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UNIVERSIDADE D
COIMBRA

Arturo Osorio Gutiérrez

THE FEMALE SOCCER PLAYER

Thesis for the degree of Doctor of Sport Sciences in the branch of Sports Training supervised by Prof. Dr. Manuel J. Coelho-e-Silva, Prof. Dr. João Alberto Valente Dos Santos and Prof. Dr. Joaquim Moreira Castanheira, submitted to the Faculty of Sport Sciences and Physical Education of the University of Coimbra.

July of 2023

Faculty of Sport Sciences and Physical Education

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Abstract

This thesis consists of three parts. Part I functions as an introduction where fundamental concepts are presented. This section covers the basic terms of growth and maturation, along with the methods used to assess biological maturation. It also delves into the techniques employed to appropriately adjust morphological variables influenced by body size, such as Left Ventricular Mass. Furthermore, it explores the potential of organized sports, particularly soccer, in providing health benefits to young females. In addition, this introductory section encompasses a description of the samples included in the studies and provides an overview of the methods, materials, and instruments employed throughout the three studies that comprise the thesis. Part II consists of the three studies that form an integral part of the thesis. The objectives were: (i) to examine growth and maturation among female soccer players and to test the hypothesis of maturity-associated selection in adolescent female soccer; (ii) to analyze the influence of skeletal age, chronological age, fat free mass, fat mass, stature and sitting stature to inter-individual variability in Left Ventricular Mass in young female soccer players, using allometric modeling; and (iii) to compare the amount of bone mass in adolescent female athletes involved in sports with different weight-bearing impact. In general, the studies highlighted that: (i) Soccer tends to retain female adolescents who are classified as early maturing or already mature (63.6% among 12-13 years; 62.7% among 14-15 years) with a minority of players classified as delayed (5.9% and 13-1% respectively among initiates and juveniles); (ii) Body mass and FFM, are fundamental contributors to explain the inter-individual variability on Left Ventricular Mass in adolescent female soccer players, according to proportional allometric modeling; (iii) Female adolescent soccer players present higher values of bone mineral density (BMD) and bone mineral content (BMC) in the pelvis and lower limbs compared to their swimmers counterparts. Part III encompasses the general discussion, which presents a concise summary of the key findings and practical implications derived from the three studies included in this thesis. Overall, this research underscores the need for equitable practices in women's soccer, reevaluation of categorization regulations, individualized analysis for addressing body composition and cardiac structure variables, and recognition of the positive effects of soccer practice on bone health. These findings provide valuable insights for coaches, trainers, and policymakers in the development of strategies to support and enhance the participation and performance of female soccer players.

Keywords: Growth status, Left ventricular mass, Bone health

Resumo

Esta tese consiste em três partes. A Parte I funciona como uma introdução onde são apresentados conceitos fundamentais. Esta seção abrange os termos básicos de crescimento e maturação, juntamente com os métodos utilizados para avaliar a maturação biológica. Também explora as técnicas empregadas para ajustar adequadamente as variáveis morfológicas influenciadas pelo tamanho corporal, como a Massa Ventricular Esquerda. Além disso, explora o potencial dos esportes organizados, especialmente o futebol, em fornecer benefícios à saúde para jovens mulheres. Ademais, esta seção introdutória abrange uma descrição das amostras incluídas nos estudos e fornece uma visão geral dos métodos, materiais e instrumentos utilizados ao longo dos três estudos que compõem a tese. A Parte II consiste nos três estudos que formam uma parte integral da tese. Os objetivos foram: (i) examinar o crescimento e a maturação entre jogadoras de futebol feminino e testar a hipótese de seleção associada à maturidade em adolescentes do sexo feminino no futebol; (ii) analisar a influência da idade esquelética, idade cronológica, massa livre de gordura, massa gorda, estatura e estatura sentado na variabilidade interindividual da Massa Ventricular Esquerda em jovens jogadoras de futebol feminino, utilizando modelagem alométrica; e (iii) comparar a quantidade de massa óssea em atletas adolescentes do sexo feminino envolvidas em esportes com diferentes impactos de suporte de peso. Em geral, os estudos destacaram que: (i) o futebol tende a reter adolescentes do sexo feminino que são classificadas como precocemente maduras ou já maduras (63,6% entre 12-13 anos; 62,7% entre 14-15 anos), com uma minoria de jogadoras classificadas como atrasadas (5,9% e 13,1% respectivamente entre iniciantes e juvenis); (ii) a massa corporal e a FFM são contribuidoras fundamentais para explicar a variabilidade interindividual da Massa Ventricular Esquerda em jogadoras adolescentes de futebol feminino, de acordo com a modelagem alométrica proporcional; (iii) jogadoras adolescentes de futebol feminino apresentam valores mais altos de densidade mineral óssea (DMO) e conteúdo mineral ósseo (CMO) na pelve e nos membros inferiores em comparação com suas colegas nadadoras. A Parte III abrange a discussão geral, que apresenta um resumo conciso dos principais resultados e implicações práticas derivadas dos três estudos incluídos nesta tese. No geral, esta pesquisa enfatiza a necessidade de práticas equitativas no futebol feminino, reavaliação das regulamentações de categorização, análise individualizada para abordar variáveis de composição corporal e estrutura cardíaca, e reconhecimento dos efeitos positivos da prática do futebol na saúde óssea. Essas descobertas fornecem informações valiosas para treinadores, preparadores físicos e formuladores de políticas no desenvolvimento de estratégias para apoiar e melhorar a participação e o desempenho das jogadoras de futebol feminino.

Palavra Passe: Estado de crescimento, Massa ventricular esquerda, Saúde óssea

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List of Abbreviations

ACSM	American College of Sports Medicine
APHV	Age at peak height velocity
BM	Body mass
BMAS	Pediatric Bone Mineral Accrual Study
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BQ1	First birth quarter (January – March)
BQ2	Second birth quarter (April – June)
BQ3	Third birth quarter (July – September)
BQ4	Fourth birth quarter (October – December)
BSA	Body surface area
CA	Chronological age
CI	Confidence intervals
CL	Confidence limits
CV	Coefficients of variation
DXA	Dual energy X-ray absorptiometry
ES-r	Effect size correlation
Fat Free Mass Index	FFM/height^2
FFM	Fat-free mass
FIFA	Fédération Internationale de Football Association
FM	Fat mass
GH	Growth Hormone
GK	Goalkeepers
HRmax	Maximum heart rate
IGF-1	Insulin Growth Factor
k	Allometric coefficients
LBM	Lean body mass
LDL	Low-density lipoprotein
LLLM	Lean lower limbs mass

LLTS	Leuven Longitudinal Twin Study
LST	Lean soft tissue
LST	Lean soft tissue
LVEDD	Left ventricular end diastolic diameter
LVIDd,	Left ventricular internal dimension at end-diastole
LVM	Left ventricular mass
LVM/body surface area	Left ventricular mass index
MESA	Multi-Ethnic Study of Atherosclerosis
MO	Maturity offset
P	Significance level
PAS	Percentage of Adult Stature
PHV	Peak height velocity
PMS	Predicted mature stature
PPAS	Predicted percentage of adult height
PWTd	Posterior wall thickness at end-diastole
PWTd	Posterior wall thickness at end-diastole
R	Model correlation coefficients
r	Pearson’s product moment correlation coefficient
R2	Adjusted coefficients of determination
RAE	Relative age effect
RUS	Radius-Ulna-Short Bone
SA	Skeletal age
SA-CA	Subtracting to the value of SA the value of CA
SEE	Standard error of estimate
SEM	Standard error of measurement
SGDS	Saskatchewan Growth and Development Study
SWTd	Septal wall thickness at end-diastole
TO	Take off of the pubertal spurt
TW3	Second revision of the Tanner-Whitehouse method
VO2 max	Maximal oxygen uptake
WHO	World Health Organization

Part I

INTRODUCTION AND METHODS

Chapter I

1. General introduction

1.1. Basic concepts

The biological changes from birth until adulthood are complex and interact with factors such as nutrition and physical activity. It is not possible to properly describe and explain the effects of physical activity in the organism of children and adolescents without considering growth, maturation and development.

Development and growth

The process of formation and enlargement of tissues, organs and systems take place during various stages in life and it is accomplished by complex biological mechanisms. During the prenatal period, cells proliferate dramatically to settle the bases for tissue formation, starting in the germinal phase and advancing during the embryonic phase when organs and systems commence their development by differentiation of pluripotent stem cells. The process of cellular differentiation and specialization continues in the fetal stage toward the attainment of functionality of body systems. During the embryonic and mainly fetal stages, the already differentiated cells divide (hyperplasya) and increase their size (hypertrophy) resulting in growing of tissues and organs. Thus, development implicates cell differentiation; in other words, development refers to the cellular processes that the embryo and fetus experience in order to gain cell type identity, whereas growth implies the enlargement of formed structures conducted by hyperplasia, hypertrophy and accretion. The biological definition of development points out the early biological events occurring during the embryonic, fetal and a short postnatally period, whereas growth continues to be an important process in postnatal life for about two decades (Falkner Frank & Tanner J.M., 1986).

Growth during postnatal life continues its way fundamentally through hypertrophy and accretion, with the enlargement of tissues and organs, until adulthood is attained. In simple terms, growth implies the increment of the total body size concurrently with its components and segments. As time advances, the most evident sign of growing is the increase in the child's stature and weight. According to Bogin (1988), growth is “a quantitative increase in size…” (Cameron, N.D., 2012).

Some anthropometric variables are widely used to assess the growth and maturation of an individual or a group of individuals. The most common body dimensions used to determine growth status are height, body mass, and sitting height. Other measurements are occasionally considered, such as upper and lower limb lengths, or breaths of bony structures (eg, biacromial and biliocrystal) (Cameron et al., 1982). Height and body mass are variables very appreciated in the clinic context due to their feasibility; they are easy to take, can be measured in a very short period of time, do not require complex technical skills, and, controlling for diurnal variations and using suitable instruments, they offer an appropriate degree of accuracy and reliability. Body mass, however, does not discriminate for the type of tissue (e.g. muscle mass, fat mass, bone mass), which consequently present limitations, especially when we try to describe the growth status in athlete populations whose body composition is constantly altered by sport training.

One way to analyze growth and maturation based on body dimensions is by using Reference Data. According to the World Health Organization, Reference Data is a tool used to group data for the purpose of having a common basis for comparing populations. Reference Data are usually presented as curves representing different percentiles that establish a range of normal variability between children of the same chronological age (Malina R.M. et al., 2004). The data used to establish such percentiles are derived from a representative sample of clinically normal children. There are Reference Data for various anthropometric dimensions, these references are known as growth charts (https://www.cdc.gov/growthcharts/clinical_charts.htm) or growth standards (https://www.who.int/publications/i/item/9789241547635). Distance Curves are often used to assess growth status. On the other hand, the Growth Velocity Curve indicates the growth rate (cm/year) and can be used to analyze the tempo of growth (Cameron N.D., 2012; Hauspie et al., 2004). They are useful to evaluate trajectories and patterns of growth, and are plotted by locating the value of the anthropometric dimension (height or weight, usually) on the Y axis and the value of the chronological age on the X axis. A large amount of cross-sectional data from representative samples for each of the different chronological age groups, spanning from infancy to adulthood is required. In order to generate the 5th and 95th percentiles, a sample size of over 500 individuals is necessary for each age group (Malina R.M. et al., 2004). Centiles are calculated within each age group and mathematically smoothed across ages (Hauspie et al., 2004). The Growth Distance Curves allow describing the basic pattern of the anthropometric dimension that has been reached at a certain age, and the cumulative sum of previous growth. In this way, growth distance curves can be used to analyze the growth status of an individual or a sample of children (Malina R.M. et al., 2004).

In order to structure the Growth Velocity Curves, longitudinal data are necessary to clearly observe the different phases of growth and the relationship between them, in addition to being able to identify the moment in which the adolescent spurt begins (the acceleration of growth during adolescence) and when it occurs the maximum rate of growth during the spurt. Growth velocity decreases rapidly and steadily after birth to two years of age (individual grows all the time but at a slower rate as time goes on); then, from the third year of age until just before puberty, there is a period of a more or less constant growth rate that is interrupted at some point by a small spurt called "mid-growth spurt" (the mid-growth spurt is not seen in all individuals and it is more common in boys than girls); after a “latency” phase, the pubertal spurt starts and the growth rate presents a constant increase until it reaches its maximum at a moment known as age at peak height velocity (APHV). Finally, after PHV, the rate of growth decreases rapidly until adult height is reached and growth stops (Hauspie et al., 2004; Sherar et al., 2005).

The growth velocity curve makes it possible to identify various parameters or milestones that can be used as maturity indicators, such as, the age at which the maximum growth velocity occurs during the mid-growth spurt; the age at which the minimum growth velocity occurs at the onset of the pubertal spurt (Take off (TO)); the age at which the maximum height velocity occurs during the pubertal spurt (APHV); and the age of termination of growth or age of adult attainment stature (Hauspie et al., 2004; Malina R.M. et al., 2004).

