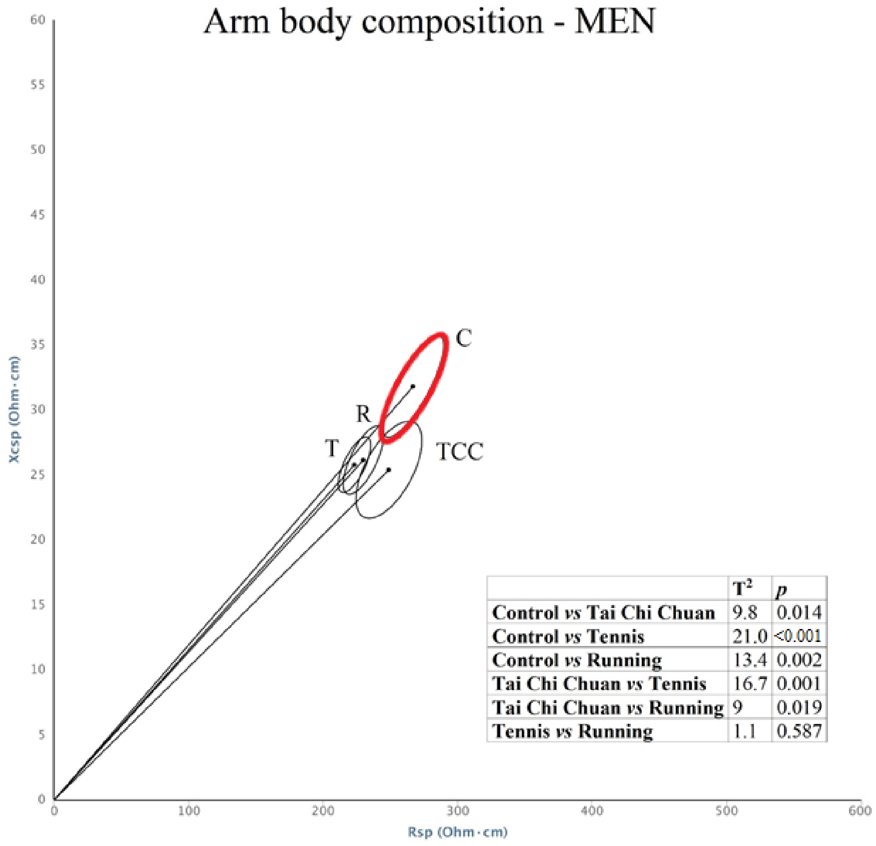
RIGHT SIDE	**Men**								
	Control (27)		**TCC (14)**		**Tennis (31)**		**Running (27)**		One-way ANOVA
ARM	Mean	DS	Mean	DS	Mean	DS	Mean	DS	
Rsp (ohm·cm)	266.9	44.67	248.8	31.70	223.2	25.65	229.8	29.25	_p< 0.001 (CR*; CTn*)_
Xcsp (ohm·cm)	31.8	7.65	25.4	4.81	25.8	4.53	26.1	5.16	_p< 0.001 (CR*; CTn*; CT*)_
Zsp (ohm·cm)	268.8	45.03	250.1	31.81	224.7	25.84	231.3	29.49	_p< 0.001 (CR*; CTn*)_
PhA (°)	6.8	1.12	5.9	0.95	6.6	0.85	6.5	0.89	_p= 0.039 (CT*)_
LEG									
Rsp (ohm·cm)	255.2	44.42	254.6	17.36	243.7	25.88	255.2	22.69	_p= 0.452_
Xcsp (ohm·cm)	31.3	9.63	27.3	3.60	28.4	5.63	32.1	6.37	_p=0.087_
Zsp (ohm·cm)	257.2	45.00	256.1	17.41	245.4	26.17	257.3	23.12	_p=0.444_
PhA (°)	6.9	1.40	6.1	0.77	6.6	0.96	7.1	1.02	_p=0.041 (TR*)_
TRUNK									
Rsp (ohm·cm)	520.0	196.78	492.9	142.46	360.9	91.13	378.5	58.12	_p< 0.001 (CTn*; CR*; TTn*; TR*)_
Xcsp (ohm·cm)	63.8	25.08	68.1	24.07	59.4	18.02	73.4	23.30	_p= 0.147_
Zsp (ohm·cm)	524.0	197.97	497.7	144.06	365.9	92.56	386.0	59.11	_p< 0.001 (CTn*; CR*; TTn*; TR*)_
PhA (°)	7.1	1.42	7.8	1.44	9.4	1.21	11.0	3.052	_p< 0.001 (CTn*; CR*; TTn*; TR*, TnR*)_

Rsp: specific resistance; Xcsp: specific reactance; Zsp: specific impedance; PhA: phase angle. CT: comparison between Control and TCC; CTn: comparison between Control and Tennis; CR comparison between Control and Running; TTn: comparison between TCC and Tennis; TR: comparison between TCC and Running; TnR: comparison between Tennis and Running. Tukey's b test was used for post hoc comparisons. The accepted level of statistical significance was p < 0.05 (*).

Table 4.6 - Descriptive statistics and comparison of segmental body composition among sports and control in women.

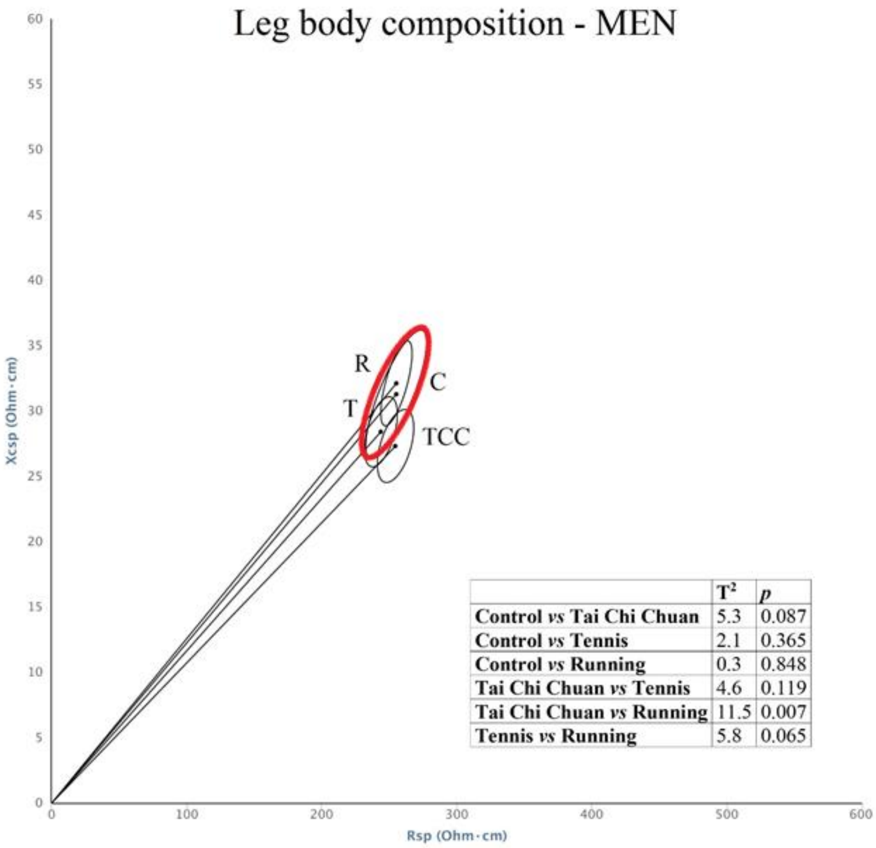
RIGHT SIDE	**Women**						
	Control (32)		**TCC (20)**		**Running (10)**		One-way ANOVA
ARM	Mean	DS	Mean	DS	Mean	DS	
Rsp (ohm·cm)	330.0	61.89	285.4	47.88	249.1	42.39	_p< 0.001 (CT*; CR*)_
Xcsp (ohm·cm)	31.2	7.99	24.7	5.17	26.3	7.26	_p= 0.006 (CT*)_
Zsp (ohm·cm)	331.6	62.08	286.4	48.05	250.5	42.69	_p= 0.000 (CT*; CR*)_
PhA (°)	5.4	1.05	5.0	0.65	6.0	1.17	_p= 0.023 (TR*)_
LEG							
Rsp (ohm·cm)	297.9	58.52	287.0	34.23	266.4	18.30	_p= 0.185_
Xcsp (ohm·cm)	31.7	9.92	29.6	7.99	33.5	6.14	_p= 0.498_
Zsp (ohm·cm)	299.7	58.94	288.7	33.66	268.6	18.63	_p= 0.194_
PhA (°)	6.0	1.33	6.0	2.25	7.2	1.10	_p= 0.156_
TRUNK							
Rsp (ohm·cm)	506.7	159.34	460.8	82.07	463.1	76.72	_p= 0.418_
Xcsp (ohm·cm)	49.0	22.57	47.3	9.05	72.4	16.61	_p= 0.003 (CR*; TR*)_
Zsp (ohm·cm)	509.2	160.37	463.3	82.15	468.9	76.90	_p= 0.437_
PhA (°)	5.4	1.42	5.9	1.01	9.0	2.01	_p< 0.001 (CR*; TR*)_

