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BODY COMPOSITION AND HUMAN MOVEMENT: FROM EXPERIMENTAL APPROACHES TO INTERVENTIONAL BENEFITS

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“One never notices what has been done; one can only see what remains to be done”

Maria Skłodowsa Curie

1. INTRODUCTION

The study of the human body finds its origins in ancient populations. Our earliest ancestors observed and examined human body shapes and their mutation for several purposes, such as aesthetics and medicine (Luximon and Zhang, 2006). The evolutionary application induced the birth of a new research field called anthropometry. Although that term is derived from two Greek words (Ánthrōpos = human, Métron = measure) and considered a subset of physical anthropology, anthropometry methods are applied in many scientific, clinical and industrial actual contexts. For example, auxology is an offshoot covering the investigation of body growth, while ergonomics studies develop and improve the behavioural human-environment interaction (Marmaras et al., 1999). Worldwide, societies and organisations care about implementing strategies and technologies to promote healthy life expectancy rather than simpler longer-life concepts (WHO, 2024), and anthropometric methods appear essential for evaluating and monitoring population prospects. Individual (community) well-being needs routine anthropometrical measurements and many of these are standard in epidemiological and clinical applications (Bauman et al., 2017; Stanley, 1997). However, the so numerous research fields involve different materials and methods that should vary in terms of population types and characteristics, investigation goals, evolutionary aspects and methodological features. For example, paediatrics focuses on anthropometric measurements that provide nutritional status and growth information (Sullivan et al., 1991), whereas sports coaches and technicians are interested in body composition and mechanical structure affecting physical performance and sport-specific demands (Santos et al., 2014). In addition, at the net of an anthropometric field, an investigator could keep her attention on a specific chapter in space (sample)-time-related (prevalence), its occurrence in some historical frames (incidence), its relationship with other biological or socio-demographical markers (cause-effect exposure), its chronological change (time-effect), its evaluation and assessment (science methodology and engineering), its internal and external validation (statistics), and so on (Lohman,1988). Contributing to one application field-specific is crucial for eliciting human biology knowledge, and the information evidence-based network can link investigators all over the world. Only the traits observed in the experimental trials could provide information that converges from samples to populations' statements.

1.1 Body Composition

Body composition is a dynamic area of anthropometrical application and research that targets the spectrum of body component characteristics, from anatomical to individual level (Stewart & Sutton, 2012). The origin of body composition belongs to Hippocrates, who about 2400 years ago theorized that the basic constituents of the environment (air, earth, fire and water) are the elements of the human body and only the perfect balance of body fluids leads to health. Despite the evolution of the concept in Greek civilisation embracing fascination with the body form and aesthetic perfection, the most important contribution ever may be attributed to Archimedes (287-212 BC), who opened the doors to the modern science of densitometry through his principle:

"A body immersed in a fluid is subjected to an upward force equal to the weight of the fluid displaced".

Archimedes' view was so far-sighted that he might metaphorically wait for 1800 years when Pascal modulated his formula allowing the conceptualization of modern laboratory applications (Biran and López-Pulido, 2014). Farer, only the pioneer studies of the so-considered "body composition fathers" Albert Behnke, Josef Brožek and William Siri signed the implementation of recent gold-standard methods (Behnke and Wilmore, 1974; Brožek, 1953; Siri, 1961).

One of the first systematic endorsements came in September 1921, when the director of the Prague Anthropological Institute, Jindřich Matiegka, published the results of a brilliant method to determine anthropometrically the extent of the bone, muscle and skin tissues (Matiegka, 1921). He estimated the skeleton's mass by measuring the maximum transversal dimensions (transverse diameter) of humeral and femoral condyles, and the ankles and wrists. Then, the weight of the skeleton was computed as follows:

$$O = o^2 \cdot L \cdot k_1,$$

where o^2 was the squared average of the bones' thickness, L was the stature and k_1 was a coefficient. Concerning skin and subcutaneous fat, he collected five skinfold thicknesses. Then, the body fat (D) was obtained as:

$$D = d \cdot S \cdot k_2,$$

where d was the one-half of the skinfold thicknesses average, $S = 12{,}312\sqrt[\frac{3}{2}]{body\ mass}$ was the body surface and k_2 was a coefficient.

Finally, for the estimation of the muscle mass, he used the formula:

$$M = k_3 \cdot c^2 \cdot L,$$

where c was the average circumference of the extremities (arm, forearm, thigh and calf) without subcutaneous fat and k_3 was a coefficient. All the coefficients k_1, k_2, k_3 need to be calculated very carefully concerning sex, age, stature and control of corpses. Table 1.1 shows the results obtained on 12 individuals:

"*The method will doubtless have to be perfected and differentiated to meet different wants, but the writer feels convinced that the measurements and determinations of physical anthropology will in future prove of considerable industrial and social utility*"; the words of Matiegka remarks its forward-looking vision, but its studies have never become so popular (Stewart and Sutton, 2012).

Table 1.1. Body components masses on 12 apprentices. Adapted from Matiegka, 1921.

Subject	stature (cm)	body mass (kg)	skeleton (kg, %)		muscles (kg, %)		skin & fat (kg, %)		remainder (kg, %)	
3	165.5	51.2	9.82	19.2	21.37	41.74	9.99	19.51	10.02	19.57
3	169	58.7	10.17	17.33	25.38	43.24	10.48	17.85	12.67	21.58
6	166.6	59.7	10.78	18.06	25.61	42.9	11.55	19.35	11.76	19.7

However, evidence from Matiegka's study underlines that body composition analysis is multifactorial and complex. This results in the need to simplify investigations and reduce the information provided to individuals. The idea of fat as the complementary unit part of active tissues, allows us to easily distinguish between fat mass and fat-free mass and to dive into the last component for a better understanding of more complex biological models. Despite body composition investigators look at body fat as a single component, it is important to specify that it encompasses interstitial and depot fats, fat in cell walls and the nervous system. Differently, the fat-free mass, generally considered as the metabolizing mass given by water and proteins, encloses many levels of classification, treated in the next paragraphs. The ratio of fat mass and fat-free mass largely determines the body structure and affects the psycho-emotional dimension (Keys and Brožek, 1953). The only unbiased way to directly measure body composition is possible in rare instances (unethical) and many of the cornerstone assumptions for indirect methods have arisen from cadaveric analysis (Mitchell et al., 1945; Widdowson et al., 1951; Forbes et al., 1953). Table 1.2 reports the results from an adult male cadaver 35 years old analysed by Mitchell and colleagues (1945). Unfortunately, most cadaveric analyses reported too small sample sizes and it appears necessary considering many articles to provide useful evidence. The Brussels Cadaver Analysis Study (CAS; Clarys et al., 1987) is one of the major research investigating cadaveric body composition on 34 corpses (CAS 1 n=12 male and n=13 female, 16-80 years old, 1979-1980; CAS 2 n=9, 1983). The investigators dissected cadavers regionally into six segments (head and neck, limbs, and trunk) and analysed skin and adipose tissues, musculoskeletal components, organs and viscera (table 1.3) by densitometry, radiography, osteometry and anthropometry.

Table 1.2. Chemical Composition of Adult Cadaver Human Body. Adapted from Mitchell et al., 1945.

		Chemical composition						
Parts	Total body (%)	Water (%)	Ether extract (%)	Crude protein (N X 6.25, %)	Ash (%)	Calcium (%)	Phosphorus (%)	The heat of combustion (calories)
Skin	7.81	64.68	13	22.19	0.68	0.0205	0.06	2.292
Skeleton	14.84	31.81	17.17	18.93	28.91	11.02	4.83	2.497
Teeth	0.006	5.00*		23*	70.9	24.42	11.81	
Striated muscle	31.56	79.52	3.35	16.5	0.93	0.0099	0.116	1.239
Nervous system	2.52	73.33	12.68	12.06	1.37	0.0188	0.352	1.905
Liver	3.41	71.46	10.35	16.19	0.88	0.0102	0.148	2.196
Heart**	0.69	73.69	9.26	15.88	0.8	0.0078	0.113	1.824
Lungs°	4.15	83.74	1.54	13.38	0.95	0.0116	0.114	0.985
Spleen	0.19	78.69	1.19	17.81	1.16	0.0079	0.217	1.193
Kidneys	0.51	79.47	4.01	14.69	0.96	0.013	0.174	1.326
Pancreas	0.16	73.08	13.08	12.69	0.93	0.0143	0.155	1.979
Alimentary tract	2.07	79.07	6.24	13.19	0.86	0.0125	0.115	1.339
Remaining tissues	13.63	50.09	42.44	7.06	0.51	0.0116	0.048	4.165
Liquid	3.79	93.33	0.17	5.68	0.94	0.0054	0.066	0.382
Solid	13.63	70.4	12.39	16.06	1.01	0.0675	0.053	2.04
Contents of alim. Tr.	0.8							
Bile	0.15							
Hair	0.03							
Total body (70.55 kg)	100	67.85	12.51	14.39	4.84	1.596	0.771	1.93

*Assumed; **Somewhat enlarged; °Somewhat congested

Table 1.3. Description of male and female CAS 1 subjects (Adapted from Clarys et al., 1987)

	n	age (year)	body mass (kg)	Skin (kg)	adipose (kg)	muscle (kg)	bone (kg)	organs (kg)
Female	13	80 ± 7	62.5 ± 9.4	3.4 ± 0.4	25.8 ± 7.8	17.8 ± 3.0	7.7 ± 0.8	7.9 ± 1.3

Male	12	72 ± 8	66.2 ± 12.5	3.7 ± 0.9	18.5 ± 4.6	25.1 ± 7.4	9.3 ± 1.4	9.5 ± 1.4

Note: values are assumed as mean ± standard deviation

Results provided by these relevant studies help us to select the best skin sites as predictors of adiposity, to know the adipose tissue segmental distribution for females (head ~ 2.3%, trunk ~ 43.99%, upper limb ~ 4.82%, lower limb ~22.03%) and males (head ~ 3%, trunk ~ 47.22%, upper limb ~ 4.49%, lower limb ~20.49%), and the relationship between skinfold sites and internal and external adipose tissue. Also, other important relations have been discovered, as the total body water (TBW) and fat-free mass (FFM) ratio equals 0.732. So, it is still clear that mass ratios and tissue or chemical proportions rest the fundaments of modern body composition. However, a further step is mandatory before exposing the latest classification. One of the most impressive works came from William Siri (1956), who provided some easy algebraic formulas to answer the following questions:

a) *Assuming that total body water, extracellular fluid space and corpora density are the only quantities that can be measured, how are fat, protein and mineral estimated from any one or a combination of such measurements?*
b) *What are the underlying assumptions in these methods and their range of validity?*
c) *What uncertainty does biological variability as well as experimental error introduce into the final estimate?*
d) *For practical purposes, what experimental accuracy is desirable in a given method?*

He divided the total body mass and volume into four components (fat, water, proteins and minerals), and assumed that the sum of both component masses and volumes must equal a unit ($f + w + p + m = 1$), and mass, density (d) and volume are related by the expression:

$$\frac{1}{d} = \frac{f}{d_f} + \frac{w}{d_w} + \frac{p}{d_p} + \frac{m}{d_m},$$

where f consists of triglycerides, w pure water, p and m proteins and minerals contained in the fluid spaces and cells, whereas the densities are expected to be constant at 37°C ($d_f = 0.9\frac{g}{cm^3}, d_w = 0.993\frac{g}{cm^3}, d_p = 1.34\frac{g}{cm^3}$ and $d_m = 3.0\frac{g}{cm^3}$). Assuming that the above-mentioned densities are likelihood, Siri used Behnke's *lean-body mass* (1953 and 1954) and Brožek's *standard man* (1952 and 1953) as best-defined reference bodies for answering the previously mentioned questions. Although question a is bridged in the next paragraphs and b has been previously filled, Siri demonstrated that a residual uncertainty due to biological variation of about 3.8% of body mass remains even if the best method is applied. The biological variability, due to variation in body constituents, and uncertainty in establishing the composition of adipose tissue and reference man, sets the accuracy limit that is desirable in determining density and fluid spaces.
Nevertheless, these pillar studies are the foundation of modern body composition.

1.1.1 Compartment Models

The complex nature of the body composition makes it hard to summarise all the possible evaluations available for early researchers, and the explanation goes step by step. The general theory hidden behind the compartmentation concept attains the reduction of applied assumptions as the number of measured components increases. This translates to a large amount and accurate pattern collected, with higher precision in classifying individual's bodies. Although body mass weighting provides useful information, it is clear that we need to fragmentize it. The question is: how and how much?

Investigators intuitively divided the number of body composition (body mass) levels from smallest (atomical) to biggest (total body). Successively, based on a molecular level, many compartments have been conceived from simple to complex, meaning that a two-component model separates molecular body mass into two parts, a four-component into four parts, and so on. Figure 1.1 shows the five body composition levels (a), from atomic to whole body, and the differentiation (compartmentation) of fat and fat-free tissue at the molecular level (b). The smallest and most cynical compartment holds about 50 elements, six of which account for more than 99% of total body mass: Oxygen (O) ~ 61%, Carbon (C) ~ 23%, Hydrogen (H) ~ 10%, Nitrogen ~ 2.6%, Calcium (Ca) ~ 1.4% and Phosphorus (P) ~ 0.83% (Wang et al., 1992). The interactions and bonds of the human body elements generate more than 100,000 chemical compounds that are embedded in five molecular components: Water ~ 60% (extracellular ~ 43.33% of TBW), lipid ~ 19.1% (nonessential ~ 89% of fat), Protein ~ 15%, Mineral ~ 5.3% and Glycogen ~ 0.57%. It is important to underline that it has been related components at different levels to each other: for example, the total body hydrogen can be estimated at the molecular level by fat, water and protein content (hydrogen= 0.122*(fat) + 0.111*(water) + 0.07*(protein); Wang et al, 1995). This allows us to classify methods according to measurable quantities (Wang et al., 1995; figure 1.8): property-based aims to distinguish between components of interest, quantifying an unknown component from a quantifiable property (examples are mass and volume to derive FM and FFM); component-based quantifies an unknown component from known property-derived components (an example is FFM derived from its relationship with TBW); combined estimates unknown components using both property and known components (an example is Siri's method).
From this classification, it is possible to derive many different equations to obtain body composition, from atomical to tissue level:

$$bm = O + C + H + N + Ca + P + K + S + Na + Cl + Mg,$$

Where K is potassium, Na is sodium, Cl is chlorine, S is sulfur and Mg is magnesium (11 components).

$$bm = Lip + Wat + Pro + Min + Gly + Res,$$

Where Res represents residual chemical compounds (<1% bm), and Min can be divided into bone and soft tissue minerals (six components). The number of elements that constitute the subject body mass represents the compartments (or components) of the model.

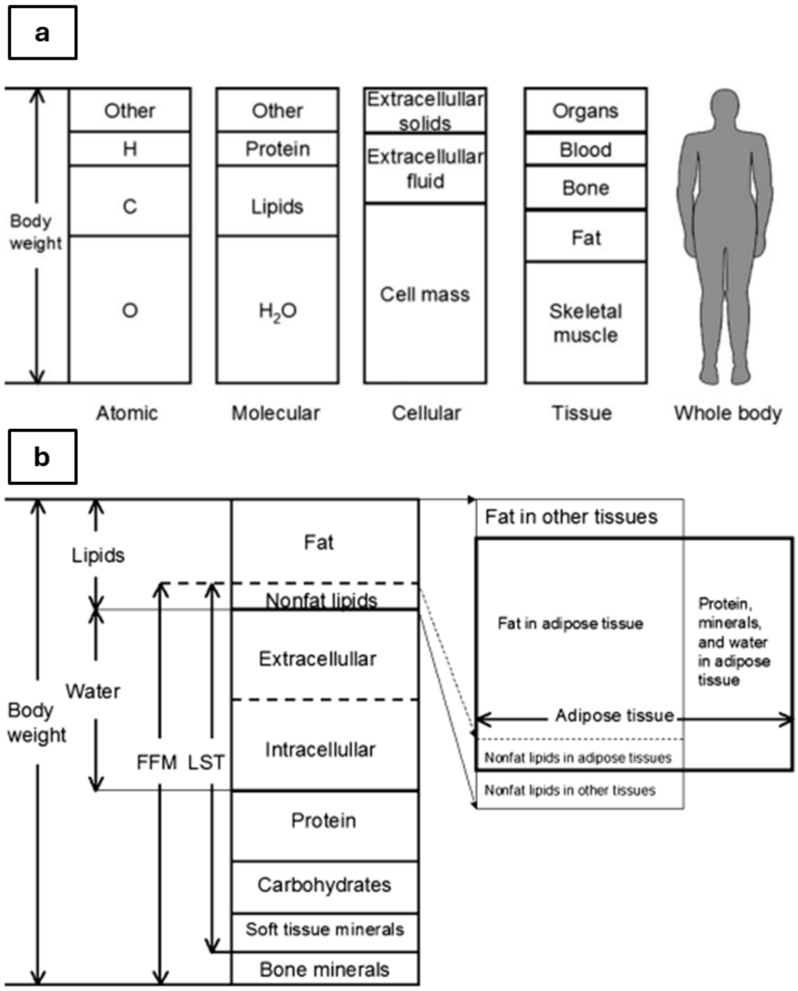

Figure 1.1. The five body composition levels (a) and the main components of the molecular level (b). Adapted from Fosbøl and Zerahn, 2015.

Modern sciences and technologies provide more than ten *in vivo* methods for estimating the total body fat, subdivided into descriptive and mechanistic (Wang et al., 1995). The fundamental concepts of the in vivo methods can be summarized as a mathematical function that relates the unknown components (investigated) and the measurable quantity (Wang et al., 1995). Evidence enhanced two types of mathematical functions that may be combined with three types of measurable quantities, resulting in six categories embracing all the available in vivo methods. Concerning mathematical functions, type I included functions statistically derived such as regression models, which depend on both internal (gender, age, disease, etc.) and ancillary (ethnicity, etc.) variables. Type II gathers functions that incorporate mathematical proportions relating an unknown component to the measurable property or known component. Type II functions are assumptions-based and time-dependent.

Thus, we explain the rationale behind the "simplest" division (two-compartment), which describes body mass by FM (fat mass) and FFM (Behnke et al., 1942; Brožek et al., 1963; Siri, 1961). For example, Behnke and colleagues' method reported two properties (body volume and body mass) and assumed that fat and fat-free mass densities were constant (0.9 g/cm^3 and 1.1 g/cm^3). In addition, considering that water, protein and all the FFM components provide a stable proportion, body fat has been derived as $4.95 * \ body\ volume - 4.50 * body\ mass$. Note that, although FFM and lean soft tissue (LST) are often red as synonyms, LST does not include bone mineral content (figure 1.1, b; Wang et al., 1992).

A simpler methodical classification sets descriptive in vivo measurements that include all the statistically derived estimations, such as anthropometry (Lohman, 1988), ultrasound (Wagner, 2013) and BIA (Piccoli et al., 1994) that depend on a reference method by which to develop a prediction formula. Differently, mechanistic methods collect all the model-dependent methods derived from stable body composition components relationships, such as densitometry with underwater weighing (Brožek et al., 1963; Siri, 1961), total body potassium (^{40}K; Forbes et al., 1961), body water and nitrogen content (Pace and Rathbun, 1945), total body fat and bone mineral content with dual X-ray absorptiometry (DXA, Jensen et al., 1993), total body elements such as ^{13}C, Ca^{+}, Na^{+}, etc. with neutron activation analysis (NAA, Cohn, 1992), computer tomography (CT; Sjöström, 1991), magnetic resonance imaging (MRI, Thomas et al., 1998), air displacement plethysmography (ADP; Millard-Stafford et al., 2001) and multi-compartment models (Wang et al., 1998). Of these mechanistical methods, some such as ADP and underwater weighing need participants' ability to test for tidal, inspiratory lung and expiratory reserve volumes, which implies some validity variability. In addition, the investigator has to perform an indirect analysis to estimate the residual volume (Wilmore, 1969). Differently, methods such as DXA, CT or MRI allow to differentiate between subcutaneous and internal fat reserves, but these are very expensive and invasive, reducing the number of administered experiments during follow-ups. In addition, these methods may account for both single (total body) or multiple-slice acquisition over selected body shapes. If we consider that we are unrevealing just body fat, it is implied that multi-compartment models are often fool in longitudinal studies. In addition, for ethical statements, we do not consider in vitro measurements of components that require cadaveric or excised tissue (Figure 1.2).

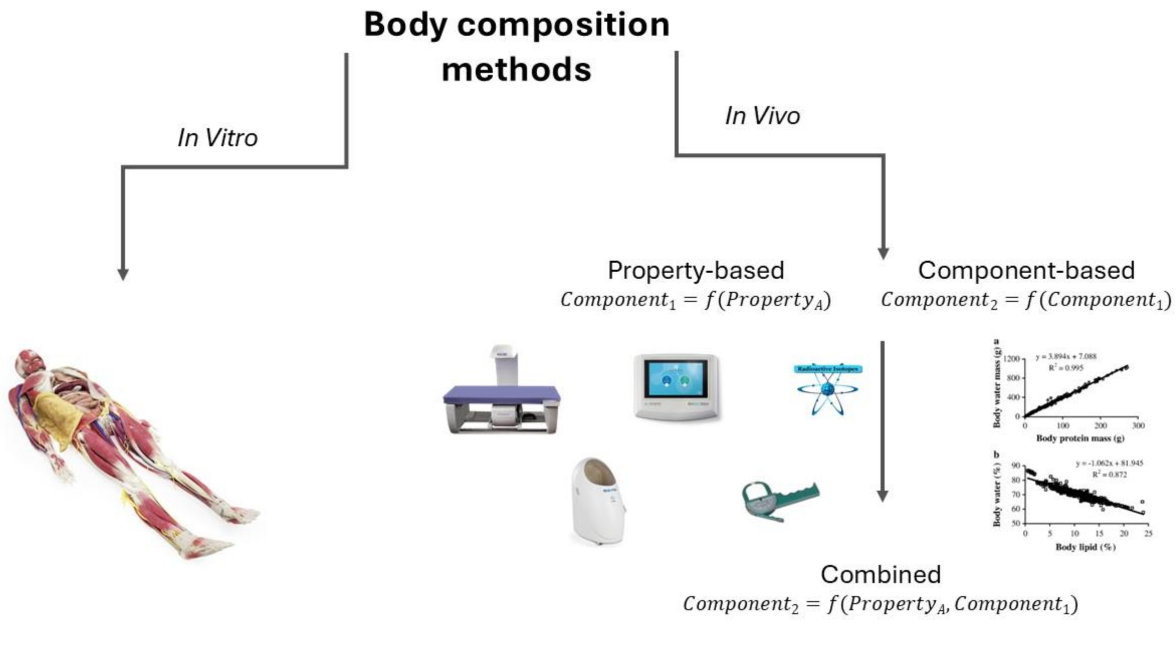

Figure 1.2. Classification of body composition methods. Adapted from: Wang et al., 1995.

Independently of classification, a wider number of components reduces the number of assumptions to meet. A step forward in Behnke's method was made by Siri (1961) who added the water component generating a new three-compartment model that eliminates the individual hydration error. Other investigators upgraded the Siri method by measuring the bone mineral, protein and/or glycogen content, reducing the assumptions but increasing costs, time and spaces needed (Wang et al., 1998). To conclude this paragraph it is crucial to underline that

a) Methods biases depend on the assumption number, sampling, validity and reliability.
b) The errors reduce as the number of measurable components increases.
c) Methods are population and condition-specific, so they must be validated every time internal variables vary.
d) Investigators should account for costs, instruments and time available for the experiments.
e) All in vivo methods are based on relationships between unknown components and known quantities. Property-based methods are fundamental for body composition evaluation.

1.1.2 Property-Based in Vivo Anthropometry

Before concerning method validation, it is crucial to describe some of the most important non-invasive methodologies proposed. Anthropometrical measurements such as circumferences and lengths (figure 1.3) are used to estimate segmental and body dimensions of specific populations, which can be applied for the development of biomechanical models, clothing design and sizing, sports equipment and workplace design, growth and nutrition (Li and Dai, 2006).

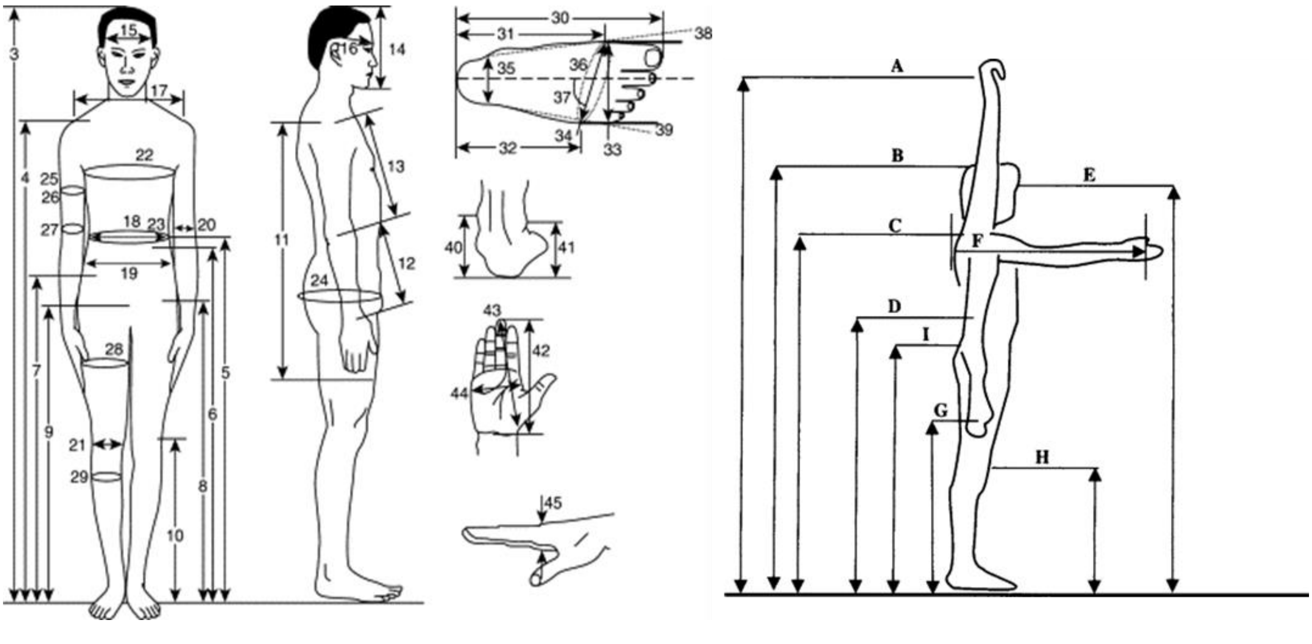

Figure 1.3. Anatomical location of anthropometrical circumferences (left) and length (right). Adapted from Luximon and Zhang, chapter 6.6 (Lin and Dai, 2006).

Of these, the skinfold thickness assessment (figure 1.4) is one of the most used and debated methods in body composition for the easy accessibility of the subcutaneous layer, its non-invasive nature and its central factor in adipose tissue patterning (Clary et al., 1987). Since 1970 more than 503 articles on Pubmed included body composition evaluation with skinfold thicknesses, of which 346 are clinical trials (Pubmed, 2024). Martin and colleagues (1985) stated the steps needed to reach body fat from caliper measurement and the assumptions to meet. To make sense of the skinfold thickness application, it is assumed that subcutaneous fat constitutes a constant proportion of body fat over body mass and that skin sites detected are representative of all subcutaneous fat (figure 1.5). Even if many assumptions are raised and the inter-variability of compressibility (16-51%) depends on skin site, sex, age and level of hydration (Clarys et al., 1987), investigators all over the world use and provide new skinfold thickness equations (Kasper et al., 2021).

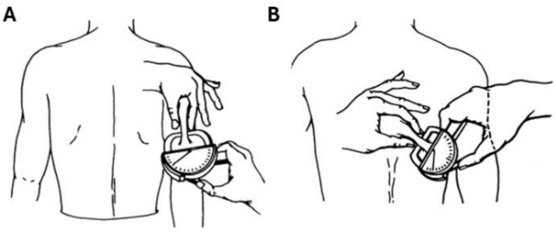

Figure 1.4. Skinfold thicknesses of A) triceps and B) subscapular sections. Adapted from Eaton-Evans (Caballero et al., 1998).

It is now clear that assessing just a single measurement is not enough to describe the individual body composition, and only combining the above-mentioned methods (and others) leads us to insight knowledge and a multi-component view. Sometimes the choice of a method is constrained by the instruments and funding availability, otherwise by time or experience. At all events, any investigator must know and explore how methods are valid and reliable.

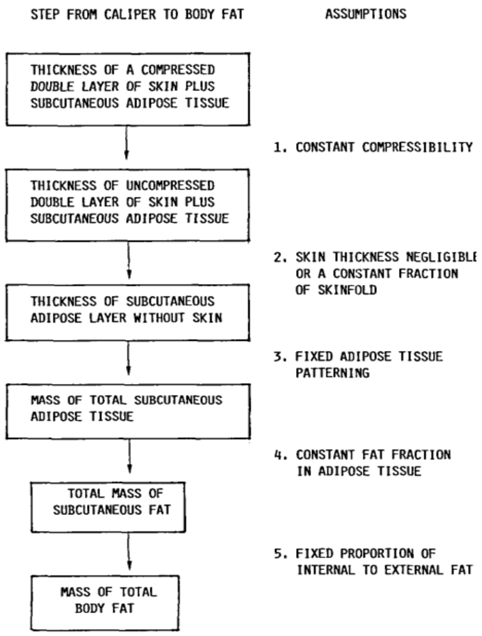

Figure 1.5. Steps and assumptions from caliper to body fat. Adapted from Clarys et al., 1987.

1.1.3 Property-Based in Vivo Bioimpedance Analysis

The Bioimpedance Analysis (BIA) has become very popular during the last two decades for its simple application and portability. Starting from physics electrical models, it aims to transduce electrical tissue properties to clinical information (Piccoli et al., 1994). Technically, BIA's principles are developed on the conductivity properties of biological tissues, quantified as impedance, a combination of electrical resistance and capacitative reactance occurring in response to current flow through the conductors of the human body. Bioelectrical resistance describes how conductors oppose alternate current flow and exhibit a negative relationship with the volume of intra/ extracellular ionic solution, whereas capacitative reactance represents the properties of the cell membrane and its time variation, and possesses a direct relation with the amount of soft tissue structures. As an arctangent of the BIA components ratio, the phase angle is a derived measure widely used in clinics. BIA assumes that the human body possesses a normal ~73% hydration, and could produce biased estimations with abnormalities (Piccoli et al., 1994). In addition, BIA generally assumes that different human shapes, considered conductive cylinders, are arranged in series with each other. As in skinfold thicknesses, many regression models have been developed to predict one or more human body components from the hydration property, in several populations (Campa et al., 2024). However, many instrument characteristics are detectable even if the most used and debated technologies refer to foot-to-hand and segmental applications. In addition, the degree of accuracy and precision of the most popular predictive equations is far from reference methods (Matias et al., 2016) and the estimation may result in bias. Despite this evidence, the advantage of a free-use instrument that does not require highly-trained personnel leads to a worldwide BIA application (Campa et al., 2024). In addition, the vectorial properties developed by Piccoli and colleagues (1994) allow to use of vector BIA (BIVA) as a stand-alone qualitative procedure based on patterns of direct impedance measurement. The described method plots a two-dimensional vector whose displacement represents the ability of body soft tissue to generate impedance (figure 1.6).

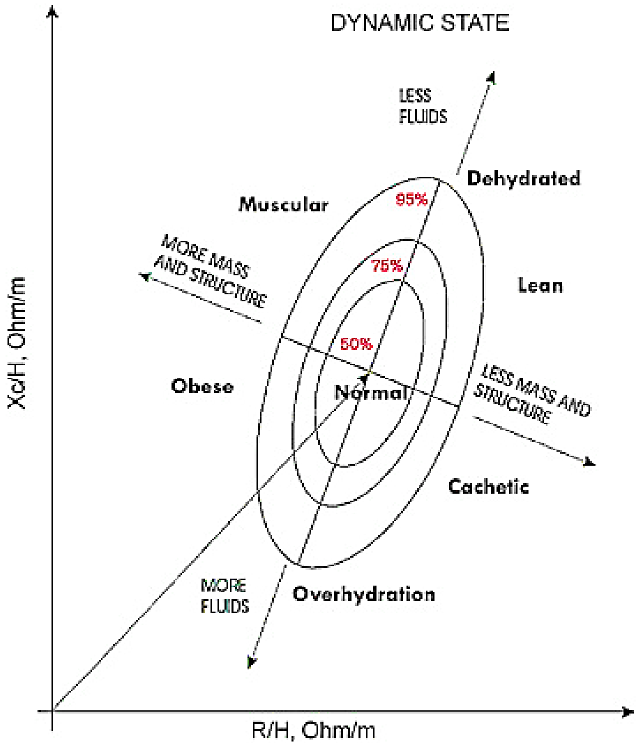

Figure 1.6. BIVA patterns with 95%, 75% and 50% tolerance ellipses. Adapted from Nwosu et al., 2019.

1.1.4 Methods Validity and Reliability

One of the most relevant points to countenance in body composition analysis embraces error sources. In biological systems such as the human body, several sources of variability could influence components, properties and mathematical functions. Before diving into bias details that affect the method validity, it appears crucial to distinguish between two types of errors involved in measurements: the first group includes the random error, whereas the second group is the systematic error (Barraza et al., 2019). Random error is associated with variations resulting from chance and can therefore influence results; it cannot be eliminated but its fluctuation around the real value can converge in mean to zero. It is associated with three factors such as individual inter and intra-variability, sample size and the effect magnitude, and mainly compromises the precision (interval estimation) of a measure and the reliability of the investigation. So, it could be controlled through a correct sample size estimation and an efficient statistical analysis (p-value and confidence intervals). Differently, a systematic error, also called bias, is a measurement tendency to under or overestimate the estimate of real value (point estimate) that can be caused by a deficiency in designing or executing the study. It influences the experiment's internal (study parameter ≠ population parameter) and external validity (study parameter cannot be extended to other populations). Although several biases exist, many of these occur during the first phase of the experiment such as selection bias (selection pressure), allocation bias (absence of randomization) and information bias (misclassification). Also, we can meet bias during the evaluation (measurement bias) and the analysis due to a lack of appropriate confounding variables control (confusion bias). All the systematic errors can be avoided with appropriate study design and expert investigators. Given that random error cannot be missed and depends on chance, we first focus on bias and how to address it in body composition research. For an easier and smoother insight, two sub-paragraphs concerning separately cross-sectional and longitudinal designs follow. Before jumping next, it is useful to report that when it is looking for one measure close to an unknown real value, multicomponent models show accuracy and precision to within 2-4%, while "field" methods account for 3.5-5% (Gatterer et al., 2017). This situation, however, is less relevant if the purpose sets longitudinal changes, where even simpler and cheaper methods can be valuable for monitoring (Marini and Toselli, 2021).

1.1.3.1 Cross-sectional design

The cross-sectional design is extremely used in body composition studies because of its ability to provide an instant picture of some sample (population representative) characteristics. As previously mentioned, the first type of mathematical function involved in body mass compartmentation refers to regression models performed in cross-sectional studies. A recent systematic review (Campa et al., 2024) highlighted 64 studies concerned with body composition estimation by BIA, providing more than 150 equations for segmental and foot-to-hand BIA technology. If we sum the number of models estimated by anthropometrical or other assessments, it is clear the high interest of new validated and reliable methods. However, a rationale question arises: why do investigators need new methods if about more than half past thousands of equations are already available? The answer conceals to every one of us. Each individual reveals internal such as gender, age, ethnicity, and disease and external (ancillary) features such as education, environmental and social influences. In addition, many of these factors can be combined to account for revealing biological characteristics modified by ancillary influences such as diet and physical exercise. For example, the body fat of caucasian adolescent females who played volleyball may differ from that of caucasian adolescent females who played basketball, that of caucasian adult females who played volleyball, and that of Caribbean adolescent females who played volleyball, and so on. If we consider the multifactorial combination of all variables related to biological differences, it appears clear the necessity for new specific methods. Thus, sample traits are valuable in planning experiments. Usually, in cross-sectional design specific common features are shared by the involved participants to limit the inter-subject variability. The most relevant drawback of "prevalence" studies lies behind the sample size. When investigators search for the general population, it is simpler to include many participants and then adjust the regression estimates for variables such as sex and age (Durnin & Womersley, 1974; Sun et al., 2003). It could be more difficult if it is investigated for a specific disease or clinical condition (Potter et al., 2024). The same is faced when looking for general athletic conditions or sport-specific equations (Campa et al., 2023). Milestone studies have been conducted on athletes of various disciplines (Matias et al., 2021; Evans et al., 2005), collecting information from more than one hundred participants. Although external validity was high reducing the random error occurrence, a wider problem of misclassification bias and inter-variability emerged due to the number of different disciplines included. Even if it has been evaluated best basketball, tennis and swimming players (these are just three of about 12 sports) ever, it is not difficult to see differences connected with each physiological demand sport-related. Differently, it is very difficult to control for external validity and obtain a wide sample size when the purpose is to estimate sport-specific equations, but the higher internal validity

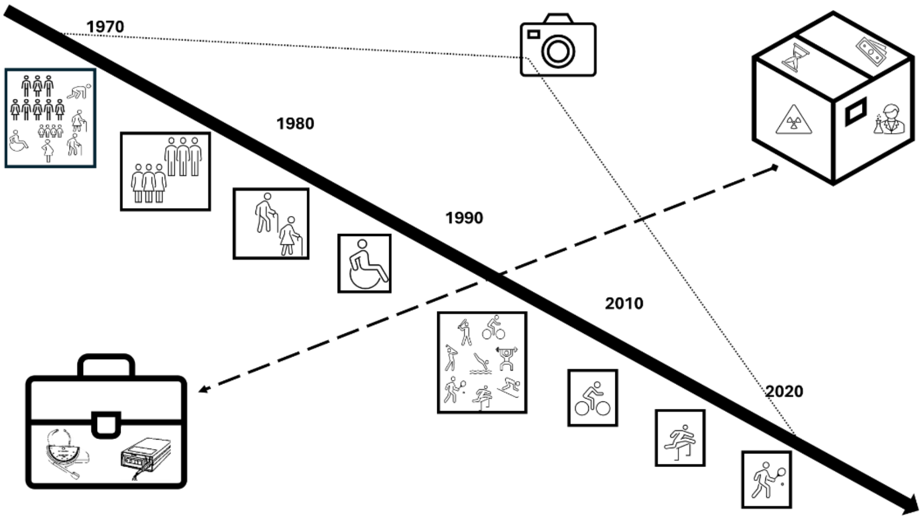

Figure 1.7. Cross-sectional design evolution in the science of body composition.

allows the extension of the results for sport players of the same sex and levels (Giro et al., 2022; Matias et al., 2022). Research is compounded accounting for higher-level compartments that require cheaper laboratories and long assessments. Although the final aim of any research is investigator-dependent, the literature trajectory suggests a trend toward highly specific models (Figure 1.7).

1.1.3.2 Longitudinal study design

Clinical studies collect several types of designs that can be described following a hierarchical scheme, from less to more quality of evidence (Burns et al., 2012).

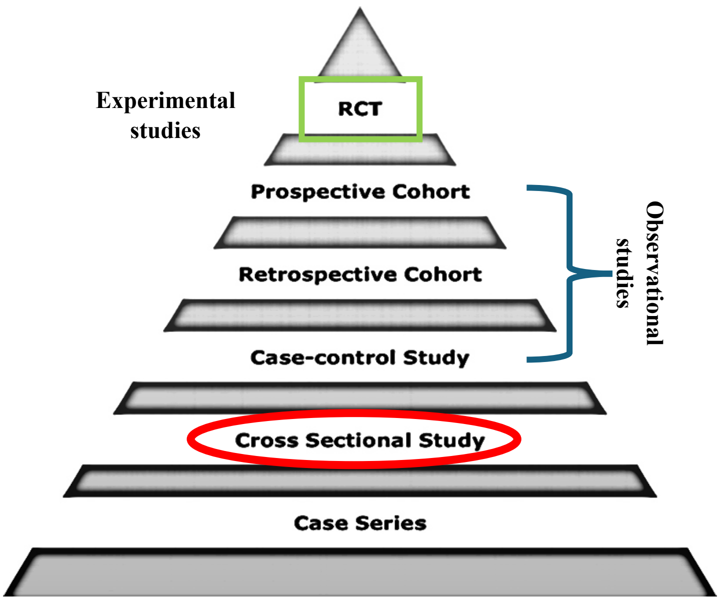

Figure 1.8. The pyramid of evidence. Adapted from Ho et al., 2008.

Figure 1.8 shows that the case report and series deal with the lower step of the pyramid. The main drawback of a case series lies behind its validity and reliability, providing biased and unreplicable pieces of evidence. The absence of a control group under or overestimates the treatment effect for three main reasons: a) regression to the mean, b) participant bias (placebo effect) and c) investigator bias (optimism). The second step holds the cross-sectional study that has previously been commented. Then, it is three levels of observational studies. A case-control study is retrospective and can evaluate the past exposition to some factor outcome-related. It can estimate the association between the selected factor and the event occurrence. It includes a hybrid design (bi-directional) and a case-crossover. However, a case-control study takes advantage of its low costs, small sample size needed, and fastness but it assumes that case and control participants come from the same cohort. Differently, Cohort studies reach for etiological, diagnostic, prognostic and efficacy outcomes and they allow us to select cases and control from different cohorts. In addition to simple factor-event association, cohort studies detect survivorship likelihood and event rate. The difference between retrospective and prospective cohort studies consists of follow-up concurrency, which is expensive and long. Finally, excluding evidence coming from the meta-analysis review, the experimental design ensures the highest quality of investigation. The randomization process resolves the external validity problems held by the selected control and the blinded strategy accounts for the placebo effect. The randomized control trials (RCT) set embraces crossover (within effect), between effect, and within-between effects with parallel harms, with stratified blocks and factorial designs. RCTs are always prospective and require high costs and long follow-ups.

In body composition studies it is often used observational and experimental designs. For example, cohort studies have been performed to find the association between body composition and risk factors for cardiovascular diseases (Carter et al., 2023), while RCT reveals how nutrition and exercise interventions affect body tissues (Eglseer et al., 2023). Although the main healthy treatment affecting individual body composition is retaining lifestyle habits such as diet and physical exercise, many internal and ancillary factors could confound its image or variation over time and need to be accounted for. Thus, the sampling process is fundamental in clinical trials and investigators may have a large experience in evaluating and modelling protocol design. The aim of this thesis is beyond the analysis of diet and pathological conditions, and hereafter the effects of physical exercise and sports on body composition are discussed. A small frame concerned about differences in active or sportive samples is shown.

1.2 Physical Exercise

Human movement is one of the physiological necessities of all living organisms, from cells that migrate to differentiate and reach specific potency (Binder et al., 2009) to humans who look for environmental well-being (McNeill, 1984). Although movement is a general term that englobes thousands of shades, human movement refers to space-time interactions between bodies and world shapes (Klette and Tee, 2008). Each adolescent interacts with nature during her walking to school and her processor (nervous system) instantaneously receives and elaborates information that acts on her next and future behaviours. In this context, human sciences provide several spheres of knowledge from body kinematics to hormonal responses (McArdle et al., 2014). It is well-stated that any movement form requires energy expenditure that is biochemically translated as oxygen consumption, which leads to substrate mobilisation and lysis, reducing organs and muscle reserves (fat, glucose and protein). So, one step more compared to baseline activity needs a metabolic cost (kcal) that is a function of gender, age, anthropometrical traits such as body mass and stature, mechanical features such as arm length and walking yield, environmental characteristics such as weather and field surface, physiological aspects such as hormones and stressors, clothes tips such as shoes and shirt, activity parameters such as intensity and duration, and so on. It follows that a greater amount of kcal consumption modifies individual body composition, but the insight into all possible ways and effects of variable interaction deserves a deeper focus. Before reporting specific responses and adaptations, it is advisable to underline that physical activity is globally renowned as a key preventive treatment factor against cardiovascular and chronic obstructive pulmonary disease, cancer, obesity, diabetes and hypertension, musculoskeletal health and senescence, psychological and mental health (Dhuli et al., 2022). For example, an increase in just one MET (metabolic equivalent, one MET ~ 3.5 ml/kg*min) reduces the hazard of death by 10-25% (Kaminsky et al., 2013), while a decrease of 2.5 standard deviations from the age-specific handgrip strength average is associated with a high risk of sarcopenia (Cruz-Jentoft et al., 2019). It makes sense to ask how an individual could increase her metabolic request or strength. Is any form of physical activity enough?

The correct answer is: no! Despite an active lifestyle being considered better than a sedentary one, smarter is still always the perfect answer. Hereafter, the concept of physical activity needs to evolve into physical exercise, the wise brother of human movement. It refers to any form of planned movement aiming to induce specific benefits. For example, aerobic exercises ameliorate lipid metabolism and increase cardiovascular system efficiency (Wahid et al., 2016), whereas resistance exercise increases protein synthesis and strength (Westcott, 2012). A set of exercises organized and repeated for an established time makes a training session, which induces physiological acute responses. A series of repeated responses divided by adequate recovery generates long-term adaptations that require central and peripheral changes. Both response and long-term adaptations are time-dependent and need progressive stimuli to improve physical abilities. The scheme stress-recovery-adaptation (Figure 1.9) shows that a baseline ability needs new progressive stress over time to induce adaptation (Mukhopadaway, 2021). This mechanism prepares individuals to receive more intensive stimuli and achieve greater abilities. In this application, the stimulus may be translated into training load, while new ability refers to performance. Individuals respond subjectively to the

Figure 1.9. The stress-recovery-adaptation scheme. Adapted from Mukhopadhyay, 2021.

magnitude of the stimulus with an internal and external load. External load means ancillary variables that can be administered to generate the desired stress, such as distance to cover, speed to run or swim, weight to lift or move, number of basket shots, and so on (Stanley et al., 2019). Differently, the internal load describes the individual perception of the external load and depends on exercises and physiological and psychosocial factors. Given that the same external load may trigger differently two individuals, only the rationale combination of external and internal load with recovery translates into positive adaptations and performance improvements. However, during the training periodization, a coach can induce the desired adaptation by modifying the training load (quantitative) or changing exercises (qualitative), but the same exercises with an unvaried training load (retaining load) over time do not improve performance. This leads to the accommodation phenomenon, which states that the response of a biological organism to a constant stimulus decreases over time (Turner, 2014). Beginners may improve with a low training load, whereas experts can exhibit no adaptation even if they train with a heavy training load (principle of diminishing return). The cause-effect relationship between training load and fatigue, recovery and internal balance, and adaptations leads to the super-compensation theory (Zatsiorsky and Kraemer, 1995). In this theory, the response to a training session is seen as a depletion of biochemical substrate that varies according to the substance availability (preparedness). After the restoration period (supercompensation phase), the substrate concentration is expected to enhance above the preparedness level (supercompensation, Figure 1.10 A, b). When intervals are too short (Figure 1.10 A, a), fitness decreases due to accumulated fatigue, while if intervals are too long no adaptation is inducible (Figure 1.10 A, c). If the training load is

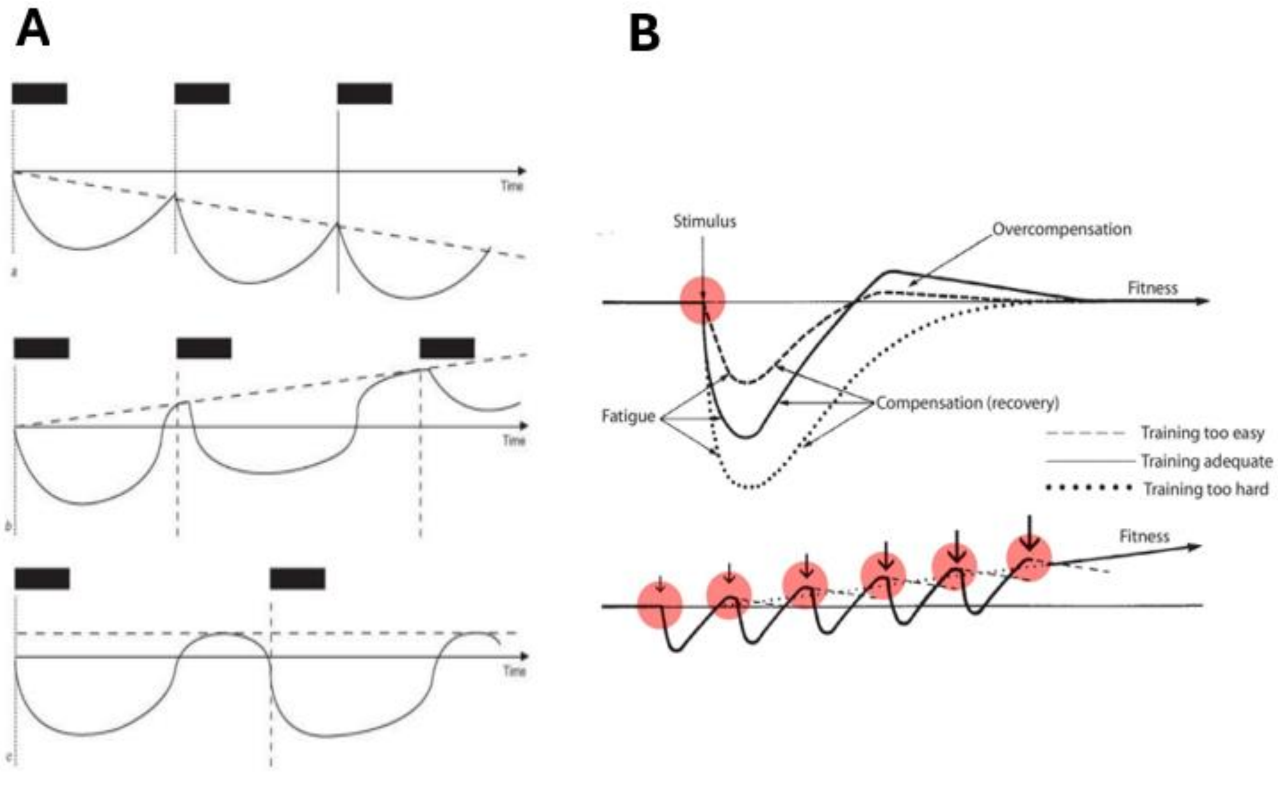

Figure 1.10. The supercompensation theory. Arranged from Zatsiorsky and Kraemer, 1995; Mukhopadhyay, 2021.

inappropriately managed detraining (low load magnitude) or over-training (not tolerable load magnitude) can occur (Figure 1.10 B). Although the supercompensation theory settled the basis for sport and exercise sciences, more sophisticated models have been developed (Rhea and Alderman, 2004). For example, the relationship between phases of fitness and fatigue reported a one to three-factor effect duration, meaning that if fatigue lasts 12 hours, fitness will last for 36 hours (Lorenz et al., 2010), and making individual preparedness as a function of its level before the workout, fitness gain and fatigue effect, and time.