Biological maturation

While growth refers to change in size, maturation can be defined as the process in which organs and systems progress toward maturity, when they attain their functional plenitude. As it can be noticed from the last sentence, maturation and maturity are not equivalent terms; maturation is a process and maturity is a state. Maturity of organs and systems of the human body differs in time and is sex dependent. For example, skeletal maturity (which is reached when the cartilage of the growth plates in the metaphysis of long bones completely ossifies) is completed later than sexual maturity (which is reached when the individual is capable of reproducing). Also, it is important to note that there is considerable interindividual variation in maturity; It is not uncommon to identify individuals who reach skeletal maturity at the chronological age of 14 years, while in others it may take longer than 20 years. (Malina R.M. et al., 2004).

The process of maturation does not follow a rigorous chronological course, rather it is conducted in a fluctuating rhythm that is controlled by genetic and environmental factors (Czerwinski et al., 2007). To better understand how maturation works, its components (timing and tempo) must be described. Timing refers to the occurrence of unique and specific events (as menarche in girls, voice change in boys, or the appearance of pubic hair in both), and tempo indicates the gradual changes occurring during a determined period (as development of breast in girls, and genitals in boys, or skeletal development in both). Each person presents a particular trend in timing and tempo; differences between individuals are expected and sometimes are quite extensive. For instance, while the average age for menarche is 12.4 years, it can occur as early as 10 years or as late as 16 years in different girls. The rate of change also varies considerably between individuals; in some boys, for example, the pass-through puberty may take only 2 years and start in a relative advanced chronological age, e.g. 16 years, meanwhile in other boys puberty may start at 13 years and last longer (Malina R.M. et al., 2004).

1.2. Assessment of biological maturation

Owing to the considerable inter-individual variation of maturation and growth in children and adolescents its assessment becomes indispensable when we try to explain the effects of physical exercise; an accurate measurement and interpretation will allow us to distinguish the morphological and physiological adaptations derived from physical training from that changes related to the natural process of growth and maturation.

Essential considerations for the assessment

The assessment of biological maturation, in some manner, is about to identify and describe timing and tempo (Hauspie et al., 2004; Malina R.M. et al., 2004). For this purpose the chronological age (CA) is commonly used as a referent point in association with different maturity indicators. An indicator of maturity can consider specific features of particular body structures or may include perceptible biological events. There are some fundamental requirements that maturity indicators must accomplish in order to be valid; the indicators must be universal (they must appear in all individuals); they must be independent of chronological age; and they must reflect the continuity property of maturation and exhibit the end of the process (Cameron N.D., 2012).

Each maturational process (e.g., skeletal maturation, sexual maturation) is independent within the individual. Although there may exist common mechanisms promoting the development of different tissues and systems each one has its own response and advances following its singular condition. Although some indicators of different maturational processes may coincide, it is not possible to establish with an appropriate degree of confidence that their relationship is consistent (Hauspie et al., 2004). Disparity in the progress of structures is natural within a particular maturational process. For example, the maturation of ulna and radius, carpals, metacarpals and phalanges does not constantly follow the same level of maturation (Malina R.M. et al., 2004).

Differences in timing and tempo of biological maturation between boys and girls are natural. In general, girls tend to attain maturity before males, for example, girls begin puberty at ages 10–11 and complete puberty at ages 15–17, and boys start puberty at ages 11–12 and complete it at ages 16–17 (Calfee et al., 2010; Georgopoulos et al., 2004).

Skeletal maturity

Skeletal maturity can be measured through radiographs of specific anatomic regions. The most accepted methods use the bones of the hand-wrist, although techniques have also been proposed for knee, foot and ankle. The choice of a specific region supposes particular advantages. For example, for the hand-wrist are included a variety of bones (ulna, radius, carpals, metacarpals and phalanges) that can better represent the maturation of the entire skeleton since the different bones that compose this structure progress at different rates (Cameron N.D., 2012); on the other hand, the rationale for the assessment of skeletal maturity using the knee bones relies in the assumption that the maturation of this region relates with growth in height, offering a more appropriate estimation of skeletal maturity in children with growth disorders (Hauspie et al., 2004).

The assessment of skeletal maturity is not exempt from disadvantages. For example, the reading of the radiographs requires special training from the evaluator and it is time consuming; a radiograph is relative expensive if we compare it with the requirements from other methods, besides, the settings were radiographs are taken imply the dislocation of the subjects from their habitual venue. Owing to the exposure to radiation, this method could be considered invasive, however, the dosage for a radiograph of the hand and wrist (0.01 milliSieverts(mSv)) implies a minimal risk; The radiation at which the child is exposed during the radiograph is equivalent to the amount of natural radiation that she/he receives in 1.7 days (Hauspie et al., 2004).

Skeletal age (SA) is the central value used to assess skeletal maturity. According to Malina (2004), SA "corresponds to the level of skeletal maturity attained by a child relative to the reference sample for the method employed" (Malina R.M. et al., 2004). There are three methods that have been commonly used in different contexts to obtain SA. Each method includes different reference samples, uses distinct criteria for the analysis of the indicators, and their score system is not related to each other, so SA is not equivalent among them.

Greulinch and Pyle (1959) developed the first method that acquired an extensive use, especially in the clinical setting. This method uses an atlas made up of a series of standard radiographs. The skeletal age is obtained by comparing the radiograph of the child to be evaluated with the radiographs that integrate the atlas. The skeletal age assigned to the child corresponds to the chronological age of the individual from the standard radiograph that better matches. The main weakness of this method is that it does not consider the uneven development of the hand and wrist bones; maturation of each bone may progress differently between subjects, so comparing the structure as a whole could not offer an actual appreciation of maturity. Another remarkable deficiency is that this method implies the use of chronological age to measure maturity which is not desirable since, as it was exposed before (section 1.2.1), CA and maturation are not necessarily related (Reynolds, 1950).

In order to solve the problems of the atlas technique, J.M. Tanner and R.H. Withehouse (1962) proposed a method based on the assignment of individual scores for 20 bones of hand and wrist (TW1 method). The rate for each bone is based on the appearance of eighth maturity indicators (for radius are nine indicators) that reflect their particular course from an immature state to maturity. The TW method, unlike the Greulich-Pyle, considers a written description rather than visual illustrations, and does not rely on CA but considers the progress of each single bone to obtain the skeletal age.

Twenty years after the publication of the TW1 method the authors revised and modified it to give place to the TW2. The indicators remained the same. The main actualization consisted in splitting the 20 bone score into radius, ulna and short bones (RUS), and carpals (CARPAL), so, in addition to 20-bone, it was possible to assess the skeletal maturation by RUS and CARPAL scores separately. The elaboration of this alternative was motivated by some concerns regarding the carpal bones. The assessment of carpal indicators is less reliable than the other bones; carpals do not relate to growth in height; and they generally complete the maturation process before the other bones of the hand and wrist (Bull et al., 1999).

In 2001, the authors (Tanner et al., 2001) made the last modification of the method (TW3). The descriptions of the indicators and the instructions for rating remained the same. The assessment of skeletal age by the 20-bone (that mixing CARPAL and RUS) was canceled. Taking in account the secular changes in population, authors updated the sample and included populations from europe and north america .

In 1988 Alex F. Roche, Cameron Chumlea and David Thissen produced the Fels hand-wrist method (Chumlea et al., 1989). This method was elaborated using 13,823 serial radiographs from the FELS Longitudinal Growth Study (Chumlea et al., 1989). The authors analyzed 130 indicators from which 98 were selected to construct the method (the indicators were selected on the basis of their ability to discriminate between children at the same chronological age, its universal appearance, reliability, validity, and completeness). Each indicator is composed of specific grades which describe the shape and ossification of the correspondent bone. For radio, ulna, metacarpals and phalanges, are also assigned indicators that consist in metric ratios of their epiphysis and metaphysis widths. Once the indicators are grated, the information is processed in a computer program (FELShw) that provides an estimated skeletal age and an estimated standard error. The Fels method is the only one that provides a standard error.

Once calculated, the SA can be expressed using the chronological age (CA) as reference in order to establish maturity status. Subtracting to the value of SA the value of CA (SA-CA) it is possible to have an easy idea about the status of the skeletal maturity of the child. For example, if a boy gets an SA of 13 years and he is 12 years old, it could be say that his skeletal maturity is advanced for one year relative to his CA, or on the other hand, if another boy gets an SA of 14 years and he is 15 years old, the results is negative (-1), and it could be say that he is delayed by one year in his skeletal maturity relative to his CA. SA can also be divided by CA, to establish maturity status; the advantage of this approach is that the omission of negative values facilitates statistical analysis. It is important to note that the terms "delayed", "on time" and "advanced" are quite arbitrary and do not offer direct information about the fundamental factors that control skeletal maturity (Malina R.M. et al., 2004).

Sexual maturation

An individual is sexually mature when she/he attains the capability to reproduce. Disregarding the psychological aspect, the sexual maturity in females implies the capacity to produce ovum that can be fertilized, sustain a healthy pregnancy, give birth, and breast-feed the baby. On the other hand, sexual maturity for males implies the capability to produce sperm that can fertilize an ovum and rise the offspring (Malina R.M. et al., 2004). The assessment of sexual maturity relies on indicators of secondary sex characteristics. The process of sexual maturation is quite indistinguishable during childhood; the indicators can be perceived only until puberty, which is a period of dramatic physical change that include the growth spurt and the appearance of secondary sex characteristics.

In 1962 Tanner, based on the work of Reynolds and Wines (1948), and Nicholson and Hanley (1959), developed a scale for the assessment of secondary sex characteristics (Hauspie et al., 2004). The method uses different maturity indicators to establish discrete stages. Tanner established 5 stages for breast in girls and 5 for genitalia in boys, 5 stages for pubic hair in both girls and boys, and 3 stages for axillary hair in both. The stages between the indicators of each secondary sex characteristic are not equivalent and it is recommended that they be identified and analyzed separately. For example, in a girl, stage 4 for breast (B4) does not necessarily correspond to stage 4 for pubic hair (PH4). The development of secondary sex characteristics is uneven; for example, the advancement through the stages of pubic hair may not correspond to the progress through the stages of breast. Further, the tempo in the stages of one secondary sex characteristic can differ drastically in the same individual; an individual does not spend the same time in stage 1, 2, 3 and so on, but can pass rapid through one stage and stay a longer period in other (Hauspie et al., 2004).

One disadvantage of the method is related to the discrete characteristic of the stages. It is not possible to know the precise moment when individuals enter a stage or when they leave it. Thus, a subject that just enters, for example, to stage 2 of pubic hair will be equally classified to an individual who is about to leave the same stage 2 of pubic hair (PH2, for both). Another inconvenient of the method relates to the invasion of privacy of the participants; the evaluator, who must be a trained clinician, must directly inspect the secondary sexual characteristics of the participant. In order to solve this problem, in research settings, it has been used a self-assessment in which the individual enters alone to a cubicle, inspects himself/herself using a mirror on the wall and compare her/his secondary sexual characteristics with a serie of photos or drawings hanging on the wall that describe each stage of each characteristic. Finally, other disadvantage of the method is that, since secondary sexual characteristics manifest only during puberty the assessment is limited to this phase, so if we want to evaluate biological maturation in prepubertal phases it is necessary to consider other methods (Hauspie et al., 2004).

Menarche

Age at menarche (age at the first menstrual period) is an indicator of gonadal maturation that is commonly used to analyze biological maturation in female adolescents. In the research context, menarche can be studied by three methods (prospective, status quo, and retrospective) (Claessens et al., 2003; Hauspie et al., 2004).

In the prospective approach the premenarchal girls (individuals in whom menarche has not occurred) are monitored individually asking whether or not she has started her periods. This method is the most accurate if during the years of the study the girl is interviewed regularly; the shorter the interval between interviews the better the accuracy. Interviews every three months is a common interval (Hauspie et al., 2004).

The status quo (the current situation) is used to determine the mean or median age at menarche of a specific population. In order to obtain useful data the cross sectional sample must include girls from 9 to 17 years of age. The researcher registers the CA of the individual and asks her if the menarche occurred. Data can be analyzed through probit or logit models (N. D. Cameron, 2012).

The retrospective method is probably the most commonly used owing to its simplicity. In this technique the girl responds to the question When did you have your first period? Evidently the accuracy in this approach depends on the recall of the individuals. A negative association has been observed between age at the moment of the evaluation and the age at which menarche is indicated (older subjects report lesser age at menarche) (Cameron N.D., 2012)

Every method presents its own weaknesses. The prospective requires repeated interviews, and there is a risk to exclude early or late maturing girls depending on the age at the study start or finish; the status quo needs large samples in order to create different age-groups. Besides, girls who have not attained menarche are excluded from the analysis (some may not attain menarche until 15 or 16 years). The retrospective method depends on the recall of the participants (as longer the recall the accuracy diminishes), individuals usually do not report specific dates but the whole year, and the method can be limited to individuals in a period of age before early adulthood (Malina R.M. et al., 2004).

Age at PHV and predicted maturity offset

The maximum rate of growth in stature during the adolescent spurt is known as the peak height velocity (PHV), while, on the other hand, the age at which the PHV is reached is called the age at peak height velocity (APHV). The PHV indicates the intensity of the spurt (tempo), while the APHV provides us a measure of the timing of the spurt and is an individual characteristic that is commonly used as an indicator of somatic maturity (Malina R.M. et al., 2004).