Rsp: specific resistance; Xcsp: specific reactance; Zsp: specific impedance; PhA: phase angle. CT: comparison between Control and TCC; CR comparison between Control and Running; TR: comparison between TCC and Running. Tukey's b test was used for post hoc comparisons. The accepted level of statistical significance was p < 0.05 (*)



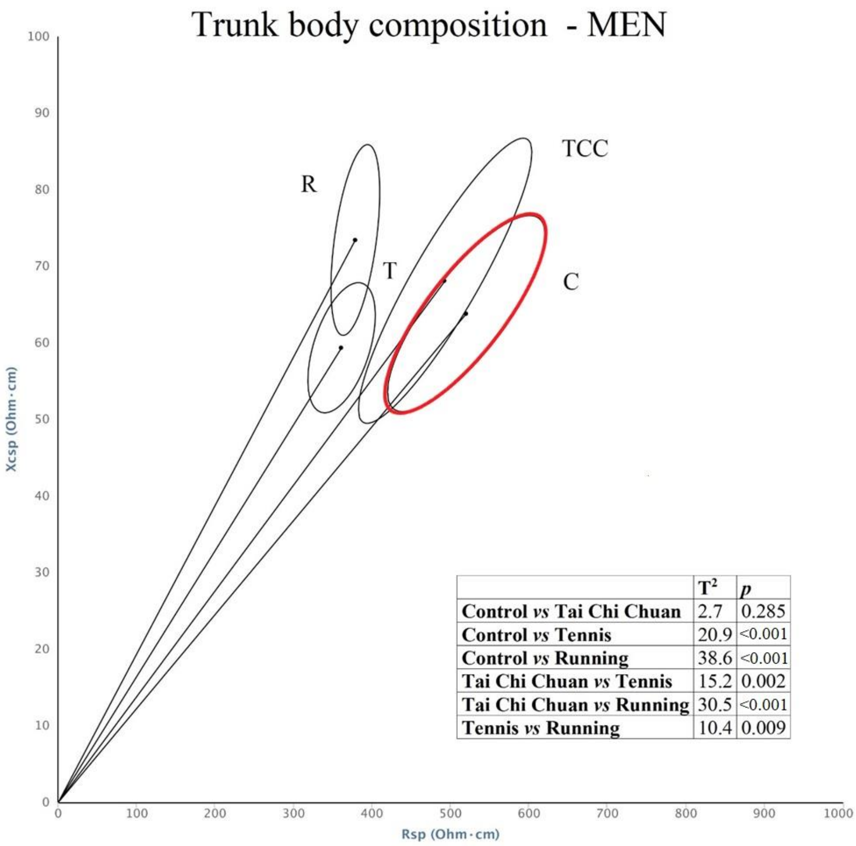
	T^2	*p*
Control *vs* Tai Chi Chuan	9.8	0.014
Control *vs* Tennis	21.0	<0.001
Control *vs* Running	13.4	0.002
Tai Chi Chuan *vs* Tennis	16.7	0.001
Tai Chi Chuan *vs* Running	9	0.019
Tennis *vs* Running	1.1	0.587

Figure 4.8 - Confidence ellipses of segmental body composition of the arm of all group of men.



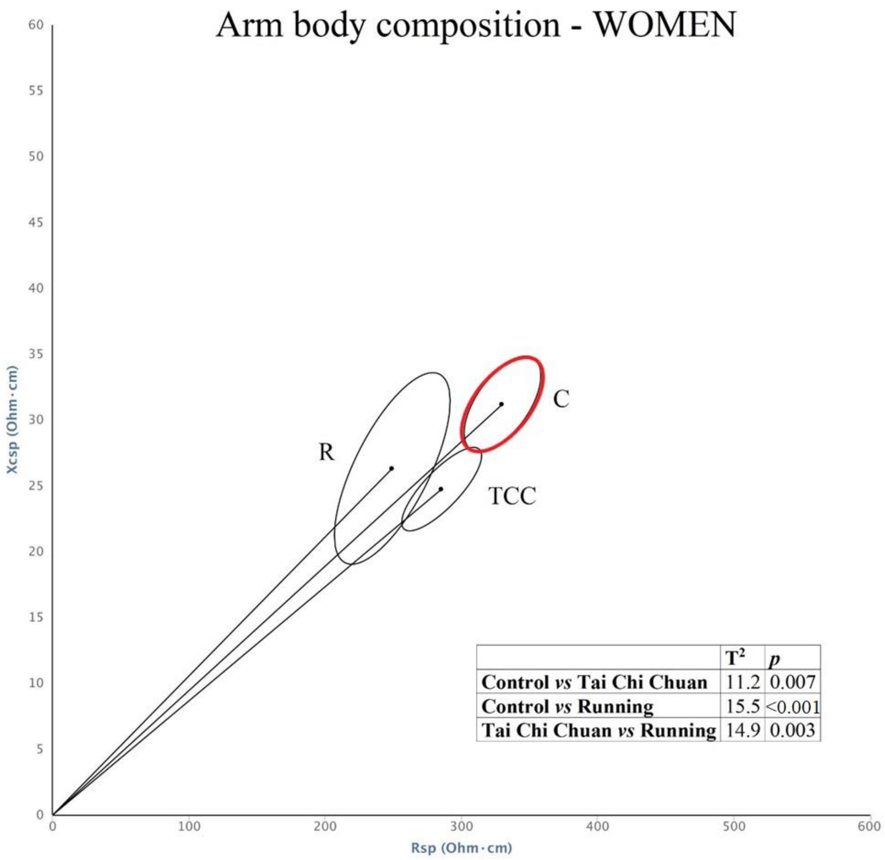
	T^2	*p*
Control *vs* Tai Chi Chuan	5.3	0.087
Control *vs* Tennis	2.1	0.365
Control *vs* Running	0.3	0.848
Tai Chi Chuan *vs* Tennis	4.6	0.119
Tai Chi Chuan *vs* Running	11.5	0.007
Tennis *vs* Running	5.8	0.065

Figure 4.9 - Confidence ellipses of segmental body composition of the leg of all group of men.



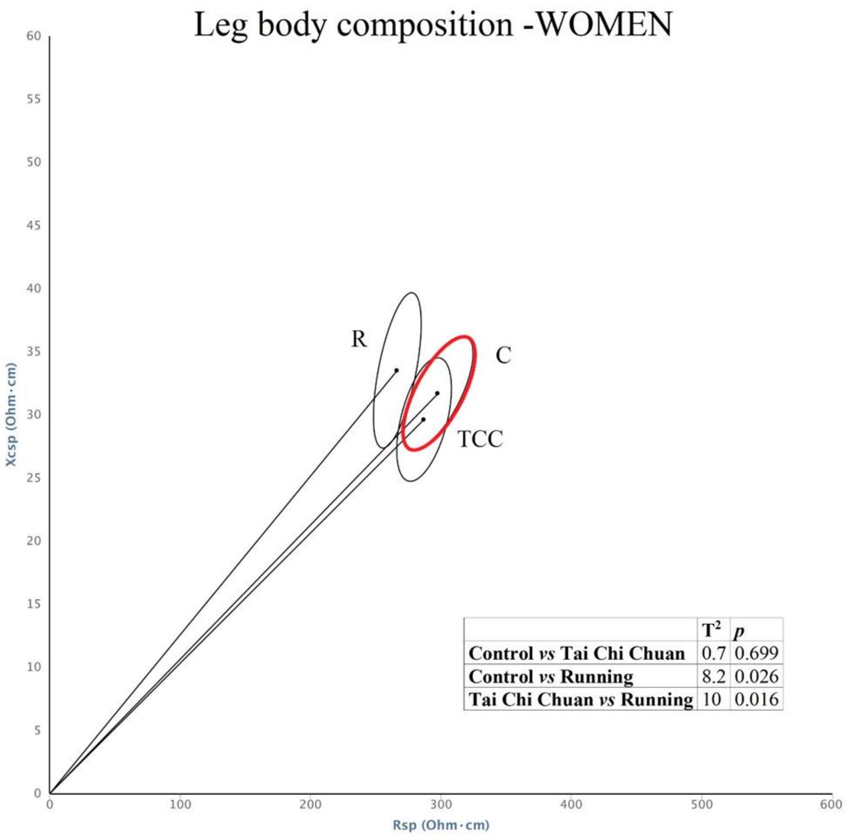
	T^2	*p*
Control *vs* Tai Chi Chuan	2.7	0.285
Control *vs* Tennis	20.9	<0.001
Control *vs* Running	38.6	<0.001
Tai Chi Chuan *vs* Tennis	15.2	0.002
Tai Chi Chuan *vs* Running	30.5	<0.001
Tennis *vs* Running	10.4	0.009

Figure 4.10 - Confidence ellipses of segmental body composition of the trunk of all group of men.