However, during the exercise-induced fatigue phase, both central and peripheral signals can run (Tornero-Aguilera et al., 2022):

- Reduce neural-muscle function and transmission with increased levels of brain serotonin and catecholamine secretion
- Accumulate adenosine in brain regions
- decrease of Ca^{2+} release by the sarcoplasmic reticulum
- depletion of glycogen and phosphocreatine (PCr)
- increase of blood lactate and reduction of contractile properties and pH levels
- decrease in circulating insulin levels
- Increase of reactive oxygen species and hydroxyl radicals that damage myocyte molecules
- increase of muscle inflammation, damage, and pain.

During the compensation phase, relevant time-dependent mechanisms appear (Mukhopadhyay, 2021):

- ATPs are completely stored within five minutes
- PCr is reassembled within 15 minutes
- Muscle damage is stored within two hours to eight days (depending on load)
- Muscle Glycogen is restored within one day, while oxygen consumption following exercise (EPOC) could last up to 38 hours
- Baseline rate of energy expenditure increases up to two days
- Protein synthesis increases
- Thermogenesis increases
- Force-generating capacity and muscle soreness returned within three days.

So, time intervals between consecutive training sessions are selected to reduce the residual fatigue while gaining fitness (periodisation). In conclusion, only adequate training load regularly applied through rationale phases can induce positive metabolic, cardiovascular, respiratory, and neuromuscular adaptations.

1.2.1 Energy metabolism

The aim of the following short paragraph lies beyond a biochemical analysis of human metabolism. It just explains basic information that allows us to link exercise metabolism and body composition adaptation exercise-induced.

Exercise physiology is underpinned by the energy systems that provide the needed energy in response to specific demands. The main molecule involved in energy fuelling is Adenosine triphosphate (ATP), which can be replenished by PCr (phosphagen) shuttle, anaerobic (glycolytic) and aerobic (mitochondrial respiration, O_2 dependent) energy systems (figure 1.11 A). During rest, a human consumes about 1.6 kg of ATP per hour, while strenuous exercise could increase its rate up to 30 kg (De Feo et al., 2002). Due to the low content of ATP stored by each cell (~8 mmol/kg wet weight of muscle), ATP may be regenerated rapidly. The first and most rapid energic source englobes the reaction that transforms PCr, ADP and H^+ into ATP and Creatine (Cr). Any reduction in muscle ATP coincides with the development of fatigue linked to the reduced production of force and power (Baker et al., 2010). Although the high production of protons increases acidosis, the residual AMP increases glycogenolysis and the rate of glucose production, relevant fuel for glycolytic systems. Many sports

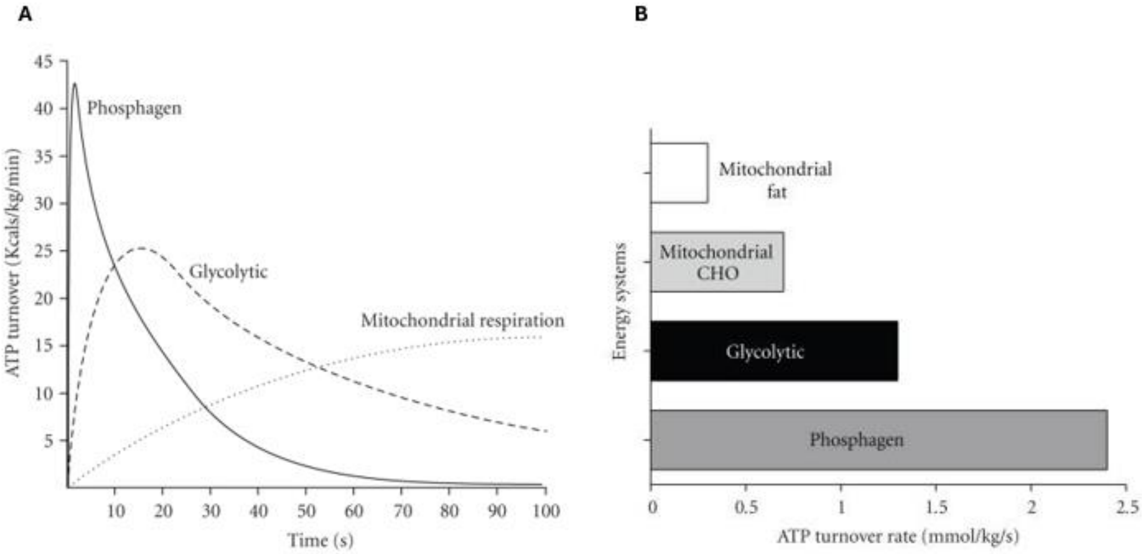

Figure 1.11. A) Energy system interaction and the differences in rates of ATP turnover during exercise and B) ATP regeneration rates from energy systems. Adapted by Baker et al., 2010.

and exercise activities depend on the phosphagen system such as power and team sports. ATP-PCr is considered the main responsible for ATP regeneration during the initial 10-15 seconds of exercise. When the bout is longer than 10 seconds, ATP is increasingly derived from blood glucose and glycogen stores (figure 1.11 A, B). During a 30-second sprint, the phosphagen system accounts for 23% of energy provision, 49% comes from glycolysis and 28% from mitochondrial respiration, whereas during a 10-second maximal sprint, energy is provided by 53% phosphagen, 44% glycolysis, and 3% mitochondrial respiration (Beneke et al., 2002). When glucose is catabolised rapidly (high-intensity exercise) just partial mitochondrial respiration occurs, pyruvate production occurs at a higher rate than pyruvate take-up, and some pyruvates may be converted to lactate (from 2 up to 18 mM). Blood lactate is one of the metabolic residuals used in exercise research to quantify physical fatigue. However, when exercise intensity decreases and is prolonged over time, sufficient oxygen availability allows cells to restore ATP through mitochondrial respiration. The fuel can be obtained from muscle fatty acids, glycogen, adipose tissue fatty acids, or blood glucose. Also, not all pyruvate is converted to lactate, but some are involved in the TCA (tricarboxylic acid) cycle in the inner mitochondrial membrane.

1.3 Sports

Sport has retained the most sophisticated form of human movement. Although physical exercise is a subset applied during sports activities and training, the final goal of any sport is competing to win. The competition requires great physical abilities, conditional capacities, psychological and emotional requirements, and technical-tactical skills. Only the perfect mix of these features leads athletes to discipline success. Sports demands may require specific energy metabolism and body movements (techniques) as physical characteristics are needed (McArdle et al., 2014). Performance in sports characterized by high objects such as the basket (basketball) or net (volley) is elicited by longer arms and taller bodies that make it easy to reach specific goals (Teramoto and Cross, 2017). In addition, leaner composition smoothens speed and jumps (Miller, 2012), generating more force in a restricted time (impulse). Power and strength are two physical conditional abilities highly related to muscle size and length (Balshaw et al., 2021). In sports that require repeated fast movements and low physical contact, where the environment does not require extreme conditions, excessive nonessential body fat provides greater resistance and inertia to motion, reducing the body yield, speed, agility and endurance. The research for the best strength and power-to-mass ratio is exacerbated in sports where

athletes compete in weight classes such as combat disciplines and weightlifting. Differently, sports in thermal extreme conditions and with hard body contacts such as open-water swimming and rugby could advantage of fat to reduce heat dispersion and absorb physical bumps. Apart from body mass proportions, relevant aspects to consider for sports success at all ages are linked to anatomical dimensions and geometrical features. For example, a higher ratio of the tibia to femoral length is correlated with better scores in 400-m international sprinters (Tomita et al., 2020), while longer feet or tibia (relative to height) may be at a mechanical disadvantage for vertical jumping potential (Black et al., 2010). In addition, even when comparing athletes from the same sport, morphological differences appeared among roles and in determining success (Joksimovic et al., 2019).
In synthesis, physical exercise, sports performance and the body structure share unremovable bonds that interact and interfere with human evolution. Evolution depends on adaptability, and only humans can choose what to adapt. Many coveted changes are genetically disallowed, and people cannot train to become 15 cm taller or 50 kg leaner. This puts the focus on what and how we can change. Improving requires tolerance, pain and effort and only with scientific-based evidence can humans gain perfect adaptations.

1.4 Adaptivity: from exercise to body composition

This represents the last paragraph of the introduction, which explains the interaction of all the above-debated factors and time. Although energy metabolisms and training types are often divided for debating scopes, it takes advantage of analysing separately the adaptations induced by the most applied exercises in both healthy and sportive contests. It is clear that in response to exercise, humans alter the phenotype of their skeletal muscle, and the final shift depends on time, training parameters such as intensity, frequency, density and duration, age, gender, experience, diet and genes. The last two factors lie behind the aim of the following thesis and are not debated further. Experience plays a relevant role in the magnitude of changes because sedentary and untrained people report the greatest adaptivity potential, showing the most rapid gains in neuromuscular and cardiovascular profiles (McArdle et al., 2014). A decline in hypertrophy (muscle fibre cross-sectional area) and cardio-respiratory efficiency with advancing age has been well documented (Stathokostas et al., 2004; Paterson et al., 2007), but this does not annihilate the adaptivity of human cells. Periodization is effective in inducing neural and peripheral positive adaptation from adolescence to senescence (Moesgaard et al., 2021). This is evident for both older men and women, despite sex differences have been explored in fatigability (Hunter, 2014). The main differences are linked to body proportion and muscle structure given that male generally possesses a great amount of faster muscle fibres due to different gene expression (Maher et al., 2009). Consequently, men are usually more powerful than women. As previously described, many responses arise after stressors training-induced and the degree of tolerance (fatigability) is stated to be wider in women due to contractile properties (Keller et al., 2011). However, this trend is not linear and depends on movement task (type of fibre contraction), and physiological mechanisms such as muscle mass and strength, blood flow and muscle perfusion, fibre type and muscle metabolism, and neural and hormonal features (Hunter, 2014).

As regards age, several differences appeared from infant to senescent ages. Older adults' physiology goes behind the focus of this thesis, but a quick shade about adolescence and maturation is due. Fast changes in body composition are supposed during the juvenile period and the presence of the "pubertal growth spurt" widely affected them (Marini and Toselli, 2021). From 10 and 12 up to 14 years both girls and boys started a rapid increase in body mass and body dimension, which generally tends to be body fat for females and muscle mass for males. However, two adolescents with the same chronological age may mature differently in terms of biological and somatic features. The range of variability between them is large accentuated around the adolescent growth spurt (Mirwald et al., 2001). These dispersions occur not only in terms of body composition but also cover all the developing physical abilities and capabilities involved in teen ageing. Thus, it is essential to attempt to control for maturity in adolescent studies. Despite the best techniques to assess maturation being not feasible in daily context and exercise practice monitoring, previously validated predictive equations are extremely useful when youths compose the target (Mirwald et al., 2001; Moore et al., 2014). It simply consists of predicting the maturity offset through anthropometrical evaluations and then using it to compute discrepancies among chronological age and age supposed at peak height velocity (growth spurt).

Once the main internal confounders have been accounted for, the last insight is devoted to training parameters and time. It is mandatory to explain that resistance and strength training refers to exercise eliciting neural and musculoskeletal systems, improving the fast fibre recruitment and cell size (hypertrophy), while endurance training refers to exercise eliciting cardiovascular and musculoskeletal systems, improving the body's ability to transport and use oxygen for generating ATP. Resistance training increases the central nervous system's skills in muscle coordination and motor unit synchronization and recruitment and favours peripheral metabolic adaptation and protein synthesis that contributes to fibre cross-sectional area increase. Figure 1.12 shows how neural and structural factors contribute to an increase in maximal strength and its dependence on time (Fleck and Kraemer, 2014). Neural adaptation is fast and can occur after a few weeks of training, providing early improvement in strength (Sale, 1988). Differently, hypertrophy and cell dimension occur after at least four weeks and contribute to strength improvements during the latter period. Generally, eight weeks is considered the lower threshold to induce long-term training adaptations (Folland and Williams, 2007). Resistance training has classically varied the external load and volume to enhance either the neuromuscular drive or muscle CSA, with a load between 1 RM (repetition maximum) to 10 RM and a volume of four to 12 repetitions (Hughes et al., 2018). Generally, a low number of repetitions at maximal external load (1-4 RM) is recommended to enhance neural adaptations, while prolonged mechanical efforts help to stimulate hypertrophy. Despite the main effect on lean tissue and muscular mass, resistance training is effective in reducing body fat (Wewege et al., 2022). The benefits of performing resistance training are numerous for all ages and sexes, positively affecting performance and body composition.

However, the human body undergoes profound adaptation to endurance training. The main effect is direct to muscle oxygenation and the ability to use oxygen for producing energy and delay fatigue.

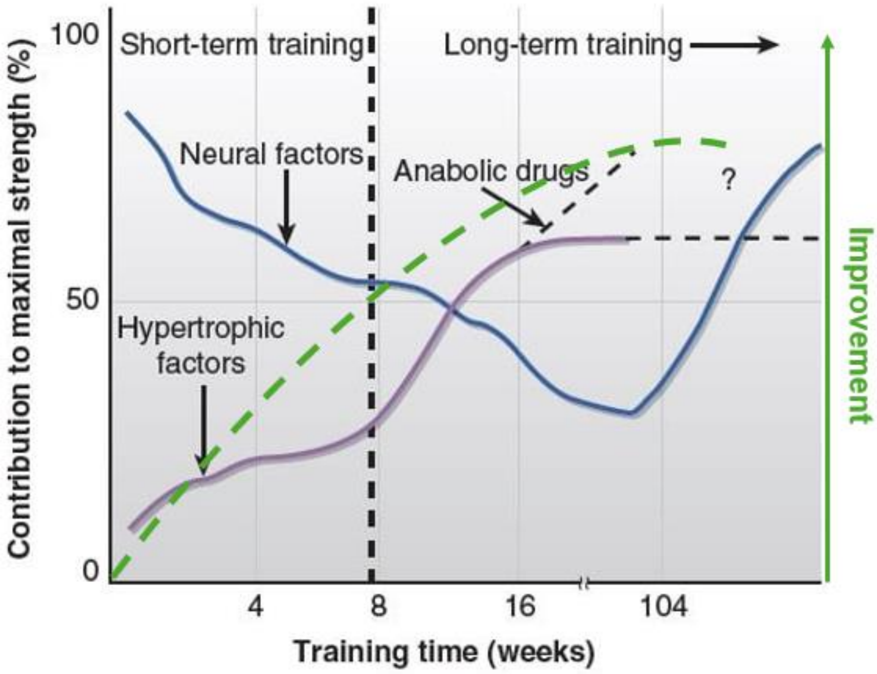

Figure 1.12. The dynamic interplay of neural and hypertrophic factors in improving strength over time (weeks). Adapted by Fleck and Kraemer, 2014.

The availability of oxygen depends on ventilation and only low-intensity contractions allow for constant oxygen consumption (McArdle et al., 2014). Endurance training contributes to an increase in muscle size of its effect on mitochondrial number and dimension (Serpiello et al., 2012). This translated into wider storage and mobilization of energy. Although endurance training progression has been based on volume modulation for a long time, recent studies revealed that low-volume high-intensity aerobic training can elicit cell adaptation similar to high-volume low-intensity one (Daussin et al., 2008). In addition, interval training has been found effective in inducing adaptations earlier (MacInnis et al., 2017). Generally, mitochondrial and capillary changes appear faster (2-4 weeks) than structural heart and myofibrillar (≥8 weeks; Zhou et al., 2024). The biggest percentage change in VO_{2peak} and oxidative potential of fast-twitch fibres is reached after 12 months of endurance training (+ 40% from baseline), while metabolic features such as enzymes involved in the aerobic processes and glycogen availability adapt up to 24 months (Fleck and Kraemer, 2014). The constant or alternate oxidative stress has a direct effect on body composition modelling, and it is supposed the best training for reducing fat mass (Zhou et al., 2024). Differently, the endurance training effect on muscle hypertrophy is limited and related to mitochondrial biogenesis and capillarization.
Can concurrent or combined training elicit both resistance and endurance adaptations? Many researchers investigated this topic and the answer seems to lie behind training intensity and volume (Hughes et al., 2018). Intensity higher than 70% of $V0_{2peak}$ interferes with normal muscle growth for greater caloric deficit that reduces protein synthesis. Figure 1.13 shows the effect of resistance training and concurrent training (combined with endurance) on maximal strength and energy expenditure. It is clear that the right dosage is a mathematical function of the desired goals, and more emphasis could be attributed to neural or mitochondrial changes. Sports coaches modulate training caring about seasonal periods, while trainers enshrine customers' needs. This puzzle question bridges a thousand shapes that only scientific and empirical evidence can smartly connect. Human body composition and performance are two inseparable units, which run on parallel platforms consuming energy from the same powerhouse (cell).

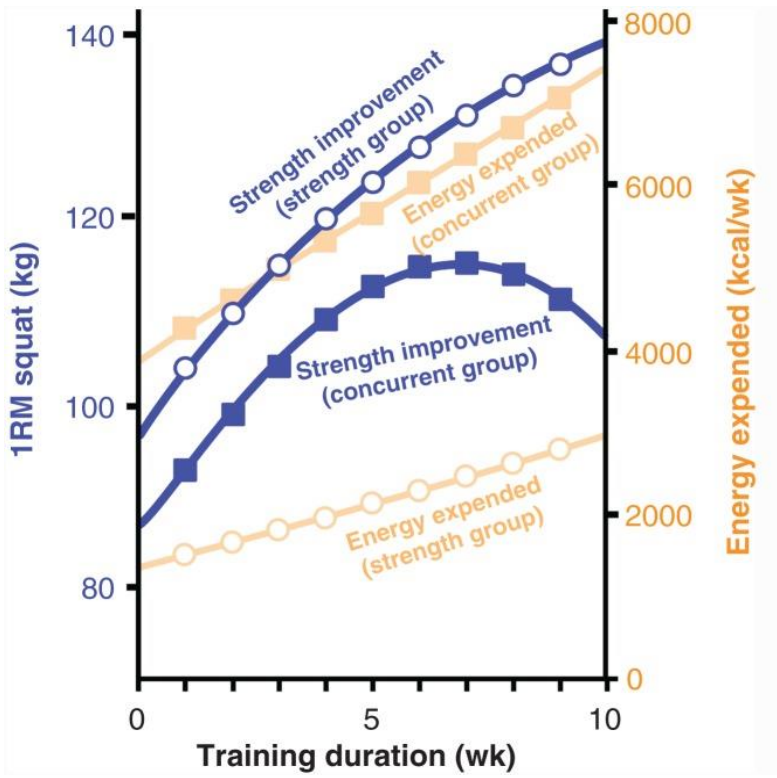

Figure 1.13. Effect on strength and energy expenditure induced by strength and concurrent training. Adapted by Hughes et al., 2018.

"I would rather have questions that cannot be answered than answers that cannot be questioned"

Richard Feynman

2. JUSTIFICATION AND AIMS

The previous introduction details the colours that make a rainbow, a set of spots that may be ordered together. Each investigation scope requires a specific plan supported by an appropriate study design. Studying body composition in sports populations and active people could vary from instantaneous frames that describe the sample characteristics or validate a new method, to longer reels that explore the interaction between time and treatment on biological adaptations. A single-moment picture is informative and supported by differences appearing in individuals who practice the same sport. For example, as previously mentioned, goalkeepers and mid-field players take advantage of different anthropometrical and morphological features and the quantitative dimensions collected and debated can improve the work of football coaches and trainers. In the last century, elite athletes of many disciplines have been tested to report essential physiological and biochemical contents. To date, annals including information about the main adult athlete's body features of the most practised sports such as football, basketball and athletics are available in famous sciences databases. This is a beacon in the veil of the night that lights the streets of thousands of worldwide sports societies, which refers to the best to aim at success. However, the highest level should deliver some biases and misperceptions in a way that involves non-elite athletes inappropriately compared to world champions or in a way that projects adolescent players in an unsuitable context. The drawbacks and relative questions follow:

1) What are the main anthropometrical, morphological and physical characteristics of some team-sport adolescent players?

2) Can these features differ according to the competitive level also in adolescent sports?

3) Can these features differ according to the somatic and biological maturation status?

4) Can these features have a role in sports selection and scouting?

Here it finds justification for the experimental purpose of four cross-sectional studies and one observational retrospective investigation reported in this thesis. In addition, the application and goal of validating a new method to predict body composition traits in a widely played sport with no previous reference for mid-level players set another cross-sectional experiment.

Switching the focus to adult individuals and looking for long-term changes and biological adaptations, the scopes assume prospective directions. The association between prospective and treatment is scientifically justified by the effect generated by the exposition of something new (treatment) prolonged for rationale time. The research questions in sports science could be interesting in comparing a new training protocol to a standard (referee) for validating an easier and/or cheaper (reproducibility) method with similar onset benefits or validating a better and unbiased (reliability) method, for investigating on time-effect, etc. It is mandatory to specify that in sports and exercise sciences one simple parameter such as the training external load or time, a different movement or technique, any supplementary food or drink, and so on, may massively affect both body composition and physical performance results. It appears evident that the contribution of a single research counts as just a drop into an ocean, which has been smaller without that drop. This explains the scope of adding some additional bricks to the wall of two long-debated training methods. The last two investigations find their justification in both body composition and exercise long-term modifications.

"It is not the knowledge, but the act of learning, not the possession but the act of getting there, which grants the greatest enjoyment"

Carl Friedrich Gauss

3. CROSS-SECTIONAL STUDIES

LIST OF PAPERS

STUDY I: *Assessment of Body Composition and Physical Performance of Young Soccer Players: Differences According to the Competitive Level*

STUDY II: *Maturation Selection Biases and Relative Age Effect in ItalianSoccer Players of Different Levels*

STUDY III: *New regression models to predict fat mass in intermediate-level male padel players*

STUDY IV: *Differences in Body Composition and Maturity Status in Young Male Volleyball Players of Different Levels*

3.1 STUDY I

3.1.1 Introduction

Soccer is practised all over the world and has been part of Olympic competitions since 1900. In this team sport there is the requirement for frequent changes in the type of movements (e.g., walking, running, sprinting, jumping, tackling), speed (e.g., accelerations, decelerations), direction, and technical tasks features an intermittent activity profile (Slimani & Nikolaidis, 2019; Stølen et al., 2005). Thus, being a multifactorial sport, players are expected to possess well-developed physical, psychological, technical, and tactical skills. The selection, at the juvenile level, is usually carried out early, with the principal aim to further develop their skills and competencies. Entering into high-level teams is an important milestone for the development of promising players, since recruited players benefit from exposure to élite level coaching, sports science and medical support, training equipment and facilities, and competition (Hill et al., 2020; Johnson et al., 2017; Meylan et al., 2010).

The assessment of the differences between athletes of different competitive levels can provide a better understanding of the specific requirements of élite soccer players, and a valuable insight into what is truly necessary for competitive success in that sport (Stølen et al., 2005). Particularly, anthropometric measures of body composition, and both physiological and physical capabilities, including cardiorespiratory endurance, muscular strength, muscular endurance, and flexibility, are generally assessed through testing of soccer players (Campa, Semprini, et al., 2019; Canhadas et al., 2010; Stølen et al., 2005; Toselli et al., 2020). These measures can complement each other, and their combination may provide soccer coaches and athletic trainers with a better understanding of those characteristics required for successful participation at the elite level. Body composition is closely related to the player's ability to achieve maximum performance in all their actions in the game since it is an indispensable factor for soccer players' physical fitness. High levels of fat act as undesirable weight in motor actions, in which the body mass must be continuously lifted against gravity and may substantially decrease the player's performance. Body fat determines the amount of non-functional inertia that a soccer player must overcome when accelerating and changing direction, thus there is an incompatibility between high fat level and competitive excellence. Low fat levels are related to quicker sprinting, acceleration, and change of direction times and are also appropriate for jumping performance (Dodd & Newans, 2018). Lower limb power is another important capacity in soccer and on average, the maximum performance that a player can achieve in the execution of the vertical jump becomes fundamental. During a game, each player performs about 15 jumps in both defensive and offensive actions (Tereso et al., 2021). In addition, the élite players, on average, travel between 9 and 12 km during a soccer game, which due to its acyclic nature and the attempt to have more ball possessions, can be considered a high-intensity intermittent sport (McMillan et al., 2005; Peñas & Rey, 2012). Thus, the anaerobic metabolism is essential for the performance during the game. The ability to perform quicker sprints and higher jumps than an opponent is crucial in determining the results of duels within a match.

Physical differences and increased aerobic and anaerobic abilities between players are an important element in player selection at the youth level (Barnes et al., 2014; Bradley et al., 2010; Carling et al., 2009; Murr et al., 2018; Reilly et al., 2000; Vaeyens et al., 2006). The differences in these characteristics are evident in the players of the different competitive levels and previous studies on soccer players have disclosed significant differences in anthropometric and fitness measures between playing levels (Gissis et al., 2006; le Gall et al., 2010; Rebelo et al., 2013; Vaeyens et al., 2006). However, the physical demands of élite senior football players have increased rapidly in recent years, and this could affect recruiters and coaches to put greater emphasis on physical fitness from an early age (Barnes et al., 2014). Thus, it is important to have updated information on the characteristics which most influence the performance and few studies have included a comprehensive test battery that evaluates a wide range of physical characteristics. It is important to know the importance of

specific variables and to improve training methods accordingly, to understand which are the most suitable actions to be then transferred to the specific situations of the game (Tereso et al., 2021).
Therefore, the main focus of this study was to value the differences in physical characteristics and physical abilities among the players of two Italian youth teams of different competitive levels, one élite and one non-elite and understand the main factors that differentiate them. This could have practical implications on the trainability or not of the identified components and on the strategies to be adopted.

3.1.2 Methods

Participants and design

A cross-sectional study was carried out on a sample of 191 pre-adolescent boys (age: 13.01±1.15 years): of 162 children attending soccer (from the Under 12 to Under 15 age categories) and belonging to two teams of different levels. The first group (n= 98) was registered with the professional Italian soccer team Bologna Football Club 1909 participating in the first division), while the second group (n= 64) was registered with the Italian soccer team U.S. Russi S.r.L. S.S.D.
The players of the élite group trained for 6 hours a week (four workouts of 1.5 hours each), while the players of the non-élite team trained for 4.5 hours a week (three workouts of 1.5 hours each).
All the subjects volunteered to participate in the study. Written informed consent was provided by the parents before the study began. The study was approved by the Bioethics Committee of the University of Bologna (Approval code: 25027).

Anthropometry

Anthropometric characteristics (height, weight, lengths, widths, circumferences, and skinfold thicknesses) were collected by a trained operator according to standardised procedures (Lohman et al., 1988). Height and sitting height were measured to the nearest 0.1 cm using a stadiometer (GPM, Zurich, Switzerland), and leg length was derived by the subtraction of sitting height from height. Body weight was measured to the nearest 0.1 kg (light indoor clothing, without shoes) using a calibrated electronic scale. Circumferences (relaxed and contracted upper arm, thigh, and calf) were measured to the nearest 0.1 cm with a non-stretchable tape and widths (humerus and femur) to the nearest 0.1 cm with a sliding caliper. Skinfold thicknesses (biceps, triceps, subscapular, supraspinal, sovrailiac, thigh, and calf) were measured to the nearest 1 mm using a Lange skinfold caliper (Beta Technology Inc., Houston, TX, USA).
Body mass index (BMI) was computed as weight (kg)/stature squared (m^2). Body composition parameters (percentage of fat mass (%F), fat mass (FM, kg), and fat-free mass (FFM, kg)) were calculated using the skinfold equations developed by Slaughter and colleagues (Slaughter et al., 1988). The total area (cm^2) of the upper arm (TUA), calf (TCA), and thigh (TTA), the muscle area (cm^2) of the upper arm (UMA), calf (CMA), and thigh (TMA), and the fat area (cm^2) of the upper arm (UFA), calf (CFA), and thigh (TFA) were calculated according to Frisancho (Frisancho, 2008). In addition, arm fat index (AFI), calf fat index (FCI), and thigh fat index (TFI) were derived.

Physical performance tests

The performance tests were implemented at the University sports centre, outdoors on a grassy surface to simulate a game condition. Measures included Yo-Yo, Countermovement Jump Test (CMJ), and 15-meter straight-line sprint. On all 4 days, the tests were preceded by a supervised and standardized warm-up consisting of 10 min of jogging, 5 min of athletic drills including Jumping Jacks, Lateral Skip, High knee walk and Backwards run, and 10 min of dynamic stretching of the lower limbs. Sufficient recovery time of 3 min was allowed between each performance trial. A photoelectric cell timing system (Fusion Sport Smart Speed Timing Gates, Brisbane, Australia) was

used to measure the run tests (Yo-Yo, 20 m sprints), while the CMJs were measured by two photocells (OptoJump®, Microgate, 11 Miller Road, 10541 Mahopac (NY) - U.S.A.).
Yo-Yo consisted of repeated 20 m runs back and forth between the starting, turning, and finish lines at a progressively increased speed, which is controlled by audio beeps from a tape recorder. When the participants failed twice to reach the finish line in time, the distance covered was recorded as the test result. This test consists of 4 running bouts at 10–13 km·h−1 and another 7 runs at 13.5–14 km·h−1, and then continues with stepwise 0.5 km·h−1 speed increments after every 8 running bouts (i.e., after 760, 1080, 1400, 1720 m, etc.) until exhaustion (Krustrup et al., 2003).
To test CMJ each participant was instructed to perform vertical jumps with (CMJ) from an erect standing position with a knee angle of 180°. A countermovement down until the knee angle was around 90° was performed (Ingebrigtsen et al., 2014). The higher values of 2 attempts were used for analysis.
The determination of 15-meter sprint times was performed on a football field and all participants wore training clothing and soccer boots, as in a previous study (Germano et al., 2015). Players were positioned behind the start line (0.5 m) and were instructed to perform the sprint with maximal effort, after a sound start signal. Two trained coaches recorded the time to complete 15 meters using two photocells (OptoJump®, Microgate, 11 Miller Road, 10541 Mahopac (NY) - U.S.A.). Each athlete performed two attempts and the mean result was gathered.
RSA test consisting of six 40 m (20+20 m sprints with 180° turns) shuttle sprints separated by 20 s of passive recovery was assessed as described by Rampinini et al. (Rampinini et al., 2007). The athletes started from a line, sprinted for 20 m, touched a line with a foot and came back to the starting line as fast as possible. After 20 s of passive recovery, the soccer players started again. Sprinting times were recorded with photoelectric cells (Fusion Sport Smart Speed Timing Gates, Brisbane, Australia). The best time (BT) in a single trial and the mean time (MT) were measured. The percentage of sprint decrement (%Sdec) was calculated as follows: 100 – (MT/BT×100).

Bioelectric Impedance Vector Analysis (BIVA)

The impedance measurements were performed with bioimpedance analysis (BIA 101 Anniversary, Akern, Florence, Italy) using an electric current at a frequency of 50 kHz. Measurements were made using four 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 cleansing the skin with alcohol, two Ag/AgCl low impedance electrodes (Biatrodes Akern Srl, Florence, Italy) were placed on the back of the right hand and two electrodes were placed on the corresponding foot (Lukaski & Piccoli, 2012). To avoid disturbances in fluid distribution, athletes were instructed to abstain from foods and liquids for ≥4 hours before the test. Athletes consumed a normal breakfast at 07:00 and the measurements were taken at 11:00. Vector length (VL) was calculated as (adjusted R2+adjusted Xc2) 0.5 and PA as the arctangent of Xc/R x 180°/π. BIVA was carried out using the classic methods, e.g., normalizing R (Ω) and Xc (Ω) for height in meters (Piccoli et al., 1994). Elite male soccer players bioelectrical specific values (Micheli et al., 2014) were used as a reference to build the 50%, 75%, and 95% tolerance ellipses on the R–Xc graph

Statistical analysis

Descriptive statistics (Mean ± Standard Deviation, SD) were calculated for each variable. Variable normality was verified with the Shapiro–Wilk test. When a variable reported a p-value (P) <0.05, a check for curve distribution skewness was assessed. Due to the common right skewed function curve, in all skinfold thickness measurements, a logarithm transformation was applied to meet the bell-shaped distribution.
The student t-test was performed on all anthropometric characteristics and physical performance trials to test the differences between sport groups, and among two categories (U13 and U15) of each soccer

team; the test value (t) and P were reported. When measurement percentage was compared, the Z test of proportion was used.
In order to describe the BIVA results, each team category was plotted in the tolerance ellipses (50%, 75%, and 95%) and 10- to 11, or 12, or 13, or 14- to 15-year-old, healthy male Italian reference population. Compared to our sample, these populations represent the closest references in terms of age (De Palo et al., 2000). Then, the BIVA confidence of each category mean was calculated to compare distances among and between two teams. A two-sample Hotelling's T^2, F, P and Mahalanobis distances (D) were reported. Furthermore, we examined the differences between every group and the Serie A elite players (Micheli et al., 2014).
To select which variable could better discriminate between the two football teams, a Linear Discriminant Analysis (LDA) through the stepwise procedure was performed. Both Fisher's and Mahalanobi's approaches were used (Fisher, 1936; Prasanta Chandra Mahalanobis, 1936). The leave-one-out average posterior probabilities classification was assessed to see how many observations were correctly classified in each group. The MANOVA statistic was performed and the values of Wilk's lambda, Pillai's trace, Lawley-Hotelling trace and Roy's largest root were reported. Due to a high Snedecor-Fisher (F) value and significant P, the univariate ANOVA was computed, and the goodness of fit (R^2 and adj. R^2) F and P were reported for all variables included in the regression model. Because we just had two groups (Bologna and Russi), only one discriminant function was produced; the canonical correlation value, eigenvalue, Likelihood Ratio, F and P were reported. To obtain a projection of the data that gave us maximal separation between the two groups, each standardized (using the pooled within-group covariance matrix) coefficient of the discriminant function was reported. These coefficients are appropriate for interpreting the importance and relationship of the discriminating variables within the discriminant functions, where a higher absolute value indicates an important role of the related variable in the discrimination function. Also, the squared Mahalanobis distance was calculated and the D^2 and P were reported.
A p-value < 0.05 was considered significant. In a within-team analysis where more than one group comparison was performed, a Bonferroni correction was applied to avoid one type-error inflation (α/m, where m = number of comparisons). BIVA software (Antonio Piccoli & Giordano Pastori, 2002) was used for all statistical calculations BIVA related. It allows to plot individuals in the tolerance ellipses (50%, 75%, and 95%) of a reference population. These ellipses are obtained from the literature using the population size, mean, and SD of both R/H and Xc/H, with their linear correlation coefficient. Furthermore, BIVA software allows the calculation of the two-sample Hotelling's T^2 test and the Mahalanobis D, using the same descriptive variables. The Other statistical analysis was performed with STATA® software for Windows 10, version 17 (Publisher: StataCorp. 2021. Stata Statistical Software: Release 17. College Station, TX, USA, StataCorp LP).

3.1.3 Results

Table 3.1 shows the mean and standard deviation of each variable for all categories and the statistical differences within them. Among the anthropometric variables, elite soccer players were generally taller than non-elite peers, with significant differences in U12 and U14.
Elite soccer players U12 presented significantly lower values than non-elite in thigh circumference and femoral diameter, in biceps, triceps and medial and lateral calf skinfolds and calf fat area and calf fat index.
The U13 represented the category which presented the most marked differences between the two groups, since BMI, circumferences (except calf), humeral diameter, skinfold thicknesses, fat mass and the majority of the limb areas significantly differed. In addition, Bologna U13 showed significantly higher PA values than Russi U13. As regards skinfold thicknesses, significant differences were observed between the two groups also in U14, except for the medial calf skinfold. In U15 the differences between the two groups were very small, regarding, in addition to the triceps skinfold, only suprailiac and medial calf skinfolds.

No significant differences result within each category in age, weight (calf circumference, and calf muscle area.
As regards body composition parameters, the fat mass showed a significant difference between elite and non-elite U13 and U14, while fat-free mass did not differ (U12: t= 1.53, P= 0.14; U13: t= -0.53, P= 0.6; U14: t= 1.35, P= 0.18; U15: t= 1.83, P= 0.08). If %F is considered, elite players of all the categories presented significantly lower values than non-elite peers.
Phase angle significantly differed only in U 13, while R/H and Xc/H only in U12.
As regards the physical performance, all the considered variables showed significant differences between the two groups in each age category: the Counter Movement Jump test (U12: t= 2.81, P< 0.01; U13: t= 2.94, P <0.01; U14: t= 3.68, P< 0.001; U15: t= 4.34, P< 0.001), 15 meters sprint test (U12: t= -8.61, P< 0.0001; U13: t= -4.81, P< 0.0001; U14: t= -9.21, P< 0.0001; U15: t= -8.73, P <0.0001), and repeated sprint ability 15 x 15 meters (U12: t= -7.27, P< 0.0001; U13: t= -3.04, P <0.01; U14: t= 2.03, P< 0.05; U15: t= -6.59, P< 0.0001). In addition, the YO-YO test reports significant differences among U14 (t= 10.21, P< 0.0001) and U15 (t= 3.87, P< 0.001) categories.
Table 3.2 shows the mean differences among the F.C. Bologna U13 and U15 and U.S. Russi U13 and U15 categories. Generally, younger categories presented higher values of skinfold measures when compared with elder soccer players. Both U15 categories reported higher values of calf muscle area and FFM than younger players, and the lowest values of fat mass percentage (%FM) and calf fat index. In addition, the elder categories showed better physical performance outcomes in countermovement jump (CMJ), 15-meter sprint and repeated sprint ability (RSA) tests.

Table 3.1. Variable statistics of Bologna and Russi Calcio for each category

Variable	Bo U12 (18)	Ru U12 (16)	Bo U13 (27)	Ru U13 (12)	Bo U14 (30)	Ru U14 (21)	Bo U15 (23)	Ru U15 (15)	Δ U12		Δ U13		Δ U14		Δ U15	
	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	t	P	t	P	t	P	t	P
Age	11.38 (0.36)	11.37 (0.28)	12.35 (0.25)	12.37 (0.33)	13.44 (0.24)	13.49 (0.26)	14.36 (0.31)	14.43 (0.34)	0.10	0.92	-0.19	0.85	-0.78	0.44	-0.61	0.54
Weight (Kg)	41.3 (7.1)	39.59 (8.41)	43.89 (6.15)	48.42 (8.35)	52.98 (8.04)	52.43 (11.08)	62.79 (9.04)	58.63 (13.19)	0.63	0.53	-1.9	0.07	0.21	0.84	1.15	0.26
Height (cm)	153 (8.2)	142.5 (4.77)	154.96 (7.63)	153.9 (9.47)	165.91 (8.28)	160.93 (8.31)	173.3 (8.99)	169.77 (7.92)	4.42	<0.001*	0.37	0.71	2.11	0.04*	1.24	0.22
BMI	17.54 (1.75)	19.42 (3.7)	18.21 (1.53)	20.36 (2.73)	19.14 (1.52)	20.11 (3.31)	20.88 (2.55)	20.21 (3.38)	-1.93	0.06	-3.15	<0.01*	-1.41	0.16	0.70	0.49
Rel. arm circum. (cm)	20.88 (2)	22.04 (3.23)	21.20 (1.58)	23.76 (2.27)	22.94 (1.94)	24.09 (2.88)	24.32 (1.73)	24.54 (3.27)	-1.27	0.21	-4.06	<0.001*	-1.70	0.10	-0.27	0.79
Cont. arm circum. (cm)	22.24 (2)	23.36 (3.25)	23.24 (1.76)	25.38 (2.04)	25.12 (2.13)	26.1 (3.42)	26.66 (3.52)	26.41 (3.39)	-1.22	0.23	-3.33	<0.01*	-1.25	0.22	0.21	0.83
Calf circum. (cm)	30.57 (2.51)	31.13 (3.29)	30.94 (4.06)	33.25 (2.28)	33.99 (4.48)	33.69 (2.93)	34.55 (2.38)	34.93 (3.28)	-0.56	0.58	-1.8	0.07	0.27	0.79	-0.41	0.69
Thigh circum. (cm)	40.86 (3.49)	44.11 (5.35)	40.84 (3.85)	46.63 (4)	45.16 (3.62)	47.6 (5.78)	46.28 (3.88)	48.33 (5.77)	-2.12	0.04*	-4.29	<0.001*	-1.86	0.07	-1.32	0.20
Humeral diameter (cm)	5.88 (0.39)	5.74 (0.42)	6.11 (0.33)	6.07 (0.5)	6.41 (0.33)	6.43 (0.42)	6.71 (0.3)	6.66 (0.41)	0.96	0.34	-4.29	<0.001*	-0.24	0.81	0.46	0.65
Femoral diameter (cm)	8.6 (0.44)	8.94 (0.57)	8.61 (0.51)	8.97 (1.02)	9.27 (0.54)	9.35 (0.55)	9.42 (0.46)	9.73 (0.58)	-1.98	0.05*	0.27	0.79	-0.53	0.6	-1.84	0.07
Biceps SK (mm)	1.67 (0.38)	1.99 (0.40)	1.5 (0.3)	1.92 (0.47)	1.48 (0.28)	1.72 (0.44)	1.28 (0.2)	1.56 (0.37)	-2.35	0.03*	-2.88	0.01*	-2.23	0.03*	-2.71	0.01*
Triceps SK (mm) #	2.13 (0.33)	2.36 (0.30)	2.09 (0.21)	2.36 (0.35)	1.9 (0.3)	2.22 (0.35)	1.85 (0.28)	2.01 (0.38)	-2.16	0.04*	-2.5	0.03*	-3.37	0.001	-1.37	0.18
Subscapular SK (mm) #	1.75 (0.27)	1.88 (0.48)	1.67 (0.17)	2.12 (0.34)	1.8 (0.16)	2.02 (0.4)	1.88 (0.21)	1.88 (0.32)	-0.98	0.34	-4.39	<0.001*	-2.42	0.02*	-0.01	0.99
Supraspinal SK (mm) #	1.79 (0.37)	1.96 (0.54)	1.54 (0.25)	2.05 (0.47)	1.57 (0.22)	1.94 (0.48)	1.67 (0.21)	1.84 (0.41)	-1.11	0.28	-3.49	<0.01*	-3.34	<0.01*	-1.5	0.15

Suprailiac SK (mm) #	2.09 (0.38)	2.29 (0.48)	1.96 (0.27)	2.41 (0.35)	2.04 (0.25)	2.3 (0.44)	2.06 (0.25)	2.25 (0.33)	-1.33	0.2	-3.97	0.001*	-2.45	0.02*	-1.9	0.07
Thigh SK (mm) #	2.35 (0.23)	2.5 (0.28)	2.27 (0.18)	2.46 (0.33)	2.2 (0.21)	2.42 (0.38)	2.12 (0.21)	2.28 (0.37)	-1.76	0.09	-1.96	0.07	-2.4	0.02*	-1.48	0.15
Medial Calf SK (mm) #	1.99 (0.39)	2.31 (0.29)	1.91 (0.28)	2.23 (0.28)	1.88 (0.28)	2.04 (0.38)	1.76 (0.23)	1.96 (0.38)	-2.76	<0.01*	-3.24	<0.01*	-1.64	0.11	-1.8	0.09
Lateral Calf SK (mm) #	2.08 (0.34)	2.34 (0.29)	2 (0.23)	2.27 (0.27)	2 (0.28)	2.17 (0.35)	1.92 (0.2)	2.03 (0.38)	-2.43	0.02*	-3.07	<0.01*	-1.84	0.07	-1.01	0.32
Total Upper area (cm^3) #	3.54 (0.20)	3.64 (0.29)	3.57 (0.15)	3.78 (0.19)	3.73 (0.17)	3.82 (0.24)	3.85 (0.14)	3.85 (0.25)	-1.11	0.28	-3.58	<0.01*	-1.51	0.14	-0.11	0.91
Upper Muscle area (cm^3)	27.98 (6.17)	32.78 (11.24)	28.61 (4.92)	38.08 (8.76)	33.75 (6.5)	39 (11.26)	38.22 (6.32)	40.17 (13.67)	-1.57	0.13	-4.33	<0.001*	-2.1	0.04*	-0.6	0.55
Upper Fat area (cm^3)	7.04 (0.74)	6.69 (0.56)	7.38 (0.68)	7.23 (1.04)	8.44 (0.91)	7.82 (0.98)	9.09 (0.81)	8.57 (0.87)	1.58	0.12	0.53	0.6	2.32	0.02*	1.88	0.07
Upper Fat Index (%) §	20.72 (4.04)	18.20 (4.95)	19.47 (3.48)	16.57 (4.15)	20.3 (2.34)	17.51 (3.95)	19.43 (2.05)	18.54 (4.03)	1.63	0.11	4.14	<0.001*	3.17	<0.01*	0.9	0.37
Total Calf area (cm^3) #	4.3 (0.17)	4.34 (0.21)	4.32 (0.26)	4.47 (0.14)	4.51 (0.22)	4.5 (0.18)	4.55 (0.14)	4.57 (0.18)	-0.49	0.62	-2.41	0.02*	0.21	0.83	-0.33	0.74
Calf Muscle area (cm^3)	52.02 (7.78)	48.09 (8.76)	56.56 (18.29)	58.6 (7.57)	70.46 (26.86)	63.59 (10.17)	74.41 (10.34)	71.85 (8.98)	1.39	0.17	-0.37	0.71	1.12	0.27	0.79	0.44
Calf Fat area (cm^3)	22.86 (7.87)	29.88 (10.78)	20.93 (5.92)	29.81 (8.76)	23.05 (7.38)	27.43 (11.21)	21.07 (5.23)	26.07 (12.34)	-2.18	0.04*	-3.72	<0.001*	-1.69	0.1	-1.73	0.09
Calf Fat index (%) §	29.95 (7.78)	37.66 (7.21)	27.6 (6.51)	33.34 (6.89)	25.01 (6.33)	29.5 (8.38)	22.03 (3.92)	25.62 (7.38)	-2.98	<0.01*	-2.5	0.02*	-2.18	0.03*	-1.96	0.06
Total Thigh area (cm^3)	133.81 (22.36)	157.02 (37.82)	133.96 (23.17)	174.31 (29.36)	163.36 (26.38)	182.96 (44.42)	171.66 (28.2)	188.47 (46)	-2.21	0.03*	-4.53	<0.0001*	-1.97	0.05*	-1.4	0.17
Thigh Muscle area (cm^3)	120.8 (22.2)	144.45 (35.58)	120.27 (22.44)	160.63 (30.37)	147.85 (25.46)	168.88 (45.21)	155.26 (27.49)	172.99 (46.97)	-2.22	0.03*	-4.64	<0.0001*	-2.12	0.04*	-1.47	0.15
Thigh Fat area (cm^3)	13.02 (1.14)	12.57 (1.4)	13.69 (1.83)	13.68 (1.99)	15.51 (1.71)	14.09 (2.88)	16.4 (1.65)	15.48 (2.18)	1.04	0.31	0.01	0.99	2.21	0.03*	1.49	0.15
Thigh Fat index (%) §	10 (1.87)	8.55 (2.5)	10.39 (1.43)	8.15 (2.22)	9.64 (1.22)	8.2 (2.56)	9.72 (1.22)	8.72 (2.4)	1.91	0.06	3.79	<0.001*	2.68	0.01*	1.7	0.1
Fat Mass #	1.72 (0.43)	1.87 (0.52)	1.74 (0.23)	2.16 (0.43)	1.77 (0.26)	2.04 (0.46)	1.76 (0.4)	1.84 (0.53)	-0.85	0.4	-3.19	<0.01*	-2.42	0.02*	-0.45	0.66

Fat-Free Mass	35.21 (5.44)	32.32 (5.58)	38.02 (5.14)	39.02 (6.17)	46.92 (7.44)	43.86 (8.67)	56.5 (7.63)	51.39 (9.53)	1.53	0.14	-0.53	0.6	1.35	0.18	1.83	0.08
%FM §	14.34 (3.94)	17.54 (5.61)	13.29 (1.98)	18.98 (5.71)	11.5 (2.73)	15.94 (5.99)	9.89 (3.11)	11.74 (4.82)	-1.91	0.03*	-3.36	<0.001*	-3.17	<0.001*	-6.94	<0.0001*
Phase Angle #	1.73 (0.1)	1.76 (0.05)	1.89 (0.14)	1.78 (0.06)	1.82 (0.08)	1.8 (0.13)	1.9 (0.08)	1.91 (0.29)	-1.26	0.22	3.54	0.001*	0.42	0.68	-0.19	0.85
R/H (Ω/m)	412.09 (64.21)	511.44 (71.74)	382.83 (49.22)	383.34 (63.66)	330.14 (48.38)	359.6 (65.37)	304.38 (45.02)	312.73 (59.93)	-3.88	<0.001*	-0.02	0.98	-1.85	0.07	-0.49	0.63
Xc/H (Ω/m)	41.73 (6.74)	51.43 (5.14)	44.42 (6.16)	40.03 (7.74)	35.55 (4.6)	38.05 (6.58)	35.36 (4.66)	36.73 (4.72)	-4.67	<0.0001*	1.88	0.07	-1.6	0.12	-0.88	0.39
YOYO test (s)	/	/	/	/	2367.4 (536.9)	787.8 (461.9)	2500 (598.9)	1702.9 (551.3)	/	/	/	/	10.2	<0.0001*	3.87	<0.001*
CMJ test (cm)	27.99 (2.88)	24.04 (5.13)	28.63 (3.86)	24.24 (4.78)	32.77 (2.99)	27.93 (6.27)	36.6 (6.15)	28.57 (4.53)	2.81	<0.01*	2.94	<0.01*	3.68	<0.001*	4.34	<0.001*
Sprint 15m test (s)	2.71 (0.12)	3.18 (0.19)	2.83 (0.11)	3.07 (0.19)	2.51 (0.93)	2.94 (0.23)	2.41 (0.12)	2.76 (0.13)	-8.6	<0.0001*	-4.81	<0.0001*	-9.21	<0.0001*	-8.73	<0.0001*
RSA 20+20m (s)	6.34 (0.22)	7.22 (0.42)	6.57 (0.19)	6.88 (0.39)	5.84 (0.19)	6.64 (0.49)	5.69 (0.20)	6.20 (0.22)	7.27	<0.0001*	-3.04	<0.01*	2.03	<0.05*	-6.59	<0.0001*

Note: Bo, F.C. Bologna.; Ru, U.S. Russi; U12, Under 12; U13, Under 13; U14, Under 14; U15, Under 15; t, student's t; P, p-value; circum, circumference; rel, relaxed; cont, contracted; SK, skinfold; CMJ, countermovement jump; RSA, repeated sprint ability; * statistically significant; Δ difference between; # logarithmic scale; § proportion analysis with the Z-test.