APHV presents on average when individuals (men and women) have reached 90% of their adult height. It is important to note that the interindividual variability of APHV is very high; in girls the APHV can cover a range from 9.0 to 15.0 years of age, while in boys the range is from 11.1 to 17.3 years of age. The APHV can also serve as a reference for other body dimensions and physical performances, for example, menarche occurs after PHV in girls, and peak strength development occurs after PHV in boys and girls (Durda-Masny et al., 2019; Philippaerts et al., 2006).

The APHV has been used to classify individuals as early, average, or late. Various longitudinal studies have reported means and standard deviations of APHV; for girls, the "median" APHV of these studies is 11.94 years ± 1.0 years. Taking these data into account, it has been proposed that the maturity status of girls who present a predicted APHV within a range of 10.94 and 12.94 years be classified as average; if the observed APHV is <10.94, the girl is classified as early maturing, while if the observed APHV is >12.94, she is classified as late maturing (Malina & Chrzanowska, 2020).

In order to measure the APHV of an individual, it is necessary to collect annual serial height measurements during a range that cover from 9 years of age to 17 years of age. This limits its use and application, fortunately; several methodological proposals have been established to predict APHV (Malina & Chrzanowska, 2020).

Maturity offset (MO) is the time before or after PHV, and it can be estimated using the chronological age of the subject and some anthropometric variables that can be easily measured (height, weight, sitting height, and estimated leg length). Predicted age at PHV is estimated as CA minus maturity offset (Malina R.M. et al., 2004). Mirwald et al. (2002) proposed a method to predict the maturity offset that has been widely used in the sports context and in scientific research (Mirwald et al., 2002a; Moore et al., 2015). The method requires only a series of simple one-time anthropometric measurements. The equations are sex-specific regression equations that incorporate three anthropometric variables (height, sitting height and leg length), chronological age (CA), and the interaction between these anthropometric dimensions and CA. Considering the fact that the timing of growth of height, sitting height and leg length is different, the authors assumed that the change in the relationship between these variables in terms of growth provides an indication of maturational status. The authors derived gender-specific equations to predict maturity offset from data from the Pediatric Bone Mineral Accrual Study (BMAS) and verified the results using samples from the Saskatchewan Growth and Development Study (SGDS) and the Leuven Longitudinal Twin Study (LLTS) (Mirwald et al., 2002b).

The authors recommend using the maturity offset as a categorical variable rather than a continuous variable (individuals can be classified as pre-PHV when the maturity offset prediction values are negative, and as post-PHV when the result is positive). Additionally, it is recommended that the method be used in populations with an age range of 8 to 16 years in women and 9 to 18 years of age in men (Mirwald et al., 2002b). Meantime, Malina et, al. (2006, 2014) indicated that Mirwald's method has some potential limitations, especially for the application in young athletes (Malina et al., 2006; Malina & Koziel, 2014a, 2014b). For example, in a longitudinal study (7years) that followed regional and national level gymnasts aged 6 to 17 years a systematic bias was found; predictions of APHV were underestimated in those gymnasts with an observed APHV greater than their predicted APHV (Malina et al., 2006). In two other longitudinal studies, it was found that in boys, the predictive equations showed greater variability in individuals classified as early and late maturing; the mean differences between the predicted and observed APHV values were negative in late maturing boys (prediction was earlier), and positive in early maturing boys (prediction was later) (Malina & Koziel, 2014a). On the other hand, in girls, the predicted MO was dependent on age at the time of prediction; this means that the prediction equation performs better when it is used before the expected APHV (Malina & Koziel, 2014b).

The predicted ages in the PHV appear to be reasonably accurate on the average of mature individuals who, at the time of measurement, are approximately ±1 year from the observed PHV. The limitations raised acquire relevance due to the practical use that is being given in physical exercise and sports (Lloyd & Oliver, 2012; Rogol et al., 2018).

Adult stature and percentage of predicted adult height

The age at attained adult stature refers to the point at which growth in height stops completely and indicates the cessation of growth. Girls reach their adult height at 15.5 years on average;

however, the variability is very high (10th=14.3 years, 90th=17 years percentiles) (Malina R.M. et al., 2004). The age at attained adult stature has been used as an indicator of the timing of the pubertal growth spurt because it correlates directly with the age at the onset of growth spurt and with the age at peak growth; girls in whom the onset of growth spurt occurs earlier usually also have age at peak growth and age at attained adult stature at a younger age.

Percentage of Adult Stature (PAS) is a somatic indicator that, based on the age attained adult stature, allows us to study the tempo of growth (Armstrong & van Mechelen, 2017); if at a specific chronological age we know the proportion in height that has been reached in relation to the final adult height (PAS), it is possible to have an idea of the growth status of the individual. For example, if we have two adolescent girls of the same chronological age (e.g. 12 years) and one of them, at the time of the measurement, has reached 95% of adult height, and the other one only reached the 90%, the former is more advanced in her growth status as she is closer to completing her growth.

Longitudinal data are required to obtain age at attained adult stature and PAS, which limits their use in exercise scientific research and their practical application in the sport field. To solve this problem, various authors proposed methods to predict adult stature. Some of them use the Skeletal Age (SA) (Bayley and Pinneau 1952; Roche et al. 1975; Tanner et al. 1983, 2001), and although they deal with the problem of obtaining longitudinal data, the SA sometimes is difficult to obtain.

There are methods to predict the percentage of adult height (PPAS) that does not consider the SA, but they add the mid-parent stature (the average height of the parents) as a variable; these methods are based on the premise that the mid-parent stature provides a target range within which the child's adult height is likely to be (Tanner et al., 1970). The first of these methods was developed from the Fels Longitudinal Study; it provides an estimate of PPAS from the chronological age (CA), height, body weight, and mid-parent height. Afterwards, Kamis & Roche (1994) proposed a method that offers specific equations for different age ranges (Khamis & Roche, 1994); the method has been widely used in various exercise science studies (Armstrong & van Mechelen, 2017), however it has limitations in the classification of maturity status; for example, in samples of young athletes it has been observed that the method presents a moderate concordance when it is compared with methods that consider SA. Finally, Benuen et al., (1997), based on data derived from the Leuven Longitudinal Study, developed a method that estimates the PPAS of boys who are in an age range of 12.5 to 16.5 years. The method includes CA, height, sitting height, and the subscapular and triceps skinfolds. It was validated and its standard error (se= 3.0-4.2 cm) is lower than that of the other methods (Armstrong & van Mechelen, 2017). Its disadvantage is that it incorporates skinfold variables that are not always accessible to measure (technical knowledge of anthropometry is required to take valid measurements) (Benuen et al., 1997).

The PPAS acquires great relevance in the sports context because, among other practical applications, it may be useful in distinguishing youngsters who are tall at a given age because they are genetically tall from those who are tall because they are advanced in maturity, however, the utility of percentage of adult height as a maturity indicator needs further validation (Armstrong & van Mechelen, 2017).

1.3. Soccer as a health-promoting activity among adolescent Females

Organized sport as a positive impact factor on health

Well-planned physical exercise performed regularly from an early age helps prevent the development of cardiovascular, osteogenic and metabolic diseases related to overweight and obesity in adult life (Warburton, 2006). A strategy that has been proposed in recent years in the fight against these diseases is the promotion of the practice of physical exercise through organized sports (Carlisle et al., 2019).

The organized sport is developed in a formal environment in which a coach directs the athlete's preparation process through a regular practice of training and competitions (Malina, 2016). And even though its main objective is to develop the physical-athletic form and the specific technical and tactical skills of the sports discipline, some of its characteristics place it as an activity that contributes to the maintenance and improvement of the health of young people. For example, its structure is based on the achievement of sports aims which lead to the practice of physical activity on regular bases; long-term planning which consists of various stages during their life allows the transfer of physical activity into adulthood; in a large number of sports disciplines, training load (volume and intensity) promotes weight regulation and body composition; and in sports in which the body weight is supported, which include explosive efforts (such as sprints, jumps, etc.) the improvement of skeletal health is stimulated (Carlisle et al., 2019).

Physical demands of female soccer

Soccer is a sport in which the body is submitted to great physical efforts that challenge both the aerobic system and the anaerobic system (Dolci et al., 2020). In a recent review study, it was found that the total distance that elite adult women players cover in a game is 10km, and that of this distance 1.7km correspond to efforts made at high intensity (speed= >19 km/h). This configuration of efforts is similar at all levels of competition and categories. For example, in a study in which a kinematic analysis of the game was performed on U9 recreational level soccer players, it was observed that the average total distance is 2943 m per game, and that there is a large amount of short, high-intensity efforts (770 speed change events are carried out, and they participate in 381 acceleration events) (Brad H. et al., 2020). Although in absolute terms there is an obvious difference in the distance covered and in the amount of high-intensity efforts, in all categories, regardless of age and competitive level, the aerobic system plays a determining role in maintaining the level of intensity during the match, while sprints and explosive actions are part of the decisive situations of the game and make up 15 to 28% of the total distance covered (Brad H. et al., 2020; Meylan et al., 2017; Vescovi & Favero, 2014).

Body composition in female soccer players

The intensity, volume, frequency and type of exercise are some factors that determine the impact of physical activity on body composition. The intensity developed in soccer matches, and in a large part of the specific exercises that make up the training, ranges between 70-90% of the HRmax (aerobic high intensity) (Alexandre et al., 2012). These intensities, which are generally maintained for a long time, lead to high fat oxidation (Bangsbo et al., 2007; Randell et al., 2019). In addition, the total energy expenditure required in training and games exceeds the minimum recommended by the World Health Organization and by the American College of Sports Medicine (ACSM) for body fat reduction (Donnelly et al., 2009). On the other hand, explosive actions in which great muscular tension is generated and frequently executed during games and training can lead to an increase in muscle mass and stimulate the acquisition of mineral bone mass (Lozano-Berges et al., 2018; Meylan et al., 2017).

It has been demonstrated that recreational soccer played by untrained male young adults enhance fat oxidation during exercise and results in greater loss of body fat, in greater strength and muscle hypertrophy, and promote the mass accumulation of bone to a greater extent than traditional interval or continuous training programs (Krustrup et al., 2010). The qualities of soccer have been used to create intervention programs aimed at reducing body fat in overweight male children and adolescents, and have proven to be as effective as programs in which there is a strict control of intensity volume, but with the important advantage of having greater adherence (Seabra A. et al., 2016; Seabra A. et al., 2014).

The changes in size and body composition that soccer players experience during puberty can influence their sports performance in different ways. For example, increased body fat causes disadvantages in situations involving body displacement such as sprinting or lower intensity running that is sustained for long periods of time. But, on the other hand, a greater weight and body size can offer advantages in actions where absolute strength is important or in contact situations such as in air or ground ball disputes (Reilly et al., 2004). The little scientific information available indicates that adiposity is heterogeneous among female soccer players (Randell et al., 2021) and that body weight is related to biological maturation status regardless of age chronological (Martinho et al., 2021).

Soccer and bone health

Osteoporosis is a disease that occurs in adulthood and although it affects men and women, the latter have a higher risk of suffering from it due to the fact that they lose bone at a higher rate after menopause and that they reach a lower maximum bone mass during adolescence. In combating this condition, experts propose two strategies; one is to minimize the decline in bone mineral density (BMD) during adulthood and the other is to maximize BMD during childhood and adolescence (Cech, 2012). The period of adolescence becomes relevant because in women the peak bone mass (the maximum acquisition of bone mineral mass) occurs at around 12 years of age (McKay et al., 1998). Various longitudinal studies indicate that more than 35% of the total body bone mineral content (BMC) accumulates during the 4 years of the circumpubertal period (Bailey, 1997); so that the period between 10 and 14 years of age is considered critical for "bone mass accrual" in women.

Physical activity can promote the accumulation of bone mass, however it is necessary that the exercise fulfills some requirements to really have a positive effect. For example, weight-bearing activities are better for this purpose than activities in which body weight is nullified (such as swimming or cycling); and within the weight-bearing activities, those that include high-impact dynamic actions are more effective (Turner, 1998). Soccer is an activity in which the body weight is supported and also involves high-impact load stimuli and muscular power actions (Bangsbo et al., 2006; Düppe et al., 1996), therefore it can be a good option to promote bone mineral acquisition.

The results of some cross-sectional studies suggest that the practice of soccer has a positive effect on bone health of young soccer players (Lozano-Berges et al., 2018; Söderman et al., 2000). The data generated in these studies indicated that both female and male soccer players at pubertal age had higher bone mass than their non-athletic counterparts, however these differences seem to be minimized during the prepubertal state (Lozano-berges et al., 2017). This relationship between biological maturation status and bone response to soccer training appears to be supported by the results of a longitudinal study in which 65 prepubertal boys (Pubertal Stage 1) were followed for three years. Forty-two of the subjects had practiced soccer for at least 3 years and the rest (23 subjects) were assigned as a control group and they only practiced the physical activity developed in the school physical education class. At the beginning of the study, when all the subjects were prepubertal, the authors found no difference in BMC between the control group and the group of soccer players, despite the fact that the latter had already practiced soccer for 3 years. At the end of the study, when all subjects were pubertal, the soccer group had higher BMC and BMD values at various sites (Zouch et al., 2015).