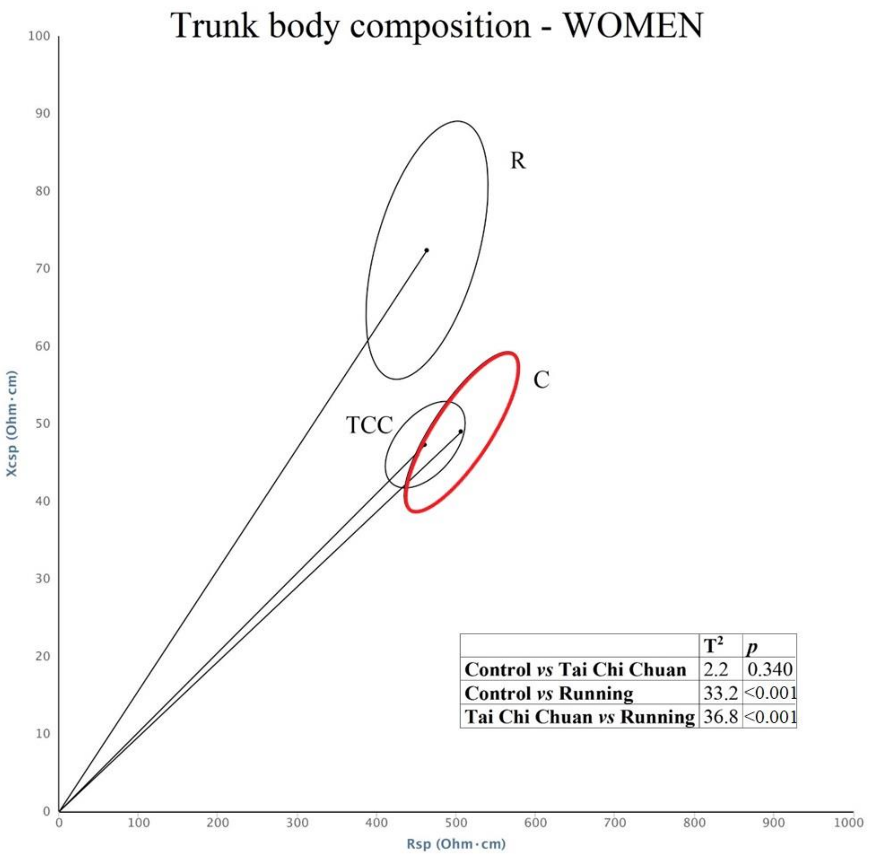
	T^2	*p*
Control *vs* Tai Chi Chuan	11.2	0.007
Control *vs* Running	15.5	<0.001
Tai Chi Chuan *vs* Running	14.9	0.003

Figure 4.11 - Confidence ellipses of segmental body composition of the arm of all group of women.



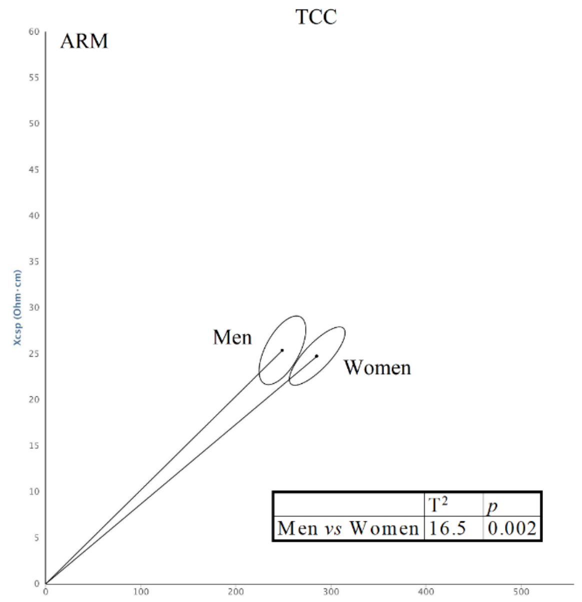
	T^2	*p*
Control *vs* Tai Chi Chuan	0.7	0.699
Control *vs* Running	8.2	0.026
Tai Chi Chuan *vs* Running	10	0.016

Figure 4.12 - Confidence ellipses of segmental body composition of the leg of all group of women.

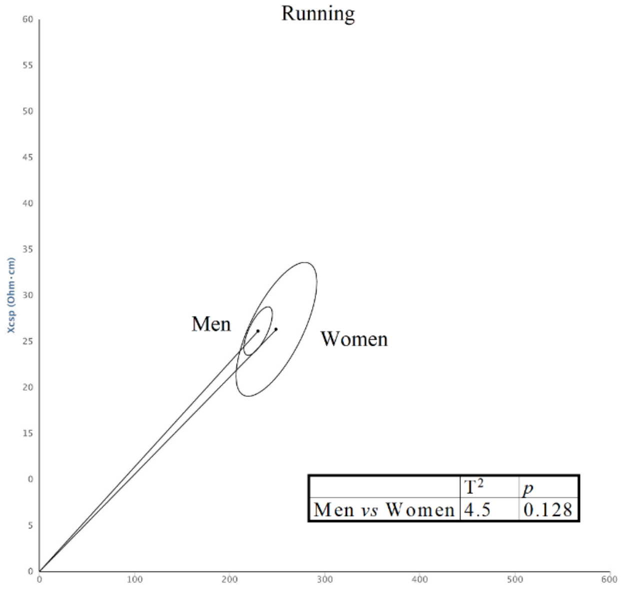


	T^2	*p*
Control *vs* Tai Chi Chuan	2.2	0.340
Control *vs* Running	33.2	<0.001
Tai Chi Chuan *vs* Running	36.8	<0.001

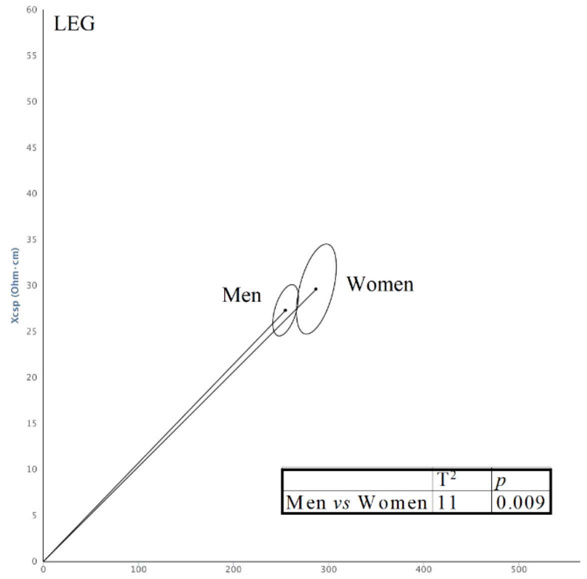
Figure 4.13 - Confidence ellipses of segmental body composition of the trunk of all group of women.