Table 3.2. Mean differences among U13 and U15 categories of each team

Variable	Bo U13 (45)	Bo U15 (53)	Ru U13 (28)	Ru U15 (36)	Δ Bologna (U13-U15)				Δ Russi (U13-U15)			
	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	t	P	95% CI		t	P	95% CI	
Weight (kg)	42.84 (6.58)	57.24 (9.73)	43.37 (9.35)	55.01 (12.22)	-8.42	<0.0001*	-17.79	-11	-4.17	<0.001*	-17.21	-6.07
Height (cm)	154.12 (7.84)	169.12 (9.28)	147.39 (9.06)	164.61 (1.53)	-8.56	<0.0001*	-18.48	-11.52	-7.49	<0.0001*	-21.82	-12.63
BMI	17.95 (1.64)	19.89 (2.19)	19.82 (3.29)	20.15 (3.29)	-4.92	<0.0001*	-2.73	-1.16	-0.39	0.69	-1.98	1.33
Rel. arm circum. (cm)	21.07 (1.75)	23.54 (1.96)	22.78 (2.94)	24.27 (3)	-6.51	<0.0001*	-3.21	-1.71	-1.99	0.05*	-3	0.004
Cont. arm circum (cm).	22.84 (1.9)	25.79 (2.89)	24.22 (2.93)	26.23 (3.36)	-5.85	<0.0001*	-3.95	-1.95	-2.5	0.01*	-3.61	-0.4
Calf circumf. (cm)	30.79 (3.5)	34.23 (3.7)	32.04 (3.05)	34.21 (3.1)	-4.7	<0.0001*	-4.89	-1.99	-2.8	<0.01*	-3.72	-0.62
Thigh circumf. (cm)	40.85 (3.67)	45.64 (3.74)	45.19 (4.9)	47.91 (5.71)	-6.29	<0.0001*	-6.29	-3.3	-2.01	0.05*	-5.42	-0.14
Humeral diameter (cm)	6.02 (0.37)	6.54 (0.35)	5.89 (0.48)	6.53 (0.42)	-7.2	<0.0001*	-0.67	-0.38	-5.72	<0.0001*	-0.87	-0.42
Femoral diameter (cm)	8.60 (0.48)	9.34 (0.51))	8.95 (0.78)	9.51 (0.59)	-7.32	<0.0001*	-0.93	-0.53	-3.29	0.001*	-0.9	-0.22
Biceps SK (mm) #	1.57 (0.34)	1.4 (0.26)	1.96 (0.42)	1.65 (0.42)	2.84	<0.01*	0.05	0.3	2.88	<0.01*	0.09	0.52
Triceps SK (mm) #	2.1 (0.26)	1.88 (0.29)	2.36 (0.31)	2.13 (0.37)	3.97	0.0001*	0.11	0.34	2.57	0.01*	0.05	0.4
Subscapular SK (mm) #	1.71 (0.21)	1.84 (0.19)	1.99 (0.43)	1.96 (0.37)	-3.17	<0.01*	-0.21	-0.05	0.22	0.83	-0.18	0.23
Supraspinal SK (mm) #	1.64 (0.32)	1.61 (0.22)	2 (0.5)	1.9 (0.45)	0.49	0.63	-0.09	0.14	0.81	0.42	-0.14	0.34
Suprailiac SK (mm) #	2.01 (0.32)	2.05 (0.25)	2.34 (0.42)	2.28 (0.39)	-0.63	0.53	-0.15	0.08	0.61	0.54	-0.14	0.27
Thigh SK (mm) #	2.30 (0.21)	2.17 (0.21)	2.49 (0.3)	2.36 (0.38)	3.11	<0.01*	0.05	0.21	1.5	0.14	-0.04	0.07
Medial Calf SK (mm) #	1.94 (0.33)	1.83 (0.26)	2.27 (0.28)	2.01 (0.38)	1.85	0.07	-0.01	0.23	3.08	<0.01*	0.09	0.44
Lateral Calf SK (mm) #	2.03 (0.28)	1.96 (0.25)	2.31 (0.27)	2.11 (0.37)	1.27	0.21	-0.04	0.17	2.41	<0.05*	0.03	0.37
Total Upper area (cm^3) #	3.56 (0.17)	3.78 (0.17)	3.7 (0.26)	3.83 (0.24)	-6.46	<0.0001*	-0.29	-0.15	-2.05	<0.05*	-0.26	-0.003
Upper Muscle area (cm^3)	28.35 (5.4)	35.69 (6.74)	35.05 (10.42)	39.48 (12.15)	-5.87	<0.0001*	-9.81	-4.86	-1.54	0.13	-10.19	1.33
Upper Fat area (cm^3)	7.25 (0.72)	8.72 (0.92)	6.92 (0.83)	8.13 (0.99)	-8.74	<0.0001*	-1.80	-1.14	-5.19	<0.0001*	-1.69	-0.75
Upper Fat Index (%) §	20.74 (3.03)	19.92 (2.24)	17.5 (4.61)	17.94 (3.96)	0.1	0.92	-0.15	0.17	-0.04	0.96	-0.19	0.18
Total Calf area (cm^3) #	4.31 (0.23)	4.53 (0.19)	4.39 (0.19)	4.53 (0.18)	-5.05	<0.0001*	-0.3	-0.13	-2.78	<0.01*	-0.23	-0.04
Calf Muscle area (cm^3)	54.75 (15.04)	72.18 (21.25)	52.59 (9.7)	67.03 (10.41)	-4.61	<0.0001*	-24.94	-9.92	-5.67	<0.0001*	-19.53	-9.35
Calf Fat area (cm^3)	21.70 (6,75)	22.19 (6.55)	29.85 (9.79)	26.87 (11.54)	-0.36	0.72	-3.16	2.18	1.09	0.28	-2.46	8.43
Calf Fat index (%) §	28.53 (7.05)	23.71 (5.57)	35.8 (7.28)	27.88 (8.1)	3.70	<0.0001*	0.2	0.3	4.11	<0.0001*	0.45	0.27
Total Thigh area (cm^3) #	4.88 (0.19)	5.1 (0.17)	5.08 (0.22)	5.19 (0.24)	-6.24	<0.0001*	-0.29	-0.15	-1.97	0.05*	-0.23	-0.001
Thigh Muscle area (cm^3)	120.48 (22.1)	151.07 (26.36)	151.39 (35.63)	170.59 (45.33)	-6.16	<0.0001*	-40.44	-20.73	-1.84	0.07	-40.05	1.64
Thigh Fat area (cm^3)	13.42 (1.61)	15.9 (1.73)	13.04 (1.74)	14.67 (2.67)	-7.3	<0.0001*	-3.15	-1.8	-2.79	<0.01*	-2.79	-0.46

Thigh Fat index (%) §	10.23 (1.61)	9.67 (1.21)	8.38 (2.35)	8.41 (2.47)	0.09	0.93	-0.11	0.12	-0.005	0.99	-0.14	0.14
Fat Mass #	1.74 (0.32)	1.77 (0.32)	1.99 (0.5)	1.95 (0.49)	-0.47	0.64	-0.16	0.1	0.31	0.76	-0.21	0.29
Fat-Free Mass	36.9 (5.39)	51.07 (8.86)	35.19 (6.65)	47 (9.67)	-9.36	<0.0001*	-17.18	-11.17	-5.52	<0.0001*	-16.08	-7.53
%FM §	13.71 (2.93)	10.8 (2.98)	18.16 (5.59)	14.19 (5.85)	4.86	<0.0001*	0.22	0.28	2.76	<0.01*	0.15	0.24
Phase Angle #	1.83 (0.15)	1.84 (0.09)	1.77 (0.06)	1.85 (0.21)	-1.12	0.27	-0.08	0.02	-2.16	0.03*	-0.15	-0.01
R/H (Ω/m)	398.48 (58.3)	318.96 (66.1)	456.54 (93.15)	340.07 (66.54)	7.35	<0.0001*	58.05	101	5.83	<0.0001*	76.56	156.38
Xc/H (Ω/m)	66.49 (9.10)	59.72 (6.03)	67.98 (9.61)	61.52 (8.63)	4.38	<0.0001*	3.7	9.84	2.82	<0.01*	1.89	11.03
CMJ test (cm)	28.35 (3.44)	34.43 (4.97)	24.12 (4.89)	28.2 (5.55)	-6.7	<0.0001*	-7.89	-4.28	-3.06	<0.01*	-6.73	-1.42
Sprint 15m test (s)	2.78 (0.13)	2.47 (.11)	3.13 (0.19)	2.86 (0.21)	12.71	<0.0001*	0.26	0.36	4.96	<0.0001*	2.91	3.04
RSA 15x15m (s)	6.48 (0.23)	5.78 (0.21)	7.07 (0.43)	6.47 (0.46)	14.93	<0.0001*	0.61	0.79	4.69	<0.0001*	0.34	0.87

Note: Bo, Bologna F.C.; Ru, Russi U.S.; SD, Standard Deviation; t, student t; P, p-value; circum., circumference; rel., relaxed; cont., contracted; SK, skinfold; CMJ, countermovement jump; RSA, repeated sprint ability; *, statistically significant; Δ, difference between; #, logarithmic scale; §, proportion analysis with the Z-test; C. I., Confidence Interval.

Bioelectrical Impedance Vector Analysis

Figure 3.1 shows the BIVA confidence outcomes among and between the categories of the Bologna and Russi Football Teams. Picture A displays the differences among each group (U12= Group 1, U13= Group 3, U14= Group 5, U15= Group 7). Only one comparison (U14 vs U15) did not show significant differences (T= 8.1, F= 4, P= 0.02, D= 0.79). A trend with increasing age was observed for the increase of cell mass and tissue hydration. Differently, the comparisons among Russi categories (picture B) reported significant differences only between the Group 2 (U12) and the other groups (vs U13: T= 25.1, F= 12.1, P< 0.001, D= 1.91; vs U14: T= 50.8, F= 24.7, P< 0.0001, D= 2.37; vs U15: T= 79.6, F = 38.4, P< 0.0001, D= 3.21), which indicates that relevant changes are visible up to 13 years old.

Figure 3.1 (C) shows the difference between all Bologna and Russi team players: the elite football team (Bologna) reported better values in terms of cellularity status (T= 15, F= 7.5, P< 0.001, D= 0.62) and hydration. In addition, Figure 3.1 (D) displayed the comparisons between Bologna U12 and Russi U13, U14, and U15, and showed only a significant difference with Russi U15 (T= 25, F= 12.1, P< 0.0001, D= 1.75).

Figure 3.2 (A) shows BIVA confidence distances between adult Serie A football players (data from Micheli) and Bologna (Group 9 vs 10: T= 727.7, F= 362.7, P< 0.0001, D= 3.29), and Russi (Group 9 vs 11: T= 803.8, F= 400.5, P< 0.0001, D= 4.03). Although large significant differences were observed in both for Bologna and Russi teams, the distance was lower for the elite players' teams. Figure 3.2 (B) displays impedance vectors of all categories of Bologna and Russi teams plotted on the 50%, 75%, and 95% tolerance ellipses of adult football players. Younger categories of the elite football team were less distant than those of the Russi team. Also, the differences among the elder and younger categories were more pronounced in elite teams where the body composition of U14 and U15 players were very close to the reference (filled square and diamond).

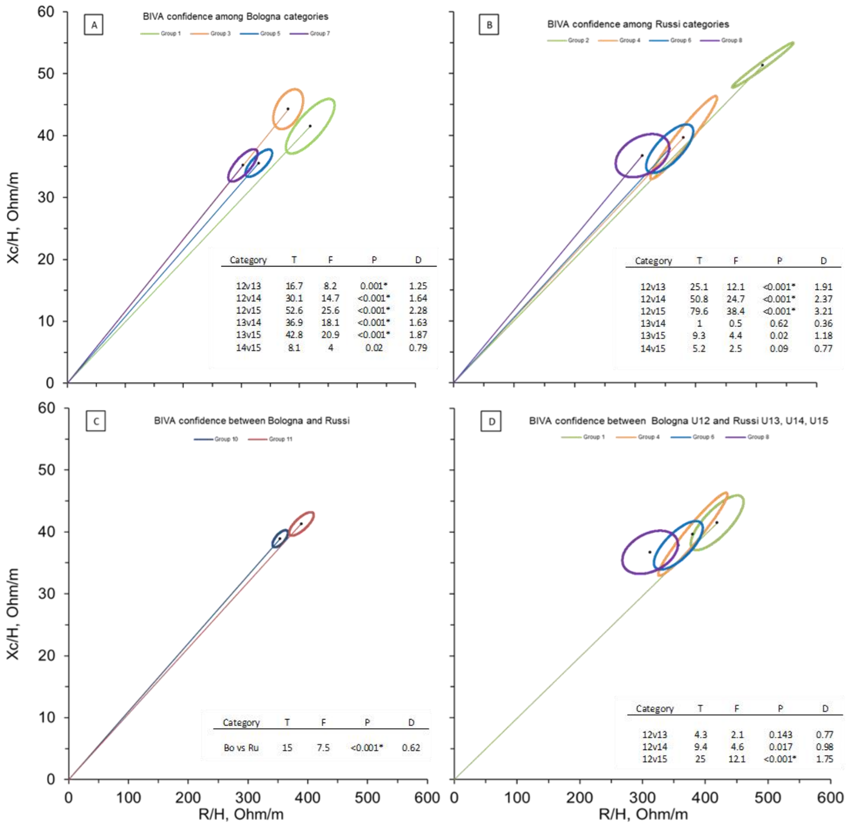

Figure 3.1. BIVA confidence among and between Bologna and Russi categories: A, among Bologna categories (Group 1 = U12, Group 3 = U13, Group 5 = U14, Group 7 = U15); B, among Russi categories (Group 2 = U12, Group 4 = U13, Group 6 = U14, Group 8 = U15); C, between Bologna and Russi mean; D, between Bologna U12 (Group 1) and Russi U13 (Group 4), U14 (Group 6), U15 (Group 8). Note: T, Hotelling T2; F, Snedecor-Fisher test; P, p-value; D, Mahalanobis distance; *, statistically significant.

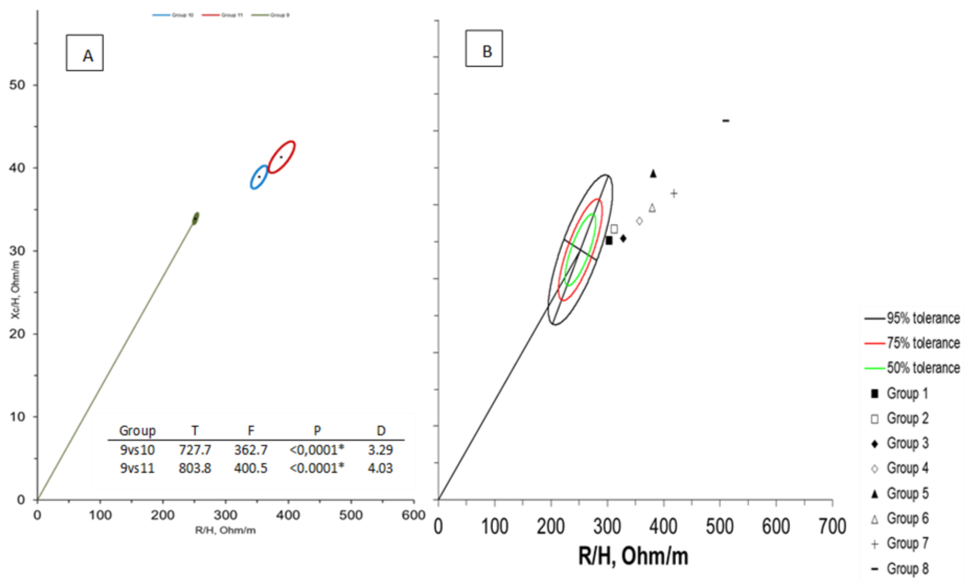

Figure 3.2. A: BIVA confidence between adult Serie A football players (Group 9, green vector), Bologna (Group 10, blue vector) and Russi (Group 11, red vector) adolescent players. B: BIVA tolerance between adult Serie A football players (Micheli vector) and Bologna U15 (Group 1), Russi U15 (Group 2), Bologna U14 (Group 3), Russi U14 (Group 4), Bologna U13 (Group 5), Russi U13 (Group 6), Bologna U12 (Group 7), and Russi U12 (Group 8).

Linear Discriminant Analysis (LDA)

To report the outcomes of the LDA, table 3.3 shows the Leave-one-out average-posterior-probabilities classification in which the total average posterior probability for the Bologna Team is 88.3% and for the Russi Team is 82.9%. Table 3.4 shows the MANOVA and univariate ANOVA summaries. Although all variables present significant outcomes, the motor tests exhibit higher values of the goodness of fit, followed by triceps, biceps, suprailiac and medial calf skinfold thicknesses. In addition, table 3.5 shows the canonical LDA function and the coefficients standardized using the pooled within-group covariance matrix. The canonical correlation equals 0.717 and the 15-meter Sprint reports the highest absolute value (-2.39) that indicates the most contributory factor in discriminating between the teams, followed by the RSA 20+20 meters test (1.26), suprailiac skinfold (-0.5) and CMJ test (-0.45) as reported by the ANOVA outcomes. Finally, we calculate the squared Mahalanobis distance which is equal to 4.49 ($F_{(7, 128)} = 19.37$, $P < 0.0001$).

Table 3.3. Leave-one-out average-posterior-probabilities classification

		LOO Classified	
	True	Bologna	Russi
Number	Bologna	72	14
Average posterior prob.		0.896	0.729
Number	Russi	8	42
Average posterior prob.		0.76	0.862
Number	Total	80	56
Average posterior prob.		0.883	0.829
	Priors	0.5	0.5

Note: prob., probabilities; LOO, Leave-One-Out

Table 3.4. MANOVA and univariate ANOVA summaries

MANOVA (n=136)				
Statistic	value	df	$F_{(7, 128)}$	P
Wilks' lambda (W)	0.486	1	19.37	<0.0001*
Pillai's trace (P)	0.514		19.37	<0.0001*
Lawley-Hotelling trace (L)	1.059		19.37	<0.0001*
Roy's largest root (R)	1.059		19.37	<0.0001*
Residual		134		
Total		135		

ANOVA (n=136)							
Variable	Model MS	Resid MS	Total MS	R^2	Adj. R^2	$F_{(1, 134)}$	P
Triceps SK	1.45	14.89	14.79	0.089	0.082	13.06	<0.001*
Biceps SK	0.88	6.64	6.6	0.117	0.111	17.78	<0.0001*
Suprailiac SK	1.6	15.19	15.09	0.095	0.088	14.09	<0.001*
Medial Calf SK	1.45	13.91	13.82	0.095	0.088	14.01	<0.001*
CMJ test	691.70	3901.16	3877.39	0.151	0.144	23.76	<0.0001*
Sprint 15m test	4.27	6.25	6.23	0.406	0.401	91.55	<0.0001*
RSA 20+20m	12.16	28.71	28.59	0.298	0.292	56.78	<0.0001*

Note: df, degree of fredom; MS, Mean Squared; Resid, Residual; n=number of observation; *, statistically significant

Table 3.5. Canonical LDA and Standardized function coefficients

Function	Canon correl.	Eigenvalue	Variance	LLR	$F_{(7, 128)}$	P
1	0.717	1.06	1	0.486	19.37	<0.0001*
Standardized function coefficients						
Triceps SK	Biceps SK	Suprailiac SK	Med Calf SK	CMJ	Sprint 15m	RSA 20+20m
0.23	0.02	-0.5	0.04	-0.45	-2.39	1.26

Note: LDA, Linear Discriminant Analysis; Canon. correl., Canonical correlation; LLR, Likelihood Ratio; df, degree of freedom; *, Statistically significant

3.1.4 Discussion

The present study aimed to value the differences in anthropometric characteristics and physical performance among the soccer players of two Italian youth teams (U12-U15) of different competitive levels, one élite and one non-elite, and understand the main factors that differentiate them, to infer the specific requirements of élite soccer players and what is truly necessary for competitive success in this sport.

As regards the anthropometric parameters, the selected players were generally taller compared to their non-selected counterparts, even is significant differences were observed only in U12 and U14. This is in line with previous research showing that adult players attaining higher levels of play were, on average, substantially differentiated from amateur players in height as well as body mass (le Gall et al., 2010; Nughes et al., 2020).

Of particular note is that U13 elite soccer players presented the most marked differences in comparison with low-level peers, since BMI, circumferences (except calf), skinfold thicknesses, fat mass, %F and the majority of the limb areas significantly differed from their low-level counterparts. In addition, Bologna U13 showed significantly higher PA values than Russi U13, indicating better cell integrity and functionality. This suggests that this category, probably because of the differences linked to the particular period of growth, is the one that deserves special attention.

Among the anthropometric characteristics, the results of this study showed that skinfolds are the parameters that differ most between the two groups. This confirms the importance of monitoring body fat, as appropriate fat levels enable players to move more effectively during training and games (Bernal-Orozco et al., 2020; Toselli et al., 2021). In particular, the triceps skinfold showed significant differences between competitive levels in each category. Apart from U13, significant differences were observed between the two groups also in U14, except for the medial calf skinfold.

In U15 the differences between the two groups were very small, regarding, in addition to the triceps skinfold, only suprailiac and medial calf skinfolds. This seems to suggest that with the maturity approach the differences between the players of the two teams become more attenuated.

Considering the difference between elite and non-elite groups, from the results emerged that all the physical performance variables showed significant differences between the two groups in each age category. This confirms that high-intensity activities are fundamental aspects of performance in soccer (Faude et al., 2012; Nughes et al., 2020). The elite players were capable of higher acceleration over 15 m than non-elite players. This is in accordance with previous studies which showed that elite players tended to present better sprint performances and change of direction than non-elite ones (Nughes et al., 2020; Rago et al., 2020; Rebelo et al., 2013). The differences in sprint time could be connected to the fact that elite players predominantly perform their high-speed runs over short distances during the match (Baptista et al., 2018). The better results in RSA showed by the elite player in comparison with non-elite is in accordance with previous studies carried out on players of different age groups (Coelho E Silva et al., 2010; Impellizzeri et al., 2008; Rampinini et al., 2007). In the current study, U14 and U15 elite players presented longer distances covered during the Yo-Yo test compared to non-elite ones.

Concerning age, both elite and non-elite players showed a growing trend for some anthropometric measurements and all the physical tests: the under-15 division registered higher physical performance and better body composition values than the other categories, especially in fat-free mass. Also, fat mass percentage (%FM) decreased with age in both groups. This trend is in agreement with the observation of Slimani and colleagues, who reported that, as compared to the older groups (U-17, U-19, and Pro2), the U-15 players have a significantly higher % of body fat (Slimani & Nikolaidis, 2019). Many authors found a negative high correlation between the body fat percentage and physical performance in elite players, which could indicate that body composition impacts performance outcomes (Aurélio et al., 2016; Leão et al., 2022; Toselli et al., 2021). Elite players of all the categories considered in the present study showed a significantly lower %F than non-elite players. Previous studies on soccer players have provided significant differences in %F between soccer players of

different levels (Slimani & Nikolaidis, 2019). The overall of % of body fat mean values reported in the scientific literature vary between 9.9 and 11.9% for the male elite, and between 12.4 and 16.5% for amateur senior soccer players (Slimani & Nikolaidis, 2019). As regards %F, elite and non-elite U13 and U14 player fall within their respective ranges.
In terms of phase angle, elite soccer players showed generally higher values than non-elite, even if, as already reported, the differences were only significant for U13. Apart from U12, the mean PA values found in elite players of the present study were comparable to those reported in prior studies with age-matched male athletes (U-13–U-17: range = 6.2–7.0°) (Campa, Silva, et al., 2019; Koury et al., 2014, 2018; Toselli et al., 2020), and higher than those reported by Martins and collaborators on U13 and U15 Brazilian professional soccer (Martins et al., 2021). It has been shown that PA is an objective indicator of cellular health, with higher values reflecting better cellularity, cell membrane integrity, and cell function, while lower PA values can indicate decreased cell integrity (Martins et al., 2021). Considering that PA is a measure derived from R and Xc, any alteration in cellular membrane integrity (Xc), body fluid (R), or a combination of both, results in changes in PA.
As regards BIVA, the present study showed significant differences in confidence ellipses among and between the categories of elite and non-elite soccer teams. Among the age categories, a trend was observed for the increase of cell mass and tissue hydration, especially in elite teams where significant distances were found in all comparisons (except between U14 and U15 groups). Differently, the comparisons among non-elite team categories reported significant differences only between U12 and the other groups, which indicates less differentiation between age categories and greater homogeneity. As regards the differences between the categories, the elite soccer team reported better values in terms of cellularity status and hydration than the non-elite team. In addition, the comparisons between elite U12 and non-elite U13, U14, and U15, carried out to understand whether BIVA outcome could be a relevant selection criteria and parameter to scout among adolescent football players, showed only a significant difference with non-elite U15, which indicates that elite adolescent players exhibit similar cellular composition when compared with elder players of the non-elite team. BIVA confidence distances between adult Serie A football players (data from Micheli, [31]) and elite and non-elite groups showed that although significant differences were observed both for elite and non-elite teams, the distance was lower for the elite players team. The younger categories of the elite soccer team were less distant from the reference ellipses than those of the non-elite team, which may indicate that the elite team has strict selection criteria or begins the scouting process earlier. Also, the differences among the elder and younger categories were more pronounced in the elite team where the body composition of U14 and U15 players were very close to the reference.
The second aim was to identify the minimum set of predictors that best discriminate the elite and non-elite groups, to provide important and useful information that may help coaches improve the development and selection of young players, as well as to increase success opportunities in their training sessions and competitions. The variables that best discriminated the two groups were Sprint 15m, RSA, and CMJ, in terms of physical performance, followed by suprailiac, triceps, medial calf and biceps skinfold thicknesses. The Sprint 15m reports the highest absolute value (-2.39) which indicates the most contributory factor in discriminating between the two groups, followed by the RSA 15x15m test, suprailiac skinfold and CMJ test. Therefore, coaches and practitioners should consider these characteristics in the talent identification and development process. It is important to note that, apart from motor tests, skinfold thicknesses may well guide training programs, having the potential to associate with competitive level and match performance. Nughes and colleagues in their study on the anthropometric and functional profile of selected vs. non-selected 13-to-17-year-old soccer players found that that dribbling skills, 15-m sprint time, and height best discriminate U17 players by competitive level (Nughes et al., 2020). Contrarily to the results of the present study, anthropometric characteristics and functional abilities could not discriminate across competitive standards between younger males (U15), but only U17 soccer players.
The results of the present study could have practical implications on the trainability or not of the identified components and on the strategies to be adopted. However, it must be taken into

consideration that the selection of soccer players is a strongly debated issue since scouts do assess and advise on the selection of players at younger ages. According to Bergkamp scouts are aware of the idea that early indicators of later performance are often lacking or hard to predict, given the difficulty of predicting future performance directly (Bergkamp et al., 2021). Scouting in the younger age cohorts could be more affected by the finding of the best current player, rather than finding the best player for the future (Ford et al., 2020). This approach seems to rely on the assumption that the best current young players are also those who have the highest potential for excellence in the future. In any case, even on the youngest, this study reveals useful suggestions on the most informative parameters for selective purposes.
The main limitation of this study is the lack of an assessment of biological maturation. This study investigated 12 to 15-year-old players who were homogeneous in terms of chronological age, and the growth and maturation process could have interfered with their anthropometric characteristics and physical test measures. Furthermore, we did not consider playing position, but it is to consider that the physical demands that characterize the specific positional roles require soccer players to adapt to meet them, influencing their characteristics. Finally, we did not compare groups with the same sample dimension and no randomized group allocation was applied during the sampling process.

3.1.5 Conclusion

Elite soccer team players present better anthropometric characteristics and higher physical performance levels than non-elite players. However, age plays a key role in increasing the body composition and capabilities of both elite and non-elite soccer players. Despite this, elite youngest players reveal BIVA outcomes closer to older groups and it may be a relevant selection criterion to scout among adolescent soccer players. Although physical performances are the most discriminant factor between elite and non-elite teams, body composition deserves a greater focus in scouting research.

3.2 STUDY II

3.2.1 Introduction

Soccer is practised all over the world, and male soccer players are among the most studied groups of athletes in sports sciences (Campa, Semprini, et al., 2019; Cossio-Bolaños et al., 2021; Mohr et al., 2021). Soccer players at a high level require highly developed physical capacities, psychological factors, and perceptual, cognitive, and motor skills such as running, jumping, heading, kicking, passing, dribbling, and balance (Akpinar, 2022; Kokstej, 2019; Rommers et al., 2019). To achieve these goals, soccer academies play a fundamental role, as they guide the long-term development of young soccer players, with the main goal of further developing their skills and competencies. Youth selections are made early, to identify and develop talented individuals to compete at senior levels (Radnor et al., 2021). The possibility of joining high-level teams is an important opportunity for developing promising players (Meylan et al., 2010), which is demonstrated by the significant differences between the elite and non-elite players in the youth category in physical and physiological characteristics (Itoh & Hirose, 2020).

Among the factors affecting both players' selection and performance in youth soccer players, two of them play a fundamental role: the relative age effect (RAE) and biological maturation.

RAE refers to the asymmetric distribution of dates of birth in favour of players born at the beginning of the reference year concerning peers born at the end of the same year; players within the same age group can be by almost twelve months apart in chronological age. RAE has been demonstrated within different elite youth soccer academies (Hill et al., 2020; Rubajczyk & Rokita, 2018; Skorski et al., 2016; Teixeira et al., 2022). Many studies affirm that a relatively greater age represents a performance advantage in experience and major physical, neural, motor and/or psychosocial maturity (Helsen et al., 2005; Hill et al., 2020; Simmons & Paull, 2001; Wattie et al., 2008). Therefore, there is an over-selection of relatively older players. These players are more likely to be identified as talented and recruited into academies and consequently provided with greater support and investment in their development (Delorme et al., 2010).

Biological maturation can be defined as the timing and tempo of progress to achieving a mature state (Malina et al., 2004; Towlson et al., 2021). Maturity status is an important factor in the physical development of young players, especially concerning their body composition, physical capacities and match-running performances (Campa, Silva, et al., 2019; Di Mascio et al., 2020; Toselli et al., 2020). Understanding the role of maturity on physical characteristics and performance in youth soccer players during adolescence is essential since this period coincides with the selection of players. Furthermore, it is important to consider that the physical demands of elite senior soccer players have increased rapidly in recent years, and this could cause recruiters and coaches to put greater emphasis on these aspects and physical fitness from an early age (Barnes et al., 2014).

As far as we know, the association between RAE and maturation and their relationship with anthropometric characteristics, body composition and physical performance during adolescence, when players are selected at various competitive levels, have not been fully evaluated. Differences in maturity status and relative age have been identified in previous investigations, along with a considerable variation in timing and rate for physical and biological maturation (Johnson et al., 2017; Towlson et al., 2017). Recent research reported that RAE and maturity status-related selection biases are separate processes and as such should be considered independently, but concluded that further research is required to better understand the nature and sources of the selection biases and how they may be used to optimize the opportunity for all youth players (Hill et al., 2020).

Thus, it is important to have an updated picture of how RAE and biological maturity affect the choices of players in teams, considering also what happens in teams of different levels, and how these two aspects affect the differences in anthropometric characteristics, body composition and physical performance of the players.

Therefore, this study aimed to evaluate the differences in RAE and biological maturity among the players of two Italian youth teams of different competitive levels, one elite and one non-elite and to understand the interaction effects amongst maturation status, and birth quartiles on physical characteristics and physical abilities of the players of the two groups. We assume that we will find a selection bias towards players who are born earlier and who are in an advanced maturity status in elite-level players than their lower-level peers. In addition, we hypothesize that they also exhibit superior physical characteristics such as body composition and performance.

3.2.2 Methods

Participants and Study Design

The design of the presented study was a cross-sectional experiment. The players of the two teams were examined on two different days in December 2021, from 10 a.m. to 6 p.m. Before enrolling the participants in the study, all the adolescents and their parents were informed about the experimental procedures and risks, and they could voluntarily decide to participate in the study. Although 191 samples were first enrolled, only 162 participants (13.01 ± 1.15 years) completed all the evaluations. No randomization was adopted. The Bologna (elite) Football Club 1990® registered 98 attending soccer players who were divided into four categories (U12= 18; U13= 27; U14= 30; U15= 23), while the Russi (non-elite) Sports Union 1925® registered 64 players (U12= 16; U13 = 12; U14= 21; U15= 15).

The researchers did not collect information on diet attitudes. Also, no further information than the hours and frequency of training were collected. The Bologna's young players trained four times per week for a total of six hours, whereas the Russi's players trained three times per week for a total of four and a half hours.

Written informed consent was provided by the parents before the study began. The study was approved by the Bioethics Committee of the University of Bologna (Approval code: 25027).

Anthropometry

Three trained researchers cooperated and assessed the anthropometric evaluations according to standardized procedures (Lohman et al., 1988). Height and sitting height were measured to the nearest 0.1 cm using a stadiometer (GPM, Zurich, Switzerland), and leg length was derived by the subtraction of sitting height from height. Body weight was measured to the nearest 0.1 kg (light indoor clothing, without shoes) using a calibrated analogue scale. Circumferences (relaxed and contracted upper arm, thigh, and calf) were measured to the nearest 0.1 cm with a non-stretchable tape and widths (humerus and femur) to the nearest 0.1 cm with a sliding caliper, both on the left side of the body. The upper arm circumference was taken at the mid-point between the shoulder acromion and the olecranon process point, with the participant's elbow relaxed along the body side (relaxed evaluation) or to be flexed 90° with palm facing upward (contracted evaluation); the thigh circumference was taken at the mid-point between the inguinal fold and the superior rotula point, with the participant in a standing position (thigh muscles relaxed); the calf circumference was taken at the bulkiest calf point, with the participant in a standing position (calf muscles relaxed); the humerus and femoral widths were taken, respectively, between the own lateral and medial epicondyles, with participants elbow and knee flexed 90°. Skinfold thicknesses (biceps, triceps, subscapular, supraspinal, sovrailiac, thigh, and calf) were measured to the nearest 1 mm using a Lange skinfold caliper at the left side of the body (Beta Technology Inc., Houston, TX, USA) at the following sites (Slaughter et al., 1988): triceps and biceps, vertically at the mid-point between the acromion process and the olecranon process, respectively, at the posterior and anterior upper arm face; subscapular, at an angle of 45″ to the lateral side of the body, about 20 mm below the tip of the scapula; sovrailiac, about 20 mm above the iliac crest (in the

axillary line); thigh, vertically at the mid-point between the inguinal fold and the superior rotula point; calf, vertically at the bulkiest calf point both medially and laterally.
Finally, many measures were derived as in the previous literature. Body mass index (BMI) was computed as the ratio between the body weight (kg) and the stature squared (m^2). Several parameters were estimated according to previous indications (Frisancho, 2008). Four measures for the upper body and eight for the lower body were calculated: the total upper arm area (TUA,cm^2), the upper mass area (UMA, cm^2), the upper fat area (UFA, cm^2) and the upper-fat index (UFI, %); the total calf area (TCA, cm^2), the calf mass area (CMA, cm^2), the calf fat area (CFA, cm^2), and the calf fat index (CFI, %); the total thigh area, (TTA, cm^2), the thigh mass area (TMA, cm^2), the thigh fat area (TFA, cm^2), and the thigh fat index (TFI, %). Also, to calculate the body composition of each player, the skinfold equations developed by Slaughter and colleagues (1988) were used and three measures were gathered: the fat mass (FM, Kg), the fat-free mass (FFM, Kg), and the percentage of fat mass (%F, %).

Bioelectric Impedance Vector Analysis

A trained researcher performed the bioimpedance analysis using the BIA 101 anniversary analyzer (Akern®, Florence, Italy). The current frequency was settled at 50 kHz. A total body patient cable with four insulated alligator clips was used for connection to proximal (black) and distal (red) electrodes (Biatrodes™, Florence, Italy). At the beginning of the evaluation day, the analyzer was tested to check its validity. To assess the evaluation, each participant was asked to lie down on a bed in the supine position, with a lower limb angle of 45° compared to the median line of the body and the upper limb angle of 30° from the trunk. Before recording the measurement, each participant waited two minutes to allow for uniform distribution of bodily fluids. After cleansing the skin with alcohol, the electrodes (Ag/AgCl) were placed homolaterally on the right hand and foot, keeping them at least 5 cm apart (Lukaski & Piccoli, 2012).
The day before the evaluation, each participant was asked to abstain from foods and liquids for at least four hours before the test.
Vector length (VL) was calculated as (adjusted R^2 + adjusted Xc^2) 0.5 and PA as

$$arctg\frac{Xc}{R}\times\frac{180°}{\pi}$$

BIVA was carried out using the classic methods, e.g., normalizing R (Ω) and Xc (Ω) for height in meters (Piccoli et al., 1994). Elite male soccer players' bioelectrical-specific values (Micheli et al., 2014) were used as a reference to build the 50%, 75%, and 95% tolerance ellipses on the R–Xc graph. BIVA plots the parameters recorded in BIA (R, Xc, PhA) as a vector within a specific tolerance ellipse (specific profile for each sport and competitive level), and it allows to evaluation of soft tissues through patterns based on percentiles of their electrical characteristics (Campa et al., 2021). A BIVA vector that falls out of the 75% tolerance ellipses exhibits an abnormal tissue impedance, while vectors that fall in the 50% represent a normal tissue impedance. BIVA outcomes could be interpreted by the vector direction to the x and y-axis: vertical displacements indicate changes in tissue hydration (dehydration with long vectors, out of the upper pole; hyperhydration with short vectors, out of the lower pole); horizontal displacements indicate changes in soft tissue mass (more soft tissue to the left pole; less soft tissue to the right pole) (Lukaski & Piccoli, 2012).

Maturity Status

An estimation of the years from peak height velocity (PHV), which is an indicator for the adolescent growth spurt, was made using the equation for boys developed by Mirwald and colleagues (Mirwald et al., 2002).
Maturity offset= $-9.236 + 0.0002708$ (leg length $*$ sitting height) $- 0.001663$ (age $*$ leg length) + 0.007216 (age $*$ sitting height) + 0.02292 (weight/height).

Since maturity offset represents the time before or after PHV, the years from PHV were calculated by subtracting the age at PHV from chronological age.
In 2014, Malina and Koziel (Malina & Kozieł, 2014) reported that the approximation of the age at PHV (APHV), based on the prediction equation used, is often lower in younger children who are not yet in their adolescent growth spurt, and higher in older and sexually mature participants who already passed their adolescent growth spurt. To overcome this potential age effect, we followed the approach proposed by Rommers and colleagues (Rommers et al., 2019), who used age-specific z-scores to classify players according to their maturity status. The predicted APHV was used to calculate z-scores within each specific age category (U10–U15, N = 6). Based on these age-specific z-scores of the predicted APHV, players were then classified as "earlier" ($z < -0.5$), "on-time" ($-0.5 \leq z \leq 0.5$), or "later" ($z > 0.5$) maturing (Drenowatz et al., 2013; Hill et al., 2020).

Relative Age Effect (RAE)

Relative age was established from the birth date of each player and the cut-off date for the respective year group (1 January). As such, January was selected as the first month of the selection year and December was the last. The birth month of each player was compiled to define the birth quarter (Q), and four birth quartiles were designated: Q1= January to March; Q2= April to June; Q3= July to September; Q4= October to December.

Motor Tests

The performance tests were implemented at the University sports centre. All participants performed three motor tests: the countermovement jump (CMJ), the 15 m straight-line sprint, and the repeated sprint ability (RSA). In addition, the soccer players who were 13 or older performed the Yo-Yo intermittent recovery test (Toselli et al., 2022). All the tests were preceded by a supervised and standardized warm-up consisting of 10 min of jogging, 5 min of athletic drills including jumping jack, lateral skip, high knee walk and backwards run, and 10 min of dynamic stretching of the lower limbs. A rest period of at least 3 min was allowed between different trials. Two electric photocells estimated the distance from the field through the jump duration during the CMJ test (OptoJump®, Microgate, 11 Miller Road, 10541 Mahopac, New York, NY, USA). Also, a photoelectric cell timing system (Fusion Sport Smart Speed Timing Gates, Brisbane, Australia) estimated the time and distance covered during the 15 m sprint, RSA, and Yo-Yo tests.
The CMJ was assessed according to previous authors (Ingebrigtsen et al., 2014). Before the evaluation, each participant was instructed to start from an upright position, making a rapid downward movement to a knee angle of 90° and simultaneously beginning to push off. The foot position coincided with the fitted acromion vertical line, with an extra-rotation at most of 15°. The hands were maintained on the waist for the entire trial. One minute of rest was allowed between the two attempts and the higher value was gathered.
The time to cover 15 m was detected on a football field and all participants wore technical clothes (Germano et al., 2015). Players were positioned behind the start line (0.5 meters) and were instructed to perform the sprint with maximal effort, after a sound start signal. Two trained coaches recorded the time to complete 15 meters. Each athlete performed two attempts and the mean result was gathered.
The repeated sprint ability (RSA) consisted of six shuttle sprints of 40 meters (20 + 20) with one change of direction (180°), as previously described (Rampinini et al., 2009). Each shuttle was separated by 20 s of rest, after which the soccer player sprinted for 20 meters, touched a line with a foot and came back to the starting line as fast as possible. One trial was assessed for each player and the best time (BT) in a single trial was measured and reported.
The Yo-Yo intermittent recovery test consisted of repeated 20 m runs back and forth between the starting, turning, and finish lines at a progressively increased speed, which is controlled by audio beeps from a tape recorder. When the participants failed twice to reach the finish line in time, the

distance covered was recorded as the test result. This test consists of 4 running bouts at 10–13 km·h^{-1} and another 7 runs at 13.5–14 km·h^{-1}, and then continues with stepwise 0.5 km·h^{-1} speed increments after every 8 running bouts (i.e., after 760, 1080, 1400, 1720 meters, etc.) until exhaustion (Krustrup et al., 2003). One trial was assessed for each player.

Statistical Analysis

The descriptive statistic was calculated and reported as mean ± standard deviation (SD) for continuous variables, while the frequency of appearance (percentage, %) was determined for qualitative variables (RAE and maturity status). The variables' distribution was previously checked through graphics such as scatter plots, histograms, and box plots, and then verified with the Shapiro-Wilk test. When a variable showed a non-well-shaped distribution, a check for curve skewness and kurtosis was assessed. When the curve functions appeared right skewed, a location and scale (logarithm) transformation was applied.
The inference statistic was performed. Differences in frequencies were tested by the chi-squared (χ^2) test and the Z test of proportion. In addition, the Risk Ratio (RR) was assessed and reported.
The two-way ANOVA was performed to compare differences between elite and non-elite players' categories among RAE groups and between elite and non-elite players' categories among maturity status groups. A p-value (p) < 0.05 was considered significant. In addition, when an F value was significant, a post hoc Tukey evaluation was assessed to investigate among categories. However, only the F value (with its degrees of freedom) and the p-value (p) were reported.

3.2.3 Results

Table 3.6 shows the prevalence differences in maturity status and RAE between Bologna F. C. and Russi U. S. in each category. The comparisons in maturity status between the two teams did not report significant differences, while the number of Bologna's youngest players who were born between January and March was greater than those of Russi players. Despite several significant outcomes not arising, figure 3.3 shows that the percentage of Bologna players who belonged to the first quartile (Q1: n = 51, 52.04%) was higher than that of other quartiles in each Bologna's category (Q2: n = 19, 19.39%; Q3: n = 19, 19.39%; Q4: n = 9, 9.21%), and than the Q1 of Russi players (n = 18, 28.12%; RR = 1.85). However, most of the Russi players also belonged to the first quartile (Q2: n = 17, 26.56%; Q3: n = 14, 21.87%; Q4: n = 13, 20.31%).

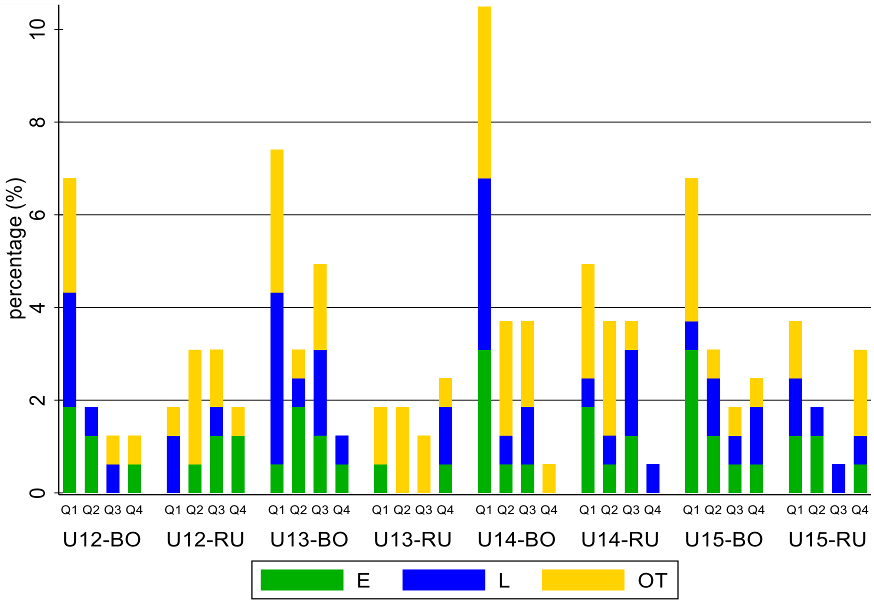

Figure 3.3. Percentage of soccer players for each team's category over the maturity status and RAE.

Table 3.6 Analysis of maturity and RAE proportions among categories of each football team.

	Δ Bologna- Russi U12			**Δ Bologna- Russi U13**			**Δ Bologna- Russi U14**			**Δ Bologna- Russi U15**		
Maturity	Z or χ^2	*p*	RR	Z or χ^2	*p*	RR	Z or χ^2	*p*	RR	Z or χ^2	*p*	RR
E	0.232	0.817	1.067	1.065	0.287	1.556	−0.423	0.6725	0.817	0.359	0.717	1.174
L	0.752	0.452	1.778	1.630	0.103	2.444	0.11	0.912	1.050	−0.482	0.630	0.783
OT	−0.424	0.671	0.667	0.178	0.859	0.500	0.269	0.788	1.089	0.092	0.926	1.043
Total	1.263	0.532		3.871	0.144		0.181	0.913		0.2531	0.881	
RAE												
Q1	2.505	0.012 *	3.259	1.152	0.249	1.778	1.302	0.192	1.487	0.474	0.635	1.196
Q2	−1.007	0.317	0.533	−0.463	0.644	0.741	−0.710	0.478	0.7	0.128	0.898	1.087
Q3	−1.449	0.147	0.355	0.856	0.392	1.778	−0.710	0.478	0.7	0.626	0.531	1.956
Q4	−0.628	0.530	0.593	−2.071	0.038 *	0.222	−0.259	0.796	0.7	−1.129	0.258	0.5
Total	6.462	0.091		5.161	0.160		1.705	0.636		1.462	0.691	

Note: E, early; L, late; OT, on time; RAE, relative age effect; Q1, quartile 1; Q2, quartile 2; Q3, quartile 3; Q4, quartile 4; Z, the test of proportion Z; χ2, Pearson chi-squared test; p, p-value; RR, risk ratio; *, statistically significant; Δ, difference

Maturity Status (MS)

Generally, significant differences resulted among each category for height and trunk height. The maturity status effect was greater with ageing, especially for weight, leg length, relaxed arm circumferences, humeral diameter, femoral diameter, total upper-body area, upper-body mass area, and fat-free mass. The maturity status had significant effects on the 15-meter sprint in U12, U13 and U14, while it affected the CMJ only in U12. Few measures were not affected by the maturity status in all categories such as subscapular, suprailiac and thigh skinfolds, calf, and thigh fat indexes, and the Yo-Yo IRT (U14 and U15).

Concerning the interaction between the maturity status and team membership, it was significant in several measurements among the U13 category trunk height, BMI, relaxed arm circumference, thigh circumference, femoral diameter, subscapular skinfold, total upper-body area, upper-body mass area for U13, upper-body fat index, calf fat area, total thigh area for U13, thigh mass area, fat percentage, and fat mass. No significant results emerged among the other categories.

Figures 3.4 and 3.5 show the interaction between the maturity status, team membership and categories on better physical performance and anthropometric competition level discriminants, respectively. Regarding physical performance, the interaction differences between Bologna F.C. and Russi U.S. soccer players were significant on the 15-meter sprint in U13, on the CMJ test in the U12 category, and RSA in U13 (figure 3.4). Concerning body composition, the interaction comparisons resulted in significance only on the medial calf skinfold in U13 (figure 3.5).

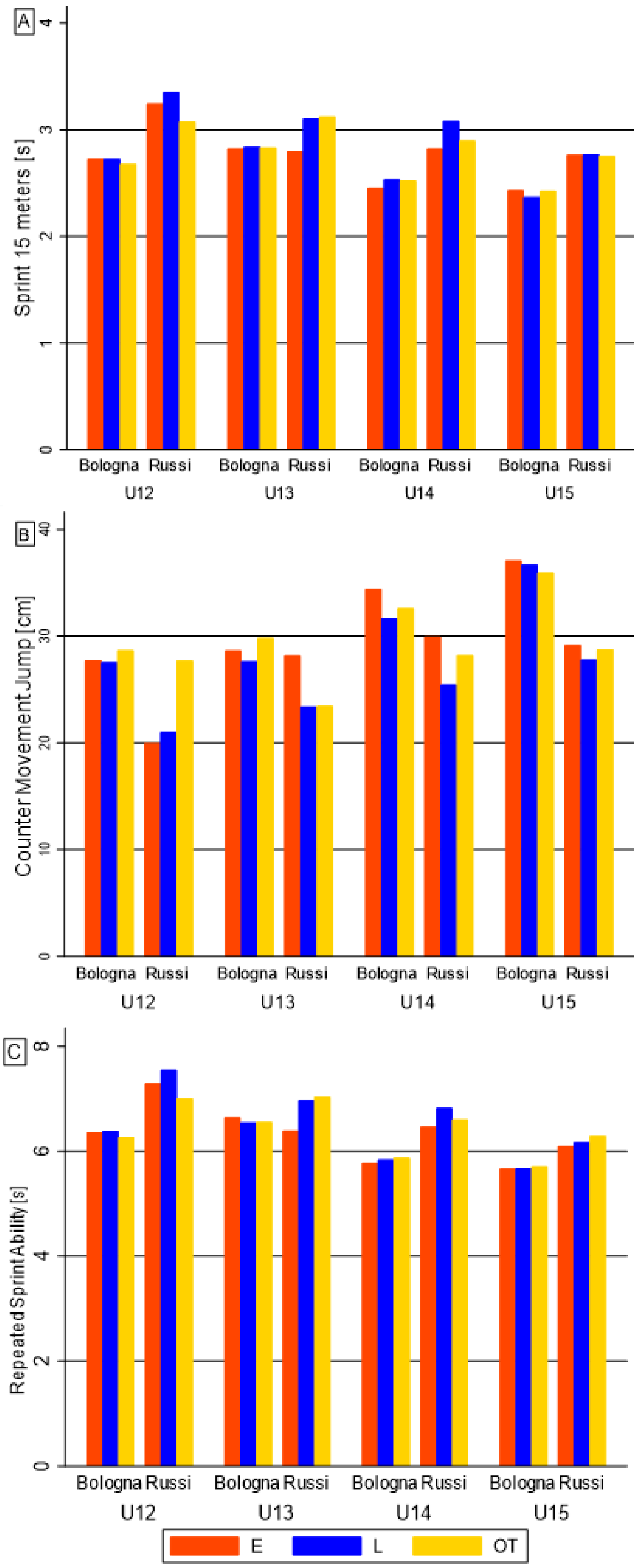

Figure 3.4. Bar graph of the maturity status effect on performance competition level discriminants, among teams and categories: (A) 15-meters sprint; (B) CMJ; (C) RSA.