Most studies, regardless of gender and age, have shown that the effect of soccer on increasing BMC and BMD is localized and adaptations occur only at weight-bearing sites such as the lumbar spine, hip, the femoral neck, the trochanter, the intertrochanteric region and the two legs (Calbet et al., 2001; Lozano-berges et al., 2017; Vicente-Rodriguez et al., 2004; Zouch et al., 2014).

The practice of soccer during childhood and adolescence can contribute to maximizing bone mineral mass and diminish the decrease that occurs in adulthood and therefore reduce the risk of fracture in dangerous bone sites. To see more details about Bone Mineral Density the reader can go to section 1.7 of this chapter.

Cardiovascular adaptation in youth soccer

When played recreationally, soccer has been shown to have a positive effect on the cardiovascular health of men and women of various ages. Its attributes as physical activity have been used to develop programs that have shown favorable effects on the risk of cardiovascular profile in hypertensive men and women (Krustrup et al., 2010, 2013), and in overweight adolescents of both sexes (Andersen et al., 2014; Ørntoft et al., 2016). Some of the benefits that have been observed in cardiovascular health are a decrease in arterial hypertension, a decrease in plasma LDL cholesterol and triglycerides. In addition, improvements in cardiac function have been observed, such as increases in left ventricular end-diastolic volume, decrease in isovolumetric relaxation, and increase in left ventricular systolic and diastolic performance (Andersen et al., 2014).

As it could be expected, higher workloads in competitive sport elicit deeper adaptations in the cardiovascular system when compared to recreational practice. In adult athletes of various sports disciplines, it has been described a cardiac remodeling which consists in a harmonic increase of the diameter of the cavities and the thickness of the myocardial walls. These adaptations in the heart, induced by high-intensity training, are known as "athlete's heart" (see section 1.6 of this chapter for more details) and differ from the changes caused by diseases such as aortic stenosis or arterial hypertension which cause a constant overload of pressure on the internal walls of the left ventricle and result in their thickening, hardening and loss of elasticity (Golbidi & Laher, 2012; Kramann & Kindermann, 2002; Morganroth et al., 2016).

In order to assist cardiologists in making an accurate diagnosis, many studies have focused on characterizing training-induced cardiac remodeling to differentiate it from that caused by hypertrophic heart disease (Lauschke & Maisch, 2009). A correct diagnosis allows, on the one hand, to prevent athletes who do present pathological cardiac changes, from suffering sudden cardiac death during sport practice, and on the other hand, give peace of mind to athletes with the heart of an athlete (harmless changes) to continue competing safely. Most of these studies have been conducted in adult athletes, and few data are available regarding cardiac remodeling in adolescent female soccer players (Baggish & Wood, 2011; Pelliccia et al., 1996).

When studying cardiac adaptations associated with sport training in adolescents it is particularly important to choose the best method to cancel the effect of body size, since the dimensions of the heart are strongly related to this variable (Dewey et al., 2008). Even adolescent athletes with similar chronological ages exhibit substantial variation in body size related to differences in biological maturation, so misinterpretations can be made if not properly adjusted (A. L. Claessens et al., 2006; Erlandson et al., 2008; Myburgh GK, Cumming SP, Coelho E Silva M, Cooke K, 2016; Nevill et al., 2005; Robert M. Malina, Claude Bouchard, 2004). For more details, see sections 1.5 and 1.6.

1.4. Categories in soccer

In soccer, and in many other team sports, the structural arrangement to establish categories most widely used throughout the world uses chronological age (CA) as a reference (Cobley et al., 2009). In some sports organizations the categories are divided into age groups based on an annual calendar, as in the Netherlands or Belgium where the cut to establish the categories starts in January and ends in December. For example, considering the current year (2021), the U13 category includes players born on or after January 1, 2008; the U14 category includes players born on or after January 1, 2007; and so on. In other countries sports programs use a bi-annual calendar (Helsen et al., 2005). So that, for example, a soccer player born in December 2009 could, in his own category (U12&U13), team up or compete against athletes almost two years older (those born in January 2008).

The wide difference in chronological age within categories results in disadvantages for athletes who are relatively younger (those born at the end of their category). Generally, older athletes are also more advanced in terms of maturation and show greater development in some morphologic and physiologic characteristics (e.g., greater strength, VO2max and height). Moreover, they have a better development of their technical skills which allows, temporarily, to perform better than their younger mates (Kelly Adam L. et al., 2021). This may lead to negative aspects in sports participation; on one hand trainers often mistakenly interpret the advantages of the older athletes as talent, give them more participation during competitions and promoted them to teams of higher levels, meanwhile, on the other hand, the relatively younger athletes receive less attention, which leads to stagnation in their athletic development, demotivation and finally drop out (Eime et al., 2019).

1.5. Scaling for body size

Definition of scaling

When biological responses and adaptations to physical exercise are studied in children and adolescents, body size must be considered. Many physiological and morphological parameters associated with physical performance are size dependent. In order to cancel the effect of body size, mathematical adjustments must be carried out. The technique used to make these adjustments is called scaling (Welsman, 2007). Once physiological and performance variables are scaled, it is possible to properly assess the effect of training during growth. There are various scaling methods, and each one has its advantages and limitations. Selection of the scaling method depends on the research question and whether available data accomplish certain statistical requirements (Nevill et al., 1992).

Simple ratio standard

One of the most commonly used scaling adjustment methods in the context of sport training is the simple ratio standard (Y/X) (REF). It consists of dividing the size-dependent variable (Y) by the body-size variable (X) (Eston & Reilly, 2009). Maximal oxygen uptake (VO2 max) is a good example of this adjustment; VO2 max is commonly expressed relative to body size dividing the VO2 in ml by body mass in kilograms (ml/kg/min). In practice this can be useful for comparisons between athletes of different sizes and to monitor their aerobic performance. However, it has been stated that the simple ratio standard is not a valid method to cancel the influence of body size either in VO2 max or in many other physiological variables. According to Tanner (1949), the use of the simple ratio standard method is proper only when this “exceptional circumstance” is satisfied:

$$Vx/Vy = r$$

In this equation Vx= coefficient of variation of x; Vy= coefficient of variation of y; and r= Pearson's product-moment correlation coefficient. This implies an intercept of 0, and a slope equal to the ratio standard. This brings two problems, in the first place, this circumstance practically never occurs, and secondly, a value of 0 for physiological and size variables does not correspond to reality (a living mammal has a minimum VO2 at rest and always has body mass above 0) (Tanner, 1949).

Linear regression

Linear regression has been proposed as a more effective approach to cancel the effect of body sizes than simple ratio standard (Tanner 1964). One advantage of this method is that it integrates an intercept term:

$$y=a+b\cdot x+\varepsilon \text{ (equation 1)}$$

In the equation, “a" represents the intercept; “b” the slope, and ε the residual error. Here, it is possible to construct adjusted scores (regression standards) by adding the residual error of the individuals to the group mean score. A straight regression standard line is obtained and can be used as reference to evaluate the results of the individuals; if the subject´s score is above the line, the result is above average, and if the result falls below the line it is lower than the average (Eston & Reilly, 2009). Some researchers have reported different results using ratio standard and linear regression scaling with the same set of data.

Allometric Scaling

A relationship between a body size variable and another biological variable is referred to as allometric. The term "Allometry" was introduced by Julian Huxley and George Tessier in 1936, it means "different measures" and refers to the differences in growth rate between parts of the body and the body as a whole. The mathematical approach that includes power function to explain structure and functions of organisms of different sizes is denominated allometric, and the general equation is represented as follow:

$$Y = Y0Mb \cdot \varepsilon \text{ (equation 2)}$$

In this basic equation Y is the dependent variable (e.g., VO2max, Left Ventricular Mass), Y0 is a normalization constant, M is the independent variable (usually body mass, stature, or body surface area), and b is the scaling exponent (power function). An advantageous characteristic of this method is that the association between the body size and the physiological variable is not expected to be strictly linear; when data is plotted, it draws a curve rather than a straight line, which is more similar to that which is observed in nature (Welsman & Armstrong, 2013). The term in the equation that describes the curvature of the line and the effect of body size on the dependent variable is the power function, or the "b" exponent (equation 1). The exponent can be used to partition out the influence of body size by computing power function ratios (Y/Xb). In addition, the "b" exponent exhibits the extent and the direction of the relationship between the performance (Y) and body size (X) variable. If b < 1.0, the dependent variable (Y) increases at a lower rate than the independent variable (X). Conversely, when the b exponent is greater than 1, it indicates that the dependent variable (Y) increases faster than the independent variable (X). Finally, an exponent of 1.0 indicates that the relationship is linear, and, on the other hand, if b=0 it means that the body size has no effect on Y. In order to obtain Y0 and the exponent "b" it is necessary to solve the linear form of the allometric equation. To do this, it is necessary to transform the data of the body size and the performance variable using natural logarithm, and then apply standard least square regression to the logarithmically transformed data. The result is the linear form of the equation 1:

$$loge = logea + b \cdot logeX \cdot log \cdot log\varepsilon \text{ (equation 3)}$$

An advantage of this procedure is that the log of the error term is independent and has a normal distribution with constant variance which accommodates the heteroscedasticity observed in the linear regression scaling. However, it is important to note that this procedure can not be performed if the variable to be adjusted does not correlate with the body size variable (Eston & Reilly, 2009).

Scaling using only one body size variable (as body mass, body surface area or height) usually is not sufficient to properly adjust performance variables. In this regard, proportional allometric models had been proposed to explore more options in which other relevant variables can be incorporated in the same equation with body size variables (Nevill et al., 1998). This scaling technique consists in applying multiple stepwise regressions (equation 4) once the allometric equation is linearized through log-transformation (equation 5) (Nevill AM, Ramsbottom R, 1992).

$$y= \text{size descriptor1k1} \cdot \text{size descriptor2k2} \cdot \varepsilon \quad \text{(equation 4)}$$

$$\ln(y)= k1 \cdot \ln(\text{size descriptor1}) + k2 \cdot \ln(\text{size descriptor2}) + \ln(\varepsilon) \quad \text{(equation 5)}$$

It is important to note that allometric exponents only describe the relationship between body size and performance variable and does not offer an explanation of the mechanisms of the relationship (Nevill et al., 1998).

1.6. Cardiac Adaptations in young athletes

The athlete's heart

The practice of systematic and strenuous physical activity, as what is performed by competitive athletes, drives diverse changes in the heart. These adaptations can be structural (eg. increase of wall thickness and chamber dilatation), functional (eg. increasing in diastolic filling and stroke volume), electrical (eg. sinus bradycardia, first degree of AV block), and peripheral (eg. enhance capillary conductance and oxidative capacity). All these changes are recognized as the athlete's heart (Kramann & Kindermann, 2002; Sharma et al., 2015)

The athlete's heart was first described by Henschen in 1899 when he studied a group of elite cross-country skiers. Since then, the athlete's heart has been a prominent research topic in sport science and medicine. After more than one century studying its physiologic and morphologic features, yet remain questions that must be elucidated about the athlete's heart. The genetic influence, the variation in the individual response of training, the mechanisms that stimulate heart enlargement, and the trainability of children and adolescent´s heart, are some subjects that are currently being investigated.

Data from literature is insufficient to affirm whether adolescent athletes follow or not a similar cardiac remodeling pattern to their adult counterparts. Probably, the first difficulty is to establish the congruity regarding the training stimulus at which they are exposed. Even being the same sport, training loads and activities performed during the sessions and the level of demand during competitions are not the same between children, adolescent and adult athletes (Perseghin et al., 2007).

Evaluation of left ventricular mass in young athletes

In addition to the related aspects associated with training load quantification, there are other facts that need consideration at the moment of explaining the adaptations in cardiac remodeling in young athletes. The heart size is directly associated with the body size so that is crucial to "normalize" the variables that represent the heart size.

The adjustment of the hearth size becomes of greater need in children and adolescents, who, due to the natural growth process, present wide variability in body size, even in similar chronological ages. This is explained by the differences in "timing" and "tempo" of biological maturation among each athlete. It has been pointed out that even within the same athletic discipline, in which a uniformity in the athlete's physical appearance would be expected, the body size varies widely during the adolescence period (Malina et al., 2015). In order to avoid misperceptions regarding cardiac adaptations to children and adolescent athletes, it is essential to use the most adequate methods to nullify the influence of body size over the heart size. Owing to the relevance of the left ventricle functionality during physical exercise effort, Left ventricular mass (LVM), which refers to the weight of the left ventricle, is a variable commonly considered to analyze the adaptation of the heart of the athletes. (Brumback et al., 2010).

For practicality, in the clinical context, it is usually recommended to adjust the LVM values on the basis of anthropometric variables such as height, body surface area (BSA) or fat free mass (FFM). The BSA has a high correlation with LVM, as a result, it has been widely used to normalize the LVM values from the simple method of ratio standard (Fuchs et al., 2016). However, this approach features some inconveniences. It has been proven that when BSA is used to normalize LVM, values on obese individuals are underestimated. Further, it has been demonstrated that the relation between heart's size and BSA is not linear but almost curvaceous or logarithmic, so that the adjustments based in allometric methods are more appropriate.