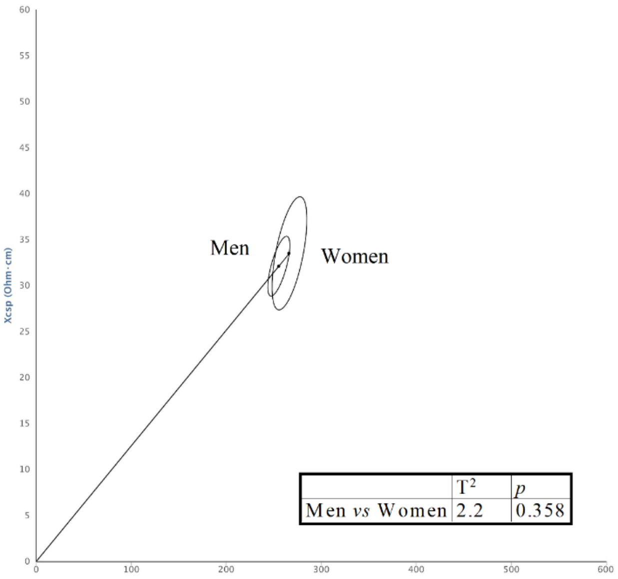
	T^2	*p*
Men *vs* Women	16.5	0.002



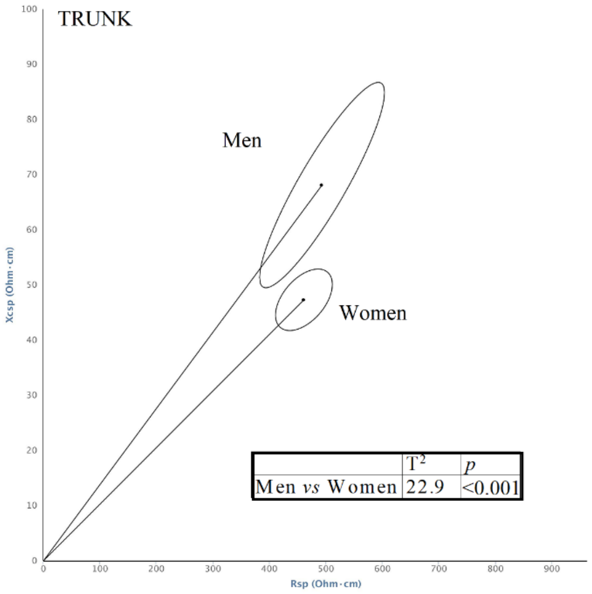
	T^2	*p*
Men *vs* Women	4.5	0.128



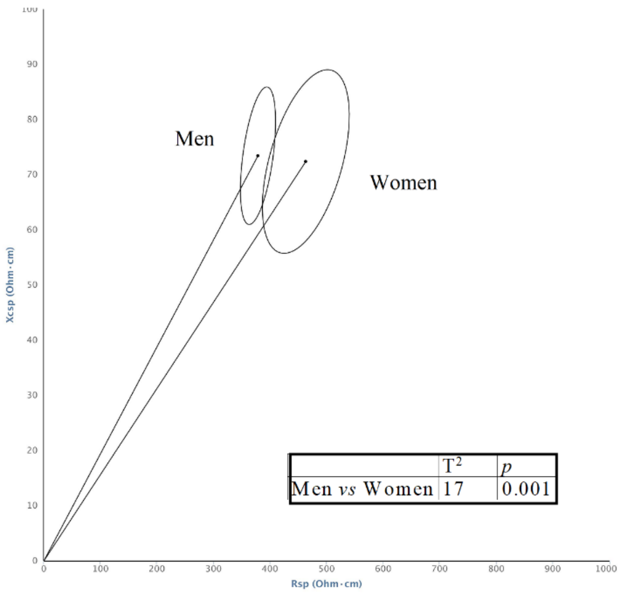
	T^2	*p*
Men *vs* Women	11	0.009



	T^2	*p*
Men *vs* Women	2.2	0.358



	T^2	*p*
Men *vs* Women	22.9	<0.001



	T^2	*p*
Men *vs* Women	17	0.001

Figure 4.14 - Confidence ellipses of segmental body composition between the two sexes. Sexual differences in TCC and Running.

Hand grip strength was correlated only with PhA in both active men and women, but not in the control (Table 4.7).

Table 4.7 - Correlation between hand grip strength and bioelectric variables of the arm.

	Men		**Women**	
	Control	Active	Control	Active
Rsptot (ohm·cm)	-0.233	0.056	-0.075	-0.006
Xcsptot ohm·cm)	-0.041	0.252*	0.133	0.315
Zsptot (ohm·cm)	-0.230	0.060	-0.074	-0.002
PhA (°)	0.190	0.380*	0.249	0.384*

Rsp: specific resistance; Xcsp: specific reactance; Zsp: specific impedance; PhA: phase angle.
* The correlation is significant at 0.05 level.

4.4.2 DISCUSSION

The results of segmental body composition analysis showed differences between sexes and sports. As in the case of total body composition, in all body districts, active (including tennis players) and control men were characterised by higher phase angles and lower vector length than women, indicative of greater muscle mass and lower %FM, whereas in the trunk no sex differences in %FM were detected. Indeed, the preferential central distribution of fat mass in men (Wells 2007) is likely the cause of the low degree of sex differences in this body district. Also, middle-age and elderly women tend to a central distribution of fat as men thus further reducing dimorphism at the trunk level. Considering the sports separately, as for the whole-body composition, a low degree of sexual dimorphism was observed among TCC practitioners and especially among runners, who showed some difference at the trunk level only. This result among TCC and runners could be due to the reduction of %FM, particularly evident among women in the arm and the leg, which led to less dimorphism in the limbs.

The active subjects of both sexes considered both at a whole and with sport separated showed a tendency to lower %FM in the arms and the trunk than the controls, while the legs did not show significant differences. In the trunk they also showed higher skeletal muscle mass than controls. TCC subjects showed higher values of %FM and lower values of PhA than runners and tennis players in all body districts.

The literature on segmental body composition in middle-aged adults and older individuals who regularly practise a sport is scarce, but the interest on this topic has been growing in recent years (Mikkelsen et al. 2013; Piasecki et al., 2019; Mitchell et al. 2020). These studies almost relate to Tennis and Running, as in this research, while TCC has been much less investigated.

To our knowledge, one study only considered regional body composition in TCC using skinfolds thickness (Yu et al., 2007), and showing, as in this study, reduced levels of FM in the central area of the body. On the contrary, the muscle mass appears less influenced by TCC, especially in the limbs (Stagi et al., 2020a).

The research on regional body composition of veteran tennis players manly concerned the bone mass (Huddleston et al., 1980; Moysi et al 2004a) or asymmetry between dominant and non-dominant arms (Piasecki et al., 2019; Ireland et al., 2014), while studies comparing elderly tennis players with sedentary subjects or with other sport practitioners are very rare. Moysi et al. (2004b) stated that in middle-aged men the regular practice of tennis can maintain or increase leg muscle mass, while Moysi et al. (2004a) found no differences in body lean mass between tennis and controls postmenopausal women, but higher values of fat in controls, especially at the trunk level.

Among old runners, a regular practice was associated with lower regional body fat (Piasecki et al., 2019; Mitchell et al. 2020) and greater lean mass at the leg (Mikkelsen et al., 2013; Piasecki et al., 2019) or trunk (Mitchell et al., 2020) level. These results are consistent with this research, and in the case of the trunk the difference observed with controls has been also detected in the comparison with the other sports.

The analysis of the association between hand grip strength and body composition showed a positive correlation with PhA, and hence with muscle mass, in sports subjects only (both sexes). A previous research (Mereu et al., 2017), that investigated the same relation in a sample of Alzheimer's older subject and in a control sample, failed to detect any association. In this research the control sample was characterised by similar PhAs of the arm to those of the active subjects, but active individuals, especially Tennis and Running, tend to higher values in hand strength, as discussed in the paragraph on whole body composition.

4.5 ASYMMETRY

4.5.1 RESULTS

Bioelectrical values of dominant and non-dominant arm, and right and left leg are showed below (Table 4.8).

Table 4.8 - Descriptive statistics of bioelectrical values of the limbs in all groups.

	Control				**Active**				**Tai Chi Chuan**				**Tennis**		**Running**			
	Men		Women		Men		Women		Men		Women		Men		Men		Women	
D ARM	Mean	DS	Mean	DS	Mean	DS	Mean	DS	Mean	DS	Mean	DS	Mean	DS	Mean	DS	Mean	DS
Rsp (ohm·cm)	266.3	44.5	330.7	62.84	231.6	29.28	274.4	48.29	248.8	31.70	287.1	46.93	223.2	25.65	230.1	28.18	249.1	42.39
Xcsp (ohm·cm)	31.6	7.61	31.3	8.13	25.8	4.81	25.3	5.82	25.36	4.81	24.8	5.11	25.8	4.53	26.2	5.23	26.3	7.25
Zsp (ohm·cm)	268.3	44.90	332.2	63.05	233.1	29.43	275.6	48.42	250.1	31.80	288.2	47.09	224.7	25.84	231.6	28.43	250.5	42.68
PhA (°)	6.7	1.12	5.4	1.05	6.4	0.92	5.3	0.97	5.8	0.95	5.0	0.64	6.6	0.85	6.5	0.91	6.0	1.17
ND ARM																		
Rsp (ohm·cm)	264.8	40.78	328.1	63.30	232.9	32.249	284.5	52.32	251.8	28.09	301.5	47.57	224.2	31.38	230.7	32.03	252.3	47.29
Xcsp (ohm·cm)	30.1	6.62	30.6	8.24	25.3	5.317	25.5	5.20	24.3	3.55	25.1	4.08	24.8	5.29	26.4	6.10	26.1	7.08
Zsp (ohm·cm)	266.6	40.96	329.6	63.59	234.3	32.43	285.7	52.43	253.0	28.14	302.5	47.68	225.6	31.58	232.2	32.37	253.7	47.65
PhA (°)	6.5	1.16	5.3	1.0	6.2	0.97	5.1	0.79	5.5	0.70	4.8	0.44	6.3	1.01	6.5	0.92	5.9	0.82
RIGHT LEG																		
Rsp (ohm·cm)	255.2	44.42	297.9	58.52	250.3	23.49	280.1	31.12	254.6	17.36	28.0	34.23	243.7	25.88	255.2	22.69	266.4	18.30
Xcsp (ohm·cm)	31.3	9.63	31.7	9.92	29.5	5.86	30.9	7.55	27.3	3.60	29.62	7.99	28.4	5.63	32.1	6.37	33.5	6.14
Zsp (ohm·cm)	257.1	45.00	299.7	58.94	252.1	23.80	282.0	30.70	256.1	17.41	288.7	33.66	245.4	26.17	257.3	23.12	268.6	18.63
PhA (°)	6.9	1.40	6.0	1.33	6.7	1.01	6.4	1.99	6.1	0.77	6.0	2.25	6.6	0.96	7.1	1.02	7.2	1.10
LEFT LEG																		
Rsp (ohm·cm)	261.2	52.30	303.4	53.67	244.9	23.53	279.3	35.04	248.3	18.93	284.5	36.86	243.0	24.87	245.4	24.68	267.9	29.28
Xcsp (ohm·cm)	30.62	9.11	33.4	9.71	29.1	6.06	31.1	7.09	26.8	4.39	29.4	6.50	28.3	5.80	31.1	6.67	35.0	7.23
Zsp (ohm·cm)	263.0	52.82	305.2	54.18	246.7	23.86	281.2	34.88	249.8	19.10	286.1	36.54	244.6	25.20	247.4	25.09	270.2	29.85
PhA (°)	6.6	1.20	6.2	1.16	6.7	1.07	6.4	1.70	6.1	0.82	6.0	1.82	6.6	0.98	7.2	1.15	7.4	0.92

Rsp: specific resistance; Xcsp: specific reactance; Zsp: specific impedance; PhA: phase angle. D: dominant; ND: non dominat.