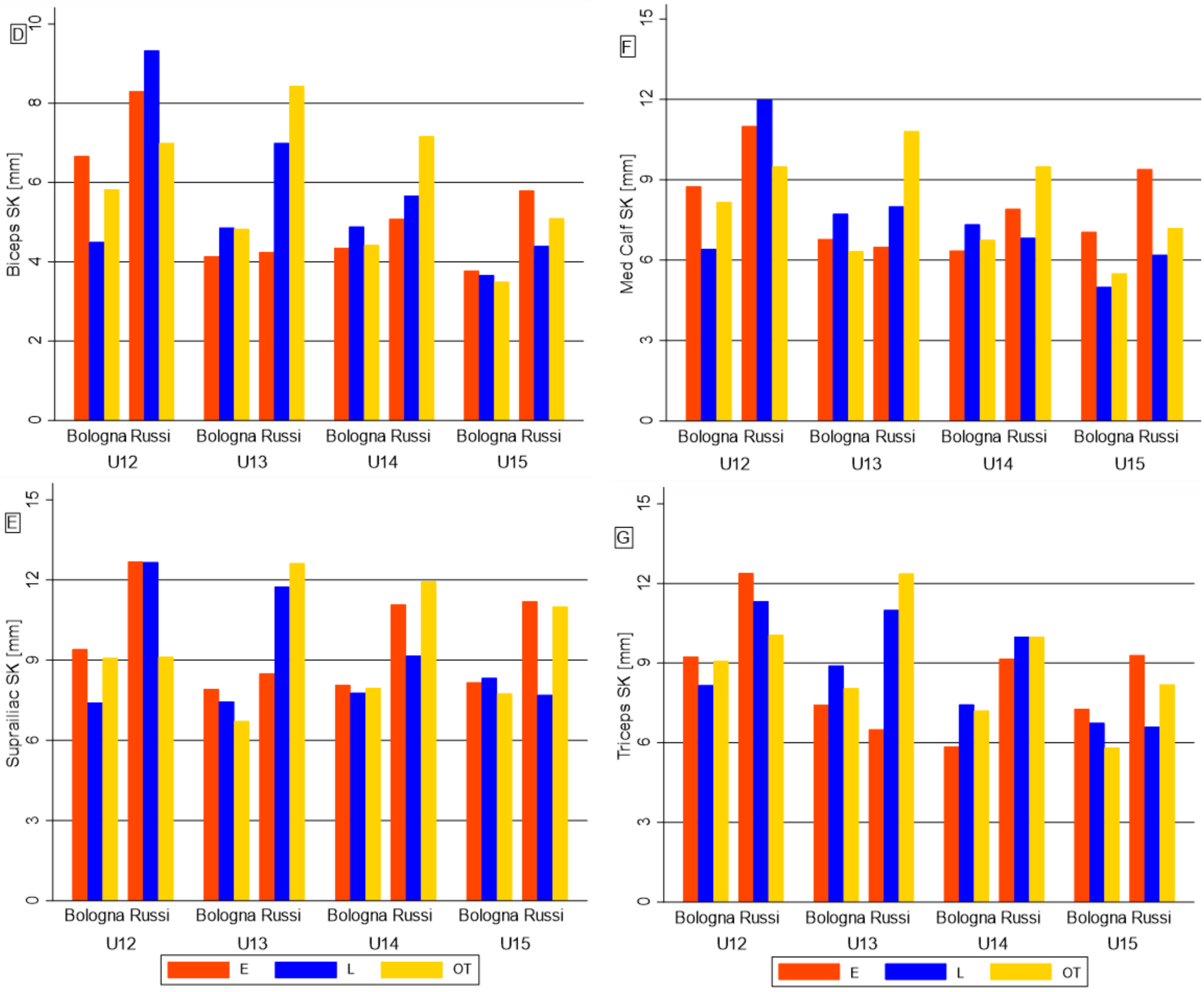

Figure 3.5. Bar graph of the maturity status effect on anthropometric competition level discriminants, among teams and categories: (A) Biceps SK; (B) Suprailiac SK; (C) Medial Calf SK; (D) Triceps SK.

Relative Age Effect (RAE)

Generally, no significant differences emerged from RAE comparisons for any parameters in all teams' categories simultaneously. Also, the youngest group did not report significant outcomes between RAE quartiles. Differently, many significant differences appeared in U13 and U15 categories for relaxed arm circumference, thigh circumference, biceps SK, supraspinal SK, thigh SK, total upper area, upper mass area, total thigh area, total mass, total fat area, total fat index, fat mass. Differences in the U14 category were found in a few measurements: the fat-free mass, the 15-m sprint, and the RSA.
Regarding the interaction between RAE and team membership, few significant differences appeared in the U13 and U15 categories for thigh SK and total fat area. A significant difference resulted in U14 on YO-YO IRT. In addition, Figures 3.6 and 3.7 show the interaction between the RAE, team membership and categories on seven variables that previously discriminated among team levels (Toselli et al., 2022). Concerning physical performance (figure 3.6), the interaction significantly differed for CMJ in U13 and for RSA in U13 and U14. Regarding body composition (figure 3.7), the interaction comparisons resulted in significance for biceps SK in U13 and U15, and triceps SK in U13.

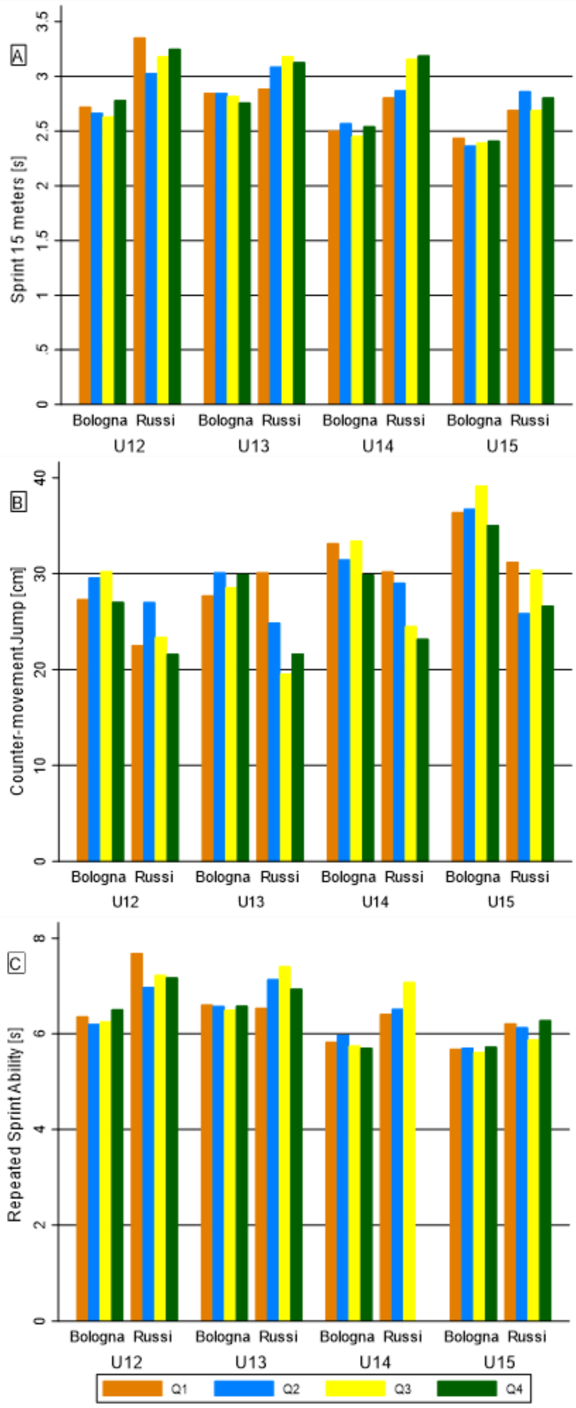

Figure 3.6. Bar graph of the Relative Age Effect on performance competition level discriminants, among teams and categories: (A) 15-m sprint; (B) CMJ; (C) RSA.

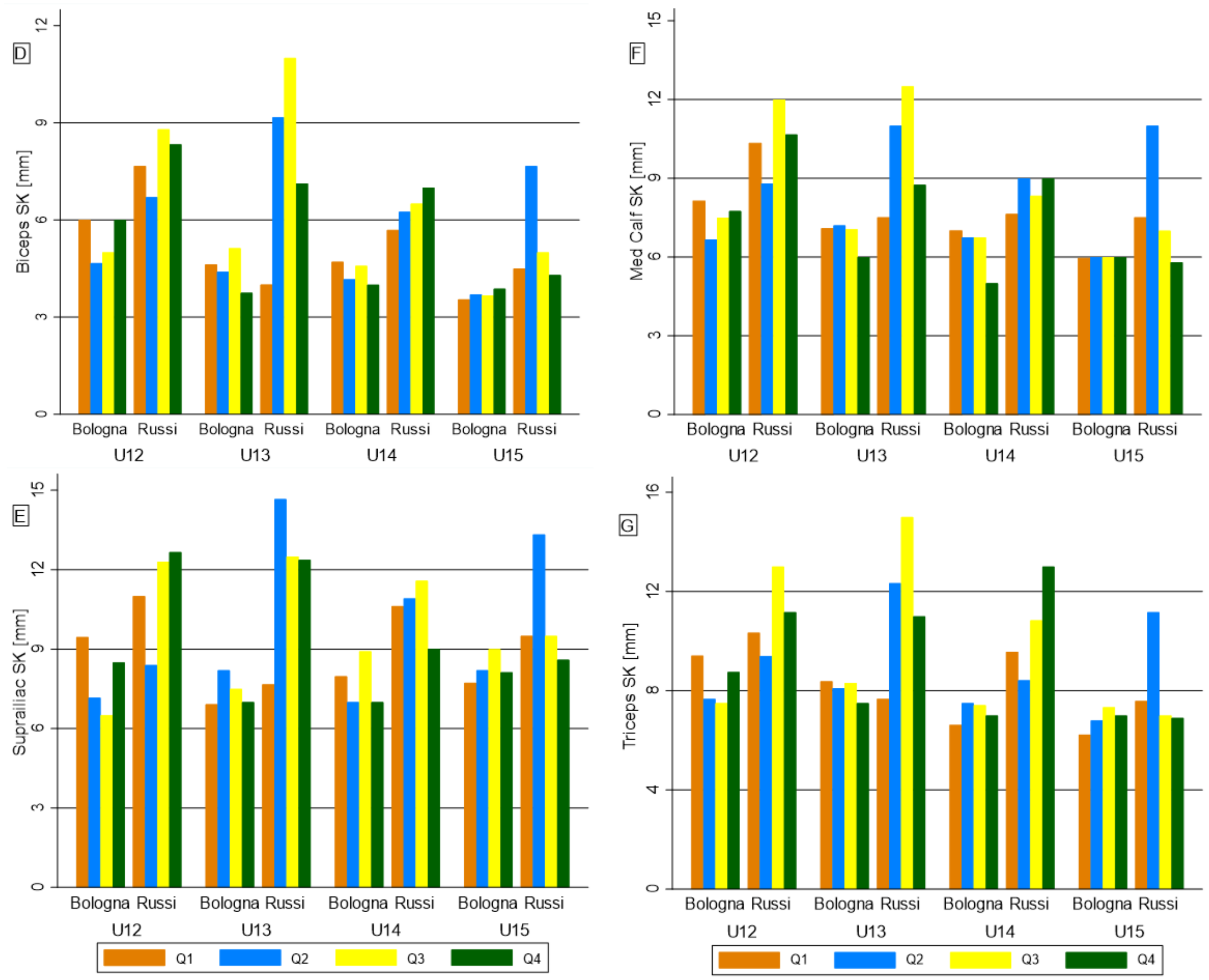

Figure 3.7. Bar graph of the Relative Age Effect on anthropometric competition level discriminants, among teams and categories: (A) Biceps SK; (B) Suprailiac SK; (C) Medial Calf SK; (D) Triceps SK.

Bioimpedance Vector Analysis (BIVA)

Figure 3.8 shows BIVA results in U12 soccer players of both teams for Maturity Status (left side) and RAE (right side) considering two different reference populations (A and B). Generally, elite U12 players reported greater cellularity than non-elite. As regards maturity status, several differences appeared within and between teams' comparisons. Firstly, the 12-year-old white-male ellipse appeared the most adequate for the elite team (figure 3.8 B, left side), while the non-elite team fell better in the 10–11 year-old white-male ellipse (figure 3.8 A, left side). In addition, when compared to the Serie A soccer players graph, Bologna soccer players, who matured earlier or on time, were the closest to the ellipse, while the later matured players were the farthest. Regarding RAE, players who were in quartile 1 of both teams showed cellularity more similar to elder reference populations, but this trend was not linear with the increasing quartiles.

Figure 3.9 shows BIVA results in U13 soccer players of both teams for Maturity Status (left side) and RAE (right side) considering two different reference populations (A and B). Generally, the means of the two teams presented close positions in the graph and the 13-year-old white-male reference ellipse

appeared the most adequate for both elite and non-elite players. As regards maturity status, earlier players' cells' characteristics resulted closer to the Serie A soccer players' ellipse. In contrast, players who matured later appeared farthest from the elite men's graph, especially in the Russi U.S.
Regarding RAE, despite players who were born in the first quartile laid on a 75% tolerance line, the graph and the 13-year-old white-male reference ellipse appeared the most adequate for both teams (figure 3.10 B, right side). However, the earlier non-elite team players were the closest to the Serie A soccer players' ellipse, while the elite team players were not affected by the RAE and showed similar cell characteristics among quartiles.
Figure 3.10 shows BIVA results in U14 soccer players of both teams for Maturity Status (left side) and RAE (right side) considering two different reference populations (A and B). Generally, most of the means of the two teams lay on the 50% tolerance line in the 14–15 year-old white-male reference ellipse (figure 3.10 B). As regards maturity status, earlier players of the two teams showed more similar characteristics to Serie A adult players, while the latter players moved up and to the right on the 14–15 years ellipses (figure 3.10 B).
Regarding RAE, the elder players (Q1 and Q2) of both teams showed similar characteristics and were nearer to elite adult players. However, the 14–15-year-old white-male reference ellipse better described the body composition of the elder U14 soccer players (figure 3.10 B), while the younger (Q3 and Q4) better laid in the 13-year-old white-male reference graph (figure 3.10 A).
Figure 3.11 shows BIVA results in U15 soccer players of both teams for Maturity Status (left side) and RAE (right side) considering two different reference populations (A and B). As regards maturity status, the earlier players were better described by the 16–85-year-old white-male reference ellipse (figure 3.11 B), while the latter players appeared similar to the 14–15-year-old white-male population (figure 3.11 A). Also, the earlier players' cells were more similar to Serie A men than the latter U15 players.
Regarding RAE, the elder players (Q1 and Q2) showed more athletic characteristics than their younger teammates (figure 3.11 B), who appeared similar to the 14–15-year-old white-male population (figure 3.11 A). However, non-elite team players who were born between January and June lay in the 95% tolerance line of Serie A adult players.

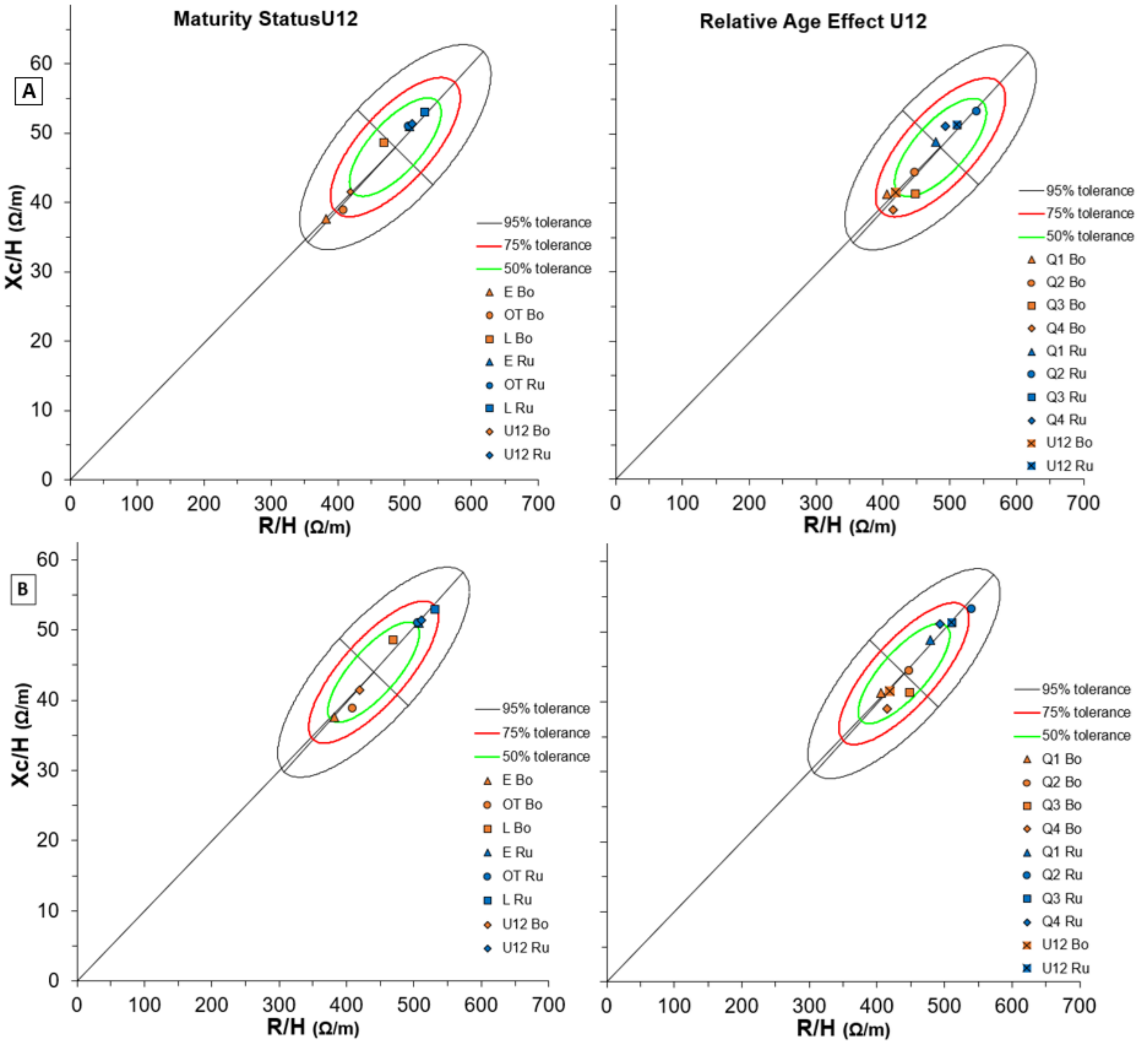

Figure 3.8. BIVA tolerance with Maturity Status (left) and Relative Age Effect (right) of both Bologna and Russi U12 groups for two reference populations: (**A**) number 112 (males, white, age 10–11 years, BMI 18, Italy, Akern-RJL Systems); (**B**) number 114 (males, white, age 12 years, BMI 18, Italy, Akern-RJL Systems). Note: U15 Bo and U15 Ru refer to team means respectively.

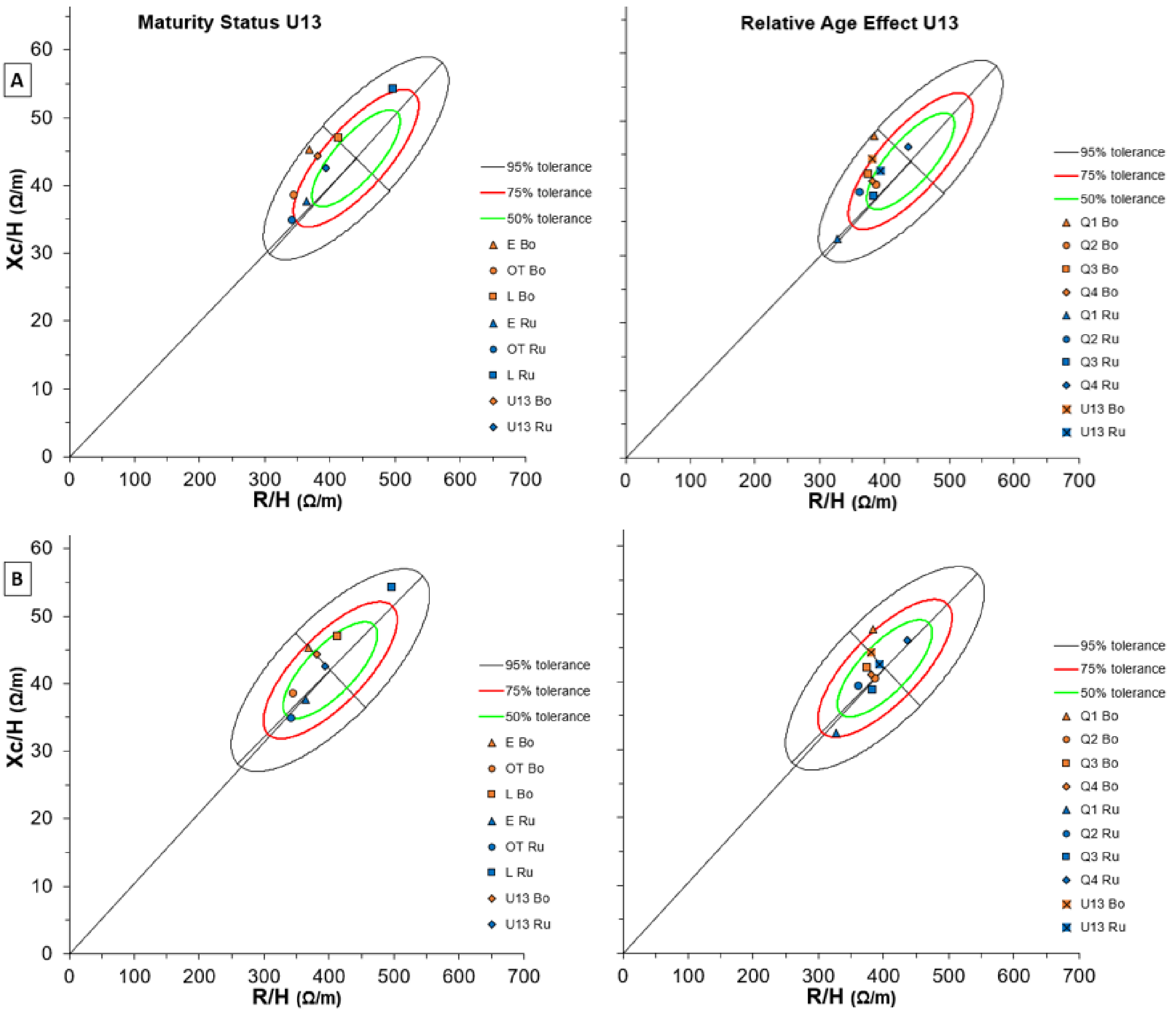

Figure 3.9. BIVA tolerance with Maturity Status (left) and Relative Age Effect (right) of both Bologna and Russi U13 groups for two reference populations: (**A**) number 114 (males, white, age 12 years, BMI 18, Italy, Akern-RJL Systems); (**B**) number 116 (Males + Females, White, age 13 years, BMI 19, Italy, Akern-RJL Systems). Note: U15 Bo and U15 Ru refer to team means, respectively.

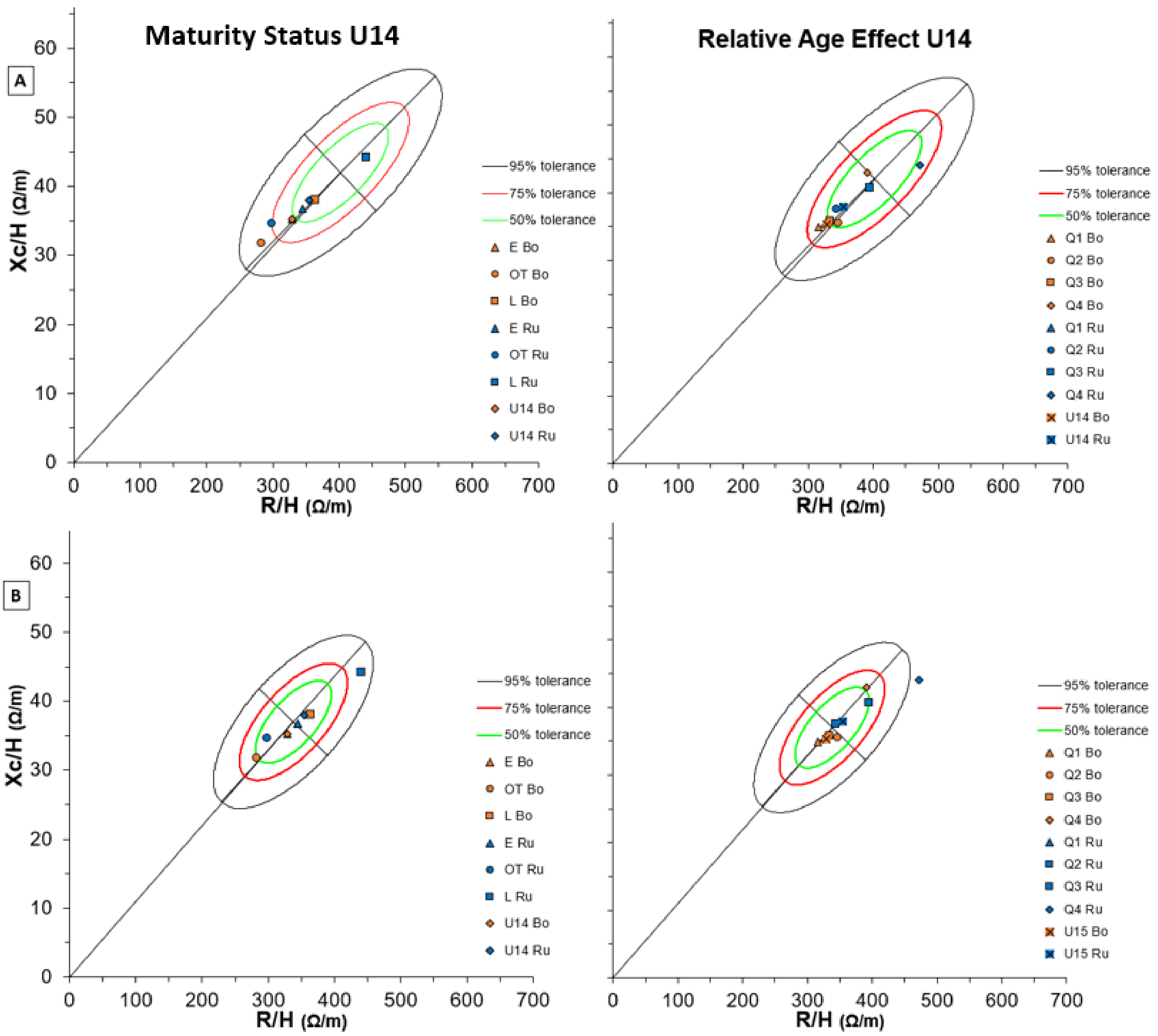

Figure 3.10. BIVA tolerance with Maturity Status (left) and Relative Age Effect (right) of both Bologna and Russi U14 groups for two reference populations: (**A**) number 116 (males, white, age 13 years, BMI 19, Italy, Akern-RJL Systems); (**B**) number 118 (Males + Females, White, age 14–15 years, BMI 20, Italy, Akern-RJL Systems). Note: U15 Bo and U15 Ru refer to team means respectively.

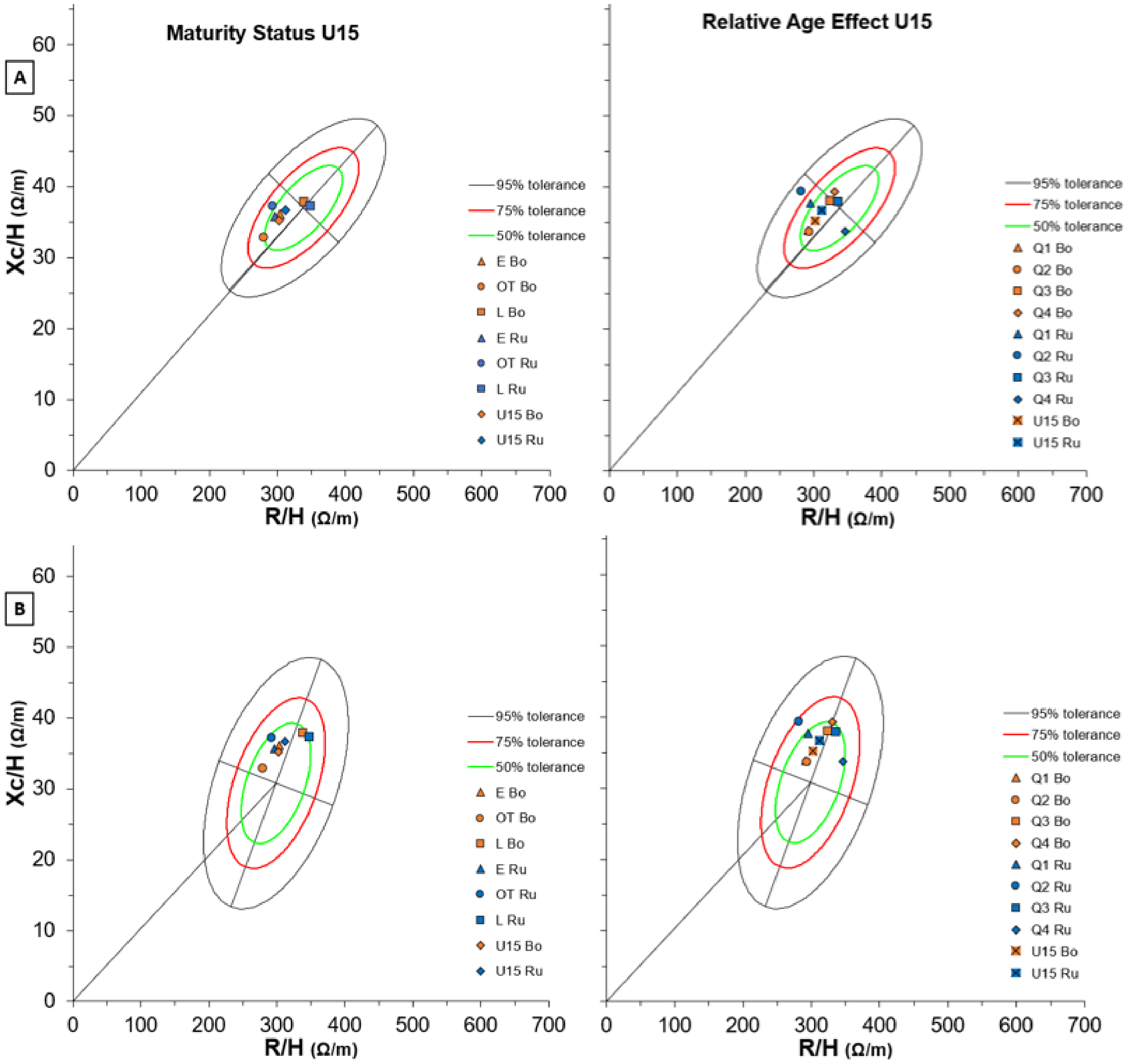

Figure 3.11. BIVA tolerance with Maturity Status (left) and Relative Age Effect (right) of both Bologna and Russi U14 groups for two reference populations: (**A**) number 118 (Males + Females, White, age 14–15 years, BMI 20, Italy, Akern-RJL Systems); (**B**) the number 1 (Males, White, 16 age 85 years, 16 BMI 31, Italy, Akern-RJL Systems). Note: U15 Bo and U15 Ru refer to team means respectively.

3.2.4 Discussion

The main aim of this study was to evaluate the differences in maturity status and relative age effect among the players of two Italian youth teams of different competitive levels, one elite and one non-elite. We found that the two teams did not show significant differences in the frequencies of maturity status, while few differences in RAE emerged. The percentage of the Bologna players who belonged to the first quartile was higher than those observed for Russi players in all age groups. Thus, the overall RAE for the elite soccer players showed that players born at the beginning of the year were consistently over-represented. These results are in line with those reported in several elite soccer leagues worldwide (Brustio et al., 2018; S. P. Cobley et al., 2008; Doyle & Bottomley, 2019; Figueiredo et al., 2021; Götze & Hoppe, 2020; Helsen et al., 1998, 2012; Jiménez & Pain, 2008; Lupo et al., 2019; Mujika et al., 2009; Musch & Hay, 1999; Rađa et al., 2018; Salinero et al., 2013; Williams, 2010). In addition, the results confirmed that RAE was more prevalent in the clubs and academies classified with the highest level of certification (Figueiredo et al., 2021; Peña-González et al., 2018). According to Figueiredo et al. (Figueiredo et al., 2021), this might suggest that clubs and academies certified as training institutions also have the means to select more players than the lower-level certification clubs and academies, thus taking advantage of the potential beneficial effect of an over-representation of the chronologically older players. In our study, the prevalence of players born in Q1 was particularly evident in U14. Prior studies have reported that the extent of the RAE decreases with increasing age, with evidence after adolescence (Brustio et al., 2018; S. Cobley et al., 2009; Doncaster et al., 2020; Helsen et al., 2005; Lovell et al., 2015).

Regarding maturity status between the two competitive levels, we did not find differences in prevalence, despite other authors reporting differences among the competitive levels (Johnson et al., 2017). However, previous studies reported that the chance of selection for relatively younger soccer players is higher only if they were early maturing whereas relatively older athletes had a selection advantage independent of their maturity status (Deprez et al., 2013; Müller et al., 2017).

The second aim was to understand the interaction effects amongst maturation status, and birth quartiles on the players' physical characteristics and abilities. The results have shown that the maturity status had greater effects than RAE in both anthropometry and motor tests. The magnitude of the effects was seen to vary with age and the maturity status effect was bigger in U12 and U14 than in RAE.

The effects of RAE in the two teams were observed only in U13 and in U15, with significant differences for some anthropometric parameters (weight, circumferences, fat parameters and many of the limb areas). In contrast, RAE was unrelated to performance tests and only significantly associated with superior sprint 15 m and RSA performance in players born in the first months of the years U14. The results follow what was reported by other authors (S. Cobley et al., 2009; Peña-González et al., 2021). Cobley et al. (S. Cobley et al., 2009) in their meta-analysis showed a small-moderate effect for individuals aged 15–18 years that declined for older individuals, while Peña-González et al. (Peña-González et al., 2021) affirmed that anthropometrical and physical performance differences observed in different competitive levels are not due to the relative age but principally to the level of competition. In addition, some authors found that earlier birthdates (quartile one) were not associated with the likelihood of being selected or promoted to a higher level in soccer players (Castillo et al., 2019).

The effects of the differences in biological maturity were evident for all the age groups, and both the anthropometric characteristics and performance tests, indicate that maturity has a greater association with physical characteristics and physical abilities than RAE in Italian male youth soccer players. The current study found that early mature subjects were taller, and heavier, and presented better body composition parameters and performance than youths who matured on time or late. Similar findings have been reported in other studies (Johnson et al., 2017; Parr et al., 2020; Peña-González et al., 2018; Radnor et al., 2021), where maturity status was shown to have a much greater influence on anthropometry and physical characteristics than RAE in young soccer players. Johnson et al. (Johnson et al., 2017) reported that maturation status had an even 10-fold stronger influence on selection in elite youth soccer than the relative age.

Maturation affected physical performance, with early maturing boys performing better than them on time and late peers, and this had a subsequent impact on match performance in soccer (Buchheit & Mendez-Villanueva, 2014). However, it should be considered that, although advanced maturity offers an initial advantage in terms of performance and selection, in the long term this can be counterproductive (Radnor et al., 2021). Players who mature early tend to overlook their technical

and tactical development in favour of the use of their physical ability (Malina et al., 2015). In elite soccer, there is the gradual exclusion of early-matured players and the selection of those who matured late with increasing age (Radnor et al., 2021). Caution must be taken in assessing relationships between RAE, maturation, and performance. Physical advantages related to age and/or maturation during adolescence are highly transitory and tend to disappear or even reverse in adulthood. Those involved in the identification and development of the academy players should be aware of and accommodate individual differences in maturation.
The last purpose of this study was to evaluate the biological maturity and the relative age effect on bioimpedance parameters. To interpret the BIVA outcomes well, one of the most relevant features is to compare the analyzed sample to an adequate reference population. In adolescent players, the faster change of maturity stages requests rigorous analysis. We found that players who matured earlier had similar cellularity to elder adolescent and adult players, independently of team level. The effect of the elite team was more evident in U12 and U15 soccer players. Although previous studies follow biological maturity influence, this effect seems to be more pronounced in soccer players' body fluids (Bongiovanni et al., 2020; Toselli et al., 2020). However, to the best of our knowledge, no authors investigated the biological maturity effect on BIVA at two competitive soccer team levels using different reference population graphs.
Regarding RAE, despite the elder reference population ellipse including most of the observations in the 50% tolerance line, the quartiles showed different trends among the categories. Players who were born in the first six months of the year exhibited greater cellularity in the U12, U14 and U15, while in U13 this discrepancy is evident only in non-elite team players. Also, elite players showed characteristics more similar to adult soccer players only in the U12 and U14 categories. To the best of our knowledge, no authors investigated the RAE on BIVA in younger soccer players and more evidence is needed.
The results found are of great importance for coaches and other professionals responsible for the process of scouting and training young soccer players. These professionals should be aware of the different stages of growth and biological maturation and their influences on different body dimensions and performance. Following our results, relative age should be considered as a secondary factor in the process of identification, selection, and development of young soccer players.
This study presented many limitations: (1) maturity was not assessed using the gold standard method of skeletal maturity; (2) due to the presence of three or four groups for maturity status and RAE respectively, a bigger sample size should evidence many differences; (3) no specific soccer performance test was assessed.
In conclusion, maturity status and relative age were differentially associated with physical characteristics and physical abilities in young soccer players. Specifically, advanced maturity was associated with better anthropometric characteristics and superior performance in most age groups, whereas relative age was, in the majority of cases, unrelated to performance.
The findings from the current study expand on this previous research, identifying that maturity influences anthropometric characteristics and performance rather than RAE between 12 and 15 years.

3.2.5 Conclusions

The main purpose of the present study was to evaluate the differences in RAE and biological maturity among the players of two Italian youth teams of different competitive levels, one elite and one non-elite and to assess the relationship between maturation, age, and relative physical and performance characteristics. The characteristics analyzed are mainly associated with maturation, while the relationship with RAE is less evident. Professionals should understand that RAE and maturity status are two distinct constructs. Coaches and other professionals involved should be encouraged to monitor growth and maturation to better interpret changes in the physical performance of young soccer players. Maturity status should be taken into consideration both in making the selections, but also to guide training, and to mitigate the differences due to the different maturity statuses.

3.3 STUDY III

3.3.1 Introduction

Padel is a racquet team sport played on a 20 x10 meters rectangular court divided in the middle by a tennis net and surrounded by a glass wall and metallic mesh area that is 3-4 meters tall (Carrasco et al., 2011; Courel-Ibáñez et al., 2019). Although padel uses the same scoring system and similar rules as tennis, it requires specific physiological, mechanical, technique-tactical and physical demands (Sánchez-Muñoz et al., 2020). Padel matches include high-intensity activities with intermittent efforts, with an average point-rest duration ratio of about 1:2 (7.24 and 14.12 seconds, respectively), and a total distance covered of 1117 meters at an average speed of 7 km/h (Castillo-Rodríguez et al., 2014). However, performance characteristics can vary depending on the competition level (Courel-Ibáñez & Herrera-Gálvez, 2020).

Speed, power, strength, and endurance are performance parameters related to athletes' physical characteristics and body composition (McArdle et al., 2014). Body size and composition play a key role in the performance of professional athletes in multiple disciplines (McArdle et al., 2014) including racket sports (Cádiz Gallardo et al., 2023). Although elite padel and tennis players exhibited similar profiles (Martínez-Rodríguez et al., 2014), different body characteristics could affect the specific sport's performance. For example, anthropometric features such as height and weight can affect the technical-tactical patterns of padel players (Courel-Ibáñez et al., 2019). Also, body composition components such as Body fat mass (FM) and fat-free mass (FFM) varied among different padel competitive levels (Marín et al., 2021; Sánchez-Muñoz et al., 2020). To date, the anthropometric characteristics, body composition and somatotype of male (Fuente et al., 2019) and female (Fuente et al., 2014, 2019) elite padel players have been described, but further information on low and mid-level players is needed.

Thus, body composition analysis and monitoring are of interest to team sports clubs participating in padel competitions. In addition, padel adherence has worldwide increased in the last decade and it could be an effective strategy to enhance physical activity practice and improve cardiovascular fitness, muscle strength and body composition in sedentary people (Sánchez-Alcaraz & Courel-Ibáñez, 2022). However, the gold-standard methods used to quantify FM and FFM require specific laboratory techniques such as hydrostatic weighing or expensive instruments such as dual-X-ray absorptiometry (DXA) (D. Stewart & Hannan, 2000; Katch & McArdle, 1973). To provide easier and cheaper methods, both bioelectrical impedance analysis (BIA) and anthropometry have been well-debated. Also, bioimpedance vector analysis (BIVA) could provide interesting details to identify the specific sport's profile in terms of body fluids and hydration status (Lukaski & Piccoli, 2012). Despite several authors having proposed equations for generally healthy adults and athletes, it is unclear which methods could better predict FM and FFM. Also, few studies have drawn sports-specific regression models to estimate body composition (Campa et al., 2023). To date, only one research evaluated players' body composition with DXA in padel (Courel-Ibáñez & Herrera-Gálvez, 2020), and all the regression equations used in FM estimation were derived from the unspecific population (Martínez-Rodríguez et al., 2014; Sánchez-Muñoz et al., 2020).

To the best of our knowledge, no study has calculated any specific regression model for estimating body composition parameters in padel. In addition, no research study has reported BIVA graphs and profiles of male padel players. Furthermore, there is no report on body composition differences between right and left players' positions. So, the first aim of the following study is to draw two different equations that may accurately estimate FM and FFM using portable and field instruments such as the skinfold calliper and the bio-impedance analyser. The second purpose is to provide the specific BIVA characteristics of male padel players, which allows us to compare them with other sports athletes and generate a reference ellipsis for future investigation. The final aim is to compare anthropometric and body composition features between right and left players.

3.3.2 Methods

Study Design

This study used a cross-sectional experimental design. One racket sports association with an intermediate-level padel team of 16 players, who competed for the Italian third category, was selected for the experiment. Due to the lack of a female competitive team, only male players were included in the study. All the players were examined on one day in September 2022, from 10 a.m. to 2 p.m., at the Medical University Centre of Bari. Before enrolling the participants in the study, they were informed about the experimental procedures and risks, and they could voluntarily decide to participate in the study. For minor participants, their parents were informed and allowed them to participate. After the enrollment, one participant was excluded due to a leg injury, and 15 players completed all evaluations. The players trained for three days (two hours per training), of which two hours per week of strength and conditioning and four hours per week of technical-tactical training. In addition, all players played two matches per week. The researchers did not collect information on their dietary intake.

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Alma Master Bologna University (code 0122481, May 23, 2022). All participants or their parents gave their written consent before enrolling on the study.

Dual-energy X-ray absorptiometry (DXA)

One specialized physician assessed the DXA measurements. Each participant was asked to lie in a supine position on a bed for the evaluation. The Hologic Horizon Wi (S/N 300294M) densitometer with InnerCore^{TM} Visceral Fat Assessment was used to measure total and segmental (upper limbs, lower limbs, thorax, and head) body composition parameters such as body fat mass (FM, g), percentage of body fat (%F) and total lean mass (FFM, g). This feature uses a source that generates X-rays at two energies, and it has a switching-pulse system that alternates the voltage of the X-ray generator, producing two beams of high and low energies. The subject's differential attenuation of the two energies is used to determine the body composition within five minutes. Then, an interface with a computer system produces colour images displaying the distribution of fat, lean mass, bone, and fat mass index. The information is translated into an easy-to-interpret report for improved patient management and counselling. The Horizon DXA system incorporates the National Health and Nutrition Examination Survey (NHANES) whole-body composition reference data (Hangartner, 2007). The accuracy and precision of the instrument were previously tested (Hangartner, 2007).

Anthropometry

Two trained researchers cooperated and assessed the anthropometric evaluations according to standardized procedures (Lohman et al., 1988). Height and sitting height were measured to the nearest 0.1 cm using a stadiometer (GPM, Zurich, Switzerland), and leg length was derived by the subtraction of sitting height from height. Body weight was measured to the nearest 0.1 kg (light indoor clothing, without shoes) using a calibrated analogue scale. Body mass index (BMI) was computed as the ratio between the body weight (kg) and the stature squared (m^2).

Circumferences (relaxed and contracted upper arm, thigh, and calf) were measured to the nearest 0.1 cm with a non-stretchable tape and widths (humerus and femur) to the nearest 0.1 cm with a sliding calliper, both on the left side of the body. The upper arm circumference was taken at the mid-point between the shoulder acromion and the olecranon process point, with the participant's elbow relaxed along the body side (relaxed evaluation) or to be flexed 90° with palm facing upward (contracted evaluation); the thigh circumference was taken at the mid-point between the inguinal fold and the superior kneecap point, with the participant in a standing position (thigh muscles relaxed); the calf circumference was taken at the bulkiest calf point, with the participant in a standing position (calf muscles relaxed); the humerus and femoral widths were taken, respectively, between the own lateral and medial epicondyles, with participant's elbow and knee flexed 90°. Skinfold thicknesses (biceps, triceps, subscapular, supraspinal, supra-iliac, thigh, and calf) were measured to the nearest 1 mm using a Lange skinfold calliper at the left side of the body (Beta Technology Inc., Houston, TX, USA) at the following sites (Slaughter et al., 1988): triceps and biceps, vertically at the midpoint between the acromion process and the olecranon process, respectively, a posterior and anterior upper arm face; subscapular, at an angle of 45″ to the lateral side of the body, about 20 mm below the tip of the scapula; supra-iliac, about 20 mm above the iliac crest (in the axillary line); thigh, vertically at the mid-point between the inguinal fold and the superior kneecap point; and calf, vertically at the bulkiest calf point both medially and laterally.

Bioelectric Impedance and Vector Analysis

Two trained researchers performed the bioimpedance analysis using the BIA 101 BIVA® PRO (Akern®, Florence, Italy). The current frequency was settled at 50 kHz. A total body patient cable with four insulated alligator clips was used for connection to proximal (black) and distal (red) electrodes (Biatrodes™, Florence, Italy). The analyser was tested at the beginning of the evaluation day to check its validity. To conduct the evaluation, each participant was asked to lie down on a bed in the supine position, with a lower limb angle of 45° compared to the median line of the body and the upper limb angle of 30° from the trunk. Before recording the measurement, each participant waited two minutes to allow for uniform distribution of bodily fluids. After cleaning the skin with alcohol, the electrodes (Ag/AgCl) were placed homolaterally on the right hand and foot, keeping them at least 5 cm apart (Lukaski & Piccoli, 2012). The day before the evaluation, each participant was asked to abstain from foods and liquids for at least four hours before the test. The raw parameters (R, resistance; Xc, reactance; PhA, phase angle) were measured and gathered.
BIVA was carried out using the classic methods, e.g., normalizing R (Ω) and Xc (Ω) for height in meters (Lukaski & Piccoli, 2012). Due to the lack of padel players' ellipses, the Elite male tennis players' bioelectrical specific values were used as a reference to build the 50%, 75%, and 95% tolerance ellipses on the R/H–Xc/H graph. BIVA plots the parameters recorded in BIA (R, Xc, PhA) as a vector within a specific tolerance ellipse (specific profile for each sport and competitive level), and it allows to evaluate of soft tissues through patterns based on percentiles of their electrical characteristics. A BIVA vector that falls out of the 75% tolerance ellipses exhibits an abnormal tissue impedance, while vectors that fall in the 50% represent a normal tissue impedance.

Statistical Analysis

The descriptive statistics were calculated and reported as mean ± standard deviation (SD) for continuous variables. The variables' distribution was previously checked graphically and then verified with the Shapiro-Wilk and the Kolmogorov-Smirnov tests. When a variable showed a non-well-shaped distribution, a check for curve skewness and kurtosis was assessed. A location and scale (logarithm) transformation was applied when the curve functions appeared right-skewed. The one-way ANOVA was performed to compare differences between left and right players. The stepwise backward procedure was performed to draw the best regression model, with a significant level for removal from the model equal to 0.10 and a significant level for additions to the model equal to 0.10. To meet the general linear regression model assumptions, the heteroskedasticity was checked by the White and Breusch-Pagan/Cook-Weisberg tests. The variance inflation factor (VIF) checked the multicollinearity, where a value lower than 5 was considered acceptable (moderate correlation (Dodge, 2008)). The leverage plot and Cook's distance were computed to look for the outlier presence. When the presence of some outlier affected the model, it was removed, and a new model was performed. To report the goodness of fit, the adjusted R^2 was calculated and the plot with the residuals of any regressors was computed. A *p*-value (P)< 0.05 was considered significant. In

addition, the F value (with (k-1, n-k) degrees of freedom, where k is the groups' number, and n is the sample size), the Root MSE, the regression coefficient (β), the standard error (SE), the student's t-test (t) value, the 95% confidence interval and the partial η^2 for each slope were reported. Then, the Bland-Altman plot and the pairwise correlation coefficient (r) were computed to assess the agreement between the new method and the gold standard (Bland & Altman, 1986). In addition, the concordance correlation coefficient (CCC) was computed and reported. Finally, a post hoc test was assessed to compute the achieved power given the type I error value, sample size, effect size (R^2 of the tested model) and the number of coefficients tested. A 1-β value ≥ 0.90 was considered optimal.
As regards BIVA outcomes, the Mahalanobis' distance (D) was computed to quantify the degrees of BIA similarity between padel and other sports.