Another suggestion that has been broadly employed to normalize cardiac dimensions is based on the simple allometric method which consists in dividing the LVM value by height to 2.7 potency. This 2.7 exponent, which shows the curvaceous relation between height and LVM, was obtained upon a diverse sample of males and females without cardiac pathologies, in a wide range of ages (children and adults), varied body mass index, and multiple ethnic groups. Despite the fact that this method has been recommended in guidelines of clinical use, the idea of this "universal exponent" has been criticized by various authors. Amongst other remarks, it has been demonstrated that the influence of body size does not vanish, inasmuch as the normalized LVM values show a negative correlation to height (Krysztofiak et al., 2019).

Other exponents have been derived from more specific samples. For example, in a recent study there were obtained exponents from a group of 791 children and adolescent athletes, male and female (Krysztofiak et al., 2019). The authors generated sex-specific allometric exponents, that demonstrated more consistent adjustment, and annulled the effect of body size (height) without inverting the relation between adjusted values and height values, as is the case when using the "universal exponent" of $k=2.7$.

Although Krysztofiak et al. managed to propose a suitable route to normalize LVM through height, several authors have agreed that other variables such as sitting height and fat-free mass (FFM), better explain the variability of LVM in children and adolescents. For example, it has consistently demonstrated that FFM is the best predictor of LVM (Janz et al., 2000; Whalley et al., 2004). The FFM represents the tissue with the most metabolic activity. During physical exercise both, skeletal muscles and myocardium, are stimulated and corresponding adaptations between these tissues may occur as the training period progresses. Several studies in which a multivariate analysis was applied, showed that when examining the most commonly used body size variables to normalize LVM values (BSA, height, body mass, FM and FFM) the only one which predicts independently the LVM, in both, adult athletes and their sedentary counterparts, is FFM. In absolute terms, the athletes showed greater LVM values than the sedentary group, however, these differences vanish when indexing LVM to FFM.

Left ventricle mass in girls and boys

When addressing the study of cardiac responses and adaptations caused by sports training, it is essential to consider gender. Scientific evidence proves that among male and females there are morphologic and physiologic differences in the heart. For example, values of left ventricular function and mass were described in a sample taken from the Multi-Ethnic Study of Atherosclerosis (MESA) that included 400 males and 400 females within a range of age of 45 to 84 years old (Natori et al., 2006). Results showed that men presented higher values of LVM and higher values of internal dimensions of the left ventricle, not only in absolute terms but also when the variables were index-linked by BSA and BMI.

In adult athletes cardiac differences between men and women are also evident. In a recent study, it was described the left ventricle geometry of 1,083 elite athletes (40% women) (Finocchiaro et al., 2017); the pattern of LV geometry was determined taking into account the values of relative wall thickness and left ventricular mass in each athlete; it was found that a higher proportion of female athletes (22% women vs 14% men) exhibit eccentric hypertrophy (dilatation of the left ventricular chamber), and fewer proportion of female athletes exhibit concentric hypertrophy (thickening of the wall within the left ventricle) when compared to their male counterparts (4% women vs 15% men). Male athletes showed higher values in LVM while female athletes had higher Left Ventricular End Diastolic Diameter; both variables were indexed to BSA. According to these results, it could be concluded that women adapt by increasing the size of the left ventricular cavity and, on the other hand, men tend to increase the thickness of the walls to a greater extent. However, in the referred study the level of training was not reported. Thus, it could be argued that, although they belong to the same sports disciplines, the athletic stimulus could not be the same, and therefore the discrepancy in cardiac adaptations could be attributed to the difference in training loads and not to gender.

In order to confirm if heart adaptations differ between females and males subjected to the same training load, a recent study evaluated 15 couples belonging to the Slovenian national dance team, with several years of experience in the discipline (9 years). In this sport both, training sessions and competitions are performed in pairs, so that in this case the level of training can be controlled or excluded as a confounding variable because women and men follow a very similar sport-training plan. Men and women of the referred study presented similar values of VO2 max, but adaptations in cardiac morphology were different. Both men and women dancers, when compared with samples of non-athletic subjects (matched for age and body composition) showed an increase in the thickness of the walls of the left ventricle within the normal limits. On the other hand, when comparing the group of male dancers with that of female dancers, the left ventricular mass index (LVM/body surface area) was significantly higher in male athletes. It has been argued that gender discrepancies in training-induced cardiac adaptations are explained by various factors such as differences in body size and composition, by different hemodynamic responses to exercise, and by the particular hormonal profile of each gender. In the case of dancers, it was found that ambulatory systolic blood pressure (measured every 30 minutes for 24 hours), which is a variable strongly associated with LV hypertrophy, differs between women and men, being higher in females.

In pre-pubertal boys and girls, when physical differences between them are not evident yet, the structural and functional parameters of the heart are also similar in both genders. This was confirmed in a longitudinal study in which heart growth, changes in body composition, and cardiac function were followed for three years in boys and girls starting at an age of 11.5 years. In the first evaluation, no differences were found in the size and function of the heart or in body composition. However, in subsequent measurements, the rate of change in body and heart variables began to differ between boys and girls and to become more pronounced as time went on. At 14.5 years of age, men had increased their LVM by 14% while women only 8.6%. These changes were consistent with Fat Free Mass (14% in boys and 10% in girls), with Diastolic Blood Pressure (9.4% in boys and 5.7% in girls), and with skinfold sum (-0.5% in boys and 12.6% in girls).

1.7. Bone mineral density and physical exercise training in youth

Physical Exercise Training and Bone Accrual

According to scientific literature, weight-bearing activities and efforts that imply compressive, bending, tension and torsion stress on bones are fundamental to promote bone accrual. Jumps, sprints, jogging, sudden change-of-direction displacements, and resistance exercises are examples of this kind of actions (Gregov, C. & Salaj, S., 2014). Biological responses to physical training are directed and controlled by the components of training load (intensity, volume, frequency and density). Training load is expressed differently according to the physical capacity. In some cases, it is not difficult to control the training load in order to address the desired adaptations. For example, for aerobic training, intensity can be established as velocity of running, or by considering some physiological parameters, such as heart rate. But to determine intensity, volume and frequency in order to generate predictable adaptations on bone is problematic. It is not feasible to quantify the mechanical forces exerting on the skeleton during a training session, and therefore it is not possible to accurately regulate bone response and establish a specific intensity. Besides, the exposure time at which bone must be submitted to any specific intensity to stimulate osteogenic activity has not been determined.

Allowing for the exposed in the last paragraph, intensity plays a fundamental role to ensure an effective osteogenic response. It seems that exercise mechanical loading needs to inflict a minimum strain on bones in order to elicit bone adaptations. According to the Mechanostat Theory (Tyrovola & Odont, 2015), the activity of osteoblasts and osteoclasts is influenced by mechanical usage at which bones are habitually submitted in daily life. When a specific site of bone is overloaded, osteoblasts are stimulated and osteoclasts are inhibited resulting in the addition of new bone and in a change of architecture (modeling). Conversely, when bone is under-loaded (for example, due to prolonged bed rest or immobilization) the process swaps and the increased activity of osteoclast incite bone removal. Forwood and Turner (1995) established 4 zones (trivial, physiological, overload and pathological) corresponding to different mechanical loads at which bone can be stimulated; these zones are determined according to strain magnitude and each one drives specific alterations on bone (Nazer et al., 2012). The mechanical load produced by exercise must provoke a strain magnitude of 2000-3000 με (overload zone) in order to induce osteogenic and increase bone mass (Turner, 1998). According to a recent systematic review that analyzed studies in which strain was measured in vivo in humans, dynamic impulsive exercise as jumps and sprints produces a strain on the tibia that correspond to the physiologic loading zone, consequently, the intensity of those kind of activities is suitable to strengthen the bone (Gregov & Šalaj). These results are supported by interventional studies in which training programs, based on strict jumping trials and varied weight-bearing activities, resulted in an increase of bone mineral density and positive changes on bone geometry (Gómez-Bruton et al., 2017). Furthermore, it was reported that 3 hours per week of football or handball practice (weight-bearing activities that include dynamic impulsive efforts) brings osteogenic benefits (Vicente-Rodriguez et al., 2004).

The increase in BMD caused by physical activity occurs in specific sites of the skeleton and it depends on the exercise performed. For example, jogging is an action that implies a constant impact between ground and feet; ground reaction force generated in each foot strike is transmitted to the bones of lower limbs and stimulates bone accrual in tibia (Bennell et al., 2004). On the other hand, jumps, like those performed in plyometric training, produce a high mechanical load in whole lower limbs but mainly in the femoral neck; high tensile force on bone is generated by the attached tendons of the muscles of the hip joint when they contract during the jump (Bonetto & Bonewald, 2019; Gregov & Šalaj). In the same way, increments in BMD on arms have been reported in sports that impose mechanical load on upper limbs, like tennis (Magkos et al., 2007), combat-sports (Nasri et al., 2015), and gymnastic (Proctor et al., 2002).

Bone accrual and biological maturation

Several studies, including cross-sectional and longitudinal observations have derived strong arguments that support the importance of increasing bone strength during early years (Jackowski et al., 2011). Epidemiological data suggests that little increments on bone mass during childhood can bring great benefits by reducing osteoporotic fractures in adult life (Heaney et al., 2001). Practice of regular physical activity is probably the best strategy to prevent bone fragility in healthy individuals (MacKelvie et al., 2002; Naughton et al., 2017).

Bone accrual response to physical activity seems to be dependent on maturity status. Growth is addressed by the action of various hormones, including Insulin Growth Factor (IGF-1), Growth Hormone (GH), oestrogen and sexual hormones. These hormones have a positive influence on bone mass gain. Release of IGF-1, GH and oestrogen start to increase at the beginning of puberty contributing to the rapid growth that characterize adolescence. Likewise, the disposal of these hormones allows the increment of bone mineral content velocity, which attains its peak in the peripubertal phase. It has been demonstrated that the presence of these hormones promotes the positive effects of physical activity on bone during adolescence (Jackowski et al., 2011).

Some events may be highlighted in the period surrounding adolescence. There has been identified a 2-years duration period of fast mineral skeletal accrual, corresponding to 11-13 years of age for girls and 12-14 years of age for boys, in which 25% of the total bone mineral content is achieved. This means that at the end of adolescence more than 95% of the adult bone has been formed (Heaney et al., 2001).

Bone and muscle are considered as a functional unit system; the mechanical interactions between them allow locomotion (Bonetto & Bonewald, 2019). In addition to weight-bearing function, the skeleton must support the force that muscles exert on it in order to generate movement. During growing, bone and muscle develop congruently, and maintain a balance between function and structure, ensuring the integrity of tissues. The association between bone and muscle has been apparent in studies in which children and adolescents were included. Research has revealed that changes in BMD related to growth and physical activity must be explained considering muscle mass. In a recent study it was compared the BMD of adolescents participating in different sports and sedentary subjects. Authors adjusted the results considering confounding variables as sex, body mass index, chronological age, lean soft tissue, and maturity offset. They found that lean soft tissue was the most relevant mediator in both female and male subjects (Maillane-Vanegas et al., 2020).

Despite all this accumulation of knowledge, many important questions regarding the specific application of training loads, the selection of the most suitable activities, and the influence of biological maturation on bone response, have yet to be answered.

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Chapter II

2. Materials and Methods

2.1. Study design and sampling

The present PhD thesis comprises three cross-sectional studies investigating various aspects of female adolescent soccer players. Prior to conducting the research, the ethical principles for medical research involving human subjects ("World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects," 2013), were followed. Accumulated years of soccer training (i.e., training experience) were recorded in studies 2 and 3, and current or previous formal experience in sport was determined through an interview with all participants. Study 1 investigated the relationship between skeletal maturation and body size, using standardized instruments and methods, and statistical analysis to test the hypothesis of maturity-associated selection in adolescent female soccer. Study 2 aimed to analyze the influence of skeletal age, chronological age, fat-free mass, and sitting stature on inter-individual variability in left ventricular mass in young female soccer players, using echocardiographic evaluation, anthropometric measures, and imaging protocol, and applying descriptive statistics and linear regression analysis with a power function model and log-transformation. Finally, Study 3 compared the amount of bone mass and body composition between soccer players and swimmers, using radiographs to assess skeletal maturity, DXA to measure bone mass and body composition, and pairwise comparisons to analyze the differences between the two sport groups.

2.2. Anthropometric characteristics and estimated body composition

Anthropometric measurements, including stature, sitting stature, and body mass, were obtained in all three studies of the present theses. All measurements were taken in accordance with the procedures outlined by Lohman et al. (1988). Stature and sitting stature were measured to the nearest 0.1 cm using Harpenden stadimeters (model 98.603 for stature, and model 98.607 for sitting stature, Holtain Limited Crosswell, Crymych, UK). Body mass (BM) was measured to the nearest 0.1kg using a digital scale (SECA, model 770, Hanover, MD, USA).