Whole active sample

Considering paired bioelectrical data and with joined sexes, the active group showed limbs symmetry (arms: T^2=6.3, p=0.1; legs: T^2=5, p=0.1), as also indicated by the position of the ellipse on the origin of the graph (Figure 4.15). The univariate analysis confirmed the results of the arm, with no significant differences in the comparison between bioelectrical values of dominant and non-dominant side, while in the legs slightly higher values of Rsp and Zsp were detected in the right side (p=0.027). On the contrary, the control group showed limbs asymmetry (arms: T^2=6.8, p<0.001; legs: T^2= 8.8, p<0.001), mainly due to the higher values of Xcsp (p= 0.018) and PhA (p=0.054) in the dominant arm, and to higher values of Rsp and Zsp in the left leg (p=0.004 in both cases) (Figure 4.15). The difference in asymmetry between active and control subjects was significant in the leg (T^2=5, p<0.001) and not significant in the arm (T^2=2.6, p=2.8).

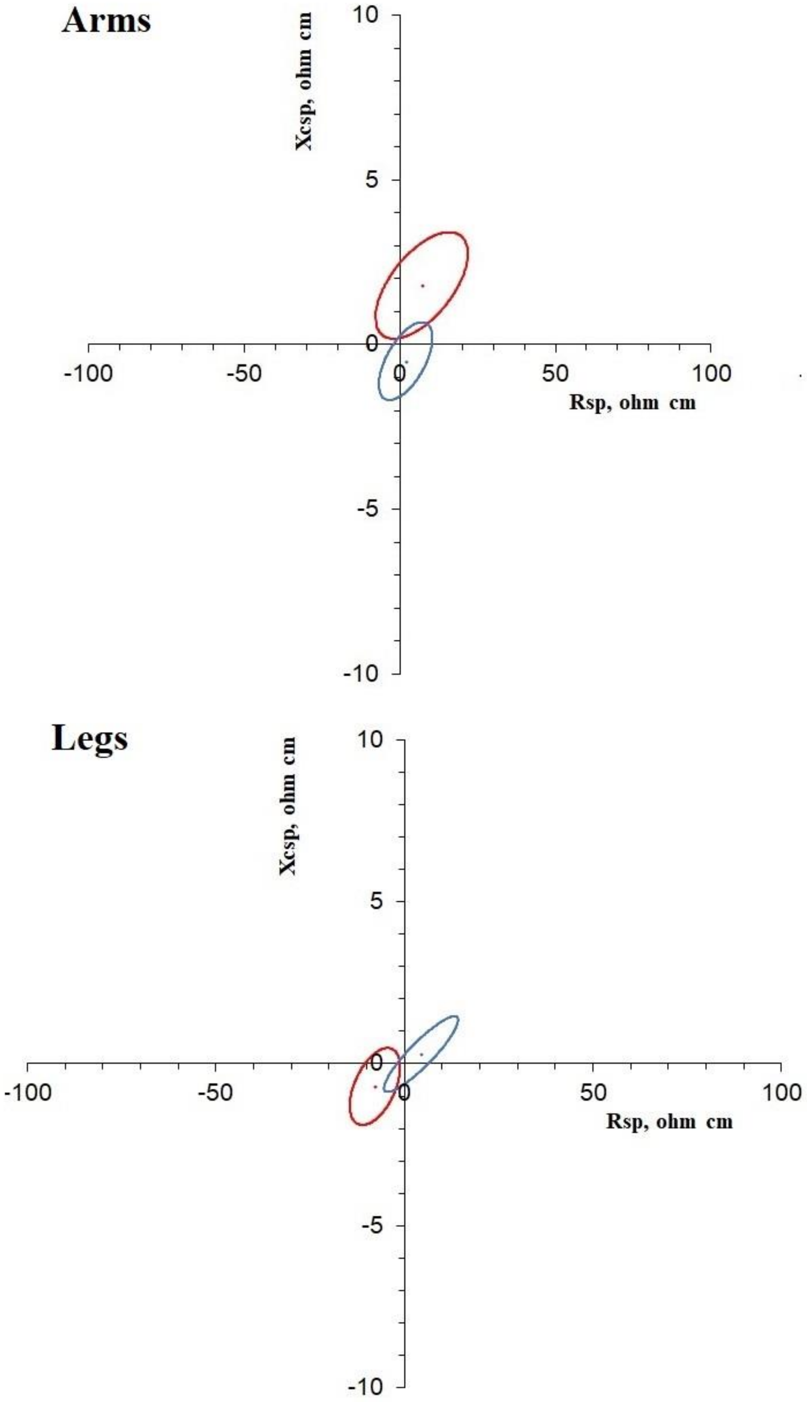


Figure 4.15 - Blue: active subjects; Bordeaux: controls.

Analysis by sport

Considering the sports separately, the paired data graphs showed symmetry in the arms of subjects practicing all sports (TCC arms: T^2 =0.3, p=0.9; Tennis arms: T^2=2.0, p=0.4; Running arms: T^2 = 0.2, p=0.9) (Figure 4.16), also confirmed by the univariate analysis. Same results were detected in the legs where all sports analysed were symmetrical (TCC legs: T^2=1.6, p=0.5; Tennis legs: T^2=0.1, p=0.9; Running legs: T^2=5.2, p=0.1), even if the univariate comparisons indicated a tendency to higher Rsp values in the right leg of runners (p=0.034) (Figure 4.17). The comparison between asymmetry levels in the different disciplines did not show significant differences.