3.3.3 Results

Participants characteristics

Table 3.7 shows the anthropometric and body composition for the whole sample and the comparisons between right and left players. As reported, no significant differences emerged between the two groups. Although two of the right players were left-arm dominant, both FM and FFM resulted similarly in the left and right halves. However, a greater variability appeared in the arm fat percentages of the left group (%FM arm left range= 29.6; %FM arm right range= 24.4). Regarding BIA, despite no significant differences appearing, right players showed a greater resistance average value than those who played at left (Δ= 18.89 Ω).

Table 3.7. Anthropometric and Body Composition characteristics.

Variable	Total sample (n=15)	Right Players (n=7)	Left Players (n=8)	Roles differences	
	Mean (±SD)	Mean (±SD)	Mean (±SD)	$F_{(1, 13)}$	P
Age [year]	26.66 (11.84)	29.31 (13.26)	24.30 (10.83)	0.65	0.435
Weight [kg]	71.6 (12.51)	70.93 (11.43)	72.19 (14.15)	0.04	0.854
Height [cm]	173.13 (7.63)	174.19 (7.71)	172.21 (7.97)	0.24	0.635
leg length [cm]	53.9 (4.3)	121.19 (5.04)	117.53 (4.63)	2.15	0.166
Trunk Height [cm]	119.23 (5.02)	53.00 (3.11)	54.69 (5.22)	0.56	0.469
BMI [kg/m^2]	23.73 (2.66)	23.23 (1.89)	24.17 (3.25)	0.45	0.514
R [Ω]	426.21 (49.46)	436.29 (50.8)	417.4 (49.89)	0.53	0.481
Xc [Ω]	55.75 (6.36)	56.14 (6.75)	55.41 (6.45)	0.05	0.834
PhA	7.43 (0.49)	7.30 (0.42)	7.54 (0.54)	0.88	0.364
Arm stretch. [cm]	28.18 (3.36)	27.57 (3.30)	28.71 (3.54)	0.41	0.532
Arm contract. [cm]	31.03 (3.45)	30.93 (3.76)	31.11 (3.41)	0.01	0.922
Calf circum. [cm]	35.69 (2.24)	34.96 (2.38)	36.33 (2.05)	1.44	0.252
Thigh circum. [cm]	50.75 (4.65)	51.21 (4.41)	50.35 (5.12)	0.12	0.734
Waist circum. [cm]	76.31 (7.7)	75.10 (7.06)	77.38 (8.56)	0.31	0.587
Pelvis circum. [cm]	93.31 (6.37)	92.14 (5.92)	94.34 (6.96)	0.43	0.526
Humeral diam. [cm]	6.41 (0.38)	6.44 (0.44)	6.39 (0.35)	0.07	0.788
Femural diam. [cm]	9.53 (0.52)	9.61 (0.55)	9.46 (0.52)	0.3	0.592
Biceps SK [mm]	4.73 (1.68)	4.64 (1.49)	4.81 (1.93)	0.03#	0.953
Triceps SK [mm]	10.93 (3.09)	11.36 (3.57)	10.56 (2.8)	0.23	0.637
Subscap SK [mm]	10.83 (3.55)	9.86 (2.48)	11.69 (4.27)	0.33#	0.563
Supra-iliac SK [mm]	10.53 (3.68)	9.71 (1.60)	8.88 (3.64)	0.40#	0.524
Supraspinal SK [mm]	8.4 (2.59)	9.43 (2.88)	9.88 (4.70)	0.05	0.831
Calf med. SK [mm]	9.73 (4.1)	10.14 (3.89)	9.38 (4.50)	0.12	0.731
Calf lat. SK [mm]	10.07 (3.28)	10.43 (3.21)	9.75 (3.54)	0.15	0.705
Thigh SK [mm]	13.9 (3.34)	14.64 (3.4)	13.25 (3.37)	0.63	0.441
FM arm left [kg]	1.04 (0.83)	0.86 (0.24)	1.20 (1.13)	0.59	0.458

FM arm right [kg]	1.02 (0.69)	0.87 (0.26)	1.16 (0.92)	0.65	0.434
FM thorax [kg]	7.04 (2.3)	7.09 (1.69)	7.00 (2.85)	0.01	0.941
FM leg left [kg]	3.14 (0.78)	3.27 (0.89)	3.02 (0.71)	0.37	0.553
FM leg right [kg]	3.23 (0.78)	3.31 (0.82)	3.15 (0.79)	0.13	0.72
FM total [kg]	16.69 (4.8)	16.62 (3.49)	16.75 (5.97)	0	0.959
%FM arm left	23.17 (6.9)	21.91 (1.85)	24.26 (9.46)	0.01#	0.91
%FM arm right	21.93 (6.05)	20.96 (2.51)	22.77 (8.13)	0.01#	0.91
%FM thorax	22.05 (3.54)	22.30 (2.69)	21.83 (4.32)	0.06	0.81
%FM leg left	24.81 (3.77)	26.14 (4.25)	23.65 (3.10)	1.72	0.21
%FM leg right	24.83 (3.53)	25.66 (3.61)	24.10 (3.53)	0.71	0.41
%FM total	23.49 (3.25)	23.77 (2.51)	23.25 (3.95)	0.09	0.77
FFM arm left [kg]	2.98 (0.81)	2.92 (0.81)	3.03 (0.86)	0.06	0.809
FFM arm right [kg]	3.21 (0.79)	3.08 (0.80)	3.32 (0.82)	0.34	0.567
FFM thorax [kg]	23.58 (3.57)	23.69 (3.11)	23.49 (4.14)	0.01	0.92
FFM leg left [kg]	13.88 (19.33)	8.61 (1.37)	8.49 (26.34)	0.97	0.342
FFM leg right [kg]	9.17 (1.39)	8.99 (1.54)	9.32 (1.33)	0.21	0.657
FFM total [kg]	50.75 (7.65)	50.24 (7.52)	51.20 (8.25)	0.05	0.82

Note: n, number of observations; SD, standard deviation; min, minimal value; max, maximal value; *F*, Snedecor-Fisher test value; *P*, p-value; R, resistance; Xc, reactance; PhA, phase angle; stretch., stretched; contract., contracted; circum., circumference; diam., diameter; SK, skinfold; med., medial; lat., lateral; FM, fat mass; FFM, fat-free mass; #, the parametric comparison was assessed on a logarithm transformed variable.

Table 3.8 shows the best regression model assessed by the stepwise method (n=14). The mean VIF was 1.49, the $\chi2(1)$ obtained by the Breusch–Pagan/Cook–Weisberg was 3.13 (P=0.08), and the F (2, 11) of the White test equalled 2.15 (P=0.159). Before this, the first model was affected by an outlier (Cook's distance), which was deleted from the latest model. Figure 3.12 shows scatter plots with the error estimation of each model regressor. Figure 3.13 shows the Bland-Altman plot (A) and the scatter plot (B) with the new calculation method and the gold standard (DXA).

Table 3.8. Regression model for FM estimation with Anthropometry

Source	SS	df	MS	$F_{(4, 9)}$	P	R^2	Adj R^2	Root MSE
Model	0.880474	4	0.220118	196.23	<0.001	0.9887	0.9836	0.03349
Residual	0.010096	9	0.001122					
Total	0.890569	13	0.068505					
ln Fat-Mass	β	SE	t	P	95% CI		η^2 [95% CI]	
Weight	0.016	0.001	15.97	<0.001	0.014	0.018	0.966 [0.867; 0.980]	
ln biceps SK	0.180	0.031	5.82	<0.001	0.110	0.250	0.790 [0.358; 0.882]	
ln suprail SK	0.255	0.039	6.57	<0.001	0.167	0.343	0.827 [0.441; 0.902]	
Thigh SK	0.012	0.003	4.02	0.003	0.005	0.018	0.642 [0.130; 0.800]	
Intercept	0.658	0.085	7.76	<0.001	0.466	0.850		

Note: SS, the sum of squares; df, degrees of freedom; MS, mean of squares; F, Snedecor-Fisher statistical test; P, p-value; R^2, the goodness of fit; Adj R^2, adjusted R^2; MSE, mean of squares error; β, regression coefficient; SE, standard error; t, student's statistical test; CI, confidence interval; ; η^2, eta-squared effect size; suprail., supra-iliac; SK, skinfold thickness

The first model generates the equation (1)

$$\ln FM = (0.016 \cdot BW) + (0.180 \cdot \ln Bic\ SK) + (0.255 \cdot \ln Sup\ SK) + (0.012 \cdot th\ SK) + 0.658 \quad (1)$$

where BW is body weight, ln Bic SK is the logarithm of the biceps skinfold thickness, ln Sup SK is the natural logarithm of the supra-iliac skinfold thickness and the SK is the skinfold thickness of the thigh; all the skinfold measures are expressed in millimetres. The exponential function is required to obtain the real FM value (e^{lnFM}). The mean for the new method was 16.917 (± 4.851), while the DXA mean was 16.925 (± 4.894; r= 0.997). The achieved power (1-β) tends to be 1.00 (Δ= 87.5).

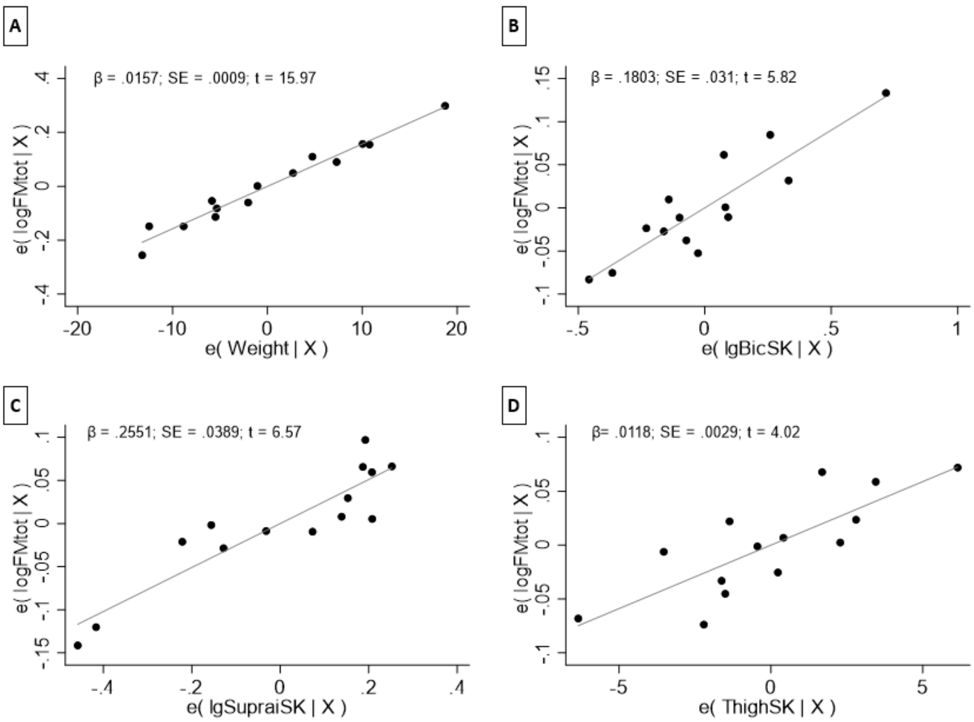

Figure 3.12. Scatter plots with the error of DXA FM and each anthropometric regressor: (A) body weight, (B) logarithm biceps skinfold thickness, (C) logarithm of supra-iliac skinfold thickness, (D) thigh skinfold thickness.

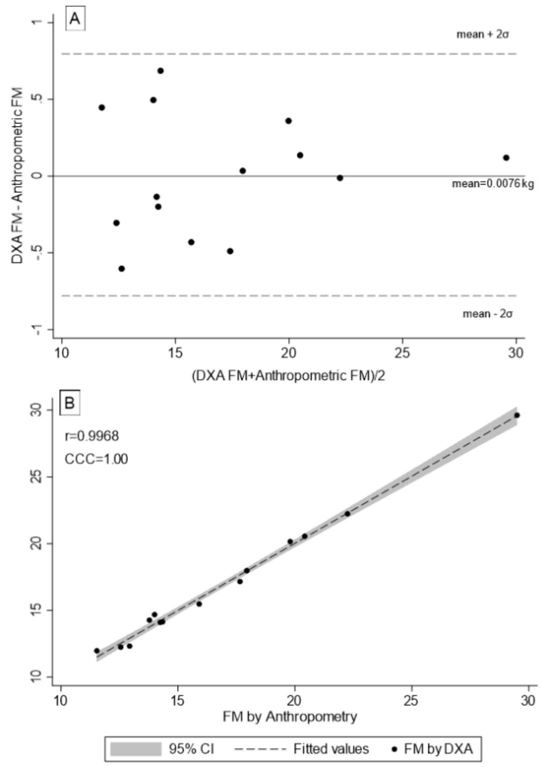

Figure 3.13. Bland-Altman plot (A) and scatter plot (B) between DXA FM and new FM estimation by anthropometry. Note: σ, standard deviation; r, Pearson correlation coefficient; CCC, concordance correlation coefficient.

Table 3.9 shows the best regression model assessed by the stepwise method (n= 14). The mean VIF was 2.97; the $\chi2(1)$ obtained by the Breusch–Pagan/Cook–Weisberg was 0.01 (P= 0.954), and the F (2, 11) of the White test equalled 2.47 (P= 0.126). Before this, the first model was affected by an outlier (Cook's distance), which was deleted from the latest model. Figure 3.14 shows the scatter plot with the error estimation of each model regressor. Figure 3.15 shows the Bland-Altman plot (A) and the scatter plot (B) with the new calculation method and the gold standard (DXA).
The model with the BIA parameter generates the equation (2)

$$\ln FM = (0.0300007 \cdot BW) + (0.006438 \cdot \frac{R}{H^2}) - 0.3035 \quad (2)$$

Where ln FM is the natural logarithm of the fat mass, BW is the body weight, R is the bio-electrical resistance and H is the height (expressed in meters). The exponential function is required to obtain the real FM value (e^{lnFM}). The mean for the new method was 16.766 kg (± 4.83; r=0.973). The achieved power (1-β) tends to be 1.00 (Δ= 22.26).

Table 3.9. Regression model for FM estimation with BIA

Source	SS	df	MS	$F_{(2, 11)}$	P	R^2	Adj R^2	Root MSE
Model	0.878596	2	0.439298	122.3	<0.001	0.957	0.9491	0.05993
Residual	0.039511	11	0.003592					
Total	0.918107	13	0.070624					
log Fat-Mass	β	SE	t	P	95% CI		η² [95% CI]	
Weight	0.030007	0.002329	12.88	<0.001	0.02488	0.035133	0.938 [0.799; 0.963]	
R/h²	0.006438	0.001205	5.34	<0.001	0.003785	0.00909	0.722 [0.296; 0.839]	
intercept	-0.3035	0.324187	-0.94	0.369	-1.01703	0.410028		

Note: SS, the sum of squares; df, degrees of freedom; MS, mean of squares; F, Snedecor-Fisher statistical test; P, p-value; R^2, the goodness of fit; Adj R^2, adjusted R2; MSE, mean of squares error; β, regression coefficient; SE, standard error; t, student's statistical test; CI, confidence interval; η^2, eta-squared effect size.

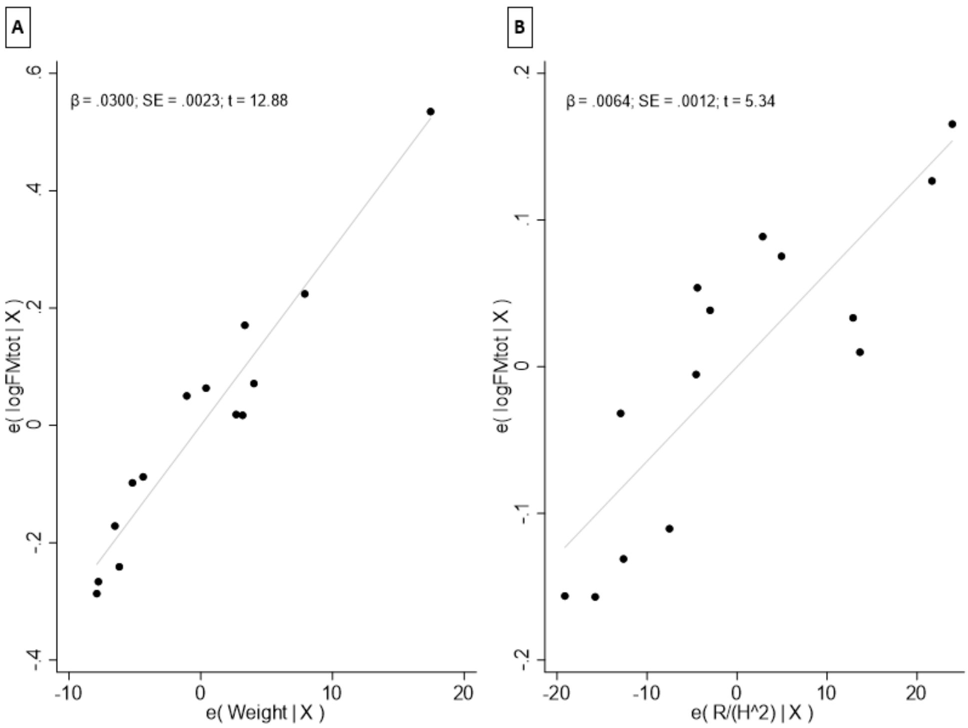

Figure 3.14. Scatter plots with the error of DXA FM and Weight (A) and Resistance/Height^2 (B) regressors.

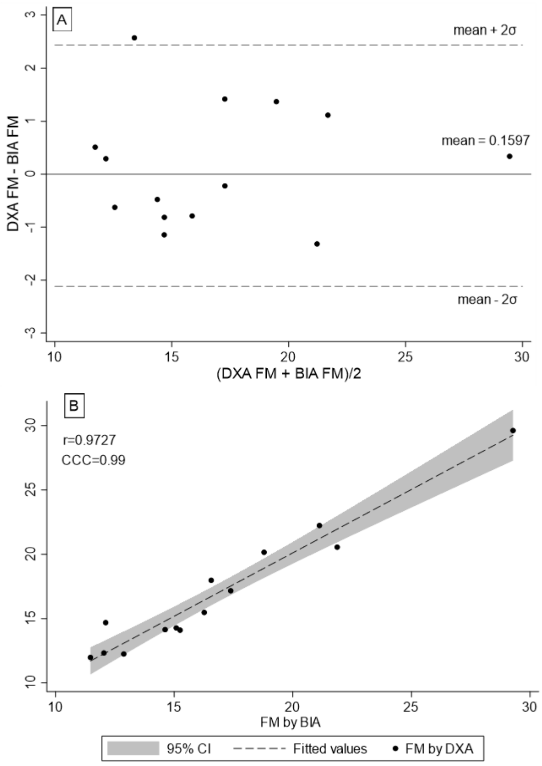

Figure 3.15. Bland-Altman plot (A) and scatter plot (B) between DXA FM and new FM estimation by BIA, Height, and Weight. Note: σ, standard deviation; r, Pearson correlation coefficient; CCC, concordance correlation coefficient.

BIVA

Figure 3.16 shows the BIVA qualitative analysis of all players (A), and the two roles mean respectively (B), where the reference ellipse referred to elite tennis players. Despite the wide variability between each participant, the means of the two groups exhibited similar vectorial characteristics and lay in the 50% tolerance of the ellipse of tennis players. In addition, Figure 3.16 (C) shows the BIVA confidence ellipses of padel, tennis, endurance, power, and elite team sport. There, padel players exhibited more similar characteristics to team sports (T2= 0.70; D= 0.22, p= 0.71) than elite tennis players (T2= 3.71; D= 0.62, p= 0.18), while significant differences appeared compared to endurance sports (T2= 8.52; D= 0.79, p= 0.01).

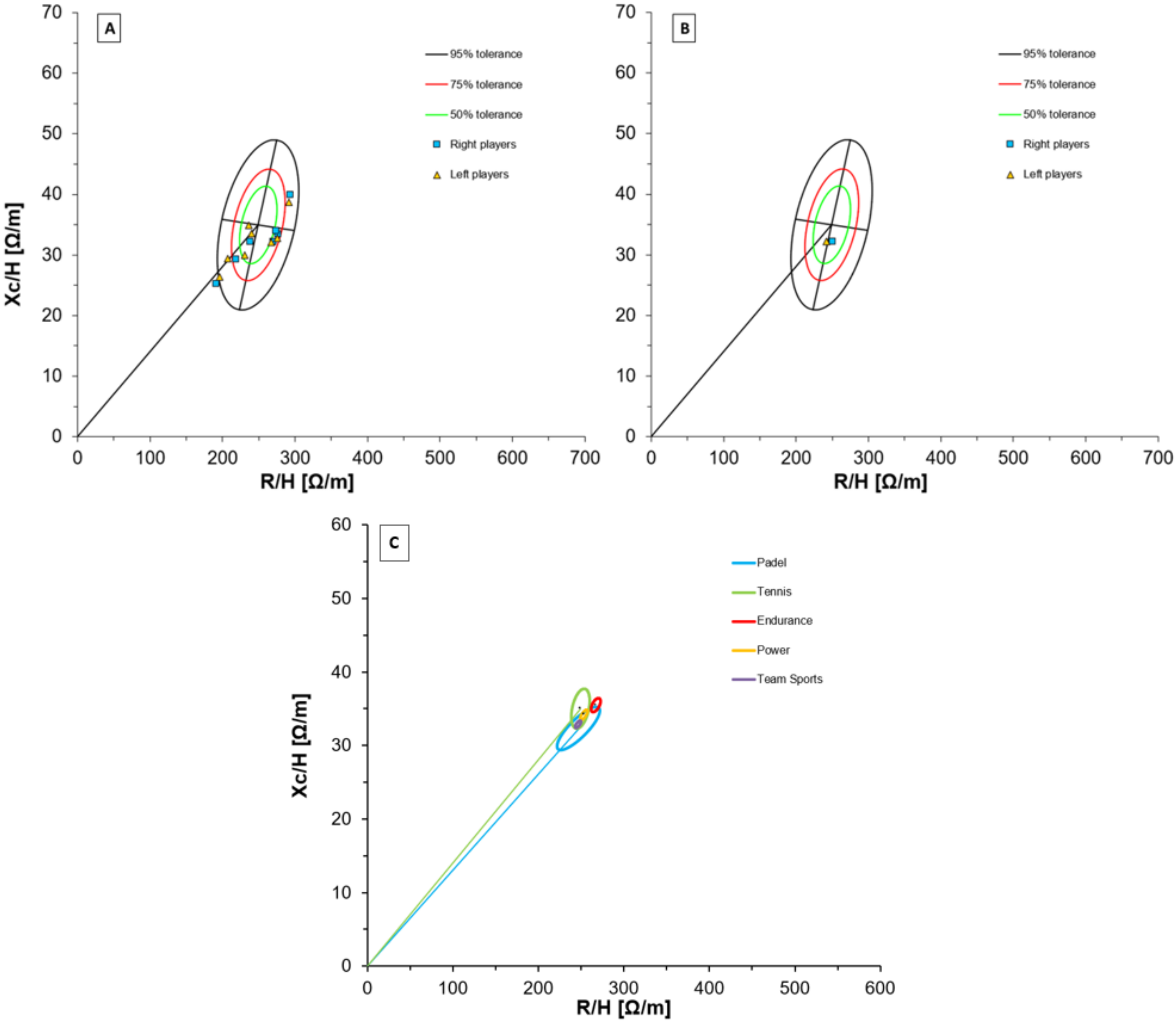

Figure 3.16. BIVA tolerance patterns of all (A) or right and left Padel players means (B) in elite tennis players reference ellipse; (C) BIVA confident ellipses of Padel and Tennis, Endurance, Power, and Team Sports elite players.

3.3.4 Discussion

This study first aimed to draw two specific linear regression models to estimate FM with simpler field methods such as anthropometry or BIA in padel players. To the best of our knowledge, the literature is lacking specific models and no previous studies have tried to fill this gap. Regarding anthropometry, previous authors (Fuente et al., 2019; Marín et al., 2021; Martínez-Rodríguez et al., 2014) who investigated body composition in padel players used classic methods such as Siri's equation (Siri, 1961) to convert the body density (BD) estimated by Withers and colleagues' model (Withers et al., 1987). Although BD conversion has been widely applied since 1961 and Withers et al. yielded their regression equation on 185 subjects, the correlation coefficient of their predicted model was less than 80% and the sample was composed of lacrosse and football players. Differently, Sánchez-Muñoz and colleagues (Sánchez-Muñoz et al., 2020) used five different equations derived from 1967 to 1974 to estimate the body density of elite padel athletes (Durnin & Womersley, 1974; Katch & McArdle, 1973; Sloan, 1967; Wilmore & Behnke, 1969; Withers et al., 1987). These pioneering studies used underwater weighting as the gold standard to calculate BD, which requires higher expertise in maximum exhalation to ensure the correct measure of participants' residual volumes. Three works assessed the analysis of healthy college and university USA students and did not report any information about their training status or sports classification; the correlation coefficients between the observed and predicted BD values were r=0.867 (16.8-36.8 years, 133 men (Wilmore & Behnke, 1969)) r=0.861(18-26 years, 50 white men (Sloan, 1967)) and r=0.86 (53 men (Katch & McArdle, 1973)), respectively. Durnin and Womersley (Durnin & Womersley, 1974) analysed 209 healthy men classified by their age (from 16 up to 72 years) and assessed many models to estimate BD by one through four skinfolds (r varied from 0.7 up to 0.9). Despite these articles reporting both the standard error and the correlation coefficient of each measurement, they lacked several statistical applications such as checking collinearity, heteroscedasticity, and the presence of any outliers, testing the reliability and the power achieved and reporting the goodness of fit of each model. Our model met all the assumptions the generalized linear model requested and reported a higher correlation coefficient value in predicting players' FM. Differently, Stewart and Hannan (D. Stewart & Hannan, 2000) used rigorous statistical criteria to draw different regression models for predicting FM and FFM through anthropometry in 106 athletes of several disciplines. Although twelve of them were racket sports players and DXA was used, the models considered the whole sample with no discipline differentiation. In addition, 24 of them did not compete in their sports and 19 were international athletes. Contrarily, our model was computed on only padel players who trained and competed at the same level. The new equation calculates precise players' body composition through field measurements such as body weight, biceps, supra-iliac, and thigh skinfolds.

Regarding Bio Impedance Analysis, a recent review showed that many BIA-based predictive equations for FM estimation in general and athletic populations are available, but sport-specific models are needed (Campa et al., 2023). To the best of our knowledge, no authors have computed any regression model in racket sports such as padel. Related to other sports, Matias and colleagues developed an accurate regression equation from DXA to BIA in elite futsal players, for predicting FFM (Matias et al., 2023). In 44 collegiate footballers, three authors compared BIA evaluation for predicting total FM with DXA measurements and found that multifrequency BIA underestimated FM and overestimated FFM (Raymond et al., 2018); however, no regression model was reported. In addition, Matias et al. reported a high goodness of fit (R^2=0.94) using the BIA resistance as a predictor to estimate FFM derived from the 4-C model, but the study included 105 male and 37 female general athletes (Matias et al., 2021). Although our model was derived from only 14 players who were male, the achieved statistical power was very high, and the explained variance equalled 95%. However, more efforts are needed for padel and other sports.

The second aim of our research was to provide a BIVA profile of padel players. Many studies observed the vector trajectory to measure hydration status in several disciples (Giorgi et al., 2018; Martins et al., 2021; Micheli et al., 2014; Reljic et al., 2013), but no references are available in padel. Martins and colleagues included seven players of badminton, tennis or tennis table in a sample of 167 university athletes (18-35 years), but they only compared team and individual sports (Martins et al., 2021). Just one study reported the BIVA comparisons between 23 sports, including tennis, which was classified as a velocity-power discipline (Campa et al., 2019). According to their classification, we compared padel players with tennis, endurance, power, and team sports vectors. Reversely to what was expected from sports features, padel players exhibited vector direction and angle closer to power and team sports than tennis, while endurance athletes appeared significantly different with a longer vector (less hydrated athletes). Despite padel and tennis sharing several rules and game situations, padel has confirmed to possess team sports body composition parameters. However, padel became widely played in the last five years and many of our participants played different team sports before its occurrence.

The final purpose of this study was to understand whether anthropometric and body composition differs among padel roles. Despite technical-tactical, physiological and mechanical demands being expected to be similar, the probability of possessing a dominant left hand on the right court side is higher. Contrary to our expectation, the sample showed no differences in BC among left and right players. Many studies exhibited how body characteristics could vary among roles of different team sports (Hogarth et al., 2021; Ramos-Campo et al., 2014; Staśkiewicz et al., 2022; Zaric et al., 2020). Due to its misleading classification close to tennis, previous authors who investigated Padel players' body composition did not discriminate between right and left positions (Martínez-Rodríguez et al., 2014; Sánchez-Muñoz et al., 2020). Left and right padel players involved in our research appeared homogeneous in all anthropometric measurements and BIVA positions. However, the sample size achieved in this study was small and a larger analysis could detect specific roles' features. In addition, more efforts are needed to explore the physiological demands of roles as done among gender (Torres-Luque et al., 2015) and competitive levels (Courel-Ibáñez & Herrera-Gálvez, 2020).

This study presents some limitations: a) the sample size was small to detect any difference between left and right players; b) the participants were male, and their ages varied from 16 up to 47 years. The equations are specific for these sex and age targets; c) although all participants played padel at least for five years, many of them practised team or individual sports such as soccer or tennis before becoming padel players, and d) no diet information was recorded, e) these equations may not be generalized.

3.3.5 Conclusions

Padel has aroused great interest in the last five years, showing increased participation in amatorial and professional competitions. This study provides for the first time two specific regression equations that allow us to precisely estimate the body composition in padel players through anthropometry instruments and BIA. Also, it adds novel to the growing literature related to padel training evaluation assessments and body characteristics, and it draws a specific BIVA profile. These methods may have practical implications for enhancing the accuracy of longitudinal player monitoring such as in a game season or a multi-season plan, and to provide a cheap and easy method to evaluate padel players. Evaluating body composition characteristics and comparing body dimensions and requests among sports ranks could increase the fundamental knowledge needed for players' best performances and sports advancement.

3.4 STUDY IV

3.4.1 Introduction

Volleyball is an intermittent sport that requires the performance of high intensity with an intermittent nature, i.e., frequent short bouts of high-intensity exercise followed by periods of low-intensity activity and brief rest periods (Chamari et al., 2001; Gabbett & Georgieff, 2007; Mendes et al., 2021). Suitable anthropometric and body composition characteristics and high technical and tactical skills are needed to succeed in this sport (Fields et al., 2018; Gaurav et al., n.d.). The frequent jumps that are usually performed during a volleyball match require specific characteristics, such as thinness along explosive muscle power. Among anthropometric variables, leg length, arm span, and height, differ between high-level players, along with physical skill, such as coordination in agility tests and vertical jump (Rubajczyk & Rokita, 2020; Zhao et al., 2019). Height, arm span, and upper and lower body power have been identified as key factors for performance in both male and female adolescent volleyball players (Tsoukos et al., 2019a, 2019b). However, few studies discussed volleyball players' physical and functional characteristics, particularly during adolescence. In addition, the available literature principally focuses on female volleyball players (Carvalho et al., 2020; Papadopoulou et al., 2019), but there are far few studies on males.

Regarding adolescence, the influence of maturity status on physical and physiological characteristics has attracted increased scientific interest, considering its relevance for sports performance. Biological maturation can be defined as the timing and tempo of progress to achieving a mature state (Malina et al., 2005). The physical development of young players is strongly influenced by maturity status, especially as regards their body composition and physical capacities (Campa et al., 2019; Toselli et al., 2020; Živković et al., 2022).

Understanding the role of maturity on physical characteristics and performance in youth athletes during adolescence is essential since this period coincides with the selection of players. Sport is selective, chiefly during adolescence, and often occurs along a maturity-related gradient. Many studies analysed the influence of maturity status on physical, physiological, and performance characteristics in soccer, basketball or handball players (Barazetti et al., 2019; Campa et al., 2019; Romero-García et al., 2023; te Wierike et al., 2015; Toselli et al., 2020, 2022), but less information exists on male volleyballers. Albaladejo-Saura and colleagues reported that volleyball players with a more advanced state of maturation exhibited higher values of height, arm span, sitting height, bone diameters, muscle perimeters and fat, muscle and bone masses, and better performance achieved in medicine ball throwing and in countermovement jump (CMJ) than their chronological age peers (Albaladejo-Saura et al., 2022). Since variables such as height, sitting height, leg length, and muscle circumferences have a high correlation with performance in physical fitness tests related to volleyball requirements, the best values obtained by volleyball players with an advanced maturity status testify how this state represents a competitive advantage in the sport performance of volleyball during adolescence.

To our knowledge, no previous studies were carried out about bioelectrical impedance vectorial analysis (BIVA) and young volleyball players. Therefore, the present study aims to (a) compare the prevalence of maturity status among volleyball players of the teams that have reached different positions in the ranking of a national tournament, and (b) investigate the relationship between maturity status and anthropometric, performance, body composition parameters and BIVA. These two aspects are strongly connected with the talent selection

It was hypothesized that players who reached a higher position in the ranking would exhibit differences in maturity status and their anthropometric and body composition profile. In particular, people with an early maturation could have better results in the final racking, and they could show higher value for some anthropometric characteristics, such as stature, circumferences, and lower value of fat mass in comparison with boys classified on time or with a late maturation.

3.4.2 Methods

Participants and Study Design

This is an observational study assessed between the 17th and 18th of June 2022, during the National Tournament "0.13 Torneo Città di Treviso", organized in Treviso (Italy) from the Volleyball Society Volley Treviso. Eight teams of 22 were randomly selected to be measured during the manifestation: Volley Treviso, La Piave Volley, Kosmos Volley, Pallavolo Sestese, Cisanonembro'thers, Gas Sales Bluenergy Piacenza, Virtus Fano and VT Personal Time. A total of 94 young male volleyball players were evaluated (Volley Treviso:11, La Piave Volley: 12, Kosmos Volley: 11, Pallavolo Sestese: 12, Cisanonembro'thers: 9, Gas Sales Bluenergy Piacenza: 12, Virtus Fano: 13, VT Personal Time: 14). Figure 3.17 shows the study design. All the evaluations were assessed within a Treviso sports centre where a private room was set up for specific environmental features such as a temperature between 22°C and 24°C and air humidity between 50 and 60%.
The volume of the weekly workouts of each team was collected from all coaches, and each player trained for about 6 hours per week (four workouts of 90 minutes each). In each training unit, 45 minutes were spent on strength and conditioning and coordinative capabilities, whereas 45 minutes looked for technical-tactical skills. No diet information was collected.
Participants were informed and volunteered to decide to participate in the study. Their parents were informed and provided written consent. This study was in accordance with the Declaration of Helsinki and approved by the Bioethics Committee of the University of Bologna (N. prot. 25027).

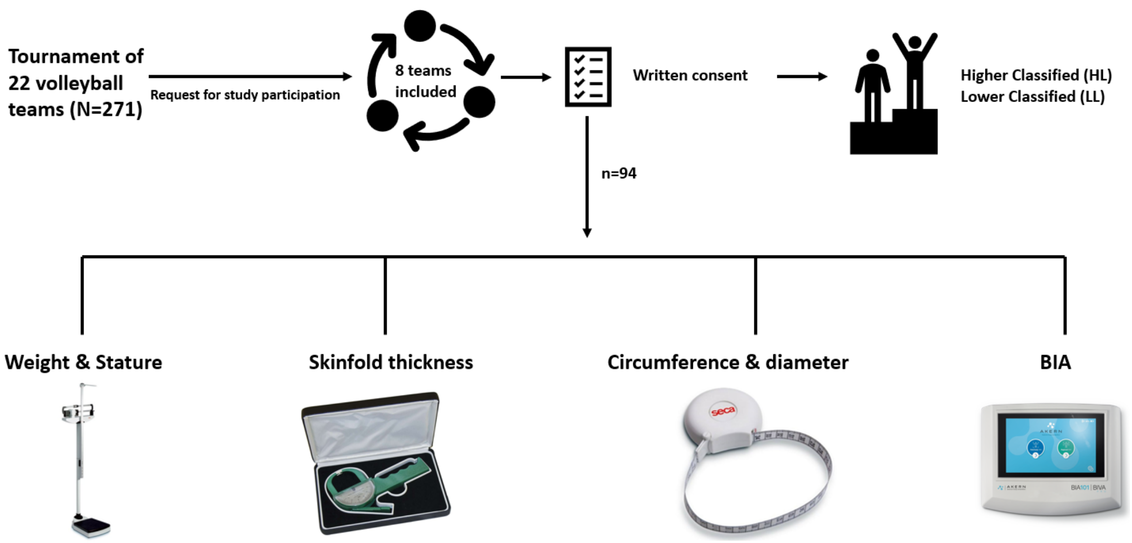

Figure 3.17. Study design.

Anthropometry

A trained operator collected all the anthropometric measurements, such as weight, height, circumferences, and skinfold thickness, according to standardized procedures (Lohman et al., 1988). The mean value of three measurements was gathered. Weight was measured to the nearest 0.1 kg using a calibrated analogue scale. Height and sitting height were collected at the nearest 0.1 cm using a stadiometer (GPM, Zurich, Switzerland). The body mass index (BMI) was calculated as the ratio between weight (kg) and squared stature converted in meters (m).
Circumferences (relaxed and contracted upper arm, waist, hip, calf) were measured to the nearest 0.1 cm with a non-stretchable tape. The upper arm circumference was taken on the subject in a standing position, at the mid-point between the shoulder acromion and the olecranon process point, with the

participant's elbow relaxed along the body side (stretched evaluation) or to be flexed 90◦ with palm facing upward (contracted evaluation); the waist circumference was taken on the subject in a standing position with close feet and arm along the trunk, at the minimum abdominal circumference line, between the inferior margin of the last rib and the iliac crest; The hip circumference was taken on the subject in a standing position with close feet and arm along the trunk, at the highest point of glutes; the calf circumference was taken at the bulkiest calf point, with the participant in a standing position (calf muscles stretched).
Diameters (humerus and femur) were taken to the nearest 0.1 cm with a sliding calliper, both on the left side of the body. The humerus and femoral widths were taken, respectively, between the own lateral and medial epicondyles, with the participant's elbow and knee flexed 90◦.
Skinfold thicknesses (biceps, triceps, subscapular, supraspinal, supra-iliac, thigh, medial and lateral calf) were measured to the nearest 1 mm using a Lange skinfold calliper at the left side of the body (Beta Technology Inc., Houston, TX, USA) at the following sites: triceps and biceps, vertically at the midpoint between the acromion process and the olecranon process, respectively, a posterior and anterior upper arm face; subscapular, at an angle of 45″ to the lateral side of the body, about 20 mm below the tip of the scapula; supra-iliac, about 20 mm above the iliac crest (in the axillary line); supraspinal, about 20 mm above the iliac spine; calf, vertically at the bulkiest calf point both medially and laterally.
Then, body composition parameters such as fat-free mass (FFM), fat mass (FM), and percentage of fat mass (%F) were estimated according to the equation developed by Slaughter et al. (Slaughter et al., 1988). According to Frisancho's equations, many body areas were estimated such as the total area of the upper arm (TUA) and the lower limb (TCA), muscle area of the upper arm (UMA) and lower limb (CMA), fat area of the arm (UFA) and lower limb (CFA) (*Anthropometric Standards*, n.d.). In addition, calf, and arm fat index (FCI and UFI) were derived.

Maturity status

Mirwald and colleagues developed a specific equation for boys to estimate the years from the peak height velocity (PHV), which is an important index of adolescent growth (Mirwald et al., 2002). Maturity offset represents the time before or after the PHV, by subtracting the age at PHV from chronological age, it is possible to estimate the year from PHV.

$$MO = -9.236 + 0.0002708\ (leg\ length\ *\ sitting\ height) - 0.001663\ (age\ *\ leg\ l007216\ (age\ *\ sitting\ height) + 0.02292\ (weight/height).$$

Children who are not yet in their adolescent growth spurt often have a lower approximation of the age at PHV (APHV) and those who have already passed their adolescent growth spurt are often higher [12]. For this reason, age-specific z-score was used to classify the young athletes. Based on the age-specific standardized Z-score of the predicted APHV, boys were classified as later (Z >1), on time (-1.0≤ Z ≤1.0), and earlier Z<1.0 maturing (Drenowatz et al., 2013).

Bioelectric Impedance Vector Analysis (BIVA)

The bioelectric impedance analysis (BIA) was used to measure the impedance. An electric current was used with a frequency of 50 kHz (BIA 101 BIVA® PRO, Akern, Florence, Italy). The participants were in the supine position, with four electrical conductors, two electrodes were posed in the right hand and two in the right foot, after cleaning the skin with alcohol (Micheli et al., 2014; Piccoli et al., 1994). Subjects were asked to put their lower limbs at an angle of 45° compared to the median line of the body and to put their upper limbs at an angle of 30° from the trunk. Athletes received instruction to abstain from foods and liquids for ≥4 hours before the test. BIVA was carried out using the classic methods, e.g., normalizing R (Ω) and Xc (Ω) for height in meters (Campa et al., 2021). Both the elite male volleyball players and the general adolescent male population bioelectrical-specific ellipses were used as a reference to build the 50%, 75%, and 95% tolerance ellipses on the R/H–Xc/H graph. BIVA plots the parameters recorded in BIA (R, Xc, PhA) as a vector within a specific tolerance ellipse (specific profile for each sport and competitive level), and it allows evaluation of soft tissues through patterns based on percentiles of their electrical characteristics. A BIVA vector that falls out of the 75% tolerance ellipses exhibits a different tissue impedance compared to the selected reference population, while vectors that fall in the 50% represent common impedance characteristics.

Statistical analysis

The eight teams were divided into two groups (Higher Level, HL; Lower Level, LL) according to their final ranking at the tournament (teams that got at least quarterfinals =HL, teams that lost before quarterfinals =LL). The mean and standard deviation (SD) of the two groups were calculated for each variable and the frequency of appearance (percentage) was determined for the maturity status. The distribution of the variables' residuals was verified with the Shapiro-Wilk test. When a variable presented a right-skewed curve, the logarithm transformation was applied to meet the normality distribution assumption. The two-tailed one-way analysis of variance (ANOVA) was performed to evaluate the differences between the two groups and among maturity statuses. When a variable's distribution could not meet the normality assumption, a non-parametric statistic test was performed (Mann-Whitney rank-sum and Kruskall-Wallis's rank tests). The probability of the type-I error was settled at <0.05. Finally, a post hoc Tukey evaluation was used to evaluate the difference between the final position at the tournament and between the maturity status when the Snedecor-Fisher statistical test probability value (*F*) was observed as significant.

3.4.3 Results

Table 3.10 shows the maturity status prevalence according to the tournament's final ranking. Three teams were classified as higher level due to the results of the tournament, and five teams were classified as lower level. Teams with a worse ranking presented a higher number of boys with later maturity status, whereas the ratio of players who matured on time was similar (HL= 69.44%, LL= 63.79%).

Table 3.10. Prevalence of maturity status among team better and worse classified.

MS (Z ± 1)	Ranking frequency		Δ Ranks		
	HL	LL	Z or χ^2	p	RR
E	7	5	1.529	0.126	2.256
OT	25	37	0.562	0.574	1.089
L	4	16	-1.901	0.05*	0.403
Total	36	58	4.98	0.083	

Note: MS= maturity status, E=early, OT=on time, L=late, Z=the test of proportion Z, X^2= Pearson chi-squared test; p=p-value; RR=risk ratio; *, statistically significant; Δ difference.

Table 3.11 shows the mean and standard deviation of each variable for both the ranking group and the maturity status, and it reports the statistical comparisons between them and their interaction.

Table 3.11. General variable statistics according to MS ± 1 year and the final ranking of the tournament.

	HL			LL			Ranking		MS		Ranking*MS	
	E (n=7)	OT (n=25)	L (n=4)	E (n=5)	OT (n=37)	L (n=16)						
Variable	Mean (± SD)	Mean (± SD)	Mean (± SD)	Mean (± SD)	Mean (± SD)	Mean (± SD)	$F_{(1, 88)}$	P	$F_{(2, 88)}$	P	$F_{(2, 88)}$	P
Age (year)#	12.49 (0.81)	12.01 (0.37)	12.75 (0.51)	11.68 (1.82)	13.04 (0.26)	12.40 (0.85)	0.354	0.552	2.865	0.239	3.680	**<0.001***
Weight (Kg)	64.07 (7.97)	52.32 (9.29)	38.00 (4.00)	59.40 (13.99)	45.78 (7.99)	49.88 (9.19)	0.010	0.931	14.540	**<0.001***	1.910	0.154
Stature (cm)	175.89 (7.29)	161.79 (5.30)	148.98 (3.48)	162.36 (16.84)	155.83 (6.71)	159.91 (8.83)	1.580	0.212	13.330	**<0.001***	4.970	**0.001***
Trunk Height (cm)	86.81 (3.21)	79.24 (2.65)	70.80 (1.60)	85.68 (8.30)	74.15 (1.46)	80.35 (4.24)	1.130	0.291	43.020	**<0.001***	1.130	0.327
Leg lenght (cm)	89.07 (5.30)	82.55 (3.74	78.18 (2.32))	76.68 (8.95)	81.68 (6.83)	79.56 (6.39)	5.720	**0.019***	0.800	0.454	5.710	**0.005***
BMI (kg/m^2)	20.64 (1.43)	19.99 (3.49)	17.13 (1.74)	22.24 (1.69)	19.18 (3.26)	19.37 (2.42)	1.730	0.192	4.860	**0.010***	1.560	0.216

Note: E=early, OT=on time, L=late, MS=maturity status, SD=standard deviation, F=Snedecor-Fischer statistic test, BMI=body mass index, , %F=fat percentage, FM=fat mass, FFM=fat free mass, R=resistance, Xc=reactance, PA=phase angle, *=statistical significant, #=Mann-Whitney rank-sum test and Kruskall-Wallis rank test.

Regarding the differences linked to the ranking position (tables 3.12 and 3.13), better teams exhibited significantly higher values in leg length and femoral diameter, and lower amounts of fat on the most informative skinfolds, and in fat percentage. On the contrary, boys who stopped before the quarterfinals showed significantly higher values in arm circumference, arm and calf skinfold thicknesses, and fat area or percentage on their lower and upper limbs (TUA, UFA, UFI, CFA, and CFI). Also, players clustered in the HL group showed a wider skeletal robustness in their lower limb (femoral diameter).

Several statistically significant anthropometric differences were relative to maturity status. Boys classified as early showed better values in many important anthropometric characteristics such as height, weight, all the circumferences, calf muscle area, and body composition parameters such as fat mass and fat-free mass than on-time and later youths.

Finally, regarding the interaction effect between ranking and maturity status, the earlier young players classified as higher-level showed significantly wider values in height, leg length, and femoral diameter than the earlier young players classified as lower level. In addition, the earlier boys ranked between the lower level presented higher values in parameters related to the local (triceps, subscapular, supraspinal, suprailiac, and lateral calf skinfolds, UFI, CFI) and total body fat mass (%F, FM) than earlier players classified in the first positions. Finally, although players who matured on time showed better characteristics in HL than LL teams in body composition (%F, FM), the LL players were taller and exhibited longer low limbs.

Table 3.12 Anthropometric statistics according to MS ± 1 year and the final ranking of the tournament

Variable	HL E (n=7) Mean (± SD)	HL OT (n=25) Mean (± SD)	HL L (n=4) Mean (± SD)	LL E (n=5) Mean (± SD)	LL OT (n=37) Mean (± SD)	LL L (n=16) Mean (± SD)	Ranking $F_{(1, 88)}$	Ranking P	MS $F_{(2, 88)}$	MS P	Ranking*MS $F_{(2, 88)}$	Ranking*MS P
str. arm circ. (cm)	24.97 (2.68)	20.98 (2.12)	22.48 (2.18)	26.90 (2.72)	22.93 (2.96)	23.60 (2.54)	5.420	**0.022***	8.460	**<0.001***	0.230	0.796
con. arm circ. (cm)	26.66 (2.34)	22.00 (1.92)	23.85 (2.34)	27.76 (2.40)	23.71 (3.01)	24.61 (2.68)	2.610	0.110	9.230	**<0.001***	0.180	0.832
Calf circ. (cm)	35.69 (1.99)	29.88 (0.38)	32.61 (2.44)	35.06 (3.77)	31.33 (3.65)	32.32 (2.40)	0.060	0.813	9.890	**<0.001***	0.630	0.533
Waist circ. (cm)	70.24 (6.69)	60.55 (1.97)	65.90 (5.87)	73.82 (5.82)	64.74 (6.42)	66.19 (5.22)	2.800	0.098	8.740	**<0.001***	0.860	0.428
Hip circ. (cm)	89.89 (6.60)	71.95 (7.48)	82.62 (6.51)	91.16 (8.20)	80.32 (7.21)	82.72 (6.27)	2.950	0.089	14.240	**<0.001***	2.030	0.137
Humeral diamet. (mm)#	6.79 (0.59)	6.15 (0.44)	6.34 (0.38)	6.50 (0.44)	6.62 (1.47)	6.43 (0.39)	0.001	0.991	6.015	**0.050***	0.740	0.595
Femoral diamet. (mm)#	9.50 (0.58)	8.10 (0.48)	9.40 (1.28)	8.56 (0.85)	8.35 (0.60)	8.59 (0.55)	17.366	**<0.001***	12.647	**0.001***	5.610	**<0.001***
Triceps SK (mm)	10.14 (3.53)	9.25 (2.06)	12.44 (4.3)	16.40 (1.52)	12.31 (4.88)	11.68 (4.12)	5.680	**0.019***	1.160	0.318	3.950	**0.023***
Subscapular SK (mm)	9.29 (2.69)	6.00 (2.16)	10.40 (4.61)	11.20 (1.79)	9.94 (3.02)	8.82 (2.89)	4.400	**0.039***	2.870	0.062	5.600	**0.005***
Supraspinal SK (mm)	11.71 (5.68)	6.50 (2.65)	12.80 (7.05)	15.00 (3.32)	11.06 (4.37)	10.22 (5.10)	2.850	0.095	3.010	0.054	3.970	**0.022***
Suprailiac SK (mm)	13.00 (6.32)	9.25 (3.69)	14.40 (6.84)	16.60 (2.30)	14.31 (5.87)	11.95 (5.34)	2.510	0.117	1.150	0.320	3.350	**0.039***
Medial Calf SK (mm)	10.86 (4.02)	11.25 (1.71)	12.68 (4.43)	15.60 (1.52)	13.44 (3.61)	12.65 (3.81)	4.440	**0.038***	0.170	0.846	2.060	0.133
Lateral Calf SK (mm)	10.57 (2.15)	12.25 (1.26)	12.80 (3.54)	15.60 (1.34)	12.88 (3.40)	12.97 (2.87)	5.100	**0.026***	0.100	0.909	3.140	**0.048***
TUA (cm^2)	50.11 (10.41)	35.28 (7.22)	40.56 (7.90)	58.05 (11.83)	42.50 (10.82)	44.81 (9.81)	5.680	**0.019***	8.790	**<0.001***	0.260	0.768
UMA (cm^2)	38.11 (7.77)	26.13 (4.67)	27.66 (5.07)	38.11 (9.56)	29.25 (6.34)	31.88 (6.06)	1.950	0.166	9.140	**<0.001***	0.680	0.508
UFA (cm^2)	12.00 (4.77)	9.15 (2.78)	12.91 (5.02)	19.94 (2.90)	13.25 (6.26)	12.93 (5.58)	6.450	**0.013***	2.710	0.072	2.810	0.066
UFI (%)	23.59 (6.36)	25.59 (3.49)	31.20 (8.32)	34.82 (4.14)	30.10 (8.55)	28.15 (7.12)	3.980	**0.049***	0.320	0.729	5.100	**<0.001***
TCA (cm^2)	101.61 (11.08)	71.03 (1.80)	85.09 (12.66)	98.72 (20.35)	79.11 (17.11)	83.58 (12.16)	0.100	0.749	10.940	**<0.001***	0.730	0.483
CMA (cm^2)	67.23 (12.97)	40.31 (3.46)	48.74 (10.7)	51.62 (14.34)	43.09 (10.46)	47.33 (9.14)	2.670	0.106	9.480	**<0.001***	2.880	0.061
CFA (cm^2)	34.37 (8.26)	30.72 (1.80)	36.34 (10.83)	47.10 (7.34)	36.02 (10.22)	36.25 (8.98)	4.340	**0.040***	1.700	0.189	1.910	0.155
CFI (%)	34.06 (8.24)	43.31 (3.46)	42.57 (9.88)	48.32 (4.88)	45.64 (7.99)	43.27 (7.93)	5.980	**0.016***	0.470	0.624	3.240	**0.044***

Note: E= early, OT= on time, L= late, MS= maturity status, SD= standard deviation, F= Snedecor-Fischer statistic test, BMI= body mass index, circ= circumferences, str= stretched, con= contracted, SK= skinfold thickness, TUA= total upper limb area, UMA= upper limb muscle area, UFA= upper limb fat area, UFI= upper limb fat index, TCA= total calf area, CMA= calf mass area; CFA= calf fat area, CFI= calf fat index, *= statistical significant, #= Mann-Whitney rank-sum test and Kruskall-Wallis rank test.