In studies 1 and 2, triceps skinfold, subscapular skinfold, and calf skinfold thicknesses were measured to the nearest 0.5mm using a Lange Skinfold Caliper (Beta Technology Incorporated Cambridge, Maryland). Percentage body fat and fat-free mass were calculated from triceps and subscapular skinfold thicknesses using the protocol of Slaughter et al (1988). Percentage fat mass was estimated from the sum of two skinfolds using an equation proposed by Slaughter et al (1988): % Fat mass = 0.735 x (triceps skinfold + medial calf skinfold) +1.0. Fat mass (FM) and fat-free mass (FFM) were subsequently derived.

2.3. Skeletal maturity

In all three studies included in this thesis, skeletal maturity was assessed using the Fels method (Roche, Chumlea, & Thissen, 1988). This method entails obtaining a posterior-anterior hand-wrist radiograph, which is then read and interpreted for specific indicators in the 22 bones of the hand and wrist, as well as the ratios of measurements of epiphyseal and metaphysical widths as outlined in the method. To determine skeletal age and the standard error of estimate, all ratings and ratios were processed and computed using the Felshw 1.0 software. A trained observer who was the same for all radiographs analyzed the images. In Study 1, skeletal maturity status was expressed as the difference between skeletal age and chronological age (SA minus CA), which was used to classify each player as late (SA younger than CA by more than 1.0 year), on time (average, SA within ±1.0 year of CA), or early (SA older than CA by more than 1.0 year) maturing (Malina, 2011).

2.4. Echocardiographic assessment of left ventricular morphology and mass

In Study 2, it was evaluated heart morphology using resting echocardiography. The transthoracic examination was conducted with the participant lying in the left lateral decubitus position. It was used a Vivid 3 ultrasound machine with a 1.5 to 3.6 MHz transducer from GE Vingmed Ultrasound in Horten, Norway. It was followed the American Society of Echocardiography guidelines (Nagueh et al., 2016) to measure the left ventricular internal dimension, septal wall thickness, and posterior wall thickness. Left ventricular mass was estimated using the method proposed by Devereux et al. (1986). All echocardiographic examinations were performed by the same trained operator.

2.5. Bone mineral content, bone mineral density and Body Composition by DEXA

In Study 3, dual-energy X-ray absorptiometry (DXA) was used to obtain bone mineral density (BMD; g/cm2), bone mineral content (BMC; g), lean mass (LST; g), and fat mass (FM; g). The Hologic QDR 4500A (Hologic Inc., Waltham, MA, USA) was used and bone area (cm2), BMC (g/cm2), and BMC (g) were obtained for the Whole Body, Left and Right Arm, Left and Right Ribs, Thoracic Spine, Lumbar Spine Pelvis, Left and Right Leg. Calibration of the equipment was performed following the manufacturer's instructions and practical considerations (Wilson, 2011). All scanning exams were performed by the same trained evaluator.

2.6. Food frequency questionnaire

In Study 3, it was assessed the daily calorie and calcium intake of soccer players and swimmers using a semi-quantitative food frequency questionnaire. This questionnaire is a widely used method for estimating nutrient intake and has been validated in previous studies (Lopes 2000; Lopes et al. 2007). The questionnaire was composed of food items commonly consumed in the participants' diets and nine categories of frequency of consumption ranging from "never or once a month" to "six or more times per day." For each food item, participants were asked to indicate their average consumption frequency over the past year. The questionnaire also included questions about portion sizes and cooking methods. Dietary data were analyzed using a computerized program to estimate nutrient intake. The analysis included the calculation of the total daily calorie intake and the daily calcium intake for each participant.

2.7. References

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Part II

STUDIES

Chapter III

3. Growth, maturation and playing position in female youth soccer

3.1. Abstract

This study aimed to examine growth and maturation among female soccer players and to test the hypothesis of maturity-associated selection in adolescent female soccer. A total of 194 female junior soccer players aged 12.0-16.7 years were examined, and their stature, body mass, skeletal age, and maturity status were assessed. It was observed a large spectrum of inter-individual variability in stature for chronological age and also regarding body mass for chronological age when compared against US reference percentiles. Estimated fat mass was consistently nearby 25% of whole body mass across age groups and for the total sample. Results of this study suggested that soccer tends to retain female adolescents who were early maturing or already mature (63.6% among 12-13 years; 62.7% among 14-15 years) with a minority of players classified as delayed (5.9% and 13-1% respectively among initiates and juveniles). The data confirmed that adolescent soccer players advanced in skeletal maturation dominate the game as already noted for Portuguese male youth soccer (Malina et al., 2000) using the same protocol of the current study to estimate skeletal age.

keywords: skeletal age, growth female soccer players, body composition

3.2. Introduction

Soccer is probably the most popular sport. In Portugal, sport participation from 1996 to 2014 raised from 95.746 to 158.738. This volume of sport participation is offered by 1.976 clubs including medical examination, medical insurance, supervised coaching on regular training sessions and organized competitions. The total number of registered sport participants are 546.348 (males: 404.623; females: 141725). It is being assumed that girls are increasingly participating in sport and soccer is no exception. The literature in female soccer is lacking. For example, the total distance covered during a match ranged from 9.7 to 11.3 km (Krustrup et al. 2005). Another study used computerized tracking system evidenced that the highest total covered distance was performed by midfielders and the lowest by central backs, respectively 9.5±0.6 km and 11.0 ± 0.7 km (Datson et al., 2016). Additional data suggested variation by performance levels (Mohr et al., 2008; Andersson et al., 2010) and playing position (Vescovi, 2012).

The long-term athletic development plan for female youth soccer needs to consider information about the most effective ages for sport initiation, transition from small sided games to full field. For the purpose of the previous questions, studies about growth, maturation and their independent and combined effects on talent identification are needed. Growth refers to intra-individual changes in body size with implications in proportions and body composition (Malina et al. 2004b). Inter-individual variation in biological maturation is a common theme in research pediatric exercise sciences. Available studies of youth soccer players including systematic information on skeletal age are mainly in males from Portugal (Malina et al. 2000), Serbian (Ostojic et al. 2014), Belgium (Vaeyens et al. 2006), Brazil (Teixeira et al. 2015). The two most commonly used indicators of maturity status are skeletal age (SA) and stage of development in secondary sex characteristics. As repeatedly mentioned, correspondent information on female youth soccer is not as extensive and is lacking. The current study was aimed to examine growth and maturation among female soccer players and to test the hypothesis of maturity-associated selection in adolescent female soccer.

3.3. Methods

Participants included 194 female junior soccer players aged 12.0-16.7 years (14.5±1.1 years). The sample corresponds to players examined in Porto Institute of Sports Medicine.

Stature was assessed using a stadiometer (Harpenden stadiometer, model 98.603, Holtain Ltd, Crosswell, UK) to the nearest 0.1cm. Body mass (BM) was measured using a digital scale (SECA balance, model 770, Hanover, MD, USA) to the nearest 0.1 kg. Percentage fat mass was estimated using an equation (% Fat mass = 0.735 x (triceps skinfold + medial calf skinfold) +1.0) as proposed by Slaughter et al (1988). Fat mass (FM) and fat-free mass (FFM) were subsequently derived.

The Fels method for determination of skeletal age (SA) was used (Roche, Chumlea, & Thissen, 1988). The method provides an estimate of skeletal age in addition to standard error associated with each assessment, providing a greater degree of confidence regarding the predicted values (Malina et al., 2004). Positioning of the hand-wrist for the radiograph required the placement of the forearm, palm and fingers in contact with a cassette with the middle finger positioned aligned with the forearm. The fingers were splayed and totally extended. Bone-specific assessments (grades) were performed and widths measured to produce ratios. Indicators are specific to sex and age and were entered into the Fels software program (Felshw 1.0 Software) that computed the skeletal age and related standard error. Skeletal maturity status was expressed as the difference between skeletal age and chronological age (SA minus CA). The SA-CA difference was used to classify each player as late (SA younger than CA by >1.0 year), on time (average, SA within ±1.0 year of CA), or early (SA older than CA by >1,0 year) maturing (Malina, 2011).

Descriptive statistics were calculated for chronological age, stature, body mass, skeletal age, and SA-CA. Stature and body mass of individual players were also plotted relative to reference data. The interrelationship among competitive age groupos and maturity status was tested using chi-squared test. Body size and skeletal maturation were compared by playing position according to competitive age groups. For each dependent variable, the effect was interpreted using eta derived from the analysis of variance (root square of eta squared that corresponds to the ratio of the sum of squares of the treatment, that is, age group, and the total sum of squares) and it was interpreted as follows (Hopkins et al. 2009): trivial (<0.100), small (0.100 to 0.299), moderate (0.300 to 0.499), large (0.500 to 0.699), very large (0.700 to 0.899), nearly perfect (0.900 to 0.999). Absolute and relative frequencies for birth quarters were calculated per competitive age group in order to examine relative age effect phenomenon.

3.4. Results

Table 3.1 summarized the characteristics of the sample. Figure 3.1 plotted each individual player against reference data given by percentiles, and it was clearly evidenced a substantial inter-individual variance of body size at all ages. As presented in Table 3.2, the distribution of female soccer player by the three categories representing the maturity status is not similar across age groups (χ^2=61.294, p<0.01).

Table 3.3 compared female soccer players on continuous variables including chronological age (CA), skeletal age (SA), discrepancy between ages (SA-CA), body size given by stature and body mass, measurements of adiposity (skinfold thickness) and estimated fat and fat-free mass components (Slaughter et al. 1988). Differences by playing position within age groups were uniquely considering outfield players (defenders, midfielders, forwards). However, descriptive statistics were added for the limited number of goalkeepers (infantiles: n=02; initiates: n=09; juveniles: n=02). Playing position corresponded to a small effect among the youngest age group (infantiles: 12-13 years) and that mean differences were negligible for players contrasting in playing position at age 14-15 years. However, among the oldest group (juveniles: 16-17 years), playing position contributed to large magnitude of mean differences for body mass and fat mass.

Table 3.1. Descriptive statistics for the total sample (n=147)

Variable (X_i)	units	Range		Mean			Standard deviation
		min	max	Value	SE	(95% CI)	
Chronological age	years	12	16.71	14.57	0.79	(14.21 to 14.73)	1.10
Skeletal age*	years	11.46	17.92	15.27	0.13	(15.01 to 15.52)	1.57
SA-CA*	years	-2.54	5.15	1.02	0.12	(0.77 to 1.26)	1.49
Stature	cm	136.0	182.2	161.0	0.5	(160.0 to 162.6)	6.9
Body mass	kg	29.5	101.0	56.4	0.8	(54.9 to 57.9)	10.8
Skinfold triceps	mm	5.1	35.0	16.5	0.4	(15.71to 17.3)	5.5
Skinfold calf	mm	3.7	40.1	16.4	0.5	(15.4 to 17.3)	6.9
Fat mass	%	10.5	50.9	25.1	0.5	(24.1 to 26.2)	7.2
	kg	4.0	51.4	14.7	0.5	(13.7 to 15.7)	7.1
Fat free mass	kg	25.5	60.8	41.7	0.4	(40.8 to 42.5)	5.7

95% CI (95% confidence interval) *Statistics for SA are based on players who are were not skeletally mature (n=147), SA (skeletal age), CA (chronological age)

Table 3.2. Distribution of female soccer players by maturity status in age groups considered (12-13 years, 14-15 years, 16-17 years).