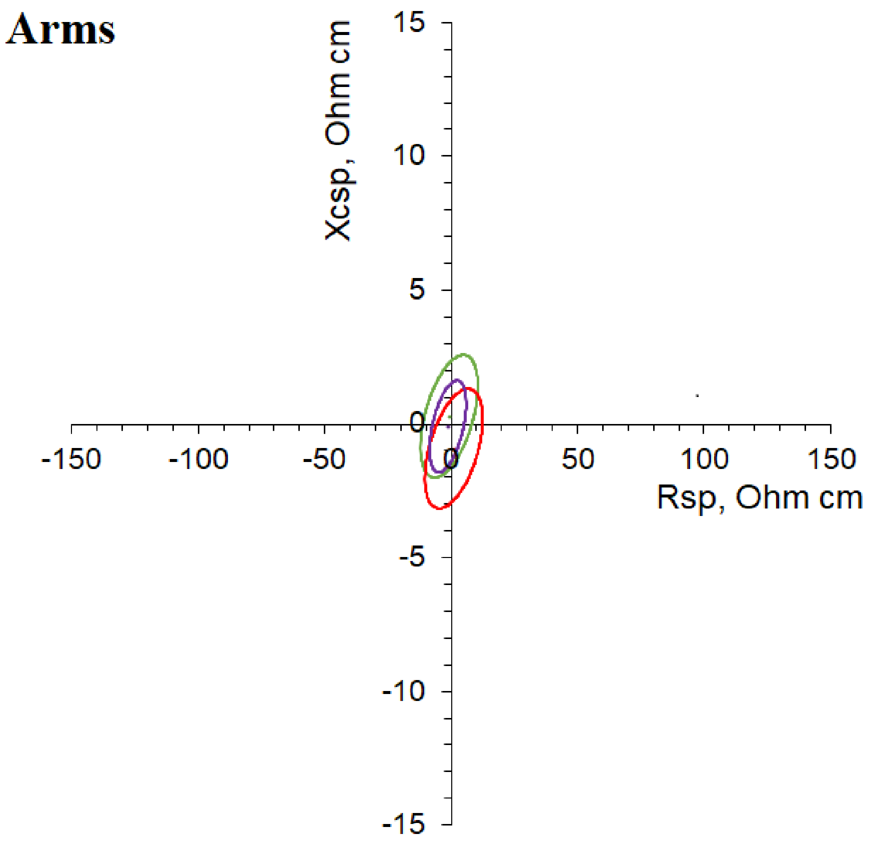
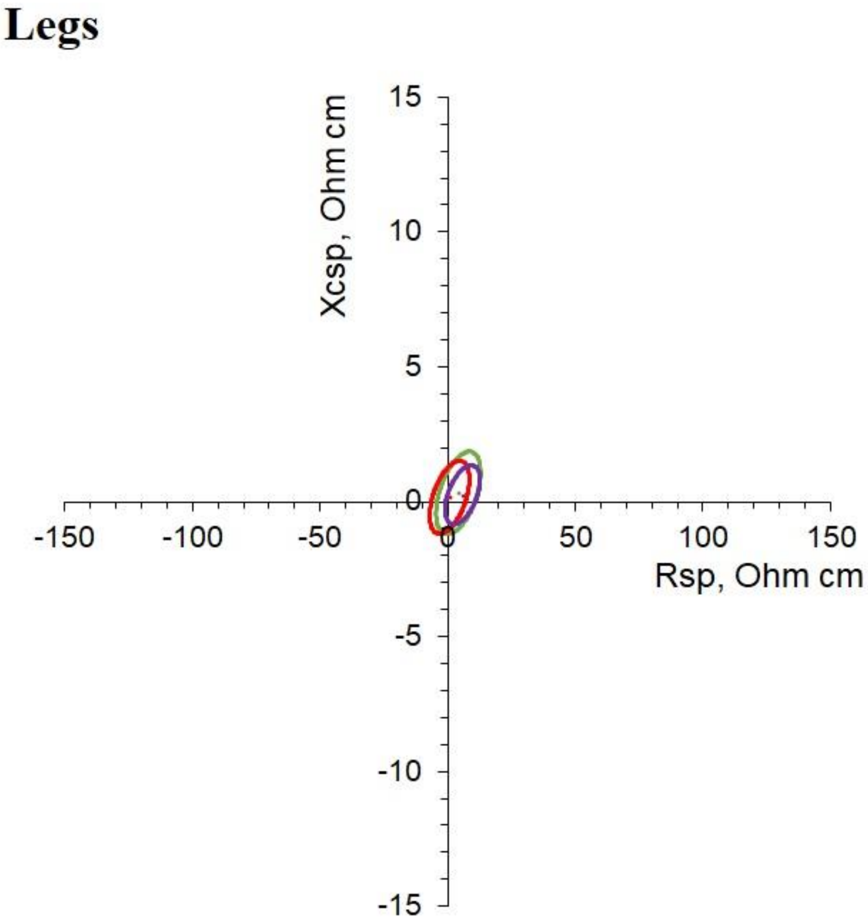


Figure 4.16 - Green: TCC; Red: Tennis; Purple: Running.



Legs

Figure 4.17 - Green: TCC; Red: Tennis; Purple: Running.

In all groups, the hand grip strength was significantly higher in the dominant hand (Table 4.9).

Table 4.9 - Descriptive statistics and paired data test between dominant and non-dominant arm.

	Dominant		**Non-dominant**		**Paired t-test**	
	Men	Women	Men	Women	*t*	*p*
	Mean (DS)	Mean (DS)	Mean (DS)	Mean (DS)		
Control	38.1 (9.36)	23.5 (5.45)	36.9 (8.49)	22.6 (5.45)	2.885	0.005
Active	40.6 (7.67)	25.3 (5.09)	38.0 (7.29)	22.3 (5.72)	7.080	<0.001
TCC	39.4 (8.67)	23.9 (4.77)	38.4 (8.74)	20.8 (4.80)	3.328	0.002
Tennis	40.8 (8.1)	-	37.3 (7.40)	-	4.236	<0.001
Running	41.1 (6.7)	28.0 (4.8)	38.6 (6.56)	25.0 (6.52)	5.431	<0.001

4.5.2 DISCUSSION

It is well known that the human body possesses laterality. However, body asymmetry of body composition and strength in the elderly is a subject poorly analysed, especially among active subjects. The literature discussing the role played by body composition asymmetry in maintaining body balance and preventing falls is still scarce, while the relation with strength asymmetry has been more deeply analysed.

In the present research, the analysis of body composition showed that active subjects were symmetrical in the upper and lower limbs, while the control subjects were characterised by limbs asymmetry, with higher values of muscle mass in the dominant arm and higher values of %FM in the left leg. The pattern of limbs symmetry was similar in the different disciplines, even if the runners showed higher %FM (tendentially higher Rsp and Zsp values) in the right leg. In all active and control subjects the dominant side showed higher hand grip force.

As in this study, a previous research investigating body composition asymmetry in the general population and the whole life cycle showed that the dominant leg and arm were characterised by higher values of lean mass, while the differences in the FM were less accentuated (Hinton et al., 2017). As regards the sports, to our knowledge, body composition asymmetry in older people has been studied in tennis players only, and the results of these studies are not consistent each other. In line with this research, Piasecki et al. (2019) detected symmetrical muscle size in the arms of older male tennis players. However, other authors observed greater lean mass in the dominant arm of young (Moysi et al. 2010) and veteran (Ireland et al. 2014) tennis players.

The asymmetry of hand grip strength has been more extensively studied. Differences between dominant and non-dominant sides have been frequently observed in healthy adults (Coren et al., 1979; Incel et al., 2002; Petersen et al., 1989), and estimated about 10% (Petersen et al., 1989). Consistently, in this study the dominant arm showed higher values than the non-dominant one in all cases: the control, the active group as a whole, and each sport separately. However, according to some authors, healthy elderly subjects show a less homogeneous pattern, with cases of symmetrical strength too

(Koda et al., 2018; LaRoche et al., 2012; Skelton et al., 2002). To our knowledge, the literature on hand grip strength asymmetry in elderly active subjects, again, considered tennis players only, where a greater strength in the dominant arm was observed (Maughan et al.,1986; Vodak et al. 1980; Ireland et al., 2014; Piasecki et al. 2019), as in this study.

The literature clearly shows a positive correlation between strength asymmetry and health outcomes in the elderly, in particular with functional disabilities, body balance, falls (Portegijs et al., 2005; LaRoche et al., 2012; Chon et al., 2018; McGrath et al., 2020a), and ultimately increased risk for early cases of mortality (McGrath et al., 2020b). Nevertheless, the role of body composition symmetry in the health of elderly people has not yet been defined.

According to recent studies, lower limbs body composition symmetry is less relevant (Lee et al., 2019) than strength symmetry (Lee et al., 2019; McGrath et al., 2020a), or than good levels of muscle mass in both limbs (Mertz et al., 2019) in order to maintain functional capacity or prevent risk of falls.

The results of this research showed that subjects practicing tennis, TCC, or running showed hand grip asymmetry, but had symmetrical body composition, especially in the lower limbs. Hence, along with the general positive effect on nutritional status and muscular strength, physical activity seems to play a role in maintaining the symmetry of body composition. Our results suggest that a symmetrical body composition would represent a positive aspect, and, contrary to the results of Lee et al. (2019), it could contribute to maintaining better mobility. This hypothesis should be further analysed.

4.6 BODY IMAGE AND PSYCHOLOGICAL WELL-BEING

4.6.1 RESULTS

Whole active sample

The comparison between active subjects and control has shown differences in body image perception. In fact, active men and women identified themselves with smaller silhouettes (men: numbers 6-7 more frequently chosen; women: numbers 3-7) than the control (men: numbers 7-8; women: numbers 4-9) (Figure 19). No significant differences were found as to the ideal silhouette, that in both groups was identified more frequently with the silhouette number 3 among women and the number 5 among men (Figure 4.18). Consequently, the percentage of body dissatisfaction was higher, in both sexes, in the control with respect to the active sample (Figure 4.18).