Table 3.13. Body composition statistics according to MS ± 1 year and the final ranking of the tournament

	HL			LL								
	E (n=7)	OT (n=25)	L (n=4)	E (n=5)	OT (n=37)	L (n=16)	Ranking		MS		Ranking*MS	
Variable	Mean (± SD)	Mean (± SD)	Mean (± SD)	Mean (± SD)	Mean (± SD)	Mean (± SD)	$F_{(1, 88)}$	P	$F_{(2, 88)}$	P	$F_{(2, 88)}$	P
%F	18.54 (5.21)	14.79 (3.88)	21.23 (6.65)	25.56 (1.95)	20.87 (5.97)	19.40 (5.35)	6.510	**0.012***	2.160	0.121	4.900	**0.010***
FM (kg)	11.94 (3.79)	5.71 (2.05)	11.54 (5.49)	15.29 (4.09)	9.85 (4.06)	9.90 (3.99)	3.350	0.071	6.980	**0.001***	3.780	**0.027***
FFM (kg)	52.13 (6.88)	32.29 (2.32)	40.78 (5.13)	44.11 (10.05)	35.93 (5.05)	39.98 (6.70)	0.690	0.410	14.450	**<0.001***	2.590	0.081
R (Ω)	458.14 (50.11)	578.60 (74.45)	505.38 (54.37)	526.92 (43.87)	520.34 (71.54)	529.48 (63.68)	0.440	0.510	2.670	0.075	3.520	**0.034***
Xc (Ω)	61.66 (12.79)	68.63 (18.91)	61.72 (7.59)	59.70 (5.15)	61.21 (8.97)	63.21 (9.55)	0.570	0.453	0.460	0.631	0.840	0.437
PA#	7.73 (1.93)	6.50 (0.97)	6.74 (0.83)	6.48 (0.39)	6.61 (0.85)	6.78 (0.70)	0.318	0.572	3.022	0.221	1.880	0.106
R/H (Ω/cm)	260.88 (30.86)	388.98 (55.30)	428.79 (582.32)	329.06 (57.49)	335.26 (53.83)	333.65 (55.06)	0.100	0.758	0.390	0.681	0.350	0.708
Xc/H (Ω/cm)	34.89 (5.83)	46.03 (12.45)	38.18 (4.77)	37.20 (6.07)	39.45 (6.77)	39.74 (6.90)	0.240	0.627	3.270	**0.043***	2.180	0.119

Note: E= early, OT= on time, L= late, MS= maturity status, SD= standard deviation, F= Snedecor-Fischer statistic test, %F= fat percentage, FM= fat mass, FFM= fat-free mass, R= resistance, Xc= reactance, PA= phase angle, *= statistical significant, #= Mann-Whitney rank-sum test and Kruskall-Wallis rank test.

Bioimpedance Vector Analysis (BIVA)

Figures 3.18 and 3.19 show BIVA results regarding both the final ranking of the tournament (on the left) and the maturity status (on the right).
Figure 3.18 shows significant differences in BIVA vector distance according to the final ranking (A) and between the boys classified as early and late (B).
Figure 3.19 shows different vector placements in the ellipses following the reference population. Compared to the general adolescent reference population (A), only the boys who matured on average were included in the 50% tolerance, while the early matured exhibited a lower level of biological electric resistance. The early boys belonging to winning teams showed a wider displacement to leaner cell mass (left size vector position). In addition, they had a body composition more akin to the elite population of male adult volleyball players (B). Differently, players of the HL teams who matured on average or later exhibited the greatest BIVA differences compared to elite volleyball players (figure 3B, blue triangle and diamond), especially in hydration and lean mass. As regards LL teams, all the maturity categories showed wide displacement against both the general adolescent population and the

elite volleyball reference group. However, they were closer to the adolescent reference population than the adult elite volleyball players.

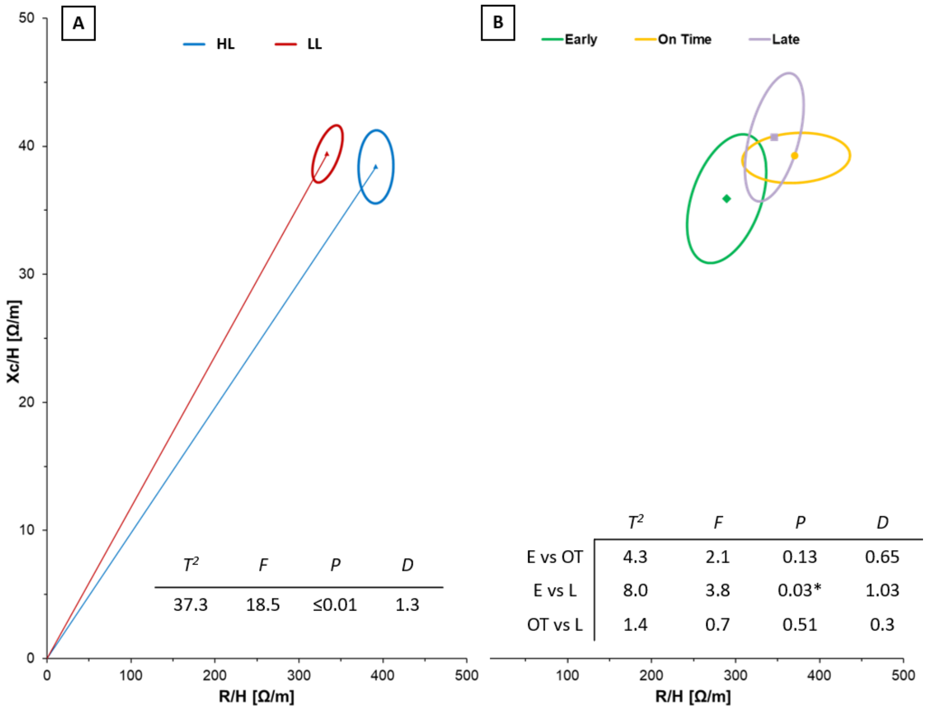

Figure 3.18. Paired graphs for the multivariate changes in classic resistance and reactance are shown depending on the ranking (on the left) and the maturity status (on the right). The mean vector displacements with 95%, confidence ellipses, and results of Hotelling's T2 test are shown. E=early, OT=on time, L=late, HL= higher level, LL= lower level.

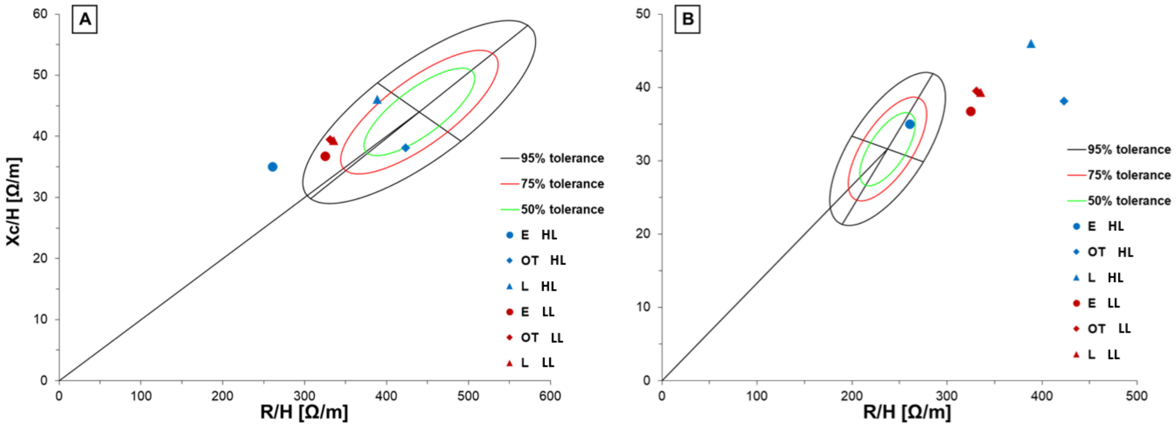

Figure 3.19. BIVA graphs for the multivariate changes in classical resistance and reactance are shown. The bioimpedance data are plotted on the tolerance ellipses of the general adolescent reference population (on the left) and of the elite volleyball players population (on the right). E=early, OT=on time, L=late, HL= higher level, LL= lower level.

3.4.4 Discussion

The present study had two aims: (a) to compare the prevalence of maturity status among volleyball players of the teams that reached different positions in the ranking of a national tournament and (b) to investigate the relationship between maturity status and anthropometric, body composition parameters and BIVA. Our beginning hypotheses include that players who reached a higher position in the final ranking would exhibit differences in maturity status and their anthropometric profiles. Also, we believe that players who mature earlier show better body composition.

A lot of studies regarding the influence of maturity status on the body, physical performance, and physiological characteristics on the growing and scouting of adolescent soccer, basketball or handball players and less information exists on male volleyballers. The elite players have rapidly increased their physical demands in recent years, and, for this reason, recruiters and coaches put greater emphasis on physical fitness, and talent selection, from an early age [16, 26]. In fact, in recent years the identification of adolescent talent grew of interest for both the scientific community and sports managers (C et al., 2014). The implementation of early talent identification programs could bring advantages to the teams that carry them out, both in economic and sporting terms (Pion et al., 2015).

Regarding the prevalence of maturity status, in the present study, significant differences were observed in the boys classified as late maturing in comparison with those who were early or on time. In the teams that achieved the higher position of ranking, only four boys were classified as late, while in the teams ranked between the lower level, there were 16 of them. This is in accordance with previous studies that demonstrated that maturity status has an important role in performance in adolescent males (Johnson et al., 2017; Lohman et al., 1988; Parr et al., 2020; Peña-González et al., 2018; Radnor et al., 2021). Romeo-Garcia and colleagues found that young male handball athletes, who presented an early biological maturation achieved higher values in anthropometric characteristics and physical test (Romero-García et al., 2023). They observed significant differences in basic measurements, such as weight, height, fat-free mass, BMI, and Cormic Index and in some physical tests, such as medicine ball throw and squat jump, with the group of early maturers which had the highest values. On the contrary, Toselli and colleagues did not find any differences in maturation category prevalences between elite and non-elite adolescent soccer teams from 11 to 14 years old (Toselli et al., 2022). However, having boys classified as late in the team reduces the possibility to win, and to have good performance in a short time. Despite this, the immediate advantage of premature maturation could not be associated with great future performance and talent expression. The role of coaches and trainers is fundamental for enhancing and scouting hidden talents.

According to the above-mentioned results, we found that teams that did not reach the quarterfinals showed higher values in several parameters linked to body fat and worse body composition. They exhibited higher values in several skinfold thicknesses, in body fat percentage, and the fat area of the limbs. These results are in accordance with a previous study that investigated the effect of team level, maturation, and interaction in adolescent soccer players (Toselli et al., 2022). Many parameters fat-related differed between elite and non-elite players such as triceps, biceps, subscapular, suprailiac and thigh skinfold thicknesses, and arm, thigh, and calf fat indexes. However, both young and adult volleyball players must make explosive movements and they may be powerful, agile and rapid; for this reason, low body fat is required, also for young volleyball players (Toselli & Campa, 2018). Teams classified between the higher levels showed significantly higher values in leg length and femoral diameter, which are two important characteristics in volleyball. Height and leg length are fundamental in volleyball, due to the height of the net (2.43 meters for elite volleyball players, 2.15 meters in U-13 competitions) (Pocek et al., 2021).

Regarding the differences due to maturity status, the present results are in line with the study conducted by Albaladejo-Saura et al. (Albaladejo-Saura et al., 2022). The authors found higher values in several anthropometric characteristics (such as height, diameters, trunk height, etc), in volleyball players with a more advanced state of maturation, akin to what emerged in the present study. Among the anthropometric characteristics, the greatest differences between the two groups were found for skinfolds. This is in line with previous studies regarding soccer, which showed the importance of monitoring body fat, since appropriate levels of fat permit the players to move more effectively during training and games (Bernal-Orozco et al., 2020; Toselli et al., 2021).

Regarding the results of the BIVA graphs, it is interesting to notice that early boys classified in the first positions had a body composition like the elite population of volleyball players, showing a lower level of resistance and leaner body. In addition, their vector characteristics differed against the general adolescent reference population. The premature growth of the muscle cells and the reduction of the inactive mass (fat mass) are relevant parameters in fast and power sports such as volleyball (Campa et al., 2021; Gabbett & Georgieff, 2007). This could explain the better performance of these teams and could also be an important factor to consider and monitor the BIVA parameters changes over time for talent selection. At the same time, it is interesting to notice that the boys in teams classified as lower levels had a similar position in both the BIA vector graphs, independently from the maturity status. The boys classified on time and in the first position were plotted out from the tolerance ellipse of the elite volleyball players' population. This could be justified by the maturity status because they are near the PHV, which is a moment of big changes for the body. Also, this information could confirm that maturation in adolescence could widely affect changes in anthropometry and body composition, impacting physical performance and team scouting. Although only seven players out of 36 were classified as early maturing in high-level teams, volleyball involves six players on the court for any action and two boys having improved body and physical characteristics could lead to winning.

Previous studies reported that the chance of selection for relatively younger soccer players was widely affected by maturation status, physical performance, and anthropometric characteristics, whereas relatively older athletes had a selection advantage independent of their maturity status (Deprez et al., 2013; Müller et al., 2017). It is difficult to provide an exhaustive comparison, but it seems that the influence of this aspect is the same in this sport.

The present study has several limitations. The study design included only one period of evaluation and longitudinal research with several follow-ups could enrich the specific literature. The teams were randomized and selected to be measured during the tournament, and it was not possible to evaluate all the teams involved. It could have been interesting to measure all the teams participating in the manifestation, to have a wider sample size and to collect more data for maturation states comparison. Also, the participants were only thirteen-aged males; many investigations considered both sexes and different ages are suggested. In addition, it was not possible to collect information about the diet habits of the young male volleyball players. No data were given about the years of experience of the players, which could influence the final ranking, or about the time on the court of each player. Finally, physical tests (for example jumping test, or speed test) were not performed and no data related to match results and skills were collected. Future investigations could draw more complete study designs to evaluate the correlation among physical performance, match analysis, anthropometry and body composition, match level and biological maturation.

3.4.5 Conclusions

In conclusion, in the present study, young male volleyball players classified as early had higher values of the anthropometric characteristics linked to better performance (represented by the final ranking of the tournament). In fact, among the eight teams, two of them that presented the most early maturing boys were ranked in the top places of the tournament (1st -8th place). Anthropometric characteristics, maturity status, and body composition variables significantly influence the final ranking of the tournament. Further studies are needed to better evaluate this relationship in volleyball.

Niels Bohr

“An expert is a person who has made all the mistakes that can be made in a very narrow field”

Niels Bohr

4. LONGITUDINAL STUDIES

LIST OF PAPERS

STUDY V: *Effects of Eight-Week Circuit Training with Core Exercises on Performance in Adult Male Soccer Players*

STUDY VI: *Anthropometric, Body Composition and Physical Performance of Elite Young Italian Football Players and differences between selected and unselected talents*

STUDY VII: *Long-Term Physiological Adaptations Induced by Short-Interval High-Intensity Exercises: an RCT comparing active and passive recovery*

4.1 STUDY V

4.1.1 Introduction

Circuit training (CT) is a popular methodology in fitness and wellness programs, as well as in sports because its modulation induces physiological benefits such as strength, power and cardio-vascular-respiratory adaptations (Alcaraz et al., 2008; Anitha et al., 2018; Giménez & Gomez, 2019; Marín-Pagán et al., 2020; Paoli et al., 2010). A circuit includes a variable number of exercises that come in succession with a specific time adaptation-relate (MacInnis & Gibala, 2017). Each exercise should be general and/or sport-related and can involve the whole body or just a specific body compartment (Adamson, 1959; MacInnis & Gibala, 2017).
The core exercises have been widely promoted in the last 25 years. The role of the core musculature and several training methods have been investigated in sport, fitness and rehabilitation to understand how trunk conditioning could affect performance and health (Akuthota et al., 2008; Borghuis et al., 2008; Brumitt et al., 2013; Hibbs et al., 2008; Prieske et al., 2016; Rommers et al., 2019; Willardson, 2007; Wirth et al., 2017). The core region, also identified as the lumbo-pelvic-hip complex and scapular stabilizing system, is the central part of the body that connects the trunk with the upper and lower limbs and represents the centre of myofascial kinetic chains (Akuthota et al., 2008; Hibbs et al., 2008; Rommers et al., 2019; Willardson, 2007). Several studies showed that core exercises improve stability and neuromuscular control between the spine and pelvis, and increase endurance, strength and power in trunk muscles (Prieske O et al., 2016; Saeterbakken et al., 2022; Willardson, 2010). Also, they could facilitate the force transfer between the upper and lower body, increase static and dynamic balance and improve the execution of specific sports skills (Hibbs et al., 2008; Prieske O et al., 2016; Rommers et al., 2019; Willardson, 2007). Consequently, a proper level of core stability and strength could improve sports performance and physical fitness, prevent several musculoskeletal injuries, and optimize training adaptations in athletes (Borghuis et al., 2008; Hibbs et al., 2008; Luo et al., 2022; Myer et al., 2006; Saeterbakken et al., 2022).
Despite the large diffusion and benefits of core exercises in training programs for individual and team sports, the scientific debate about their efficacy is still open (Luo et al., 2022; Saeterbakken et al., 2022; Wirth et al., 2017; Zemková & Zapletalová, 2022). Some authors highlighted how the core exercises induced positive effects on neuromuscular control, postural stability, trunk muscle strength and endurance, but it remains unclear whether these improvements could be transferred into sport-specific performance (Zemková & Zapletalová, 2022). Differently, a recent review showed that the core program could improve specific skills in team and individual sports if practised at least twice a week for a month (Luo et al., 2022). In addition, a meta-analysis exhibited several benefits for both general physical fitness and sport-specific performance after more than 18 short sessions of core training (Saeterbakken et al., 2022).
As regards team sports, soccer has been classified as an intermittent sport that requests many types of physical abilities such as strength, power, endurance, balance, and several coordinative and technic-tactical skills (Stølen et al., 2005; Toselli et al., 2022). The CT and core exercises performed by soccer players showed several positive effects on static and dynamic balance (Imai et al., 2014), lower limbs strength (Afyon, 2019; Atli, 2021; Bayrakdar et al., 2020; Hoshikawa et al., 2013), speed and agility (Afyon, 2019; Afyon et al., 2017; Atli, 2021; Bayrakdar et al., 2020; Brull-Muria & Beltran-Garrido, 2021; Doğanay et al., 2020; Imai et al., 2014; Prieske O et al., 2016; Vigneshwaran G., 2017), trunk muscularity and cross-sectional area (Afyon, 2019; Bayrakdar et al., 2020; Hoshikawa et al., 2013; Kubo et al., 2011; Prieske O et al., 2016), and flexibility (Atli, 2021). However, some authors reported that both static and dynamic core exercises enhanced trunk stability

without positive effects on speed, agility, quickness, and lower limb strength and power (Sever & Zorba, 2017).

Although the current literature explained conflicting results of the addition of core exercises to general and specific soccer training in all levels of players, we hypothesize that a proper CT including core exercises could report positive effects on balance, strength, and power. So, this study aims to analyze the effects of a specific CT protocol performed during off-season time, on trunk, upper and lower body strength, dynamic balance, and speed in competitive amateur soccer players.

4.1.2 Methods

Subjects and study design

This is a longitudinal study design of eight weeks with two evaluation times (pre and post), from May up to July (off-season period). The study was conducted at the Sports Science Institute of Bologna, Italy. The participant's enrolment was conducted within the soccer team Madonnina Calcio (Modena, Italy) at the end of the regular season (Emilia Romagna Regional League, Italy). The Madonnina Calcio team consists of 23 amateur soccer players. Players were defined as amateurs whether they trained less than three times per week and played not more than one match per week. Also, their performances were not compensated. The eligible criteria were: (a) no history of musculoskeletal, neurological or other orthopaedic disorders in the last 6 months, (b) age range 18-30 years, and (c) no previous experience with core training exercises. In the beginning, 23 players were considered to participate in the study. Of these, three players were injured, whereas one player cannot guarantee their participation due to holidays. Nineteen participants volunteered for the study and completed all the evaluations (figure 4.1). No randomization was possible because many participants could not guarantee to perform at least 12 CT training sessions. Participants were allocated to one of two groups: The experimental group (EG, n=11, age 22 years, weight 71.2 ± 4.8 kg, height 174 ± 5,8 cm) and the Control group (CG, CG, n=8, age 22 years, weight 73.2 ± 4.1 Kg, height 176 ± 6,3 cm). No nutritional information was collected. Written informed consent was provided before the beginning of the study. The research was approved by the Bioethics Committee of the University of Bologna (Approval code: 25027).

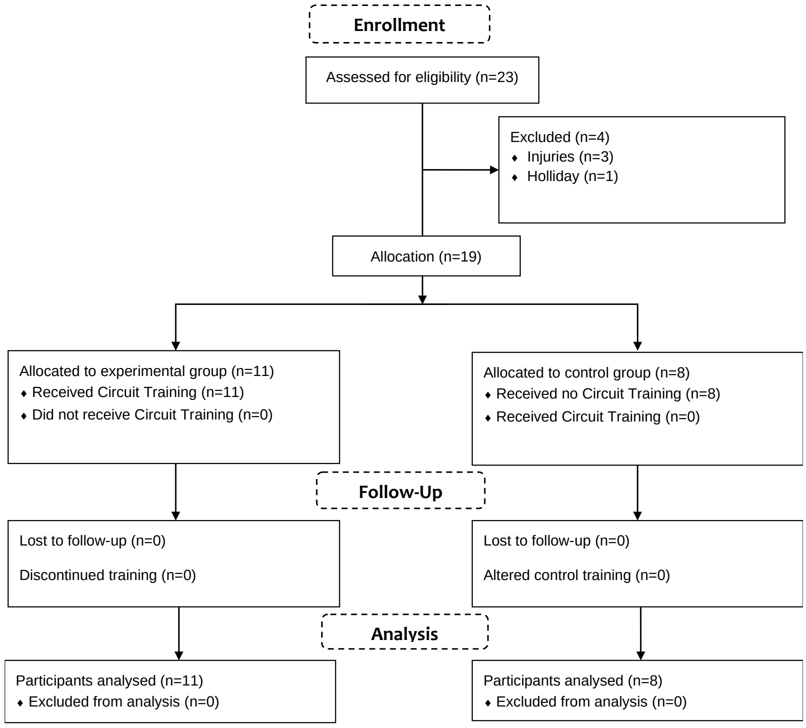

Figure 4.1. Participants' flowchart.

Training in EG and CG

EG was submitted to an eight-week core and functional training protocol (Afyon, 2019; Brull-Muria & Beltran-Garrido, 2021). Training frequency was two sessions per week and the global number of sessions was 16, according to previous research that demonstrated benefits in neuromuscular control and performance measures with this similar conditioning period (Distefano et al., 2013). The time of rest between each training session was 72 hours to optimize the physiological recovery (training days were Monday or Tuesday and Thursday or Friday).

Figure 4.2 shows the training protocol progression. It was focused on 7 exercises performed in the following way:

Session 1: 4 core training and 3 upper body strength exercises

Session 2: 4 core training and 3 lower body strength exercises.

Core training exercises protocol (figures 4.2 and 4.3) was gradually focused on trunk stability, endurance and strength, while load intensity and volume were weekly increased following previous guidelines (Behm et al., 2010; Brumitt et al., 2013; Prieske O et al., 2016; Willardson, 2007, 2010). The four exercises performed were plank, crunch, supine bridge and side bridge (Oliva-Lozano & Muyor, 2020).

Upper and lower-body functional exercises were defined as sports movement patterns (Boyle, 2016). The three upper body functional movements executed were push, pull and press, while lower body movements were squat, lunge and deadlift (Afyon, 2019; Brull-Muria & Beltran-Garrido, 2021; Distefano et al., 2013).

All movements were progressed using specific equipment (unstable surfaces, sling tools, sandbags, weight plates, dumbbells, barbells and kettlebells, medicine balls, elastic bands) and training load parameters were increased similarly to core training exercises (figures 4.2 and 4.3). During each exercise, execution participants were supposed to reach maximum effort supported by a trained kinesiologist.

Each session was organized using the circuit interval training method with a ratio between work and rest equal to 1:1, of 30” respectively. This ratio was selected to enhance the stimulation of both anaerobic systems (glycolytic and neuromuscular) and to induce the neuromuscular learning effect (improvement in body efficiency and the movement economy) (Bonacci et al., 2009; Tabata et al., 1997). Specifically, the 30” work period aimed to maintain a selected level of mechanical force (static exercise) and/or power (dynamic exercise) for a short interval (critical power) and to increase the anaerobic energy source (W’) (Jones et al., 2010). The passive 30” recovery period aimed to dilate the exhaustion time and induce a higher phosphoryl-creatine resynthesis (Dupont et al., 2004). Static exercises were performed by holding isometric positions for 30”, whereas dynamic movements requested the execution of the maximum number of repetitions with resistance tools at the same time. Participants completed 3 (weeks 1, 2, 5, 6) or 4 circuits (weeks 3, 4, 7, 8) without additional recovery and the working time was 21 or 28 minutes, respectively.

Before starting each training session, 10 minutes of dynamic warm-up with a focus on cardiovascular activation, joint mobility and flexibility were performed (bodyweight exercises). Once circuit interval training was completed, 10 minutes of cool down with stretching and myofascial release exercises concluded each session.

All training sessions were led by a kinesiologist with at least 3 years of experience in core and functional training. Before starting the CT protocol, each participant was instructed to learn the correct exercise execution and breathing.

Contrary to EG, CG performed recreational activities like running, biking, and futsal for the same period. CG participants were not allowed to perform any kind of resistance training exercises or bodyweight exercises.

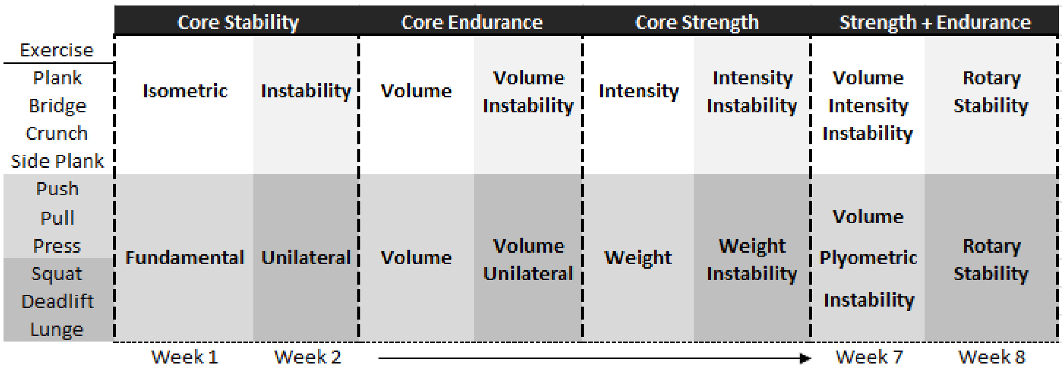

Figure 4.2. Training protocol progression.

EXERCISE	WEEK 1		WEEK 2	
	① CORE STABILITY UB (3 CT)	② CORE STABILITY LB (3 CT)	③ CORE STABILITY UP (3 CT)	④ CORE STABILITY LB (3 CT)
1	PLANK (FOREARMS)	PLANK (FOREARMS)	PLANK WTH FITBALL	PLANK WTH FITBALL
2	PRESS WITH WEIGHT PLATE	SQUAT WITH WEIGHT PLATE	UNILATERAL PRESS WITH WEIGHT PLATE	SIDE SQUAT WITH WEIGHT PLATE
3	SUPINE BRIDGE	SUPINE BRIDGE	1 LEG SUPINE BRIDGE	1 LEG SUPINE BRIDGE
4	PUSH UP	DEADLIFT WITH ELASTIC BAND	1 LEG PUSH UP	DEADLIFT WITH WEIGHT PLATE
5	CRUNCH	CRUNCH	CRUNCH WITH FITBALL	CRUNCH WITH FITBALL
6	TRX BACK ROW	BACK LUNGE	TRX SINGLE ARM BACK ROW	SIDE LUNGE
7	SIDE PLANK (FOREARM)	SIDE PLANK (FOREARM)	1 LEG SIDE PLANK (FOREARM)	1 LEG SIDE PLANK (FOREARM)
	WEEK 3		WEEK 4	
	⑤ CORE ENDURANCE UB (4 CT)	⑥ CORE ENDURANCE LB (4 CT)	⑦ CORE ENDURANCE (4 CT)	⑧ CORE ENDURANCE (4 CT)
1	PLANK (HANDS)	PLANK (HANDS)	PLANK WITH FITBALL (HANDS)	PLANK WITH FITBALL (HANDS)
2	PRESS WITH SANDBAG	SQUAT WITH SANDBAG	UNILATERAL PRESS WITH SANDBAG	SIDE SQUAT WITH SANDBAG
3	SUPINE BRIDGE (FEET ON MEDBALL)	SUPINE BRIDGE (FEET ON MEDBALL)	1 LEG SUPINE BRIDGE WITH MEDBALL	1 LEG SUPINE BRIDGE WITH MEDBALL
4	PUSH UP (HANDS ON MEDBALLS)	DEADLIFT WITH WEIGHT PLATE	PUSH UP (1 HAND ON MEDBALL)	CLEAN WITH MEDBALL
5	SIT UP	SIT UP	SIT UP WITH FITBALL	SIT UP WITH FITBALL
6	TRX PULLDOWN	TRX BACK LUNGE	TRX SINGLE ARM PULLDOWN	TRX SIDE LUNGE
7	SIDE PLANK (HAND)	SIDE PLANK (HAND)	1 LEG SIDE PLANK (HAND)	1 LEG SIDE PLANK (HAND)
	WEEK 5		WEEK 6	
	⑨ CORE STRENGTH (3 CT)	⑩ CORE STRENGTH (3 CT)	⑪ CORE STRENGTH (3 CT)	⑫ CORE STRENGTH (3 CT)
1	MOUNTAIN CLIMBER	MOUNTAIN CLIMBER	MOUNTAIN CLIMBER WITH FITBALL	MOUNTAIN CLIMBER WITH FITBALL
2	PRESS WITH BARBELLS	OVERHEAD SQUAT WITH WEIGHT PLATE	UNILATERAL FORWARD PRESS WITH BARBELL	SINGLE LEG OVERHEAD SQUAT WITH WEIGHT PLATE
3	REVERSE MOUNTAIN CLIMBER	REVERSE MOUNTAIN CLIMBER	KNEE TUCK WITH FITBALL	KNEE TUCK WITH FITBALL
4	SPIDERMAN PUSH UP	DEADLIFT WITH BARBELLS	SPIDERMAN PUSH UP WITH MEDBALLS	CLEAN WITH BARBELL
5	SIT UP WITH WEIGHT PLATE	SIT UP WITH WEIGHT PLATE	SIT UP WITH MEDBALL	SIT UP WITH MEDBALL
6	TRX BACK ROW (TRUNK EXTENSION)	BACK LUNGE WITH WEIGHT PLATE	TRX BACK ROW (FULL TRUNK EXTENSION)	TRX BACK LUNGE WITH WEIGHT PLATE
7	RUSSIAN TWIST WITH MEDBALL	RUSSIAN TWIST WITH MEDBALL	CROSS OVER CRUNCH	CROSS OVER CRUNCH
	WEEK 7		WEEK 8	
	⑬ CORE COMBO (4 CT)	⑭ CORE COMBO (4 CT)	⑮ CORE COMBO (4 CT)	⑯ CORE COMBO (4 CT)
1	PIKE WITH FITBALL	PIKE WITH FITBALL	1 LEG PIKE WITH FITBALL	1 LEG PIKE WITH FITBALL
2	PRESS WITH DUMBELLS	JUMPING BACK LUNGE	ASYMMETRICAL PRESS WITH BARBELL	FRONT LUNGE AND TWIST WITH WEIGHT PLATE
3	OVERHEAD SANDBAG ROTATION	OVERHEAD SANDBAG ROTATION	1 LEG OVERHEAD SANDBAG ROTATION	1 LEG OVERHEAD SANDBAG ROTATION
4	PLYOMETRIC PUSH UP	JUMPING SQUAT	PUSH UP AND TRUNK TWIST	SQUAT AND TWIST WITH MEDBALL
5	SIT UP WITH MEDBALL THROW	SIT UP WITH MEDBALL THROW	SIT WITH MEDBALL LATERAL THROW	SIT WITH MEDBALL LATERAL THROW
6	JUMP AND PULL WITH BAR	SWING WITH KETTLEBELL	TRX SINGLE ARM ROW AND TRUNK TWIST	SINGLE CLEAN WITH KETTLEBELL
7	WOODCHOP WITH MEDBALL	WOODCHOP WITH MEDBALL	WOODCHOP WITH LOOPBAND	WOODCHOP WITH LOOPBAND

Figure 4.3. Exercise progression over 16 training sessions (eight weeks).

Motor tests

Motor Tests were implemented at the University sports centre. Each evaluation was performed before and after the training period in indoor spaces, where the temperature was at 21.5°. All testing was performed in the afternoon between 6 p.m. and 9 p.m., due to participants' availability, similar to training time sessions. Different days were selected to perform strength and balance tests, and 24 to 48 hours were given to participants to avoid biased results due to fatigue According to previous reviews, the following tests were selected [6, 11, 14, 31]: Y Balance Test (YB), Standing Long Jump (SLJ), Medicine Ball Chest Press (MBC), Curl Up (CU), Illinois Agility Test (IAT). Before performing each test, participants were instructed by a one-trained specialist who taught them the accurate and safer procedure. Each participant did a warm-up of 15 minutes with jogging, dynamic stretching, and many athletic drills such as jumps, skips, lunges, short-run shuffle, core stimulations, arm swings, and wide push-ups, after which he practised some trials to get comfortable with the specific test. Following this dynamic warm-up, a period of eight minutes was allotted to the participants. Three trials were completed for each evaluation, separated by 4 minutes of rest intervals. Only the best results were recorded.

Standing Long Jump test (SLJ)

Horizontal jump tests measure explosive strength and power in the lower limb. These aspects are fundamental to the usual movement of soccer players like sprinting, jumping, and kicking the ball (Stølen et al., 2005). The role of core musculature can optimise the performance during this test (Hibbs et al., 2008; Myer et al., 2006; Prieske et al., 2016).

For SLJ, all subjects received standardized instructions that allowed them to begin the jump with bent knees and swing their arms to assist in the jump. A line drawn on a hard surface served as the starting line. The length of the jump was determined using a tape measure, which was affixed to the floor. The distance of the best jump was measured, to the nearest 1 cm, from the line to the point where the heel closest to the starting line land-ed. If the subject fell backward, the distance where the body part closest to the starting line touched the ground was measured as the jump's length. Reliability and validity of SLJ in younger were previously reported (Almuzaini & Fleck, 2008; Fernandez-Santos et al., 2015).

Medicine Ball Chest Test (MBC)

Medicine ball throw tests have been reported to be a valid and reliable measure of upper body strength and power (Prieske et al., 2016). Furthermore, static, and dynamic throws are significantly correlated with some performance measures and can reflect the force transfer between the core and limbs (Shinkle et al., 2012). Since soccer players require strength and stability during rotary movements, the MBC was performed dynamically in a standing position. Participants kept a split stance position with the front foot in contact with a starting line on the floor and held a 6 Kg medicine ball in their hands. Then, they threw the medicine ball as far as possible with a chest pass. Participants were allowed to rotate the trunk before the throw without moving their feet. The test was performed with the right and left foot in an anterior position. The throws were marked at the first contact on the ground and the distance from the starting line was determined using a tape measure.

Curl-up Test (CU)

Curl up test measures the strength and endurance of core musculature (Baumgartner et al., 2006) and represents an assessment tool for the Fitnessgram® program (Morrow et al., 2010).

For this test, participants attempted to complete up to 75 curl-ups at a specified pace (1 curl for every 3 seconds, 20 reps per minute) using a mat with a 12 cm measuring strip. They lay flat on their backs with their knees bent at 90° and feet flat on the floor. Arms are extended and parallel to the trunk with their palms on the mat. The measuring strip is used to help participants know how far to curl up and is placed under the knees with the fingers touching the nearest edge. Participants slid their fingers from one side of the measuring strip to the other side and then curled back down. CU speed was defined using a metronome settled at 40 bpm. The test ended when participants could not keep the requested speed or their feet were moved from the mat. The final score was the number of curl-ups completed.

Illinois agility test (IAT)

The IAT was administered as previously described (Raya et al., 2013). The length of the IAT rectangle was set at 9.144 meters and the width at 5 meters. The IAT course was marked by cones, with four cones spaced 3.05 m in a central position and four corner cones positioned 2.5 m laterally from the central cones. The participant began the test lying prone on the floor behind the starting line with his arms at his side and his head turned to the side or facing forward. On the vocal starting command, the participant ascended to his feet and ran or moved quickly forward to the first tape mark. Participants were required to touch or cross the tape mark with their feet. The participant turned around and moved back to the first central cone. The participant then ran or moved as quickly as possible to the second tape mark on the far line. Again, participants were required to touch or cross the end-line tape marks with their feet. Lastly, the participant turned around and ran or moved as quickly as possible across the finish line. The time to complete each trial was recorded in seconds.

Y balance test (YB)

The YBT was administered as previously described (Shaffer et al., 2013). The test was assessed for both the right and left lower limbs. The starting position saw the participant standing on the central footplate, with the distal aspect of the right foot at the starting line. While maintaining a single-leg stance on the right leg, the subject moved the limb to the anterior, posteromedial, and posterolateral directions with the stance foot by pushing the indicator box as far as possible. To reduce fatigue that could negatively affect the test result, participants altered their right and left lower limbs between the three directions. Attempts were discarded and repeated (a maximum of six trials) if the subject failed to maintain a unilateral stance on the platform, failed to maintain reach foot contact with the reach indicator on the target area while the reach indicator was in motion, used the reach indicator for stance support, or failed to return the reaching foot to the starting position under control. The reach distance was recorded to the nearest 0.5 cm.

Statistical Analysis

Descriptive statistics (mean ± standard deviation, SD) were calculated for each variable. Variable normality was verified with the Shapiro–Wilk test. The paired student's t-test was performed to assess the within-group differences from pre- to post-evaluation. The student's t-test was performed to assess the between-group differences. To evaluate the different treatment effects with no bias due to beginning participants' heterogeneity, the difference between pre and post of each group was calculated and then the difference of these differences was inferred. A statistical type I error (p-value, p) < 0.05 was considered significant. A posthoc analysis was computed to achieve the statistical power both for matched paired and two groups t-test with G*Power 3.1.9.7 for Windows 10 (Heinrich-Heine-Universitat Dusseldorf): for matched paired comparison in EG the mean ES=1.368, α=0.05, n=11, test two-tailed, 1-β=0.982; for matched paired comparison in CG the mean ES=0.35, α=0.05, n=8, test two-tailed(Afyon, 2019; Afyon et al., 2017; Atli, 2021; Bayrakdar et al., 2020; Brull-Muria & Beltran-Garrido, 2021; Doğanay et al., 2020; Imai et al., 2014; Mendes, 2016; Sever & Zorba, 2017; Vigneshwaran G., 2017), 1-β=0.16; for two independent groups comparisons the mean ES=0.99, α=0.05, n=19, test two-tailed, 1-β=0.52.

The statistical analysis was performed with STATA® software for Windows 10, version 17 (Publisher: StataCorp. 2021. Stata Statistical Software: Release 17. College Station, TX, USA, StataCorp LP).

4.1.3 Results

Table 4.1 shows the means, standard deviations, and differences within and between the groups in pre- and post-evaluation. The EG improved significantly in all tests over time except for the Illinois test, whereas the CG did not show significant differences among pre- and post-evaluation. In within-group comparisons, greater improvements were found in the right-side (dominant body-side) tests of the experimental group. The differences of each group difference showed a positive trend for the EG, but only three tests exhibited significant differences between groups (Med Ball chest right-side and the Y-balance test). Figure 4.4 includes five graph bars (A-E), which show the means and mean differences of each test for the two groups, respectively.

Table 4.1. Summary statistics of motor test results and differences within and between groups.

	Within Groups								Between Groups	
	EG (n = 11)				CG (n = 8)				Pre (EG-CG)	Post (ΔEG - ΔCG)
Variable	Pre (Mean ± SD)	Post (Mean ± SD)	ΔEG (Mean ± SD)	p. $t_{(10)}$	Pre (Mean ± SD)	Post (Mean ± SD)	ΔCG (Mean ± SD)	p. $t_{(7)}$	$t_{(17)}$	$t_{(17)}$
SLJ	201.09 ± 11.89	214.63 ± 11.65	13.55 ± 9.33	4.814[ł]	209.5 ± 14.17	212. 87 ± 13.91	3.37 ± 12.85	0.743	-1.406	2.005
MBCr	503.64 ± 48.22	548.18 ± 41.67	44.55 ± 22.52	6.559[ł]	526.87 ± 40.61	545.62 ± 45.15	18.75 ± 32.60	1.627	-1.105	2.046*
MBCl	507.27 ± 52.74	555.45 ± 54.84	48.18 ± 46.87	3.409*	538.12 ±43.75	543.75 ± 56.29	5.62 ± 46.40	0.343	-1.348	1.962
CU	28.45 ± 12.19	49.73 ± 23.58	21.27 ± 17.31	4.076[§]	28.5 ± 10.46	35.75 ± 10.82	7.25 ± 11.77	1.742	-0.009	1.976
Ill	18.22 ± 0.82	18.13 ± 0.57	-0.09 ± 0.77	0.382	17.99 ± 0.62	18.14 ± 0.82	0.145 ± 0.61	0.667	0.635	0.709
YBr	97.05 ± 5.92	105.46 ± 5.3	8.41 ± 4.44	6.285[ł]	98.84 ± 6.15	100.84 ± 5.35	1.99 ± 3.99	1.417	-0.640	3.241[§]
YBl	97.92 ± 6.46	105.41 ± 4.45	7.50 ± 4.18	5.941[ł]	101.11 ± 3.44	102.14 ± 3.99	1.03 ± 2.04	1.429	1.267	4.016[ł]

Note: SLJ, Standing Long Jump test; MBCr, Med Ball Chest test right side; MBCl, Med Ball Chest test left side; CU, Curl Up test; Ill, Illinois test; YBr, Y balance test right side; YBl, Y balance test left side; EG, experimental group; CG, control group; n, sample size; SD, standard deviation; p. t, paired t-test; t, student test; p, p-value; Δ, difference; *, $p<0.05$; [§], $p<0.01$; [ł], $p<0.001$

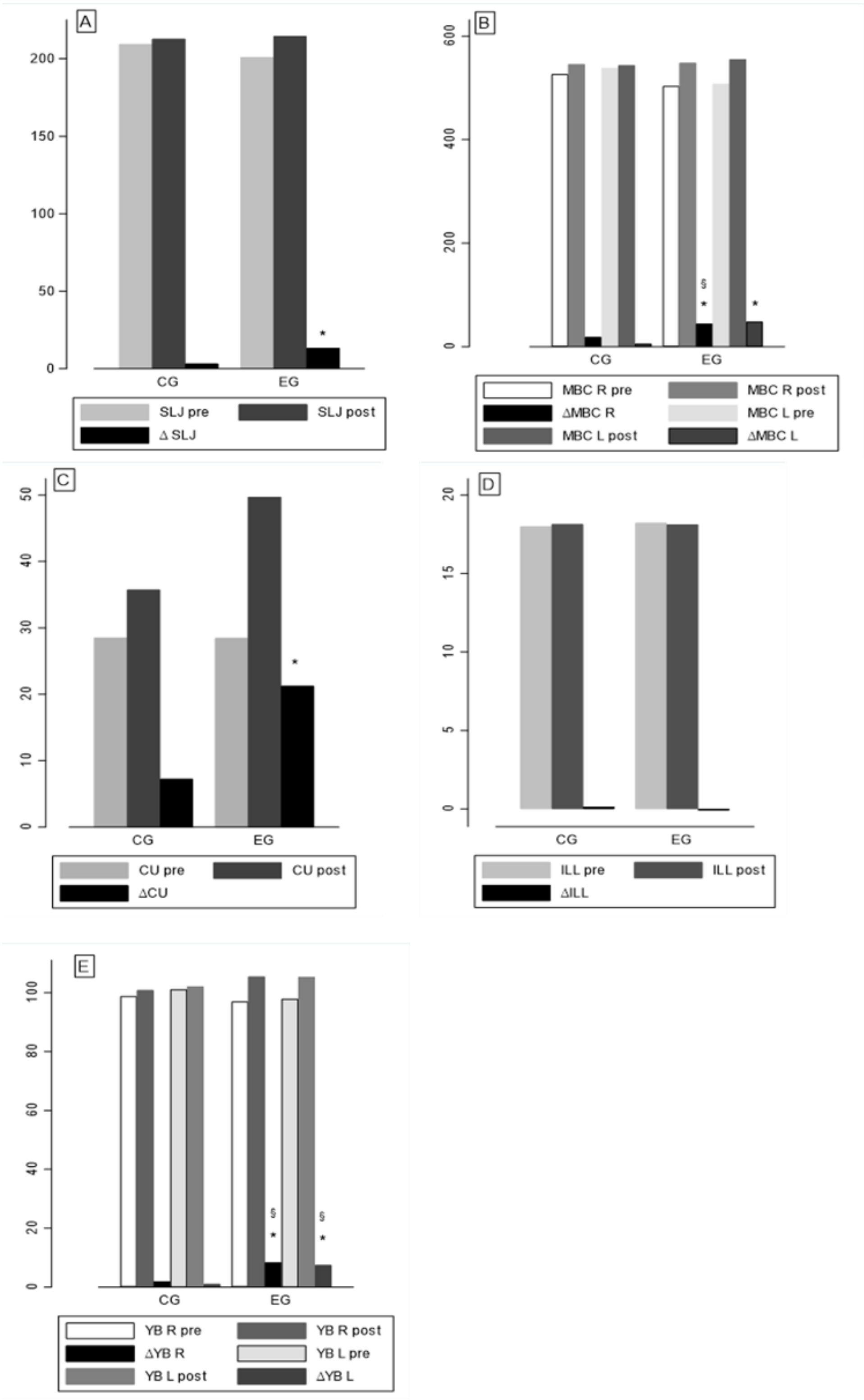

Figure 4.3. Graph bar with pre, post and post-pre of Standing long jump test (A), Medicine ball chest test (B), Curl-up test (C), Illinois agility test (D), and Y balance test (E). Note: *, significant difference within group; §, significant difference between groups.

4.1.4 Discussion

Present work aimed to investigate the effects of a circuit training with core exercises program on physical performance in competitive amateur soccer players. The training was conducted during the off-season period, two times per week for eight weeks. By our hypothesis, we found significant improvements in lower and upper body strength (SLJ and MDC on both sides, respectively), core endurance (CU) and balance (YBT on both sides) in EG. Contrary, CG did not report significant changes in the pre-posttest comparison.

The effects of core exercises on performance and physical fitness in different levels of male soccer players have been previously investigated. Training protocol duration and frequency varied normally from six to 12 weeks and from two to four times per week, respectively (Afyon, 2019; Afyon et al., 2017; Atli, 2021; Bayrakdar et al., 2020; Brull-Muria & Beltran-Garrido, 2021; Doğanay et al., 2020; Imai et al., 2014; Mendes, 2016; Sever & Zorba, 2017; Vigneshwaran G., 2017). Recently, a systematic review and meta-analysis evidenced the efficacy of short-time CT programs (< 30') performed twice a week for at least 18 sessions (Saeterbakken et al., 2022). In the current study, the CT time ranged from 21 to 28' and the total number of sessions was 16, close to the above-mentioned suggestion. Concerning duration, an 8-week integrated training protocol performed two times per week was demonstrated to enhance neuromuscular control, agility, trunk and upper body strength when compared to isolated training (Distefano et al., 2013). This training time could be long enough to provide improvements in neural aspects of strength, coordination, and motor control. Consequently, gains in strength, balance and agility can be expected. Current literature reports the efficacy of a similar training period in soccer (Afyon, 2014, 2019; Afyon et al., 2017; Bavli & Koç, 2018; Brull-Muria & Beltran-Garrido, 2021; Doğanay et al., 2020). Recently, Mahmoud found significant improvements both in the lower (SLJ and vertical jump) and upper (Medicine ball throw) arms after a 10-week core exercises program with two sessions per week, in younger soccer players (15.40 years) (Mahmoud, 2018). Al-so, two researchers showed that two different 8-week core exercise programs (static and dynamic) applied for 30 minutes two days per week increased strength levels measured by the SLJ and push-up test, in younger soccer players (15 years) (Bavli & Koç, 2018). Although the above-mentioned studies investigated a younger sample and the upper arm strength was evaluated differently, core exercises induced benefits in strength. In our study, SLJ and MBC significantly improved in EG only. The selection of exercises, load progression and training length could be the main reason for this positive change (Brull-Muria & Bel-tran-Garrido, 2021; Distefano et al., 2013). Furthermore, between-group comparisons highlighted differences in MBC for the right side only. This result can be related to the physiological discrepancy in the right and left parts of the body and specific movement patterns during throwing (Talukdar et al., 2015).