Age group	Late	On-time	Early	χ^2	P	Total
12-13 years	5	16	37			59
14-15 years	14	26	42	61.294	<0.001	107
16-17 years	0	5	2			28

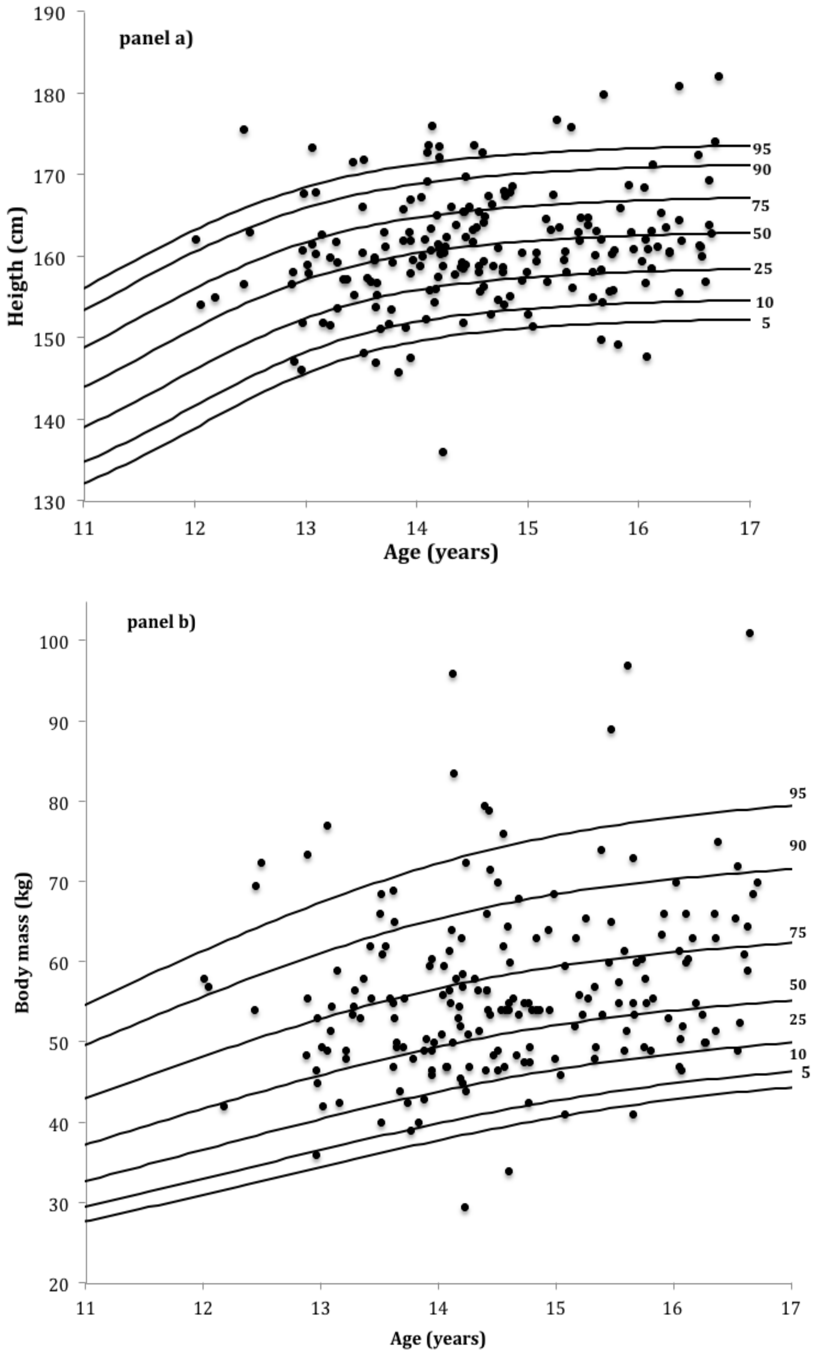

Figure 3.1. Stature (panel a) and body mass (panel b) of each soccer player plotted on the centiles of American population reference.

Table 3.3. Descriptive statistics for age group and playing position and results of ANOVA to test the effect of playing position within each age group

Age	Yi: dependent variable	units	Xi: Variation by playing position							
				Outfield players						
				Means ± standard deviations			ANOVA			
			Goal keepers	Defenders (n=73)	Midfielders (n=42)	Forwards (n=66)	F	p	ES-r	(qualitative)
12-13	(n=59)		(n=2)	(n=15)	(n=19)	(n=23)				
	CA	years	13.17±1.03	13.30±0.52	13.30±0.52	13.39±0.43	0.221	0.80	0.10	(small)
	SA*	years	15.34±0.68	14.84±1.98	14.58±1.21	14.92±1.74	0.228	0.87	0.11	(small)
	SA-CA*	years	2.17±0.34	1.56±2.20	1.28±1.02	1.53±1.72	0.230	0.87	0.11	(small)
	Stature	cm	154.05±3.75	157.45±7.81	158.68±7.09	159.57±5.90	0.436	0.64	0.17	(small)
	Body mass	kg	60.00±13.44	54.93±11.05	52.05±8.32	52.48±8.30	0.479	0.62	0.18	(small)
	Fat mass	%	24.77±0.47	27.09±7.17	23.31±5.86	23.70±4.44	2.169	0.12	0.27	(small)
14-15	(n=107)		(n=9)	(n=45)	(n=19)	(n=34)				
	CA	years	14.76±0.61	14.85±0.57	14.81±0.50	14.75±0.62	0.267	0.76	0.07	(trivial)
	SA*	years	16.44±0.67	15.29±1.63	15.13±1.40	15.58±1.42	1.701	0.17	0.24	(small)
	SA-CA*	years	1.78±0.91	0.52±1.43	0.40±1.49	0.86±1.19	2.309	0.08	0.28	(small)
	Stature	cm	165.20±7.2	160.55±6.5	161.0±5.6	162.7±6.6	1.145	0.32	0.21	(small)
	Body mass	kg	60.60±17.4	57.16±10.9	54.44±5.6	57.13±11.4	0.524	0.59	0.13	(small)
	Fat mass	%	25.40±9.7	26.08±7.7	24.28±5.7	24.06±8.1	0.803	0.45	0.12	(small)
16-17	(n=28)		(n=2)	(n=13)	(n=4)	(n=9)				
	CA	years	16.40±0.34	16.29±0.22	16.37±0.25	16.35±0.25	0.269	0.76	0.16	(trivial)
	SA*	years	***	16.76±0.84	17.88**	***	1.486	0.27	0.48	(moderate)
	SA-CA*	years	***	0.48±0.76	1.82**	***	2.628	0.16	0.58	(large)
	Stature	cm	162.0±1.2	164.3±7.9	164.8±7.3	163.1±8.1	0.091	0.91	0.11	(trivial)
	Body mass	kg	82.0±26.9	61.9±9.0	57.6±8.5	56.00±6.9	1.462	0.25	0.57	(large)
	Fat mass	%	42.50±11.9	26.44±7.6	25.17±6.5	24.75±5.6	0.172	0.84	0.55	(large)

*Statistics for SA are based on players who are were not skeletally mature (n=147), ** Only 1 player was considered in analysis,
***All players were classified as mature, SA (skeletal age), CA (chronological age), ES-r (effect size correlation

Table 3.4. Distribution of the players by playing position, competitive level and birth quarter for the total sample (n=194)

	category	total		12-13 years		14-15 years		16-17 years	
		n	%	n	%	n	%	n	%
Trimester	BQ1: January – March	55	28.4	14	23.7	33	30.8	8	28.6
	BQ2: April – June	48	24.7	11	18.6	32	29.9	5	17.9
	BQ3: July – September	56	28.9	13	22.0	28	26.2	15	53.6
	BQ4: October - December	35	18.0	21	35.6	14	13.1	0	0.0
TOTAL		194		59		107		28	

3.5. Discussion

The current study described the growth status and maturity characteristics of Portuguese female adolescent soccer players aged 12.0-15.95 years. Figure 3.1 plots each individual female adolescent soccer player against US reference percentiles (Center for Disease Control and Prevention, 2000). It clearly illustrates the large spectrum of inter-individual variability in stature for chronological age and also regarding body mass for chronological age. Estimated fat mass was consistently nearby 25% of whole body mass across age groups and for the total sample. Soccer tends to retain female adolescents who were early maturing or already mature (63.6% among 12-13 years; 62.7% among 14-15 years) with a minority of players classified as delayed (5.9% and 13-1% respectively among initiates and juveniles). The data confirmed that adolescent soccer players advanced in skeletal maturation dominate the game as already noted for Portuguese male youth soccer (Malina et al., 2000) using the same protocol of the current study to estimate skeletal age.

Reports about the skeletal maturity status of youth soccer players are always from youth male soccer and includes studies of Belgian (Vrijens et al., 1985), Japanese (Atomi et al., 1986; Satake et al., 1986) and Mexican (Peña Reyes et al., 1994) players. These reports consistently indicated mean 20-bone Tanner-Whitehouse skeletal age that approximated mean chronological ages for younger ages (10-12 years) and were in advance of chronological ages after 13-14 years. Not all studies indicated, if any players in the older groups of chronological age were skeletally mature. The distribution among maturity groups at 14-15 years and 16-17 years reflected the exclusion of skeletally mature players in the current study and probably may also reflect the catch-up of late and average maturing players in late adolescence, consistent with the general growth literature (Malina et al., 2004) as well as selective dropout from the sport (Figueiredo et al., 2009). Allowing for variation among methods (Tanner-Whitehouse, Fels), the data for youth soccer players showed a consistent pattern in skeletal ages across age groups: skeletal age approximated chronological age among players 10-12 years, and a pattern of advanced skeletal age emerged among players 13-14 years. Among females, the selective recruitment, dropout and promotion of adolescent players tend to occur at younger ages, since pubertal growth spurt occurs at younger ages in girls compared to boys (Malina et al. 2004.

Female soccer players of the current study tended to be larger in terms of mean fat mass (25.1%). This value plots on the recommendation for the general population (Laurson et al., 2011). Percentage fat mass ranged 10.5% to 50.9%. Although the limitations regarding the protocol to assess body composition, data suggests that reducing body mass should be considered as a goal for the development of female soccer. Actually, mean values did not range across age groups (24.5% for 12-13 years; 25.1% for 14-15 years; 26.9% for 16-17 years). In fact, 25% of fat mass, means that present sample is above a zone with some risk, according with the cut-off points provided by *Cooper Institute* when developed *Fitnessgram program (*Welk, et al., 2011) that was developed to assess physical fitness and active lifestyle for normal school children.

Meantime, coaches and sport administrators often promote adolescent female soccer players to compete at older age group. This phenomenon apparently occurs due to small number of female soccer players to split competitions for 2-years groups and, officially, the Portuguese federations only considers Juniors (less than 18 years) and seniors (18+ years). According to the current study, this decision needs to be carefully made, since the literature noted an increase in overuse injuries with increasing training volume. Moreover, fat mass has a negative influence in sports that involve body weight displacement. Time motion analysis of elite women soccer players showed that they cover 9-10 km per game, where 7500 meters are made in walking and jogging and in the remaining 2500 meters predominate efforts of high intensity (striding and sprinting). Moreover, recovery time between sprints is done in active recovery which proves the constant displacement of players during an official match (Gabbett, JSCR, 2008). Additionally, among adolescent male soccer players, adiposity obtained by sum of five skinfolds showed a negative influence in functional and specific soccer skills at 11-12 years old (Figueiredo et al. 2009). There is evidence to support the hypothesis that higher levels of fat mass can reduce the performance and increase the risk for overuse injuries.

Relative Age Effect (RAE) has been systematically detected in soccer players from different countries in distinct levels of competition (Helsen et al., 2005; Yagüe et al., 2018). Studies have been mostly centered in males and results demonstrate a strong RAE that begins in adolescence and remains in adult categories (Helsen et al., 2005). On other hand, data from the few available studies in female soccer shows, in general, a much less pronounced RAE (Smith et al., 2018) and distinct studies have presented contradictory results (Sedano et al., 2015; Delrome et al., 2010).

In the current study the classic RAE, with an overrepresentation of soccer players born in Q1 and Q2, was not absolutely evident across the age-categories. Only the U14-15 age-group presented a greater proportion of players born in the first semester compared to those born in the second semester of the selection year (61% and 39%, respectively). Besides, a clear underrepresentation of players born in Q4 was observed in the U14-15 and U16-17 age-groups. Results suggest that at the first stage of competition, other factors than date of birth, determine the permanence in soccer; and then in posterior stages RAE is first manifested in a discrete overrepresentation of BQ1 players, provably provoked by a selection process biased by variation in physical characteristics related to their relative older age; and then in posterior age categories a possible dropout of players born in BQ4 occurs.

The weak RAE found in the female soccer players of the current study must be interpreted carefully. RAE is a consequence of a selection process biased by the physical and functional advantages presented by athletes born at the beginning of the competitive year. Although the presence of RAE was not evident across the age-groups, in all of them soccer players were mostly classified as advanced in maturity status according to skeletal age (see table 2). Players probably passed through a selection, based on attributes related to maturity (such as greater body size and physical performance). This hypothesis may be partially supported by recent research conducted in male soccer players in which it was evidenced that maturity status influences physical performance to a greater extent than RAE (Figueiredo et al., 2019; Parr et al., 2020).

Although in the current study statistical analysis indicated small differences in body size, body composition and maturity status between defenders, midfielders and forwards across the competitive age-groups, some trends must be noted. For example, it was observed a progressive increase in Fat Mass % in midfielders and forwards across the age-groups.

Defenders on the other hand did not show the same pattern; their youngest group presented the highest mean of Fat Mass %; meanwhile values of the older categories were similar (see table 3). The progressive increment of Body Fat of the midfielders and forwards is in line with regular changes in body composition that occur in females through adolescence (Malina, 2014).

The particular display of body fat in defenders across the age categories may be explained by variation in physical demands according to playing position (Datson et al., 2016), combined to the lower level of competition displayed in minor age-categories (Sausaman et al., 2019; Vescovi et al., 2010). Defenders showed higher means of Body Fat % than midfielders and forwards across the 3 age-categories. Although higher values of Body Fat in defenders compared with others outfield positions have been reported in young males (Bernal-Orozco et al., 2020; Leão et al., 2019) and in adult females (Kammoun et al., 2020; Sedano et al., 2009), body fat in defenders of the present study are particularly elevated (see table 3). These results highlight the need to pay attention from early stages of soccer competition in order to avoid risks associated with elevated adiposity, especially in defenders.