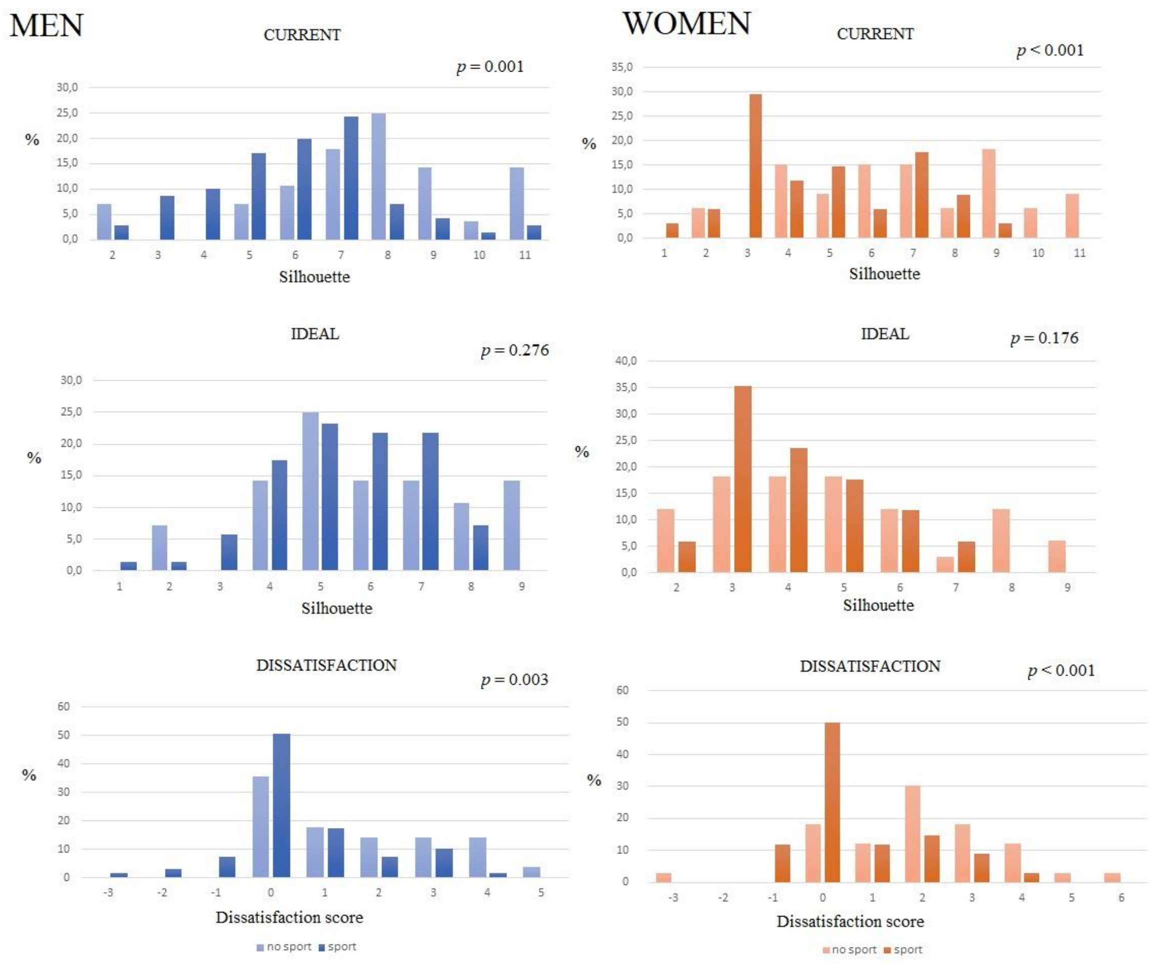


Figure 4.18 - Frequency graphs of body image variables.

Analysis by sport

Considering the sports separately, emerged that also in each of them the active subjects perceived themselves smaller than controls (Table 4.10). The difference was particularly evident among women, where the most selected current body silhouettes were the number 3 (TCC and Runners), while among men, the difference was mainly due to the runners, who chose more frequently the silhouette number 5, while TCC and Tennis chose the number 7, as the controls. Also, for the ideal body, subjects practicing different disciplines selected similar silhouettes as the controls, with the difference of male runners that chose smaller silhouettes than the others (Table 4.10). In fact, the most frequent ideal silhouettes among men were the numbers 5-7 for Tennis and TCC, as the control, while runners selected the numbers 3-6, and women selected the ideal silhouettes numbers 3-5 in all cases. Body dissatisfaction showed no differences among sports, with both sexes more satisfied than controls in all sports (Table 4.10).

Table 4.10 - Mann-Whitney U test between sports and control in body image variables.

	Control *vs* TCC	**Control *vs* Tennis**	**Control *vs* Running**	**TCC *vs* Tennis**	**TCC *vs* Running**	**Tennis *vs* Running**
C (M)	*p* = 0.078	*p*= 0.059	*p*< 0.001	*p*= 0.894	*p*= 0.002	*p*= 0.001
C (W)	*p*= 0.015	-	*p*= 0.002	-	*p*= 0.244	-
I (M)	*p*= 0.735	*p*= 0.861	*p*= 0.019	*p*= 0.593	*p*= 0.004	*p*= 0.008
I (W)	*p*= 0.602	-	*p*= 0.090	-	*p*= 0.124	-
DS (M)	*p*= 0.053	*p*= 0.073	*p*= 0.001	*p*= 0.610	*p*= 0.626	*p*= 0.220
DS (W)	*p*= 0.002	-	*p*= 0.004	-	*p*= 0.741	-

C: current; I, ideal; DS: dissatisfaction; M: men; W: women.

GDS

Both groups were within the range of normal values and therefore do not have any degree of depression symptoms, with no sex differences (Table 4.11). However, active subjects of both sexes showed lower GDS values than controls, indicative of better psychological conditions. No differences were detected among groups of sports (Table 4.12, 4.13).

Table 4.11 - Descriptive statistics and comparison between active and control groups.

	Control				**Active**				Two-way ANOVA		
	Men		Women		Men		Women				
	Mean	DS	Mean	DS	Mean	DS	Mean	DS	p_{sex}	p_{sport}	$p_{sex \cdot sport}$
GDS	1.9	2.27	2.5	1.98	1.5	1.70	1.5	1.70	0.130	0.004	0.699

Table 4.12 -Descriptive statistics and comparison among sports and control groups.

Men

	Control		**TCC**		**Tennis**		**Running**		One-way ANOVA
	Mean	DS	Mean	DS	Mean	DS	Mean	DS	
GDS	1.9	2.27	0.8	1.05	1.4	1.74	1.08	1,32	$p= 0.204$

Table 4.13 - Descriptive statistics and comparison among sports and control groups.

Women

	Control		**TCC**		**Running**		One-way ANOVA
	Mean	DS	Mean	DS	Mean	DS	
GDS	2.8	6.35	1.7	1.63	1.1	1.90	$p= 0.102$

The matrix of correlation between GDS and body dissatisfaction scores showed that a worse psychological status was associated with a lower body satisfaction in controls and TCC, and only among women (Table 4.14).

Table 4.14 - Matrix of Spearman's correlation between GDS and groups

Correlation between GDS and body dissatisfaction		
	Men	**Women**
Control	0.229	0.504**
Active	0.020	0.163
TCC	-0.030	0.473*
Tennis	-0.056	
Running	0.046	-0.044

* The correlation is significant at 0.05 level.

** The correlation is significant at 0.001 level.

4.6.2 DISCUSSION

The analysis of body image showed that active subjects involved in a long-term practice of sport perceived themselves smaller, had similar ideal body image, and were more satisfied with their appearance (50% of men and women were totally satisfied) than controls. Even dividing the group of active subjects for sport, the same results were obtained, with the only difference that the ideal body image in male runners was smaller than those chosen in other sports.

Indeed, the results of anthropometry and total body composition (section 4.3.1) indicate that the evaluation of current body size was correct, as the active sample was characterised by smaller body size with respect to the controls. The different ideal body size of runners was likely due to the peculiarities of this type of sport; in fact runners usually show a very thin body figure with respect to other athletes, as observed in this research.

Body image in active aged individuals is still little analysed in the literature, especially among men. However, as pointed out by Jankowski et al. (2016), it had great importance in the research related to this population group and should be considered in intervention studies. In fact, both physical function and physical appearance are central to body image satisfaction of older adults. Among active elderly, in line with the results of this research, Rica et al. (2018) have shown that older women who undergo resistance training are much more satisfied than the control sample. Furthermore, Condello et al. (2016) observed that physical activity at old age is important not only for the management of body weight, but also for the positive influence on body satisfaction and mental health. This last observation is consistent with the results of the present research. In fact, the analysis of GDS showed that active subjects exhibited significantly better psychological conditions, even if the whole sample was characterised by a normal status, with no depression symptoms. The analysis of sports separately showed no significant differences among groups, in both men and women. The association between psychological status and body satisfaction was confirmed by the significant correlation between worse psychological conditions and body dissatisfaction among Control and TCC women. Such association was not detected among men and this is not surprising, as body dissatisfaction is generally more pronounced among women at all ages, even at the oldest one (Feingold and Mazzella, 1998; Hetherington and Burnett, 1994; Lamb et al. 1993; Rozin and Fallon 1988; Tiggemann, 1992; Tiggemann and Stevens, 1999).

The above discussed results are interesting considering that, according to the WHO (2017), adults aged 60 or more suffering from a mental disorder correspond to the 15% of the aged population. In particular the depression, that generally has a higher incidence among women (Noble 2005), has found to be associated with disabilities or diseases in late age (Alexopoulos 2005; Taylor 2014).

Strength and limitations of this study

The main limitation of this research is the few women represented in the sample of tennis players, that did not allow the analysis of sex differences in this sport. This problem could be overcome in the future by means of a focused sampling on women tennis players, along with the analysis of other sports affordable at middle and old age, such as cycling and swimming, or other disciplines, such as yoga.