As regards core endurance and strength, this study showed a significant improvement in the experimental group after the 8-week CT with core exercises on the curl-up test. Since the focus of the training protocol was mainly on core musculature, this result was expected. Despite no significant results appearing in between groups comparison, the type one error value is near to the critical level selected and a larger sample should exhibit statistical discrepancy. However, several studies are in accordance with our results and reported improvements after different periods of the core exercises program (Afyon, 2014; Bavli & Koç, 2018; Mahmoud, 2018; Prieske O et al., 2016). Afyon et al. administered a 12-week core exercise plan to 15 younger soccer players (U-16) in addition to regular training. Compared to the control group (soccer training only), the authors reported significant improvements in the plank test and larger benefits on lower body strength (standing long jump and vertical jump), balance and speed (Afyon, 2014). In addition, Turna et al. showed significant differences in 30 abdominal crunch tests after six weeks of core training in adolescent soccer players (Turna, 2020). Prieske et al. compared two different core training programs (stable vs unstable) in U-17 elite soccer players and found similar improvements in maximal isometric trunk strength for extensor muscles (Prieske O et al., 2016). Since CU represents an easy and dynamic method to assess core endurance in a different population, this test was preferred to the Plank Test or McGill test

(Baumgartner et al., 2006). Furthermore, crunch exercise was selected within CT protocols for the muscular activation of the anterior core region (Oliva-Lozano & Muyor, 2020).

Concerning agility, EG did not show improvements after the treatment. These results are in line with the study of Server et al. (Sever & Zorba, 2017), which analyzed the effects of two different core training protocols (static and dynamic) executed three times per week for an 8-week training period in young male soccer players and did not report significant improvements in sprint and agility. On the contrary, Doganay et al. evidenced benefits in quickness and agility (measured with the Hexagon test) after eight weeks of both core and soccer training performed 3 times per week (Doğanay et al., 2020). Similarly, Akif showed enhancements in agility (IAT and T-drill agility tests) with two core exercise sessions per week in amateur players (Afyon et al., 2017). Although divergent results are present in the literature, many important elements such as training protocols (static vs dynamic exercises, bodyweight vs strength equipment), motor tests and season period (in-season vs off-season) could affect adaptations. The lack of improvements in IAT in present work could be mainly related to the off-season period: while static and dynamic exercises were added to a sport-specific routine during the regular season (Afyon, 2019; Afyon et al., 2017; Doğanay et al., 2020) our research focused on core and functional exercises alone. Probably, the absence of specific agility and quickness training in our protocol is the reason for this lack. Consequently, the specificity of core exercise programs in addition to soccer training has been related to different benefits (Brull-Muria & Beltran-Garrido, 2021).

The last evaluation of this study focused on whether core exercise protocol could improve single-leg dynamic balance measured by the Y balance test. Our results showed that eight weeks of circuit training with core exercises induced benefits in both the right and left sides of the body. YBT requires lower limb strength, neuromuscular control, flexibility, and an adequate level of core stability (Shaffer et al., 2013) (52). Consequently, this assessment tool has been widely reported as an indirect measure of core efficiency and its role in physical fitness is well documented (Borghuis et al., 2008; Prieske et al., 2016). Imai et al. compared the effects of two different trunk training programs on balance and other performance parameters (Imai et al., 2014). The authors found significant improvements in posteromedial and postero-lateral direction of YBT after 12 weeks of core training exercises (front plank, back bridge, side bridge and quadrupeds' arm-leg extension) when compared to traditional trunk exercises (sit up and back extension). Also, some researchers showed that eight weeks of core training assessed three times per week (in season) improved the balance control of 11 male soccer players measured by the sensory evaluation test (static balance) (Hung et al., 2019). Differently, one research did not report significant improvements in an experimental male soccer group on balance performance in the dominant leg (balance error scoring system) after eight weeks of core training (Aslan et al., 2018). Even if the training period was different, our results agree with Imai et al. (Imai et al., 2014). Since previous authors underlined the efficiency of an 8-week integrated training on neuromuscular control and balance, this period represented a sufficient stimulus (Distefano et al., 2013) To the best of our knowledge, no other studies analysed the effect of a specific circuit training with core exercises in soccer off-season period.

Although the heterogeneity of studies evaluating the balance performance after the core training in this player makes it difficult to obtain a statement in soccer, it seems clear that at least four weeks of core training performed twice per week could induce benefits in athletes of several sports (Luo et al., 2022). More evidence is needed to establish whether core exercises should be included in soccer season training or during the pre/off-season period.

Many limitations are presented in this study: (a) the sample size was small, so the statistical power resulted low for some comparisons, (b) no specific treatment was planned for the control group and participants were free to perform a recreational activity (c) no training assessment was provided during CT execution, so the intensity and fatigue levels were not evaluated.

4.1.5 Conclusions

The current literature highlights that training with core exercises could induce several benefits in fitness and sports. Our protocol has been effective in improving the strength, core endurance and balance of adult amatorial soccer players.

Despite study limitations, our positive results showed that circuit training with core exercises appears to be a good strategy for improving the performance of adult soccer players during the off-season period. To provide more evidence, it would be important to continue investigating this kind of exercise program's effects and to apply the intervention also to the pre and in-season periods.

4.2 STUDY VI

4.2.1 Introduction

Football is one of the most played team sports worldwide. To date, the Fédération Internationale de Football Association (FIFA) has encountered 4429 professional football clubs and more than 130,000 professional players (Home_map | FIFA Professional Football Landscape, 2024). Due to football's social, financial, and economic impact, clubs' competition has increased and talented players are already selected at the youth level. In Europe, the programs of talent identification are often integrated into "professional" academies, which spend considerable resources on identifying and developing talented young players. The goal of a club is to cultivate talent and then progress their first team (Williams & Reilly, 2000) and/or trade them players to other for-profit clubs (Neri & Rossi, 2023). The opportunities for football players to become professionals are less likely for unselected players, underlying the primary role of decision-making (Aquino et al., 2017; Nughes et al., 2020).
Since football performance depends on several features such as body profile, functional abilities, psycho-social aspects, and technical-tactical skills, individuals' characteristics provide an important multivariate framework to guide the selection process of young players and lead them to future success (Gissis et al., 2006; Huijgen et al., 2014; Meylan et al., 2010; Reilly, 2005). In recent years, the role of genes in talent selection has also received an increased interest and football players who become professionals exhibited specific polymorphism-enhancing performance (PEPs) such as the angiotensin-converting enzyme (ACE) allele I that predisposes a better endurance performance (Contrò et al., 2018). In addition, the success of young footballers is affected by external factors, such as training facilities and coaching expertise, body conditioning and injury rate, as well as social, cultural, and private influences (Reilly et al., 2000).
Football scouts generally monitor young players during friendly and seasonal matches, selecting the best ones (Huijgen et al., 2014; Nughes et al., 2020). However, due to the dynamic nature of talent, predicting future potential from current player characteristics is difficult, especially during periods of intense growth and development (Archer et al., 2016; Unnithan et al., 2012; Vaeyens et al., 2008). Collecting physiological, technical-tactical, and psychological traits resulted in a great strategy to help coaches differentiate and select talented players (Figueiredo et al., 2021; HÖner et al., 2015; Huijgen et al., 2014; Massa et al., 2022; Nughes et al., 2020; Rebelo et al., 2013). Selected players have been detected to be taller, heavier, leaner, faster, and stronger than non-selected young footballers (Figueiredo et al., 2021; Gil et al., 2007; Gravina et al., 2008; Hansen et al., 1999; Nughes et al., 2020). In addition, birth date and biological maturation strongly influence young football players' selection process (Massa et al., 2022; Toselli et al., 2022). Adolescent players who matured earlier exhibited greater strength, speed, power and endurance capabilities than later maturer teammates, spreading the selection opportunities (Coelho E Silva et al., 2010; Itoh & Hirose, 2020; Rommers et al., 2019). A common approach for identifying indicators of talent is to take a cross-section of players and to compare the characteristics of the players who are and are not selected into a talent development system, but without considering the biological maturity of the players. As football is a multifaceted sport that requires high levels of physical fitness and skill to succeed, the reasons behind progression to the elite level in youth football are multi-factorial. Therefore, identifying the characteristics that enable players to progress in football is of vital importance for coaches to optimize talent development programs. Additionally, talent identification and technical staff usually may select among already highly selected players with characteristics that may be similar (Bidaurrazaga-Letona et al., 2019). In light of this evidence, it is crystal clear that the identification of parameters able to differentiate these players would be relevant.
Therefore, the present study aims to compare biological maturation, anthropometry and body composition, and physical performance of selected and unselected adolescent football players and to identify which are the most relevant characteristics of the multivariate profiles that could better discriminate between selected and unselected elite youth Italian football players. Furthermore, to our

best knowledge, no study of this type has been conducted in Italy. Even if this scenario is only possible in professional or top-level clubs football academies, the potential conclusions drawn from the following study could also be valuable to lower-level football coaches' teams with limited resources.

4.2.2 Methods

Study sample and design

A retrospective experimental design was fulfilled with data collected on 15th September 2019 on a sample of 78 football players from the Under 10 to Under 12 age categories, registered in the professional Italian football team Bologna Football Club 1909 participating in the first division. During their young categories, the players trained for 6 hours a week (four workouts of 1.5 hours each). In 2021, 2022 and 2023 (figure 4.5), information on the selection process (Bernoulli outcome with selection and un-selection options) was gathered. Only twenty-six of them were selected for the juvenile (U18) professional team (age=11.15±0.74 years, height= 144.06 ± 6.74 cm, weight= 35.38 ± 4.56 kg), while 52 of them were unselected (age= 11.22 ± 0.83 years, height=143.06±8.34 cm, weight= 35.94 ± 6.24 kg).
All participants and their parents received and filled out a written informed consent before the evaluations. The study followed the ethical principles provided by the Helsinki declarations and was approved by the Bioethics Committee of the University of Bologna (Approval code: 25027).

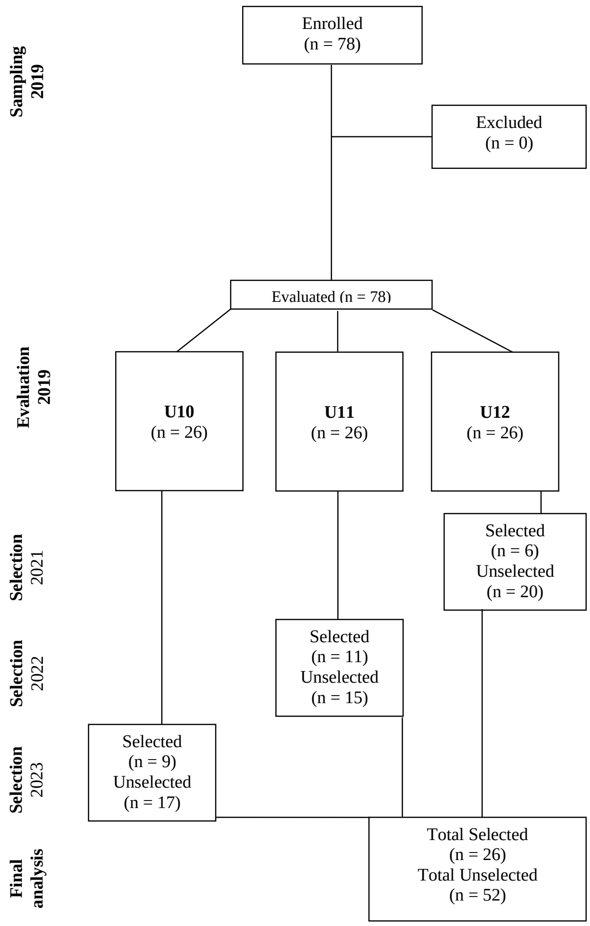

Figure 4.5. Study flowchart.

Anthropometry

All the anthropometric features were evaluated by an expert anthropometrist following standard procedures (Lohman et al., 1988). Individual stature and sitting height have been measured to the nearest 0.1 centimetres by a GPM stadiometer (Zurich, Switzerland) and a rigid seating seat with noted height (40 cm). Then, the leg length was computed by subtracting the sitting height (excluding the seat height) from stature. Body mass was measured to the nearest 0.1 kg with the participant in a standing position on a calibrated electronic scale (Seca 878 dr, Hamburg, Germany), wearing light indoor clothing and no shoes. Upper (relaxed and contracted upper arm) and lower (thigh and calf) limb perimeters, such as hip and waist circumferences, were measured to the nearest 0.1 cm with a non-stretchable tape (Seca 201, Hamburg, Germany) at the following sites: midpoint between acromion and olecranon; midpoint between the inguinal fold and patellar; maximal bulk of calf; midpoint between the last rib and the iliac crest; maximal bulk of glutes. Humeral and femoral bone widths were respectively evaluated at elbow and knee condyles by a sliding calliper (GPM Feithierenstrasse, Susten) to the nearest 0.1 cm. Skinfold thicknesses were obtained on the left side of the body to the nearest 1 mm with a Lange caliper (Beta Technology Inc., Houston, TX, USA) with a pressure of 10 g/mm^2. Each skinfold thickness assessment was the average of three site-specific values within 10% of each other. The same trained operator took all measurements. The Technical Error of Measurement, assessed before the project, was < 5% for skinfolds and < 1% for other measurements.
Body Fat Percentage (%F) was calculated using the validated skinfold equations (Slaughter et al., 1988). Then, Fat Mass (FM) was computed by multiplying participant body mass and %F; the FFM was derived by subtracting FM from body mass. The choice of the appropriate equation for each subject was based on his maturational status. The choice of the appropriate equation for each subject was based on his maturational status. In this case, for all the subjects the skinfold equation for prepubescent white males was chosen. The total area (cm^2) of the upper arm (TUA), calf (TCA), and thigh (TTA), the muscle area (cm^2) of the upper arm (UMA), calf (CMA), and thigh (TMA), and the fat area (cm^2) of the upper arm (UFA), calf (CFA), and thigh (TFA) were calculated (Frisancho, 2008). In addition, arm fat index (AFI), calf fat index (FCI), and thigh fat index (TFI) were derived.
Body mass index (BMI) was obtained as body mass (kg) and squared stature (m^2) ratio.

Maturity Status (MS)

An estimation of the years from peak height velocity (PHV), which is an indicator of the adolescent growth spurt, was made using the equation for boys developed by Mirwald et al (Mirwald et al., 2002). The participant year from PHV has been computed by subtracting the Maturity Offset (MO, computed through the Mirwald equation) from the chronological age, computed as the difference between birthdate and measurement date divided by 365.25.
To overcome the potential biases due to the age effect and subjective growth spurt (Malina & Kozieł, 2014), we followed the approach proposed by Rommers and colleagues (Rommers et al., 2019), who used age-specific z-scores to classify players according to their maturity status. All the predicted APHVs were averaged and standardized around a 0 mean value ± 1 deviation, for each category respectively. Then, players who were farther than |0.5| were classified as "earlier" (negative value), or "later" (positive value) maturing, while they were considered "on time" if $-0.5 < z < 0.5$ (Rommers et al., 2019).

Relative Age Effect (RAE)

The RAE value was computed according to the month of birth of each participant, subdividing each year into 3-month quartiles: Q1= January to March; Q2= April to June; Q3= July to September; Q4= October to December.

Physical Performance Tests

We tested the countermovement jump (CMJ), 15-meter sprint and repeated sprint ability (RSA), and the HARRE test (figure 4.6). Sprint and RSA tests were performed on a football field during the morning, with about 20°C and 40% humidity, with no rain and about 2 km/h of wind. Photoelectric cells recorded tested trial times in both RSA and sprint (Fusion Sport Smart Speed Timing Gates, Brisbane, Australia).
CMJ and HARRE tests were performed during the afternoon in an indoor Gymnasium (~21°C, 45% humidity). Photoelectric cells recorded the flight time (Optojump next, Micrograte, Bolzano, Italy). All tests were assessed with technical clothes. Parents were asked not to assist with the evaluations.
To assess explosive lower-body power, we used the CMJ test (figure 4.6, A). Each participant began the trial from an upright position, with the feet extra rotated by 15° and coinciding on the same acromion vertical line (Ingebrigtsen et al., 2014). At the sound signal, the participant rapidly fell reaching a knee angle of about 90° where he was asked to perform the maximal push-off against the field, maintaining the hands on the waist for the entire jump. Three trials were performed, punctuated by one minute of passive rest. Only the best jump was used for the analysis.
The 15-meter sprint test (figure 4.6, B) was performed on a football field with a grass surface (Germano et al., 2015). Three reference lines were marked on the field at 0 and after 15 meters (for photocells), and 50 centimetres before the starting line (for player). Each participant was positioned at the first line and was asked to run at the maximal speed possible after hearing the acoustic signal. Photocells started to record when the participant got the first line and interrupted when he reached the last line. Three trials were performed within two minutes of passive recovery. The best result was used for the analysis.
RSA's proposed test included six 40-meter football field shuttled sprints (20 + 20 m sprints with 180° turns, figure 4.6, C) with passive recovery intervals of 20 seconds (Rampinini et al., 2009). Starting from a fixed line, each participant might run at maximal speed for 20 meters, where a second line was marked. After touching the 20 m line with a foot, he might return to the starting line (0 m) as fast as possible. A 20-second passive recovery was allowed between each shuttle. All players performed three trials and the only best time was considered for the final analysis.
The Harre test (figure 4.6, D) was assessed according to a standardized protocol (Harre & Barsch, 1982). All participants were asked to complete the original circuit at maximal speed. If a participant committed a mistake the test was repeated; in case of two mistakes the test was considered unsuccessful. Three trials were performed, and the best time was collected.

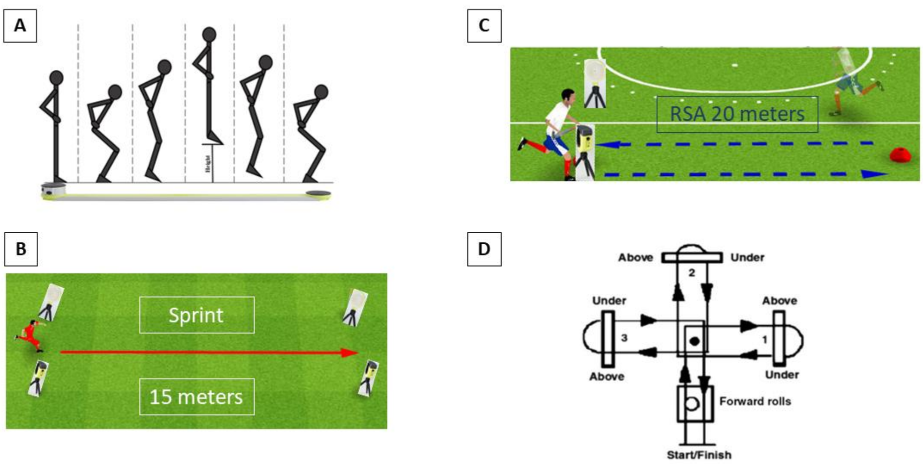

Figure 4.6. Physical performance tests: A, CMJ; B, 15 m sprint; C, 20 m RSA; D, HARRE test.

Bioelectric Impedance Analysis (BIA)

Bioelectrical impedance measurements were carried out with a body impedance analyser (BIA 101 Anniversary, Akern, Florence, Italy) using an electric current at a frequency of 50 kHz. Each participant was asked to lie on a massage bed in the supine position with a lower limb angle of 45° compared to the median line of the body and the upper limb angle of 30° from the trunk. After cleansing the skin with alcohol, two Ag/AgCl low-impedance electrodes (Biatrodes Akern Srl, Florence, Italy) were placed on the back of the right hand at the midpoint of the styloid process and 5 cm far away, and two electrodes were placed on the back of the right foot at the midpoint of the malleolus process (Lukaski & Piccoli, 2012). Two days before the evaluation, athletes were instructed to abstain from food and drink assumption for at least four hours before the test and avoid any form of physical effort. The evaluation was assessed in a quiet room, with a temperature between 20-22°C and 40% humidity.

Statistical Analysis

Descriptive statistics such as mean, standard deviation (SD), and observed frequencies (%) were calculated. For continuous variables, residual curve distribution was checked by the Shapiro–Wilk test. Whether a variable did not meet the normality assumption, its skewness was verified, and a location-scale transformation was applied.
Differences in the frequencies were tested by the chi-squared ($\chi2$) test, with Fisher's exact test. In addition, the contribution of each variable for the test statistic was reported. To compare continuous variables means between selected and unselected players for each category (U10-12), the One-Way ANOVA was assessed with the Bonferroni post hoc test. The type I error level (p) probability was settled at 5% (0.05). To check the power of the statistical tests applied, the power analysis for one-way ANOVA was computed with the following criteria: the number of subjects in each group, the significance level selected, the group means and the error variance. The mean power achieved was 0.73.
Then, the linear discriminant function analysis (LDA) through stepwise criteria was performed on anthropometric, body composition variables and motor performance parameters to classify subjects into the different sports categories, according to Fisher's approach. The MANOVA statistic was performed, and Wilk's lambda (λ) values were reported. Also, the average posterior probability classification was estimated to see the percentage of observations correctly classified in each group. Since groups were selected and unselected players, only one discriminant function was produced, and the canonical correlation, eigenvalue, F, and p values were reported. Finally, the standardized coefficient of the discriminant function was calculated to obtain a projection of the data that explained the maximal separation between the two groups.
All the statistical analyses were performed with STATA® software for Windows 10, version 18 (Publisher: StataCorp. 2023. Stata Statistical Software: Release 18. College Station, TX, USA, StataCorp LP).

4.2.3 Results

Table 4.2 shows differences in frequencies of birth quartiles and maturity status between selected and unselected players. Generally, most players were born in the first six months of the year, for selected (n= 20, 76.92%) and unselected (n= 35, 67.31%) youths. As regards maturity status, boys on time were prevalent (n=30, 40.54%), while earlier youths were 21 (28.37%). However, no significant differences appeared between selected and unselected players in both RAE ($\chi2$= 5.46, p= 0.14) and MS (χ^2= 0.74, p= 0.69).

When clustered for categories, 88.89% of U10 selected players were born in the first quartile and 88.24% of U10 unselected footballers were born in quartile one or two. Regarding maturity status, significant differences appeared only in the youngest category. However, the percentage of players who matured later was double in U11 unselected players (40% vs 20%).

Table 4.2. Differences in RAE and Maturity status prevalence between selected and unselected players.

	Selected		unselected			
	n	%	n	%	$\chi^2_{(3)}$	p
RAE						
U10					6.12	0.11
Q1	8	88.89	8	47.06	1.70	
Q2	0	0.00	7	41.18	3.70	
Q3	0	0.00	1	5.88	0.52	
Q4	1	11.11	1	5.88	0.20	
U11					2.40	0.49
Q1	7	63.64	5	33.30	1.30	
Q2	1	9.09	2	13.30	0.10	
Q3	2	18.18	5	33.30	0.50	
Q4	1	9.09	3	20.00	0.50	
U12					0.06	0.99
Q1	2	33.33	7	35.00	0.01	
Q2	2	33.33	6	30.00	0.02	
Q3	1	16.67	3	15.00	0.00	
Q4	1	16.67	4	20.00	0.03	
Maturity status						
U10					6.33	<0.05*
E	0	0.00	8	47.06	3.33	
L	5	71.43	7	23.53	3.00	
OT	2	28.57	1	29.41	0.00	
U11					1.16	0.56
E	4	40.00	4	26.67	0.30	
L	2	20.00	6	40.00	0.80	
OT	4	40.00	5	33.33	0.06	
U12					0.38	0.83
E	1	16.67	4	20.00	0.00	
L	1	16.67	6	30.00	2.00	

OT	4	66.67	10	50.00	1.80

note: n, number of observations; $\chi 2$, chi-squared statistical test; *p*, p-value

Figures 4.7 and 4.8 show some statistically significant differences that emerged in body fat between selected (right side) and unselected (left side) players. Specifically, figure 4.7 shows that selected players had a lower amount of skinfold on the triceps (8.56 ± 1.62 vs 9.61 ± 2.28, $p< 0.05$), biceps (3.94 ± 1.68 vs 5.28 ± 2.19, $p\leq 0.01$), medial (6.60 ± 2.25 vs 8.03 ± 2.73, $p< 0.05$) and lateral calf (7.27 ± 1.85 vs 8.62 ± 2.15, $p\leq 0.01$).

Figure 4.8 shows that unselected players had higher fat areas in lower limbs than selected ($\Delta CFA= -2.05$, $p< 0.05$; $\Delta TFA= -2.00$, $p\leq 0.05$). Also, significant differences emerged in percentage fat mass (selected= 13.02% vs unselected= 14.80%, $p< 0.05$).

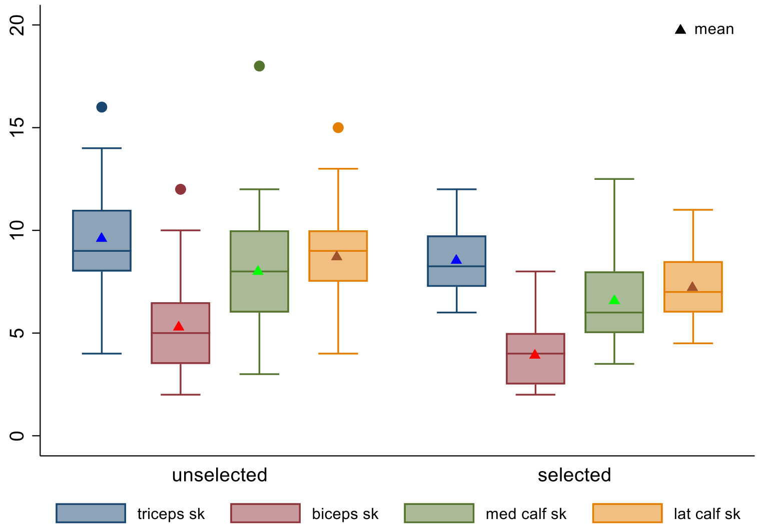

Figure 4.7. Skinfolds comparisons between selected and unselected players. Note: triangles represent mean values.

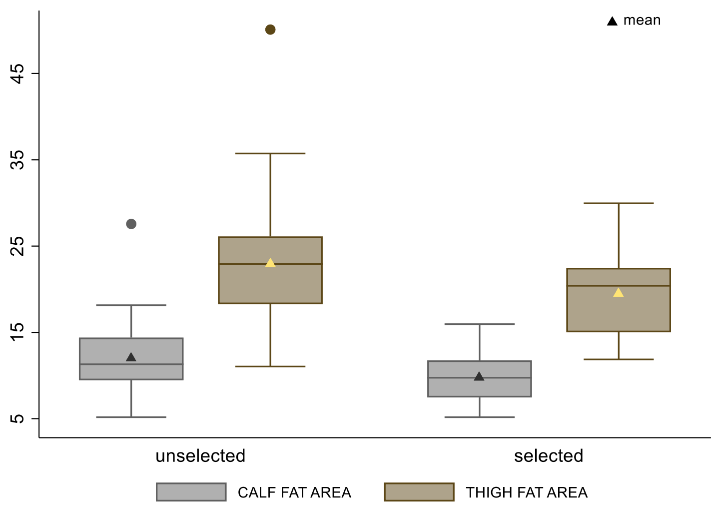

Figure 4.8. Fat areas comparisons between selected and unselected players. Note: triangles represent mean values.

Considering the differences within each age group (Table 4.3), in U10 the differences mainly concern trunk length, longer in the selected sample, maturity offset and age at PHV, which is most anticipated in the selected sample. Biceps and calf skinfolds and calf fat area were significantly thinner in the selected sample. In U11 significant differences were only observed for motor tests: the selected athletes presented a significantly better performance in the 15-meter sprint (s) and RSA. No significant differences were observed in U12.

Tables 4.4 and 4.5 show the LDA results. The stepwise procedure identified three predictor variables (Table 4.4). Repeated Sprint Ability (RSA) entered the discriminant analysis first, followed by Age at PHV and, lastly, humerus width. By this function, 42.1% of selected players and 85,3 % of unselected subjects were correctly classified.

Table 4.5 shows the standardized coefficients for the canonical variable. The canonical correlation equals 0.49 and the RSA test reports the highest coefficient absolute value (1.03) that indicates the most contributory factor in discriminating between the teams, followed by APHV (0.75) and Humerus width (0.47).

Table 4.3. Differences in anthropometric characteristics, body composition parameters and motor performance between selected and unselected players in the U10, U11 and U12 samples.

	selected U10		unselected U10			selected U11		unselected U11			selected U12		unselected U12		
Variable	mean	SD	mean	SD	$t_{(1, 24)}$	mean	SD	mean	SD	$t_{(1, 24)}$	mean	SD	mean	SD	$t_{(1, 24)}$
Body mass (kg)	32.46	4.05	31.44	3.60	0.66	36.83	4.92	35.07	4.21	0.96	40.43	6.36	37.37	2.37	1.14
Stature (cm)	139.21	6.03	137.05	6.23	0.85	146.40	6.79	142.80	3.95	1.68	148.37	8.98	147.43	3.16	0.25
Trunk length (cm)	70.30	2.60	66.61	2.78	3.01*	60.21	39.69	70.83	2.94	-1.04	73.37	4.25	72.68	2.59	0.37
Leg length (cm)	69.66	2.97	70.44	5.37	-0.36	74.25	4.84	71.97	3.25	1.42	75.52	5.17	74.75	1.49	0.35
BMI	16.96	1.78	16.88	1.22	0.12	17.21	1.52	17.13	1.48	0.13	18.91	1.79	17.62	0.96	1.68
PHV	-3.48	0.30	-3.86	0.30	2.84**	-2.87	0.35	-3.10	0.34	1.61	-2.37	0.55	-2.47	0.35	0.41
APHV	13.40	0.23	13.71	0.29	-2.49*	13.79	0.29	13.81	0.30	-0.23	14.10	0.48	14.24	0.30	-0.68
Humeral w. (cm)	5.61	0.36	5.37	0.32	1.63	5.66	0.31	5.69	0.27	-0.19	5.92	0.40	5.62	0.24	1.59
Femural w. (cm)	8.35	0.35	8.14	0.35	1.33	8.63	0.33	8.47	0.42	1.01	9.07	0.52	8.62	0.36	1.81
TUA (cm^2)	29.03	4.73	27.65	4.63	0.67	32.24	6.64	33.62	6.32	-0.52	36.75	7.17	31.17	1.57	1.70
UMA (cm^2)	21.72	3.56	19.91	3.35	1.19	24.06	5.10	24.51	4.31	-0.24	26.58	5.28	22.65	1.60	1.62
UFA (cm^2)	7.31	1.69	7.74	1.98	-0.51	8.19	2.01	9.11	2.87	-0.87	10.17	2.95	8.51	1.81	1.18
UFI (%)	25.11	3.53	27.93	4.47	-1.52	25.41	3.06	26.77	5.80	-0.67	27.56	4.82	27.24	5.26	0.13
TCA (cm^2)	62.71	9.10	63.62	7.56	-0.25	70.14	7.75	66.45	11.51	0.88	76.89	17.59	86.59	31.55	-0.91
CMA (cm^2)	54.78	7.62	52.93	5.19	0.68	59.95	7.29	55.42	9.45	1.27	63.62	15.41	74.29	32.01	-1.07
CFA (cm^2)	7.94	2.19	10.69	3.16	-2.18*	10.18	3.33	11.04	3.92	-0.56	13.27	4.35	12.29	1.81	0.48
CFI (%)	12.53	2.39	16.56	3.63	-2.79**	14.52	4.52	16.65	5.11	-1.05	17.41	4.12	15.42	4.45	0.94
TTA (cm^2)	119.61	16.82	118.71	14.60	0.13	132.81	16.74	130.20	19.33	0.34	149.09	30.00	124.36	35.37	1.57
TMA (cm^2)	101.85	14.20	98.43	11.77	0.61	112.44	13.41	107.06	13.70	0.96	124.31	23.04	104.01	31.07	1.62
TFA (cm^2)	17.76	3.89	20.28	4.46	-1.33	20.37	5.46	23.13	6.80	-1.06	24.78	9.35	20.35	6.05	1.00

TFI (%)	14.78	2.27	17.01	2.86	-1.88	15.23	3.09	17.49	3.25	-1.71	16.32	3.85	16.83	3.67	-0.26
BF (%)	12.15	2.07	13.10	2.29	-0.97	13.23	2.58	14.47	3.65	-0.92	16.37	4.06	14.02	2.67	1.21
FM (kg)	3.97	1.01	4.16	1.28	-0.37	4.92	1.32	5.20	1.83	-0.41	6.65	2.18	5.36	1.02	1.27
FFM (kg)	28.42	3.56	27.06	2.75	1.00	31.91	4.08	30.01	2.87	1.35	33.41	4.52	32.92	1.42	0.24
RX (Ω)	616.73	73.08	650.06	52.99	-1.24	622.40	55.72	641.59	56.14	-0.85	603.82	74.25	642.80	38.46	-1.12
XC (Ω)	64.69	3.26	66.96	7.57	-0.80	67.66	7.56	67.39	6.74	0.10	65.11	9.61	68.28	3.09	-0.72
PA	6.07	0.59	5.91	0.57	0.62	6.25	0.62	6.02	0.34	1.15	6.18	0.55	6.10	0.31	0.32
CMJ (cm)	24.18	4.39	23.29	3.10	0.55	26.69	3.69	24.09	3.58	1.73	28.51	4.07	27.18	3.60	0.71
15 m sprint (s)	2.97	0.13	3.02	0.07	-1.12	2.71	0.11	2.83	0.12	-2.52*	2.67	0.14	2.64	0.06	0.44
RSA (s)	6.63	0.19	6.78	0.30	-1.34	6.27	0.25	6.52	0.29	-2.08*	6.30	0.32	6.13	0.24	1.07
HARRE test (s)	14.47	1.01	14.59	0.71	-0.33	13.63	1.06	13.96	1.82	-0.54	13.50	0.91	13.34	0.84	0.36

note: w., width; sk, skinfold; SD, standard deviation; *t*, student's t statistical test; *, -value<0.05; **, p-value<0.01

Table 4.4. Selected variables by LDA

Step	Variable	Wilks' λ	$F_{(3, 74)}$	p	Tolerance
1	RSA	0.939	10.13	<0.01*	0.752
2	APHV	0.864	5.81	0.02*	0.886
3	Humeral w.	0.796	1.87	0.18	0.770

Note: Humeral w., humeral width; *F*, Snedecor-Fisher statistic's test; *p*, p-value; *, statistically significant.

Table 4.5. Standardized coefficients for canonical variables and test with successive roots removed.

Function	Canonical corr.	Eigenvalue	Variance	Wilks' λ	$\chi^2_{(3)}$	p
1	0.49	0.31	1	0.76	12	<0.01*
Standardized function coefficients						
RSA		APHV		Humeral width		
1.03		0.75		0.47		

Note: corr., correlation; χ^2, chi-squared statistical test; *p*, p-value; *, statistically significant.

4.2.4 Discussion

This study first aimed to compare maturation, body, and physical features between selected and unselected adolescent football players of an Italian elite football club, according to their age-related category. We have taken into consideration both biological (MS) and chronological (RAE) maturation methods and their prevalence in each age-related category (U10-U12).

Despite the total sample of groups did not report differences in RAE and MS prevalence, the percentage of all selected players who were born in the first quarter of the year was higher than in non-selected. Malina et al. reported that, since sport-specific skills are related to years of sports experience, footballers who were born in the first trimester could be advantaged in the selection process (Malina et al., 2007). Also, despite the wider part of early footballers in the youngest category (U10) not being promoted to professional teams, the U10-selected players were predicted to get the peak height velocity earlier than unselected teammates. It is to be noticed that the age at the PHV indicates a specific moment in time, while the classification in maturity categories indicates broader groupings. Therefore, the two things could reflect different evaluations. In the present study, the age at PHV seems to be a more indicative parameter in the selection. Although the following findings could appear in contrast with previous research showing that selected players were more biologically mature than their unselected counterparts (F. Helsen et al., 2000), the small sample size could have negatively affected our results.

Regarding anthropometry and body composition, we found that selected and unselected footballers exhibited different characteristics. Although some of our results were not statistically significant, we found characteristics that agree with previous studies (Figueiredo et al., 2021; Gil et al., 2007; Gravina et al., 2008; Nughes et al., 2020). Figueiredo and collaborators analysed data from players who were 11-12 and 13-14 years old and divided them into drop-out (players who abandoned), club (players selected for the same club), and elite (players who were selected from elite clubs) groups (Figueiredo et al., 2021). In their results, all elite players were heavier and taller than drop-out groups. Also, the sum of skinfolds appeared lower in both club and elite groups. In addition, a study in which the authors compared selected U11 players for elite vs non-elite teams of the same club showed that elite footballers were taller and had lower values for skinfold measurements (Hansen et al., 1999). Considering the club included in our study as an elite club, the selected players had less fat in the biceps, triceps, and medial and lateral calf skinfolds. Finally, the skinfold thickness in adolescent football players has been negatively correlated with cardiorespiratory fitness, especially for the triceps and calf anatomical sites (Nikolaidis et al., 2023). Although we did not test the cardiorespiratory fitness in our sample, the above-mentioned results could justify discrepancies among selected and unselected footballers.

Differently, Gravina and colleagues analysed 66 footballers divided into first-team players and reserves of four categories (U11-U14). Despite no significant differences emerging, the first-team players were taller and heavier and presented lower levels of body fat than the reserves. These results are also in line with research that investigated older adolescents.

For example, Nughes and colleagues investigated U15-U17 categories and found that selected players were taller and heavier than non-selected ones (Nughes et al., 2020). Also, Gil and colleagues highlighted similar characteristics in 14-year-old players, who collectively presented lower levels of fat mass (especially in their lower limbs) as in our selected footballers (Gil et al., 2007). In an 11-year study carried out in France, U16 players selected for international clubs were taller and heavier than footballers who did not acquire a professional contract (le Gall et al., 2010). Although no significant results appeared in body composition, players from all categories selected for international or professional clubs showed a lower level of body fat compared to amateurs. Anthropometric features such as height and weight remain relevant characteristics that could help in scouting and promotion. In addition, body composition should be more debated among football professionals. However, younger categories should be considered to better predict the anthropometric trend of talented players. Concerning physical performance, only the U11 selected players resulted faster than unselected players in both sprint and RSA tests. Differently, no significant differences appeared in jumped height (lower limb power) and the coordination test. Considering the first evaluation assessed for assigning footballers to the first-time teams or the reserves, the results of Gravina (Gravina et al., 2008) showed that the first teams' players (10-14 years) were faster than the reserves (flat sprint and sprint with cones). In contrast, no differences appeared in CMJ, squat jump and drop jump tests. Also, our results are in accordance with Figueredo et al., who found that U11-12 players selected for the elite clubs performed greater sprint and agility shuttle run tests than players who dropped out (Figueiredo et al., 2021). In addition, elite players did not significantly differ in CMJ when compared to club or drop-out categories. However, they found that both the squat jump and CMJ tests differed among elite and drop-out groups when players were older (U13-14). These discrepancies may reflect how conditional abilities development better emerges in adolescent maturation (> 13 years old; Harre and Barsch, 1982). To the best of our knowledge, just one study reported power differences already from 11 years old, but the players assessed a broad jump performance (horizontal distance; Rebelo et al., 2013). The speed may be more relevant in the youngest footballers' field performance, and the power needed for vertical jump could be offset against height discrepancies and fewer situations with air tackles. Differently, when considering older categories, better strength and speed characteristics emerged in players selected for the elite than in recreational teams (Gissis et al., 2006), in line with field-specific requests (Rebelo et al., 2013).

This study lastly aims to understand whether any anthropometric, performance or maturation feature could discriminate between selected and unselected players. According to the above-debated results, we found that the RSA test was the best predictor, followed by the age at the peak of height velocity (APHV) and the humeral width. To our knowledge, few studies computed the discriminant analysis in youth football players and age-specific-category comparisons are difficult. However, Nughes et al. who investigated U17 groups found that dribbling skills, 15-meter sprint time and height were the best predictors for cub promotion (Nughes et al., 2020). Although the RSA test requests a change of direction, the players' speed seems to be a common ability among elite footballers worldwide. Accordingly, both the 15-m sprint and RSA performance were the best discriminants among U12-U15 of elite and non-elite football players (Toselli et al., 2022). Regarding APHV, it has been demonstrated that advanced or earlier maturity could be associated with functional capacities such as speed and power, which are considered the best predictors for classification as elite and non-elite football players (Malina et al., 2007). Uncharacteristically, we found that the humeral width could be an additional useful parameter in predicting football selection. Although no previous results reported similar results for footballers, the humeral size has been correlated with stature and age development (Khan et al., 2011; Rissech et al., 2013). These characteristics are in line with Nughes' study in which the stature discriminated by competitive player level. Anthropometric features such as the skeletal width could be considered for further investigation in juvenile sports.

The main strength of this study was the description of several anthropometrical and physical profiles among Italian younger football players, and the understanding of how they could contribute to the selection process. However, this paper presents some limitations such as the size dimension sampled from only one football club, and the estimation of maturity status by Mirwald equations that may underestimate APHV with a later observed APHV and overestimate with an earlier observed APHV (Malina et al., 2015). Also, no football role information was investigated and the reasons why players were unselected have not been investigated. Finally, the statistical power achieved was lower than the desired value and the investigators did not evaluate the players' football experience due to a bias in measuring it.

4.2.5 Conclusions

Many aspects could influence the decision-making of football professionals in selecting talented players. Selected players could exhibit lower fat mass and greater physical abilities. Also, the ability to run speeder, the earlier age at growth spurt, and the humeral width discriminated among selection groups in elite football teams. These findings suggest that for adolescent categories run performance is still the best predictor, but other physical and biological features could support the selection process.

4.3 STUDY VII

4.3.1 Introduction

Factors leading to high morbidity and mortality during the COVID-19 pandemic have enhanced the worldwide interest in health and wellness (Newsome et al., 2024). To date, exercise for improved health has guided the projection of the fitness industry, and weight loss and body composition appear as top trends across the globe. Since 2014, High-Intensity Interval Training (HIIT) has been one of the most debated training modalities owing to its versatile and dynamic nature (Thompson, 2023). HIIT involves repeated short to long bouts of exercise punctuated by rest periods at intensities modulated through physiological responses such as heart rate (HR), blood lactate, velocity associated with peak oxygen consumption ($\dot{V}O_{2p}$), or rating of perceived effort (RPE) (Billat, 2001a, 2001b; Buchheit & Laursen, 2013b). The role of exercise intensity is derived from the concept that a large volume of moderate-intensity or a small volume of high-intensity training can elicit similar skeletal muscle adaptation (MacInnis & Gibala, 2017). When training is matched for volume, some metabolic enzyme mediators are greatly stimulated as intensity increases (Granata et al., 2016; Kristensen et al., 2015), eliciting gene expression (Egan et al., 2010) and mitochondrial biogenesis (Granata et al., 2016; MacInnis et al., 2017), and inducing physiological adaptations such as increasing $\dot{V}O_{2p}$ (Bacon et al., 2013; Bell & Wenger, 1988). When training is matched for high intensity, the volume increment also augments mitochondrial content (Granata et al., 2016). In addition, a single high-intensity sprint bout increased plasma catecholamine and growth hormone (GH) concentration post-exercise in both males and females, hormones involved in fat metabolism and muscle gain (Boutcher, 2011).

However, at least nine HIIT parameters such as work and rest modality, intensity, duration, number and duration of the series, time between each series, and between-series recovery intensity (Buchheit & Laursen, 2013a), can be managed to induce different physiological stimuli. Typically, work intervals shorter than 15 seconds, known as sprint interval training (SIT), allow athletes to reach a higher percentage of maximal effort, eliciting anabolic power and neuromuscular stress (Balsom et al., 1992; Dupont et al., 2004), while longer intervals of up to two minutes could favour reaching $\dot{V}O_{2p}$, improving BLa and oxidative tolerance (Seiler & Sjursen, 2004) and increasing time to exhaustion to sub-maximal effort (P. B. Laursen & Jenkins, 2002). A time series between 15 seconds and one minute aims to induce metabolic (O2 system) and neuromuscular responses (Buchheit & Laursen, 2013). Furthermore, recovery intensity and duration could be key in HIIT adaptations. Passive recovery (PR) between long work intervals facilitated training at higher power output maintaining similar session RPE (Fennell & Hopker, 2021; Stanley & Buchheit, 2014), whereas active recovery (AR) at moderate intensity (40-60% $\dot{V}O_{2p}$) was more effective in removing BLa during the session (Monedero & Donne, 2000; Sánchez-Otero et al., 2022). Differently, in short work intervals, AR elicited greater total power peak and work cost than PR with a similar RPE (Signorile et al., 1993). Despite the physiological mechanisms related to acute response in recovery intervals having been well investigated, there is a lack of evidence on the long-term adaptation induced by high-intensity exercises that combine short intervals and AR or PR (Schoenmakers et al., 2019). Furthermore, age and sex anthropometrical and biological features may be considered confounders or effectors and accounted for analysing specific physiological responses (Gibala et al., 2014). For example, females are supposed to accumulate a lower concentration of blood lactate after a 30-s sprint session, which could be associated with reduced basal activities of lactate dehydrogenase and muscle phosphofructokinase than males (Esbjorsson et al., 1996 and 2002; Jaworowski et al., 2002). It remains unclear how the HIIT long-term adaptation could affect sexes differently, and whether HIIT protocols may be administered interchangeably between females and males.

Although the origin of HIIT has been credited with running and skiing exercises (Billat, 2001a), this training modality has also been adapted to other sports (P. Laursen & Buchheit, 2018) and fitness (Machado et al., 2019). Many studies have shown that HIIT using whole-body exercises is effective in improving cardiorespiratory fitness; body composition, such as fat mass (FM) and fat-free mass

(FFM); and musculoskeletal fitness, such as strength and endurance, in healthy adults (Scoubeau et al., 2022). Similar acute (Gist et al., 2014) and long-term (McRae et al., 2012) responses appeared between whole-body and running-based high-intensity training. Still, fewer studies investigated the effect of combined running and whole-body HIIT (Eather et al., 2019).
In light of these pieces of evidence, the main purpose of this study was to evaluate the efficacy and feasibility of a novel 8-week HIIT program that combines short-term intervals with running and whole-body exercises and understand whether moderate-intensity AR (~50% HR) and PR induce different changes in body composition and physical performance between trained younger male and female adults.

4.3.2 Methods

Experimental design

A randomised clinical trial design of ten weeks was selected. The first (pre) and last (post) weeks were used to evaluate participants, whereas the HIIT treatments lasted eight weeks. Before the enrolment, a priori sample size was estimated for repeated-measures analysis of variance test for within-between, following the study parameters: Type I error (α)= 5%, Type II error (β)= 20% and statistical power (1-β) =80%, number of groups and repeated measures= 4, variance between-within expected=0.05 (Δ= 0.89), correlation between repeated measures= 0.75. The estimated sample size was 20, four males and females for each group. To prevent the sample mortality effect, the sample size has been increased by 10% (two subjects, figure 4.9).
After registration, each participant was randomly allocated to AR or PR groups, which included the same exercises, series, work-rest ratio, duration, and progression (figure 4.9). The randomisation has been processed by a statistical software-specific package (STATA 18, Windows Edition, StataCorp, Texas, USA). The AR protocol provided a walking recovery at 50% of maximal HR (Sánchez-Otero et al., 2022), whereas the PR group was requested to rest passively. Both HIIT programs included two weekly sessions, and participation in at least 15 (95%) workouts was needed. During each training session, the number of repetitions and loads per series, maximal and average HR (Polar H9 sensor and Polar beat mobile APP, Kempele, Finland), and rating of perceived effort (sRPE, 30 minutes after the workout end) were collected. Participants were tested for body composition, strength, power, agility, and maximal oxygen consumption, before (pre) and after the HIIT treatment (post). The final performance evaluations were performed 72 hours after the last HIIT session.

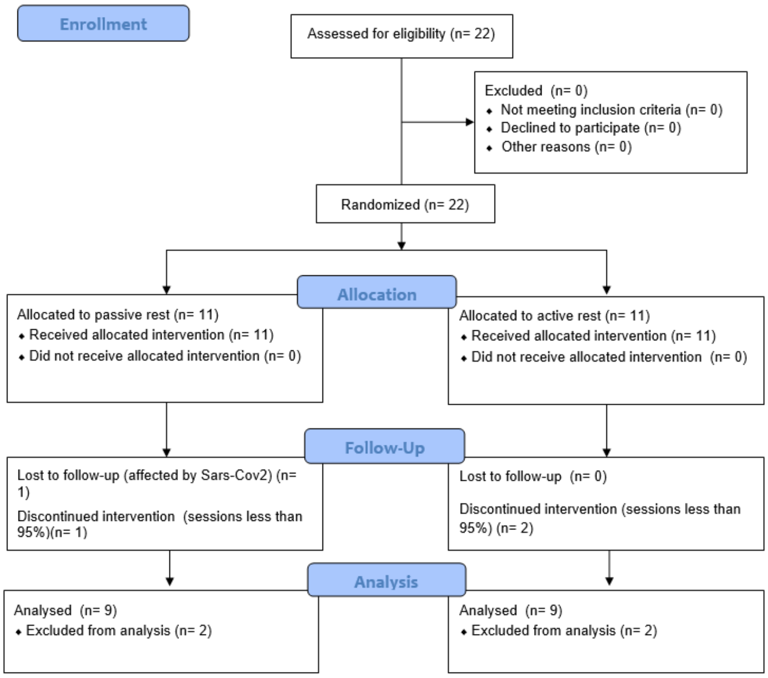

Figure 4.9. Consort flowchart.