However, this study has some important strength too. This study was one the first ones analysing segmental body composition in middle aged and elderly subjects practicing TCC, tennis, and running. Furthermore, the analysis was conducted both considering the joined sample of active individuals and each sport and sex separately. This approach allowed to clarify the homogeneity of active subjects in some aspects, such as the greater body satisfaction or the lower %FM with respect to the controls, and also the peculiarities of each sport, that highlight the risk to reach misleading results when considering different sports as a whole. Lastly, this is the first study to apply *specific* BIVA in a sample of middle-aged and elderly subjects who practised a sport over a long time.

4.7 CONCLUSIONS

The results of this study suggest that the long-term practice of sport has a positive effect on body composition, body symmetry and psychological well-being.

In fact, active individuals, considered as a whole and each sport separately, were characterised by a better nutritional status and a lower %FM in both the whole-body and the segmental level, particularly the trunk, thus with a less accentuated age-related trend toward the central accumulation of fat mass. As concerns muscle mass, athletes maintain more effectively muscle mass both at the total and the segmental level, especially the trunk.

Runners and tennis players showed lower values of %FM and higher values of muscle mass than TCC, both at the total and the segmental level. In the whole-body tennis players exhibited the highest values of muscle mass, whereas running registered the highest values in the trunk.

Long-term practice of sport appears to reduce sex differences in body composition. In fact, TCC subjects and, especially, runners exhibited less sexual dimorphism than controls in the whole body and the limbs.

As regards body composition symmetry, the active sample and all the three analysed disciplines have shown a good symmetry in the limbs, thus indicating a possible positive effect of sport on balance and mobility in old age.

Finally, the active sample as a whole and athletes of all the three analysed disciplines showed body image satisfaction and psychological well-being. The only difference among sports is related to the ideal body image, that was slimmer among runners' men.

In summary, the results of the present research suggest that the long-term practice of a sport positively influences whole and segmental body composition. Active men and women are less affected by the age-related process of %FM increase, muscle mass and strength reduction, are more symmetrical and hence are further away from the emergence of sarcopenia, sarcopenic obesity, risk of falls and frailty. All this concurs to maintain health and mental well-being and promotes successful ageing.

5. GENERAL CONCLUSIONS

This doctoral thesis was aimed to study the biology of ageing in relation with physical activity, by analysing the long-term effects of exercise on the whole and segmental body composition, on strength and body symmetry, and on body satisfaction and on mental well-being.

At this purpose, the first part of the research was dedicated to an in-depth methodological study. Such methodological section was dedicated to confirming or evaluating ex novo some concepts related to BIVA interpretation, and to the comparability among BIA devices, that frequently represent basic assumptions in the specialistic literature.

The performance of classic and *specific* BIVA in the assessment of %FM, and, for the first time in this study, of body fluids (TBW, ECW/ICW) was evaluated using DXA and dilution methods as references.

- *Specific* BIVA has confirmed to be more accurate than classic BIVA in the assessment of %FM in athletes, whereas the classic approach has shown to be more accurate in the analysis of TBW. PhA, and hence both classic and *specific* BIVA, has shown to be sensitive to ECW/ICW ratio.

A new protocol was defined for segmental body composition (trunk and limbs) evaluation, using *specific* BIVA. The performance of segmental *specific* BIVA in the assessment of %FM was then evaluated, for the first time in this study, against DXA.

- The newly defined protocol for the positioning of electrodes in segmental BIA has proven to be adequate for body composition estimation. Indeed, the sum of raw bioelectrical values at the segmental level corresponded to those of the whole-body.
- *Specific* BIVA has shown to be consistent with DXA in the assessment of total and segmental body composition. Indeed, the indices obtained with DXA (%FM and FFMI) were correlated with *specific* vector length and PhA in the whole body and each segment.

- The comparative analysis of different body segments was coherent with that of the whole body and confirmed that the adjustment of bioelectrical values for segment lengths and areas used in *specific* BIVA effectively balances the effect of body size.

Furthermore, the results of three widely used impedance devices were compared for consistency.

- The differences among all impedance devices (BIA ANALYZER^{TM} and two instruments from the same AKERN manufacturer) were significant even from a biological point of view in the case of reactance, which was systematically higher when measured with AKERN BIA 101 and BIA ANALYZER^{TM} with respect to AKERN N.E. Statistical, but not biologically relevant differences, were found for the resistance. Correction factors have been defined to amend the biases and making possible the comparison of results obtained with different devices.

Lastly, the relationship between figural stimuli (BIA-O scale of silhouettes; Williamson et al., 2000) in the assessment of current body image and body composition was investigated in samples of different age classes and in the two sexes.

- Young and elderly normal weight individuals of both sexes recognised themselves correctly. Silhouettes were strongly associated with body composition and particularly with the relative content of body fat in both sexes and until old age.

The second part of the research concerned the evaluation of total and segmental body composition using *specific* BIVA in a sample of subjects practising a sport constantly and for a long time. The effect of sport on muscle strength, body symmetry, psychological state and body image perception was also investigated.

Below the summary of the main conclusions reached in this section.

- The sample of active subjects, considered as a whole and each sport separately, was characterised by a better nutritional status and body composition (a tendency to lower %FM and higher muscle mass than controls). At the segmental level, and particularly in the trunk, where found a less accentuated age-related trend toward the central accumulation of fat mass was observed.

- Runners and tennis players showed lower values of %FM and higher values of muscle mass than TCC, both at the total and the segmental level. Tennis players exhibited the highest values of muscle mass in the whole body, and runners registered the highest values in the trunk.
- A lower degree of sexual dimorphism was found in the whole body and in the limbs among athletes (TCC subjects and, especially, runners) in comparison with controls.
- The three disciplines similarly showed stronger body composition symmetry in the limbs than controls, suggesting a positive effect of sport on balance and mobility in old age.
- Physical exercise contributed to improve body image satisfaction and psychological well-being in late age, without differences among sports, or between sexes.

In conclusion, *specific* BIVA confirmed to be a suitable tool for monitoring composition changes in sport science and clinical applications. In addition, the new segmental approach of *specific* BIVA represents a valid method for monitoring body asymmetry and performance in athletes, and to deeply investigate the regional distribution of fat and muscle mass in the elderly.

Furthermore, the association between silhouettes and *specific* BIVA showed that body perception is mainly related to %FM.

From the methodological point of view, the systematic nature of the differences found among devices made it possible to propose correction factors that allowed the comparison of data collected with different instruments.

The long-term practice of a sport confirmed to positively influence whole-body and segmental body composition, mainly at the arm and trunk level. Active men and women demonstrated to be less affected by the age-related process of %FM increase, muscle mass and strength reduction, were more symmetrical and hence were further away from the emergence of sarcopenia, sarcopenic obesity, risk of falls and frailty. Among sports the effects were found more accentuated among runners and tennis players than Tai Chi subjects.

6. ACKNOWLEDGEMENTS

A very special thanks go to Professor Elisabetta Marini for her teachings and support during these years.

A warm thanks goes to Dr. Valeria Succa and colleagues of the Laboratory of Anthropometry and Body Composition.

Many thanks also go to the students of the degree courses who have worked with me during these three years.

For the possibility of work with them in different projects I would like to thank Margherita Micheletti (University of Turin), Stefania Toselli (University of Bologna), Analiza Silva (University of Lisbon) and Stefano Cabras (University of Madrid).

A very special thanks for welcoming me to their laboratory during my time abroad goes to Prof. Esther Rebato, Dr. María Eugenia Ibáñez-Zamacona and Dr. Aline Jelenkovic from University of the Basque Country (UPV/EHU), Bilbao, Spain; and to Professor Alfredo Irurtia Amigo and Dr. Marta Carrasco Marginet from the National Institute of Physical Education of Catalonia (University of Barcelona, Spain).

For the possibility of performing the scans with DXA I would also like to thank the DXA technicians and the company of the Faixat Body Scan.

My warmest thanks also go to the all volunteers. I want to thank the:

- Tai Chi Chuan volunteers of the A.S.D. Tai Chi Chuan school of Cagliari (Italy) and La porta d'Oriente school of Quartu.
- The Tennis volunteers of the Tennis Club of Selargius and the Associazione Dopolavoro Ferroviario di Cagliari.
- The Athletics volunteers of the Atletica Fossano 75 and the ASD Polisportiva of Isili.

Thanks to Elsevier for the possibility of including in the PhD thesis the research articles published in their journals:

- Clinical Nutrition (https://www.sciencedirect.com/science/article/pii/S0261561419300706; https://www.sciencedirect.com/science/article/pii/S0261561421001321)
- Nutrition (https://www.sciencedirect.com/science/article/pii/S0899900720303130)

Silvia Stagi gratefully acknowledges Sardinian Regional Government for the financial support of her PhD scholarship (P.O.R. Sardegna F.S.E. - Operational Programme of the Autonomous Region of Sardinia, European Social Fund 2014-2020 - Axis III Education and training, Thematic goal 10, Investment Priority 10ii), Specific goal 10.5

Finally, my thanks go to all those who have stood by me during these years, my family, Antonio and all the friends who have supported me and with whom I wish to share this great achievement.

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