Participants

Eligibility criteria were as follows: (a) ages between 20 and 30 years old, (b) medical certification guarantee for high-intensity activities (exercise electrocardiography), (c) at least 5 years of adolescent sports experience with at least 2 training sessions per week during the last years, and (d) no health problems, body limitations or musculoskeletal injuries that could affect physical performance. Twenty-two subjects volunteered to participate in this study, but only 18 completed at least 95% of the training (figure 4.9; soccer= 5, basketball= 1, volleyball= 1, swimming= 2, gymnastics= 5, boxing= 1, athletics= 3). They were male (n= 8) and female (n= 10) university students (Faculty of Sports Science) who were fit (2.9 ± 0.84 sessions per week) and confident with the involved exercise techniques (figure 4.10). Subjects were randomly allocated to PR (n= 9, 66.67% female, age= 23.09 ± 2.56 years, stature= 163.69 ± 9.88 cm, body mass= 68.96 ± 14.62 kg) or AR (n= 9, 44.44% female, age= 22.05 ± 1.54 years, stature= 170.61 ± 11.5 cm, body mass= 68.78 ± 12.45 kg). All subjects were asked to abstain from any other relevant physical activity or sport not included in the program provided. Also, subjects were asked to maintain their usual nutritional behaviours and avoid new dietary supplementation or drugs that could enhance body performance. Before the evaluations, each subject was instructed to have a two-week wash-out with no physical exercise.

Treatment

High-Intensity Interval Training. Figure 4.10 A shows the first day and Figure 4.10 B the second day of the training program for both AR and PR groups. Total time, session density, and work-recovery ratio followed previous recommendations (Machado et al., 2019). The protocol included short-term series (one minute) to elicit both oxidative and neuromuscular systems response (Buchheit & Laursen, 2013). The series duration was maintained for the whole protocol, but the work-recovery ratio was modified to increase the training intensity over time. To induce mechanical tension, muscle damage and peripheral metabolic stress, factors leading to muscle hypertrophy (Egan & Zierath, 2013), each subject was asked to perform a maximal number of repetitions as possible (AMRAP) per series of each exercise, while they were asked to maintain 80-90% HR max during the work to increase central and peripheral oxidative demands. All the sessions were equal for groups regarding volume (number of exercises, series, duration) and intensity (HR interval, work-recovery ratio, and rest between series) The groups differed by the recovery within the series during each training session. AR subjects were instructed to perform active recovery between series by walking at pre-tested speed (1.89 ± 0.26 m/s) in a specific gym rectangle (10 x 2 m) with marked lines for any meter. According to the session training rest time, they covered a specific distance related to their 50% HR max. Differently, the PR group had passive rest. Recovery time between each exercise (one minute) was passive for both groups. Standardized warm-up and cool-down were provided by one of the study investigators, who supervised each training session. In addition, he recorded in a logbook the number of repetitions (or laps) per series and exercise, the external load (if used), the averaged and maximal HR, and the RPE of the session. The training progression consisted of weekly increments in volume (weeks 2-5) and intensity (weeks 7-8) as follows: week 1 (W1) included three series of seven exercises for each work-out with a work ratio recovery of 1 (30 s: 30 s, figure 4.10) and total duration of 56 min (28 per session), week 2 added one exercise in work-out 1 (push-up, Figure 4.10 A) for a week HIIT time of 60 min (32 and 28), W3 added one exercise in work-out 2 (kettlebell swing, figure 4.10 B) for a week time of 64 min, W4 increased one series in work-out 1 (4 series x 8 exercises) for a week time of 72 min (40 and 32), W5 increased one series in work-out 2 for a week time of 80 min, W6 had no increment to facilitate the adaptation, W7 changed the work- recovery ratio up to 1.4 (35 s: 25 s) in work-out 1, and the last week increased the work- recovery ratio to 1.4 (35 s: 25 s) in work-out 2.

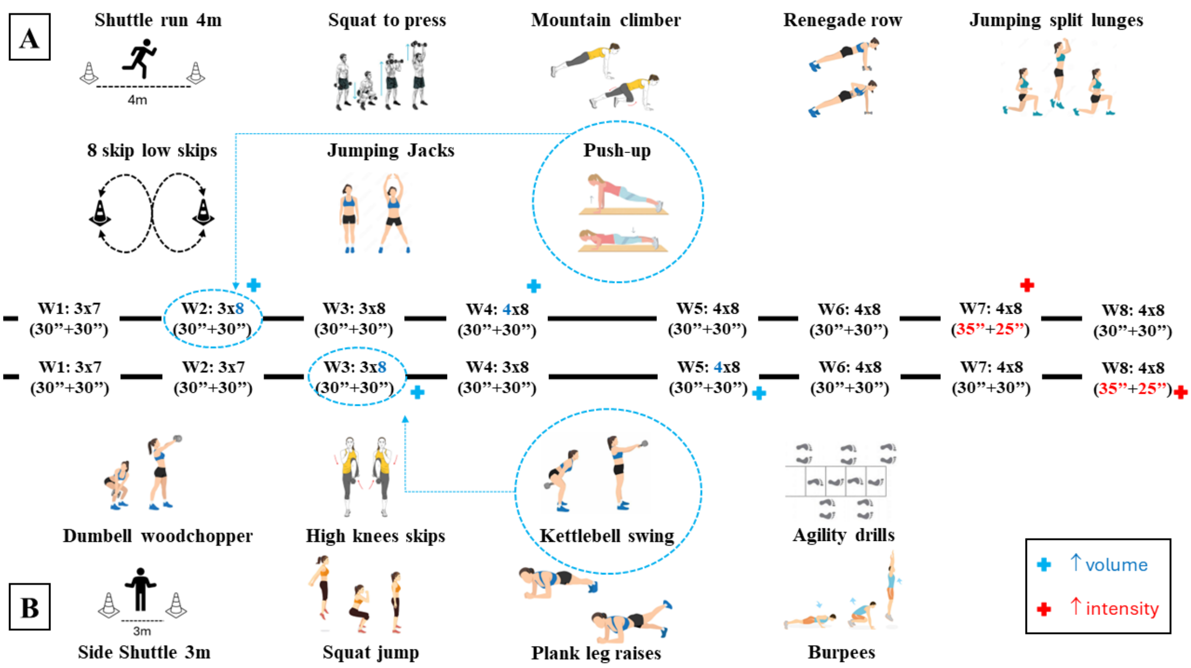

Figure 4.10. HIIT protocol exercises, parameters and progression for session one (A) and session two (B) over eight weeks (W1 - W8).

Training and Volume Load

Before the HIIT program began, each subject was instructed to rate her perceived effort using a 0-10 scale (Foster et al., 2001), where 0-1= very easy, 2= easy, 3= moderate, 4= somewhat hard, 5-6= hard, 7-8= very hard, and 9-10= maximal. Thirty minutes after the conclusion of each workout session, the investigator asked each participant to privately answer the question "How intense was your training?" and fill out the RPE 0-10 scale. The training load (TL) was computed for all sessions as the product of sRPE and the workout duration (minutes) (Haddad et al., 2017). Then, the adjusted training load (adj. TL) was calculated as follows:

$$(RPE_s \cdot time_s) \cdot \frac{\overline{HR}_s}{HR_{max}}$$

where s = session, $\overline{HR}_s$= "mean of session" HR

In addition, the volume load (VL) was computed by multiplying the number of repetitions, the number of series, and the external load weighted (if used). Finally, the covered distance (CD) and the average speed (AS) were computed for each session by multiplying the number of repetitions for the meters provided for the exercise (CD), and then by dividing it by the second of work. The TL was expressed in minutes, the VL was in kilograms, the CD was in meters and the AS was in meters/ seconds.

Anthropometry and body composition assessments

Anthropometry and body composition evaluations were assessed on day one before (pre) and after (post) the HIIT protocol. Body mass (CCC= 1.000, 95% CI: 0.999,1.000) was measured to the nearest 0.1 kg (Seca 769, Seca Scale Corp, Munich, Germany), Technical Error of measurement (TEM)= 3.18%. Arm (CCC= 1.000, 95% CI: 0.999, 1.000) and thigh (CCC= 0.999,95% CI: 0.997, 1.000) circumferences were measured to the nearest 0.1 cm with a no-stretchable tape (Seca, Seca Scale Corp., Munich, Germany), in standardised body sites (Lohman et al., 1988): the arm circumference was taken at the mid-point between the shoulder acromion and the olecranon process point, with the subject's elbow relaxed along the body side, whereas the thigh circumference was taken at the mid-point between the inguinal fold and the superior kneecap point, with the participant in a standing position (thigh muscles relaxed). Arm and thigh TEM were respectively 2.01 and 1.25% Arm and thigh muscle and fat mass areas were computed according to Lohman and colleagues (1988). Upper limb muscle area TEM= 5.29%, thigh muscle area TEM= 2.94%, while Upper limb fat index TEM= 4.56% and thigh fat index TEM= 5.61%. Triceps, abdomen, and thigh skinfold thicknesses were measured to the nearest 1.0 mm at the left side of the body (Lange, Beta Technology Inc., Houston, TX, USA), and then used to estimate body fat percentage according to Evans et al. (2005). The triceps site was marked vertically at the posterior arm face midpoint between the acromion process and the olecranon process; the abs site was marked horizontally three centimetres left and one upper the umbilicus; the thigh site was marked vertically at the mid-point between the inguinal fold and the superior kneecap point. A trained investigator assessed the evaluations, and the average value of three re-peated measures was used; their intraclass correlation coefficients (ICC) and random error of measurements (SEM) were: ICC= 0.948 (95% CI: 0.899, 0.978), SEM= 0.873 mm and TEM=7.26%, ICC= 0.981 (95% CI: 0963, 0.992), SEM= 0.969 mm and TEM=5.63%, and ICC= 0.988 (95% CI: 0.977, 0.995), SEM= 0.455 mm and TEM=5.61%, for triceps, abs, and thigh respectively. The predicted body fat, fat mass and fat-free mass reported a TEM of 5.34, 4.77 and 3.89%.

Handgrip Strength, Power, Agility, and Peak Oxygen Consumption

Handgrip strength (HGS), power (CMJ), and agility (5-0-5) were assessed on day two while the peak oxygen consumption ($\dot{V}O_{2p}$) was assessed on day three of the protocol first week and last week, at the University Sports Science laboratory (Bologna, Italy). The indoor environmental features were 20°C, 50-60% humidity, and no external music or soundtrack that could affect the performance, and they were unvaried among pre and post-tests. Before the strength, power, and agility testing session, subjects performed a standardized warm-up, according to Bartolomei et al. (2018). For the maximal oxygen consumption, a standardized warm-up of five minutes of walking was assessed, at the following speed for each minute (1% inclination): 1.25 m/s, 1.39 m/s, 1.53 m/s, 1.67 m/s, 1,81 m/s.

The handgrips strength for right (HGS r) and left (HGS l) hands were tested to the nearest 1 kg with an analogic dynamometer (Takei 5001, Takei Scientific Instruments Co. Ltd, Tokyo, Japan). Each subject stood with their arms by their sides and their elbows fully extended during evaluation. Three times of alternate measurements were made without a minute of rest among each series, and subjects were asked to squeeze the dynamometer for 3 seconds for each measurement (Gatt et al., 2018). The better result was used in the analysis. The HGS ICC were 0.983 (95% CI: 0.966, 0.993) and 0.980 (95% CI: 0.962, 0.002), while SEM were 1.315 and 1.434 kg and TEM were 4.56 and 5.14% for the right and left hand respectively.
The countermovement jump (CMJ) test was assessed by a study investigator with photoelectric cells grounded at a two-meter distance (Optojump, Microgate, Bolzano, Italy). Subjects were instructed to maximize the height of each jump while keeping their hands on their hips. Flight time was calculated as the time interval from toe-off to landing. Each subject performed three jumps with a 2-minute rest between each jump, and the best jump was used in the analysis. The CMJ intraclass correlation coefficient was 0.989 (95% CI: 0.978, 0.995), SEM= 0.909 cm and TEM= 3.27%.
The 5-0-5 agility test was set up and administered using the protocol outlined by Draper (Draper J.A. & Lancaster M.G., 1985). Two investigators assessed the evaluation with two photoelectric cells connected to a digital chronometer (Witty SEM, Microgate, Bolzano, Italy) placed 10 and 15 meters from the start line. Each subject was instructed to sprint after the acoustic signal for 15 m, turn on their preferred foot, and sprint back for another five meters. The time to cover the last five m of the 15 m straight line plus the 5m after the change of direction was recorded. Three assessments with two minutes of rest between each series were performed. The best time was used for the analysis. The 505-agility test ICC was 0.892 (95% CI: 0.790, 0.954), SEM= 0.074 s and TEM= 2.35%.
The treadmill Bruce test was set up according to Bruce protocol (1971). All subjects were asked to refrain from alcohol for 24 hours prior and caffeine for 4 hours before each trial. Also, subjects were asked to drink 500 ml of water approximately 2 hours before testing to standardize body fluids concentration. Before the trial, each subject was attached to the safety vest and was instructed to push the stop button in case of emergency. Each subject performed a continuous incremental exercise test to voluntary exhaustion on a calibrated treadmill (h/p/cosmos pulsar, COSMED, Rome, Italy). A cardiac band for heart rate monitoring was provided (Polar H9 sensor, Polar, Kempele, Finland), and the entire trial was recorded by a mobile APP (Polar Beat, Polar, Kempele, Finland). The Bruce protocol consisted of incremental seven stages: (1) 3 minutes of walking with 10%-inclination at 0.76 m/s, (2) 3 m of walking with 12%-inclination at 1.12 m/s, (3) 3 m of walking with 14%-inclination at 1.52 m/s, (4) 3 m of walking with 16%-inclination at 1.88 m/s, (5) 3 m of running with 18%-inclination at 2.24 m/s, (6) 3 m of running with 20%-inclination at 2.46 m/s, and (7) 3 m of running with 22%-inclination at 2.68 m/s. The trial ended when the subject was exhausted. The total length, average, and peak HR were collected. The Bruce equation was used to estimate the $\dot{V}O_{2p}$. The TEM of predicted $\dot{V}$O2p was 1.81%.

Statistical Analysis

For descriptive statistics, the mean was used as the central tendency measure, while the standard deviation for describing dispersion. The reliability of repeated measurements was computed as an intra-class correlation (ICC) and standard error of measurements (SEM) among baseline and follow-up and as a relative technical error of measurements (TEM) between eight weeks.
To account for both within and between-subjects correlation, the data has been analysed such as panel and the multivariate linear mixed effect model has been preferred, where both fixed (mean model) and random (covariance model) effects have been considered (Cheng et al., 2010). A full-way interaction of time, treatment and gender has been investigated. The same mean structure (fixed) has been maintained by comparing three different covariance structures (unstructured, first-order autoregressive and compound symmetry). The nested models (linear and quadratic) with different covariance structures have been fitted by restricted maximum likelihood and compared throughout

the likelihood ratio test. In addition, the Akaike information criteria (AIC) chacked for the best model. The normality assumption has been checked for marginal residuals (Jacknifed studentized). When asymmetries in curves were found, a natural logarithm transformation was applied. To infer the Wald test was assessed, and the respective χ^2 statistic with (n/2 – t) degrees of freedom has been reported, where n is the sample size and t is the number of repeated measures. Also, the marginal effects have been evaluated. The type I error probability was settled at 5%.
In addition, the percentage change was calculated as [(mean at post – mean at pre)/ mean at pre]*100. Where appropriate, the relative weighted change proportion was calculated as [(dependent var at post/ weight var at post) – (dependent var at pre/ weight var at pre)]/ (dependent var at pre/ weight var at pre). Finally, the effect size of the treatment was computed by the Hedges'g statistic.
Data were gathered in digital spreadsheets in the Excel 2023 Windows edition (Microsoft, Washington, USA) and analysed in STATA 18 Windows edition (StataCorp., Texas, USA).

4.3.3 Results

Training progression

Generally, the HR did not show significant changes over the eight weeks (79.34 ± 3.17 bpm, with a mean decrement of 0.54 bpm per week; z= -1.10, p= 0.272). When examined separately in the AR and PR groups and gender, the week progression did not significantly affect the HR variability (group: z= -0.82, p=0.414; gender: z= -0.33, p= 0.74). However, the differences in conditional means of AR vs PR are statistically significant over each week, with a mean contrast of 4.45 ± 1.53 bpm ($\chi^2_{(8)}$= 20.24, p= 0.009), while female vs male HRs differed only on W6 ($\chi^2_{(1)}$= 4.91, p= 0.027). The full-way interaction model showed significant differences in males AR vs PR at W1 (β= 4.94 ± 2.31 bpm, $\chi^2_{(1)}$= 4.56, p= 0.033), in females AR vs PR at W3 (β= 5.69 ± 2.04 bpm, $\chi^2_{(1)}$= 7.74, p= 0.005) and W8 (β= 5.35 ± 2.05 bpm $\chi^2_{(1)}$= 6.83, p= 0.009), and both sexes at W5 (♀: β= 4.33 ± 2.05 bpm, $\chi^2_{(1)}$= 4.49, p= 0.034; ♂: β= 6.96 ± 2.31 bpm,$\chi^2_{(1)}$= 9.05, p= 0.027), W6 (♀: β= 4.87 ± 2.05 bpm, $\chi^2_{(1)}$= 5.66, p= 0.017; ♂: β= 4.96 ± 2.31 bpm, $\chi^2_{(1)}$= 4.60, p= 0.032), and W7 (♀: β= 5.86 ± 2.05 bpm, $\chi^2_{(1)}$= 8.19, p= 0.004; ♂: β= 5.17 ± 2.31 bpm, $\chi^2_{(1)}$= 4.99, p= 0.026).

Figure 4.11 shows the adjusted TL (A), volume load (B), (C) coverage distance and average speed (D). As regards the adjusted TL, the overall mean was 388.54 ± 100.84 min with an average week increment of 14.13 min ($\chi^2_{(7)}$= 145.77, p<0.001). No difference appeared between AR vs PR (z= 0.26 p= 0.794) and male vs female (z=1.61, p=0.108), and adj. TL rates were significant for both groups ($\chi^2_{(14)}$= 165.87, p< 0.001) and sexes ($\chi^2_{(14)}$= 158.76, p< 0.001). Looking at marginals, the contrasts were significantly wider only in AR vs PR females at W5 (β= 117.62 ± 56.77 min, $\chi^2_{(1)}$= 4.29, p= 0.038), W6 (β= 126.27 ± 56.77 min, $\chi^2_{(1)}$= 4.95, p= 0.026), W7 (β= 156.54 ± 56.77 min, $\chi^2_{(1)}$= 7.60, p= 0.005) and W8 (β= 151.12 ± 56.77 min bpm, $\chi^2_{(1)}$= 7.09, p= 0.008).

Figure 4.11 (B) shows the VL means and trends for groups and sexes. Generally, the VL reported a mean value of 9989.412, with a within-standard deviation of 3911.01kg (r= 0.56). The baseline VL average was 3249.72 kg and the weekly effect affected it by 48.99% per week (z= 3.66, p< 0.001). The VL increments were not statistically significant just between weeks five and seven (W6 vs W5 95% CI: -628.55, 1273.55; W7 vs W7 95% CI: -186.82, 1715.27). Males reached a higher mean VL (β= 386.08 ± 194.69) than females (z= 1.98, p= 0.047), while no significant difference appeared between AR and PR over time (z= 0.64, p= 0.534). The interaction effect of groups and sexes over time detected a constant trend ($\chi^2_{(16)}$= 12.64, p< 0.699).

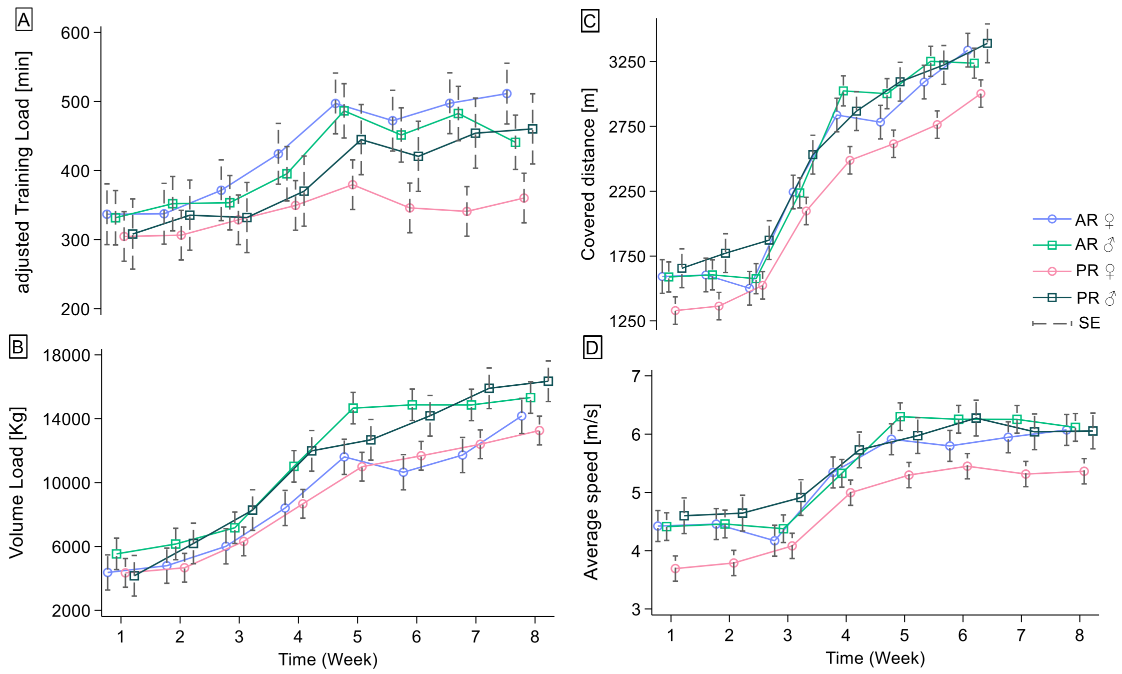

Figure 4.11. Training parameters' variations over eight weeks.

Concerning the coverage distance (figure 4.11, C), the overall mean was 2346.82 ± 685.86 m. The weekly increment was 179.72 m ($z= 4.98$, $p< 0.001$), with a 9.30% rate higher in males compared with females ($z=2.11$, $p=0.035$); no differences appeared between AR and PR over time ($z= 0.81$, $p= 0.419$). The contrast of conditional predictions showed a significant difference between AR vs PR females at W5 ($\beta= 349.33 \pm 167.36$ m, $\chi^2_{(1)}= 4.36$, $p= 0.037$), W7 ($\beta= 328.0 \pm 167.36$ m, $\chi^2_{(1)}= 3.84$, $p= 0.05$) and W8 ($\beta= 334.67 \pm 167.36$ m, $\chi^2_{(1)}= 4.0$, $p= 0.046$).

Finally, the AS reported an overall mean value of 5.18 ± 0.77 m/s, with a baseline of 4.28 m/s and a weekly rate of 0.14 m/s (95% CI: 0.00, 0.29; $p= 0.05$). However, the marginal effects detected considerable changes at weeks four (95% CI: 0.80, 1.13) and five (95% CI: 0.37, 0.70). No significant differences appeared between groups ($z= -0.16$, $p= 0.875$) and sexes ($z= 1.84$, $p= 0.065$) over time. The conditional contrast detected significant effects between AR and PR females at W1 ($\beta= 0.73 \pm 0.34$ m/s, $\chi^2_{(1)}= 4.53$, $p= 0.033$) and W8 ($\beta= 0.71 \pm 0.34$ m/s, $\chi^2_{(1)}= 4.28$, $p= 0.039$), while males speeds varied similarly.

Body Composition

Table 4.6 shows the longitudinal effects of HIIT on body composition, stratified for groups and gender. Figure 4.12 shows the body composition changes (percentage) in AR and PR females and males.

Eight weeks of HIIT reduced %BF by 10.31 ± 2.4% (figure 4.12, A) from baseline (95% CI: -15.50, -5.12), with no rate differences between groups (+ 0.23% for PR, 95%CI: -1.06, 1.52) and gender (+1.15% for females, 95% CI:-2.56, 0.26). The conditional effects reported significant slopes for PR females (pre vs post $\beta= 1.60 \pm 0.42\%$, $\chi^2_{(1)}= 14.56$, $p< 0.001$), PR males (pre vs post $\beta= 2.30 \pm 0.59\%$, $\chi^2_{(1)}= 15.01$, $p< 0.001$), AR females (pre vs post $\beta = 1.29 \pm 0.51\%$, $\chi^2_{(1)}= 6.33$, $p=0.012$) and AR males (pre vs post $\beta= 1.28 \pm 0.46\%$, $\chi^2_{(1)}= 7.77$, $p=0.005$), with no differences in rate of change (95% CI: -1.44, 2.46). However, the PR males reported the widest effect size ($g= 2.583$, 95% CI: 0.398, 4.683).

Table 4.6. Longitudinal HIIT effects on Body Composition in groups.

	PR (n=9)				AR (n=9)				Mixed Model effects (*Wald* test, χ^2 degrees of freedom)							
	Pre	Post	Pre	Post	Pre	Post	Pre	Post								
	♀ (66.67%)		♂ (33.33%)		♀ (44.44%)		♂ (55.56%)		Time		Time#Group		Time#Gender		Time#Group#Gender	
	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	z	p	z	p	z	p	z	p
BF (%)	21.65 (1.79)	20.05 (2.30)	14.16 (0.93)	11.86 (0.38)	20.83 (3.01)	19.54 (2.10)	11.04 (3.29)	9.76 (2.88)	-3.09	0.002*	0.35	0.723	-1.59	0.111	0.51	0.607
UFI (%)	37.41 (4.03)	29.65 (3.70)	28.56 (0.87)	20.71 (3.50)	31.65 (5.28)	32.64 (7.24)	24.33 (7.03)	20.36 (8.92)	-1.51	0.132	0.56	0.580	-2.18	0.029*	-0.50	0.614
LFI (%)	27.15 (6.36)	25.04 (6.21)	20.16 (2.18)	17.62 (2.16)	26.63 (5.55)	25.55 (6.37)	15.57 (3.45)	12.48 (3.93)	-2.23	0.026*	0.78	0.437	-1.01	0.314	-1.14	0.253
FFM (kg)	48.44 (7.41)	49.14 (7.39)	71.18 (10.56)	73.35 (10.62)	45.23 (1.84)	46.39 (1.02)	69.41 (7.20)	70.65 (7.58)	1.40	0.161	0.62	0.537	2.22	0.026*	-1.30	0.193
UMA (cm^2)	35.67 (9.83)	39.62 (9.49)	53.29 (12.97)	60.15 (13.31)	33.02 (2.98)	32.96 (4.05)	51.61 (13.28)	54.56 (16.37)	2.30	0.022*	-1.84	0.066	1.94	0.052*	0.06	0.952
LMA (cm^2)	70.54 (7.00)	74.82 (7.73)	89.95 (13.93)	92.73 (13.43)	65.62 (8.55)	71.38 (8.77)	88.01 (8.95)	95.55 (13.13)	1.84	0.065	0.14	0.887	0.74	0.457	0.81	0.416

Note: n, sample size; PR, Passive Recovery; AR, Active Recovery; F, Snedecor-Fisher test; p, p-value; SD, Standard Deviation; BF, Body Fat; FFM, Fat Free-Mass; UFI, Upper limb Fat Index; LFI, Lower limb Fat Index; UMA, Upper limb Muscle Area; LMA, Lower limb Muscle Area; *, statistically significant.

When observed peripherical, the UFI showed a 15.31 ± 21.27% decrement (figure 4.12, B). UFI marginal effects exhibited a greater variation in males (95% CI: -8.94, -0.47). Despite groups and their interaction with gender was not significant in mixed model rates (95% CI: -7.33, 4.33), the AR females showed a percental UFI increment by 3.13% (95% CI: -5.78, 6.40; g= 0.136, 95% CI: -1.076, 1.336) while the PR females decreased by 20.13% (95% CI: -34.17, -7.31; $\chi^2_{(1)}$= 6.42, p= 0.01; g= 1.851, 95% CI: 0.507, 3.138). Differently, the mean LFI decreased by 4.77 ± 4.41% over time (figure 4.12, B). The slopes for groups and gender interactions were: PR females pre vs post β= 2.11 ± 0.74 ($\chi^2_{(1)}$= 8.21, p= 0.004; g= 0.31, 95% CI: -0.75, 1.355), PR males pre vs post β= 2.53 ± 1.04 ($\chi^2_{(1)}$= 5.94, p= 0.015; g= 0.934, 95% CI: -0.532, 2.311), AR females pre vs post β= 1.08 ± 0.90 ($\chi^2_{(1)}$= 1.43, p= 0.231; g= 0.157, 95% CI: -1.056, 1.358) and AR males pre vs post β= 3.09 ± 0.81 ($\chi^2_{(1)}$= 14.74, p< 0.001; g= 0.754, 95% CI: -0.443, 1.91).
Concerning lean mass, FFM exhibited a mean increment of 2.09 ± 1.97% (figure 4.12, D). The marginal contrasts of AR females (β= -1.16 ± 0.54, 95% CI: -2.22, -0.11; $\chi^2_{(1)}$= 4.70, p= 0.03) and males (β= -1.24 ± 0.48, 95% CI: -2.18, -0.30; $\chi^2_{(1)}$= 6.68, p< 0.01), and PR males (β= -2.17 ± 0.62, 95% CI: -3.38, -0.95; $\chi^2_{(1)}$= 12.20, p< 0.001) reported significant slopes, whereas PR females unvaried significantly (β= -0.70 ± 0.44, 95% CI: -0.70, 0.44; $\chi^2_{(1)}$= 2.54, p= 0.11). When observed peripherical, the UMA and LMA increased by 7.74 ± 10.31% and 6.26 ± 12.79% (figure 4.12, E and F), respectively. The marginal effects reported gender differences over time are detectable on UMA: males β= 4.90 ± 1.27 (95% CI: 2.40, 7.40; $\chi^2_{(1)}$= 14.76, p< 0.001) vs females β=1.94 ± 1.13 (95% CI: -0.26, 4.15; $\chi^2_{(1)}$= 2.98, p= 0.085). However, gender and group interaction reported a significant slope in the PR females (β= 3.95 ± 1.43; $\chi^2_{(1)}$= 7.67, p= 0.006; g= 0.377, 95% CI: -0.689, 1.425). This discrepancy diverged for LMA, where PR (β= 4.28 ± 1.81, 95% CI: 0.73, 7.82; g= 0.536, 95% CI: -0.547, 1.593) and AR (β= 5.77 ± 2.22, 95% CI: 1.43, 10.11; g= 0.578, 95% CI: -0.69, 1.801) females and AR males (β= 7.54 ± 1.98, 95% CI: 3.65, 11.42; g= 0.606, 95% CI: -0.568, 1.745) increments were statistically significant, whereas PR males were not ($\chi^2_{(1)}$= 1.18, p= 0.277; g=0.162, 95% CI: -1.129, 1.434).

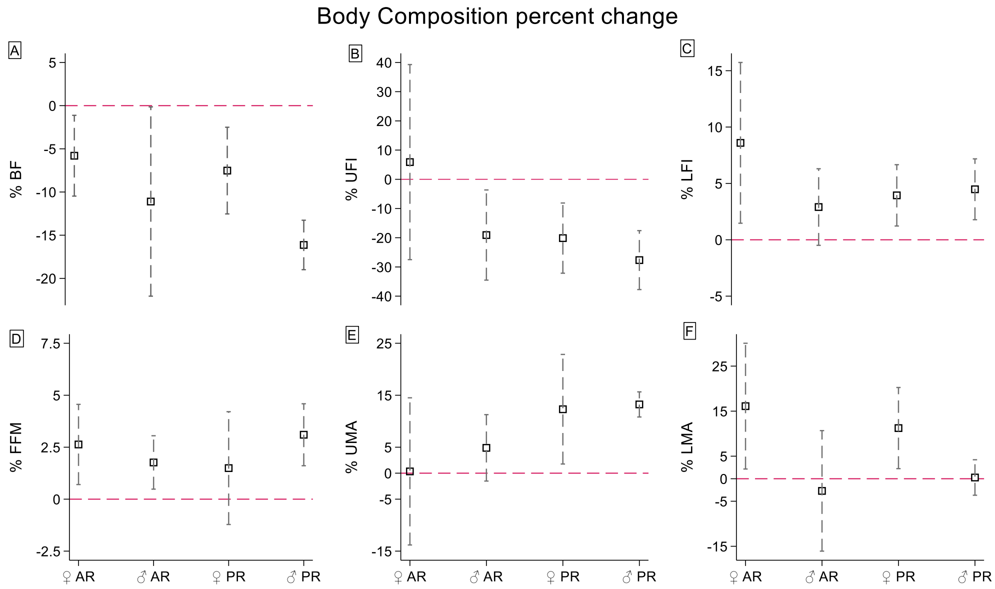

Figure 4.12. Body composition percental changes after the 8-week HIIT protocol.

Physical Performance

Table 4.7 shows the longitudinal effects of HIIT on physical performance parameters, stratified for groups and gender. Figure 4.13 shows the physical performance changes (percentage) in AR and PR females and males.

Generally, only the maximal oxygen consumption appeared to be significantly affected by the HIIT protocol (6.47 ± 4.42% from baseline, figure 4.13, E), with different slopes for group and gender interactions: PR females (β= 3.06 ± 0.74, 95% CI: 1.61, 4.51; $\chi^2_{(1)}$= 17.11, p< 0.001; g= 0.737, 95% CI: -0.372, 1.812), PR males (β= 3.68 ± 1.05, 95% CI: 1.63, 5.74; $\chi^2_{(1)}$= 12.40, p< 0.001; g= 0.618, 95% CI: -0.656, 1.847), AR females (β= 2.62 ± 0.91, 95% CI: 0.84, 4.39; $\chi^2_{(1)}$= 8.34, p= 0.004; g= 0.701, 95% CI: -0.696, 2.024) and AR males (β= 2.25 ± 0.81, 95% CI: 0.66, 3.84; $\chi^2_{(1)}$= 7.71, p= 0.006; g= 0.46, 95% CI: -0.694, 1.587).

Regarding handgrip strength and power, when looking at marginal effects, the AR protocol improved by 19.21 ± 18.64% right (β= 7.4 ± 1.81, 95% CI: 3.84, 10.96; $\chi^2_{(1)}$= 16.63, p< 0.001; g= 0.715, 95% CI: -0.476, 1.866) and by 19.04 ± 16.18% left HGS (β= 6.5 ± 1.97, 95% CI: 2.63, 10.36; $\chi^2_{(1)}$= 10.85, p= 0.001; g= 0.487, 95% CI: -0.671, 1.616) in males (figure 4.13, A and B), whereas the PR protocol increased by 16.26 ± 15.8% the CMJ in females (β= 3.87 ± 1.67, 95% CI: 0.59, 7.14; $\chi^2_{(1)}$= 5.36, p= 0.021; g= 1.024, 95% CI: -0.132, 2.136). The CMJ also positively increased in AR females (g= 0.375, 95% CI: -0.861, 1.582) by 5.06 ± 4.36% (figure 4.13, C).

In addition, the HIIT protocol enhanced agility (figure 4.13, D) in male participants of PR by 7.70 ± 3.81% (β= 0.30 ± 0.14, 95% CI: 0.03, 0.57; $\chi^2_{(1)}$= 4.80, p=0.029; g= 1.605, 95% CI: -0.114, 3.22) and AR by 6.05 ± 3.07% (β= 0.25 ± 0.11, 95% CI: 0.04, 0.43; $\chi^2_{(1)}$= 5.37, p= 0.020; g= 0.991, 95% CI: -0.251, 2.182), with an increasing rate by 72.88% compared to females ($\chi^2_{(1)}$= 8.44, p= 0.004).

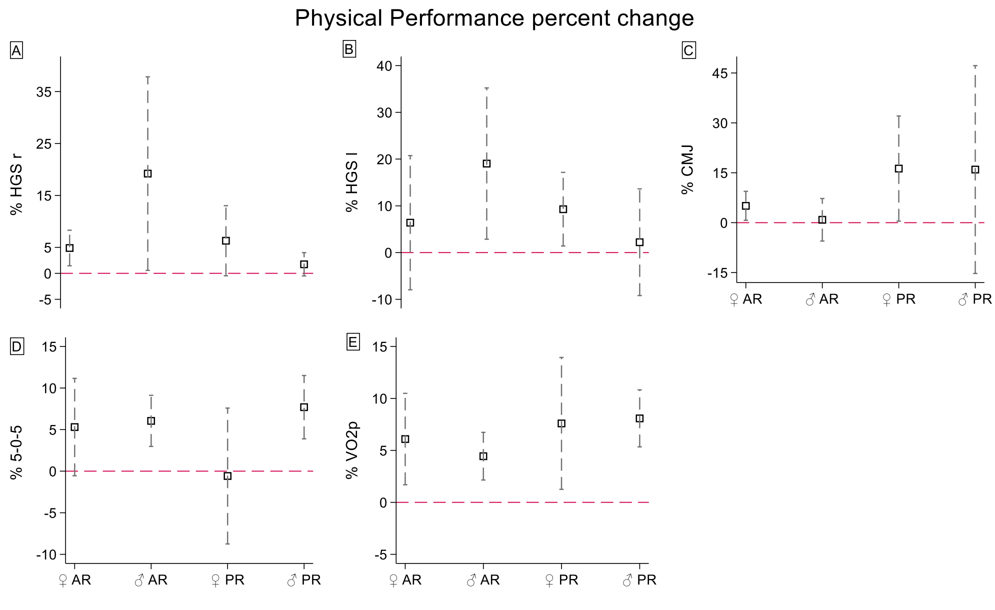

Figure 4.13. Physical performance percental changes after the 8-week HIIT protocol.

Table 4.7. Longitudinal HIIT effects on physical performance in groups

	PR (n=9)				AR (n=9)				Mixed Model effects (*Wald* χ^2 degrees of freedom)							
	Pre	Post	Pre	Post	Pre	Post	Pre	Post								
	♀ (66.67%)		♂ (33.33%)		♀ (44.44%)		♂ (55.56%)		Time		Time#Group		Time#Gender		Time#Group#Gender	
	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	Mean (±SD)	z	p	z	p	z	p	z	p
HGS r (kg)	31.00 (4.00)	32.83 (3.82)	49.17 (11.03)	50.17 (12.17)	31.63 (2.69)	33.13 (2.25)	41.20 (8.87)	48.60 (9.79)	0.25	0.804	0.03	0.977	3.11	0.002*	-0.73	0.464
HGS l (kg)	28.67 (4.93)	31.17 (4.54)	46.17 (14.89)	46.17 (11.62)	30.88 (3.42)	33.00 (6.48)	40.10 (13.25)	46.60 (10.71)	0.68	0.496	-0.09	0.926	2.20	0.027*	-0.93	0.354
CMJ (cm)	23.97 (2.48)	27.83 (4.25)	36.97 (9.90)	41.40 (8.05)	28.58 (2.99)	30.05 (3.77)	40.32 (10.05)	40.16 (8.06)	0.38	0.706	0.03	0.973	1.21	0.226	0.56	0.573
Agility (m/s)	3.89 (0.42)	3.84 (0.14)	3.98 (0.18)	4.28 (0.11)	3.81 (0.32)	4.01 (0.26)	4.08 (0.19)	4.33 (0.26)	1.42	0.154	-1.15	0.251	1.72	0.085	0.64	0.525
VO_{2peak} (ml/kg#min)	42.70 (4.42)	45.77 (3.17)	46.38 (4.43)	50.07 (3.96)	45.26 (4.37)	47.87 (2.79)	51.48 (4.51)	53.73 (4.32)	2.89	0.004*	-0.10	0.917	0.77	0.438	0.21	0.832

Note: PR, Passive Recovery; AR, Active Recovery; z, statistical test z; p, p-value; SD, Standard Deviation; HGS r, Handgrip Strenght right; HGS l, Hand Grip Strength left; CMJ; Countermovement Jump; VO2, maximal oxygen consumption; *, statistically significant.

4.3.4 Discussion

The main aim of the following investigation was to evaluate the efficacy of an 8-week HIIT program with both running and whole-body exercises in improving body composition and physical performance in trained young adults. We found that two HIIT sessions of about 36 minutes per week with short intervals (~30 s) and a work-recovery ratio of ~1 positively affected body health. The protocol was HR-based with %HR ranging from 80 to 100% on work intervals (Buchheit & Laursen, 2013b). During the follow-up, both volume (number of exercises, series, and total duration) and intensity (work-recovery ratio) were gradually increased to induce best long-term adaptations (Bacon et al., 2013; Granata et al., 2016; MacInnis & Gibala, 2017). Although the group with active recovery reached higher average HR values of 8.48 ± 2.88 bpm, sex and group characteristics did not affect the HR's variability rate over the eight weeks. This suggests that the proposed protocol maintained a comparable HR trend for both groups and active recovery helps to elicit the cardiovascular system widely. Also, the mentioned physiological pattern was more evident during the last four weeks, pointing to a human greater sensitivity in training intensity. It is in line with Plews and colleagues (2014) who demonstrated that variation in training load may influence HR responses. To evaluate both the training load and HR variation, we adjusted it by the HR mean and max ratio and detected a weekly increment of 3.65%, with a linear trend in AR and PR males and females. It accounted for steeper slopes between weeks four and five, where the protocol saw the bigger change in volume, and six and seven where participants performed greater changes in intensity. This also reflects the volume load variation, which rapidly increased between weeks three and five where 71.32% of its increment was covered. According to Granata and colleagues (Granata et al., 2016), when training is settled at high intensity a higher volume could increment the activity of citrate synthase, the protein content of electron transport system subunits, PGC-1α, NRF1, TFAM, PHF20, and p53. In addition, when the maximal amount of total volume suggested for HIIT is reached, a further increment in intensity could elicit peripheral adaptations such as a higher rate of glycogen utilization, and greater activity of AMPK, CaMKII, and ATF2 (Egan et al., 2010). All these markers of mitochondrial content and transcription factors are involved in mitochondrial biogenesis and can modify cellular energy requirements. In fact, due to mitochondrial density regulating substrate metabolism during submaximal exercise, a greater muscle enzyme and protein content could promote fat oxidation than glycogen degradation (MacInnis & Gibala, 2017). The above-mentioned physiological mechanisms accord with our results in body composition induced by the HIIT protocol progression. Although longer intervals (> 2 min) could favour triglyceride depletion due to a wider amount of time spent in oxidative metabolism, our protocol positively affected subjects' total body fat by 10.31%, showing that the increasing total volume and intensity are effective even if shorter intervals have performed. This finding is in line with Macpherson and colleagues (2011) who found that 18 SIT sessions decreased body fat by 6.4%, with a 1% increase in lean mass. However, if we focus on exercise selection, to the best of our knowledge, just two studies assessed a combination of whole-body exercise with short intervals, and the investigators did not find improvement in relative fat mass and fat-free mass (Eather et al., 2019; Evangelista et al., 2019). Although the subjects trained three times per week in both investigations, Eather and colleagues (Eather et al., 2019) subministered sessions that lasted from eight to 12 minutes, which could not be enough to elicit fat oxidation, while the HIIT protocol of Evangelista et al. (Evangelista et al., 2019) lasted six weeks and it lacked progression within weeks. The doubled weekly session duration of our protocol (~ 30 min vs ~ 70 min per week) suggested that two workouts per week are effective when the volume is appropriate.

Interestingly, the reduction of upper limb adiposity, such as its increment in muscle area, accounted for the great body composition improvement, whereas the lower limb fat increased. Female participants exhibited a general increment in the lower limb area (+13.19% muscular and +5.81% fat), while the upper limb better varied in both sexes. Differently, males worsen their lower limb mass partitioning. The observed sex differences may be justified by evidence suggesting females possess a greater predisposition for aerobic metabolism, due to a bigger (~10%) relative Type I fibre area (Hunter, 2016), with an oxidative contribution 25% higher than males during short interval exercise ($\leq$ 30 s; Hill and Smith, 1993). The sex difference occurs during HIIT recovery periods where females exhibited smaller ATP and faster restoration (Esbjirnsson et al., 2002). Also, a similar higher response in the muscular area has been detected by Esbjirnsson and colleagues (1996) who found that females greater increased in type-II fibre cross-sectional area than males, after 30 s of SIT. So, the well-stated greater muscle oxygen delivery in females may lead to higher muscle glycogen content and lipid metabolism (Hunter, 2016). The results we found agree with Hazell and colleagues (2014) who demonstrated that six weeks of sprint interval training with short work intervals (<30s) improved body mass distribution (-8% FM, + 1.3% FFM) in active women. Despite Trapp and colleagues (2008) stating that HIIT with short work intervals induced total body fat decrement, they found a wider reduction in lower than upper limbs. This discrepancy with our results can be explained by differences in training protocol since participants in the previously mentioned study performed just cycling exercises.

Concerning recovery type, PR is akin to the AR group in terms of body composition. According to previous evidence, active recovery at ~ 50% HR could not be enough to impair the energy balance and induce a wider oxygen debt (Fennell & Hopker, 2021). Compared to active rest at 80% or 110% of lactate threshold during long interval bouts, a shorter work-rest ratio at 50% HR does not appear to affect blood lactate fasting and related perceived effort. When interacting with sex, we found that passive recovery improved the female upper area better than AR. The two groups reported different slopes in the weekly volume load, covered distance and average speed, with a constant positive trend in passive recovery and some flatness in active recovery females (weeks one to three and four), which could have enhanced the mechanical cost and heat release. However, no previous study compared PR and AR between males and females to definitively state long-term adaptation; so, further investigations are needed.

As previously mentioned, modulating parameters (volume, density, intensity, etc.) involved in high-intensity interval exercise has been effective in stimulating both peripheral and central adaptation such as increased maximal blood and stroke volume, cardiac output, and other factors related to physical capacity (MacInnis & Gibala, 2017). Several studies found significant improvements in acute and long-term $\dot{V}O_{2p}$ after the HIIT protocol (Bacon et al., 2013). Our results found that eight weeks with 16 sessions of HIIT were effective in increasing $\dot{V}O_{2p}$ when progression is well monitored and the HR-based intensity ranges close to planned cut-off values. When approximatively matched for interval duration, our findings accord with Astorino et al. (Astorino et al., 2017) who evidenced how shorter bouts widely affected $\dot{V}O_{2p}$. In addition, a recent meta-analysis provided systematic evidence on how the high volume ($\geq$ 15 minutes per session) and moderate to long term (4-12 weeks of protocols) could ensure the greatest $\dot{V}O_{2p}$ improvements in healthy adults (Wen et al., 2019). However, females reported the biggest effect sizes similar for PR (+7.6%) and AR (+6.1%), whereas PR males increased by 81.90% compared to AR. Previous studies showed that active recovery in long-interval training (>2 min) favourites reaching and maintaining the VO_{2max} threshold, enhancing the metabolic responses (Spencer et al., 2006). A rationale physiological consequence of the daily metabolic peak reached could be followed by positive long-term adaptation in $\dot{V}O_{2p}$. No previous studies, nevertheless, found similar results comparing active and passive recovery in adaptive outcomes, and evidence on acute responses shows that time at $\dot{V}O_{2p} \geq 80\%$ did not differ in recovery at several intensities (Fennell & Hopker, 2021). Differently, it is well-stated that males accumulate more blood lactate after 30 seconds of repeated sprints, with a lower level of aerobic contribution, which could lead to downstream signals that regulate muscle adaptations (Gibala et al., 2014). As a direct consequence, due to ~ 25 seconds of recovery corresponding to the minimal time at which no lactic acid accumulation took place (Billat, 2001b), males could prefer passive recovery to allow partial metabolic restoration that brings to a longer time to exhaustion, higher speed and wider distance covered. Accordingly, the weekly CD and AS trends for PR males presented higher slopes that explain a constant increase in running parameters (supposed to greatly affect $\dot{V}O_{2p}$).

Although HIIT benefits on $\dot{V}O_{2p}$ have been well-stated in both athletic and healthy adults, the same could be figured out on strength, power, and agility just in competitive athletes. Stankovic and colleagues (2023) found that HIIT is a time-effective approach to moderately improve explosive strength tested by CMJ in female volleyball, soccer, and basketball adult players, whereas agility measured by the change of direction shuttle test was widely affected. We supposed combining running and whole-body exercise could enhance strength and power adaptations (Scoubeau et al., 2022). In addition, about 30 seconds of work trying to perform as many repetitions as possible could initially promote fast fibre-type recruitment increasing intra-muscular coordination, and then major eliciting slow fibre type increasing inter-muscular coordination (Lievens et al., 2020), followed by improvements in strength, power and agility. However, previous evidence suggested that "all out" bouts with long recovery is the best solution to reach the peak of power or speed because the fully restoring substrate reserves allow performing the maximal neuro-muscular effort, while long intervals are favourited to reach maximal oxygen consumption and lactate tolerance (Billat, 2001b). The last two statements rationally lead to planning exercise protocols with short work intervals and long recovery, but less contribution has been given to maximal oxygen consumption. In light of this, we implemented a protocol with whole-body and running exercises and short intervals to promote a full range of physical improvements. In terms of strength, power and agility, we found that the proposed protocol with combined exercise affected the sexes and groups differently. The passive rest protocol greatly affected power adaptive response in females (+16.26%) and males (+15.97%), and strength responses in females (+ 7.78%), than the AR counterpart. Also, despite AR males showing a good increment in speed (+ 6.05%), PR males reported a 1.27 times higher change. According to previous results, we found that the wider metabolic restoration elicits power, strength and agility improvements. Interestingly, the active recovery enhanced changes in HGS males (9.67 times higher than PR males) and 5-0-5 females (9.15 times higher than PR females). To the best of our knowledge, this study is the first study that investigated how physiological adaptations differ between active and passive recovery males and females in longitudinal high-intensity training that combines running and whole-body exercises, and it makes it difficult to report a direct comparison. Several studies discussed the benefits of HIIT protocols on strength, power and agility, but the effects of recovery type on males and females need more attention.

This study reported some limitations: a) the sample size could have negatively affected the type I error probability, reducing the statistical effects; b) the four participants excluded from the analysis impartially divided sexes into groups (PR reports a higher ratio of females); c) no gold standard instruments have been used to measure BF and $\dot{V}O_{2p}$, but have been estimated from regression models that imply a wider measurement error; d) the fatigue has been measured only by RPE without accounting for blood lactate concentration.

4.3.5 Conclusion

This study demonstrated that 8 weeks of well-monitored HIIT with two sessions per week and combined exercise decrease body fat increasing fat-free in young trained adults. Performing a combination of whole-body and running exercises included in a short interval protocol with the work-recovery ratio near the unit is effective for conditioning both males and females, especially in terms of maximal oxygen consumption. The PR, nevertheless, is suggested for improving lower limb power and female strength, while AR is more appropriate for agility and male strength. The use of different recovery types may be a practical solution in sports where the main goal is training closer to the maximal oxygen consumption threshold, but other parameters need to be elicited. Strength and conditioning trainers should be aware of the dynamic nature of HIIT, which allows them to select appropriate exercises and modulate several variables for reproducing the metabolic requirements of a specific sport, in both male and female competitions. The possibility of inducing physical adaptations with less than two hours per week of HIIT makes its utilisation optimal for each training periodisation phase.

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