Owing to few goalkeepers (GK) composing the samples in each age-group (n=2 for U12-13; n=9 for U14-15; n=2 for U16-17), we only reported descriptive statistics; nevertheless, some points should be noted. GK showed elevated values of SA-CA, suggesting more advance in maturity status than outfield positions. In the 14-15 age-group, which is the one with a larger sample, these results coincide with greater means in stature and body mass. Goalkeepers are characterized by physical attributes, like greater body mass and height, that give them advantages in game situations that imply interceptions and physical contact in ball disputes on the air (Bernal-Orozco et al., 2020). It is possible that maturity had contributed to bias the allocation of taller and heavier players in GK position in the current study. There is not data available regarding biological maturation and physical profile in female soccer, however these characteristics have been noticed in youth male soccer (Leão et al., 2019; Kammoun et al., 2020). Further research is needed to test the hypothesis that maturity influences the allocation of playing position in female youth soccer.

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Chapter IV

4. Body size, estimated body composition and left ventricular mass among adolescent female soccer players

4.1. ABSTRACT

The aim of the study was to analyze the influence of skeletal age, chronological age, fat free mass, fat mass, stature and sitting stature to inter-individual variability in Left Ventricular Mass in young female soccer players, using allometric modeling. A total of 194 female soccer players aged 12.0-16.7 years were included in the study. Anthropometric measurements were obtained according to the procedures described by Lohman et al. (1988). In order to evaluate the heart morphology, a resting echocardiography study was obtained according to the guidelines of the American Society of Echocardiography (Nagueh et al., 2016). Pearson correlation coefficients were calculated to assess the relationships between body descriptors and LVM. Linear regression analysis and allometric modeling were used to determine the best model for adjusting LVM variability. The results showed significant correlations between LVM and body size descriptors (stature, sitting stature, and body mass) and body composition descriptors (fat-free mass and fat mass). Among the body descriptors, fat-free mass exhibited the strongest correlation with LVM. Allometric modeling revealed that fat-free mass explained 37% of the inter-individual variability in LVM. Besides, when considering body mass as a descriptor, 33% of the variability was explained, whereas stature and sitting stature explained 16% and 15%, respectively. Results of the study support the use of body mass as a valid alternative to adjust LVM in adolescent female soccer players, especially when FFM is not available to properly normalize LVM.

keywords: female soccer players, skeletal age, fat free mass, left ventricular mass, allometric modeling

4.2. Introduction

Female participation in soccer has highly increased in the last decades. According to statistics reported by FIFA. In Portugal there are a total of 2.772 registered female players and it is estimated that 54% of them have an age under the 17 years. Despite the constant growing of female participation, scientific research is scarce and attention is even lesser in female adolescent players (FIFA, 2014). Long-term intensive training induces structural and functional adaptations of the cardiovascular system (Golbidi & Laher, 2012). In this way, left ventricular mass (LVM) is commonly measured to analyze cardiac adaptations in athletes (Kramann & Kindermann, 2002; Morganroth et al., 2016). The appropriate interpretation of LVM must consider body size descriptors (Nevill AM et al., 2005) since cardiac dimensions are strongly related to body size (Dewey et al., 2008). If the confounding effect of body size is not properly calculated results can be miss-interpreted (Nevill AM & Ramsbottom R, 1992). In adolescent groups is particularly important to choose the best method to remove the effect of body size because adolescent athletes with similar chronological ages present a substantial variation in body size related to differences in biological maturation (Claessens et al., 2006; Erlandson et al., 2008; Malina RM., 1994; Myburgh et al., 2016).

The appropriate approach to adjust LVM has been widely discussed (Dewey et al., 2008; A. M. Nevill, Holder, & Stewart, 2003; Alan M Nevill et al., 2002) nonetheless the theme is still under debate. Simple ratio standard is the most common method; however, it has been highly criticized (TANNER, 1949). It consists in simply dividing LVM by body mass or by body surface area assuming that there exists a linear relationship between the LVM and the body size variable. However, it is known that relationships between body size and organ dimensions are non-linear. Another unfavorable remark is that once the variable is adjusted, instead of canceling the positive relationship between LVM and body size, it turns into a negative relation, failing to produce a dimensionless variable (Nevill AM, Ramsbottom R, 1992). In other hand, many studies have demonstrated that allometric models are more appropriate to cancel the effect of body size on physiological and morphological variables (Dewey, et al., 2008; Jaric et al., 2005; Nevill, et al., 2005; Nevill & Ramsbottom, 1992). This approach allows to obtain models that can be easily interpreted, likewise, it is possible to make predictions using the available data, and present a small error of variance and therefore a better fitting when compared with other methods (Nevill et al., 2005).

Body mass and body surface area are not the only variables that must be considered to normalize LVM. It has been demonstrated that lean body mass (LBM) and fat mass (FM) are strong predictors of LVM with an independent association. Likewise, sitting stature seems to be a notable determinant of LVM (Valente-Dos-Santos et al., 2014). In addition, biological maturation is related with changes in total body size, body proportions and body composition (Malina RM, 2015). Proportional allometric modeling allows to incorporate more than one descriptor (Nevill et al., 2005; Nevill et al., 2004; Nevill & Ramsbottom, 1992). The method has been successfully used to scale cardiac dimension several body dimension variables in adolescent male athletes and non-athlete groups (Carvalho et al., 2012; Carvalho HM et al., 2011; Valente-dos-Santos et al., 2013; Valente-Dos-Santos et al., 2013; Valente-Dos-Santos, Coelho-E-Silva, Tavares, et al., 2014), however correspondent information on female young soccer players is inexistent.

The aim of the present study was to analyze the influence of skeletal age, chronological age, fat free mass, fat mass and sitting stature to inter-individual variability in Left Ventricular Mass in young female soccer players, using allometric modeling.

4.3. Methods

Procedures and procedures

The same trained operator performed all the echocardiographic examinations. The total sample of the study was composed by 194 female junior soccer players aged 12.0-16.7 years (14.5±1.1 years). All participants were registered in competitive clubs from Portugal and were engaged in formal training and competition for at least one year. All females self-identified as caucasian. None of the athletes presented any symptoms of underlying cardiovascular disease.

Anthropometry

All the anthropometric measures were obtained according to the procedures described by Lohman *et al*. (1988). Stature and sitting stature were measured to the nearest 0.1 cm.

Harpenden stadimeters were used to obtain stature (model 98.603, Holtain Limited Crosswell, Crymych, UK), and sitting stature (model 98.607, Holtain Limited Crosswell, UK). Body mass (BM) was measured to the nearest 0.1kg using a digital scale (SECA, model 770, Hanover, MD, USA). Triceps skinfold, subscapular skinfold and calf skinfold were measured to the nearest 0.5mm. Skinfolds were taken with a Lange Skinfold Caliper (Beta Technology Incorporated Cambridge, Maryland). Percentage body fat and fat-free mass were calculated from triceps and subscapular skinfold thicknesses using the protocol of Slaughter et al (1988). Percentage fat mass was estimated from the sum of two skinfolds using a equation proposed by Slaughter et al (1988) (% Fat mass = 0.735 x (triceps skinfold + medial calf skinfold) +1.0).

Imaging protocol

In order to evaluate the heart morphology, a resting echocardiography study was obtained. The transthoracic examination was made in the left lateral decubitus position. A Vivid 3 ultrasound machine with a 1.5 to 3.6 MHz transducer was used (GE Vingmed Ultrasound, Horten, Norway). Measurements of left ventricular internal dimension, septal wall thickness and posterior wall thickness were obtained according to the American Society of Echocardiography (Nagueh et al., 2016). Left ventricular mass was estimated accordingly to the method proposed by Devereux et al. (1986). The same trained operator performed all the echocardiographic examinations.

Analysis

Descriptive statistics (means, standard deviations) were calculated for chronological age, training experience, height, body mass and body composition. In order to detect the possible predictor variables to adjust LVM, correlation coefficients of Pearson were calculated. It was examined the relationships between sitting stature, fat free mass and fat mass, and chronological age with LVM. Linear regression analysis was used to detect the possible predictor variables to adjust LVM. It was followed the procedure for allometric modeling proposed by Nevill et al. (1992) and Nevill & Holder (1994) in order to obtain the best model to adjust the inter-individual variability in LVM. The following equation, based on the power function model, was firstly performed:

$$Y = a \cdot x^{k} \cdot e$$

Then in order to linearize the power function model log-transformation was used (in this equation *y* represented LVM, and *k* de body descriptor):

$$\log y = \log a + k \cdot \log x + \log e$$

A last equation was used to analyze the combined effect of body size and body composition descriptors.

$$\text{Log (LVM)} = k_1 \cdot \log(\text{body descriptor})_1 + k_2 \cdot \log(\text{body descriptor})_2 + \log e$$

4.4. Results

General characteristics and echocardiographic descriptions of the sample are summarized in Table 4.1. Chronological age ranged from 11.83 to 17.04 years. Skeletal age was higher than CA (15.29 years and 14.63 years, respectively). Means for stature and body mass were 160.8 cm (ranged from 136.0 cm to 182.2 cm) and 54.5 kg (ranged from 29.5kg to 95.2) respectively. Most athletes did not exceed the normal values for echocardiographic variables (LVIDd, SWTd, PWTd and LVM) when compare to reference values for females (Lang et al., 2006). Only 12 athletes, representing 7% of the sample, exceeded the upper limit in SWTd (6.0-9.0mm).

Table 4.1. Descriptive statistics and normality test for the total sample of adolescent female soccer players (n=164)

Variable	unit	Mean	Standard deviation
Chronological age	years	14,27	0,97
Training experience	years	5,4	1,5
Stature	cm	160,9	7,2
Sitting height	cm	84,5	4,1
Body mass	kg	54,6	10,2
Fat mass	kg	24,2	7,4
	%	13,7	6,8
Fat-free mass	kg	40,9	5,9
Left ventricular internal dimension at end-diastole	mm	44,7	3,5
Septal wall thickness at end-diastole	mm	7,6	0,9
Posterior wall thickness at end-diastole	mm	7,4	0,8
Left ventricular mass	g	104	22

Relationships between body descriptors (body size: stature, sitting stature and body mass; body composition: fat free mass and fat mass) and LVM are described in Table 4.2 Correlations ranged from 0.28 to 0.65 ($p<0.01$), corresponding to training experience and fat free mass, respectively. Linear relationships were found only between LVM and fat free mass.

Table 4.2. Correlations between physiological variable (Yi: left ventricular mass) and size descriptors for the total sample (n=164)

	correlation (X,Yi) X: LVM				
Yi	r	95% CL		p	(qualitative)
		lower	upper		
Chronological age	0,063	-0,124	0,246	0,42	(trivial)
Training experience	0,281	0,142	0,411	<0,01	(small)
Stature	0,434	0,266	0,558	<0,01	(moderate)
Sitting height	0,417	0,242	0,551	<0,01	(moderate)
Body mass	0,588	0,488	0,692	<0,01	(large)
Fat-free mass	0,658	0,529	0,744	<0,01	(large)

LVM (left ventricular mass); r (correlation coefficient); 95%CL (95% confidence limits); p (significance level)

Exponents derived from independent allometric modelling considering body size and body composition descriptors are presented in Table 4.3. The variable that better explained the inter-individual variability of LVM was fat free mass (explaining 37% of variance) while fat mass explained only the 8%. When body mass was considered as a descriptor, 33% of the variance was explained; stature and sitting explained 16% and 15%, respectively.

Table 4.3. Allometric exponents of left ventricular considering different linear size descriptors and mass for the total sample (n=164)

Yi	Simple allometric models [log a + k · log (Xi: size descriptor) + log ε]						
	constant [a_i]			beta-exponent [k_i]			
	value	SE	p	value	95% CL		p
					lower	lower	
Stature	-5,209	0,005	<0,01	1,935	1,257	2,613	<0,01
Sitting height	-2,861	1,426	≤0,05	1,687	1,052	2,322	<0,01
Body mass	1,896	0,001	<0,01	0,684	0,533	0,835	<0,01
Fat-free mass	1,217	0,001	<0,01	0,919	0,734	1,105	<0,01

LVM (left ventricular mass); ε (error); k (scaling coefficient); a (constant); 95%CL (95% confidence limits); SE (standard error); p (significance level)

Finally, Table 4.4 described the multiplicative allometric modelling combining body descriptors and chronological variables (CA and SA).

Table 4.4. Multiplicative allometric models (1) combining body size descriptors (X1i) and body composition variables (X2i) to explain variance on LVM.

Multiplicative allometric model [k1 · log (X1i) + k2 · log (X2i) + a + log ε]						models summary		
			coefficients					
Models	X1i	X2i	constant [a_i]	$K1_i$	$K2_i$	R	adjusted R^2	p
1	Statutre	Body mass	-0,697	0,573	0,604	0,584	0,332	<0,01
2		Fat-free mass	3,912	-0,643	1,073	0,616	0,371	<0,01
3	Sitting height	Body mass	0,592	0,341	0,631	0,579	0,326	<0,01
4		Fat-free mass	3,741	-0,722	1,102	0,619	0,375	<0,01

X1i (size descriptor); X2i (size descriptor); K1 (scaling exponent); K2 (scaling exponent); R (multiple correlation coefficient); p (significance level)

4.5. Discussion

There is a lacking of data regarding the independent and combined effects of body size variables, biological maturation and training experience on LVM in adolescent female athletes. In the present study, proportional allometric modelling revealed that body mass and FFM, are fundamental contributors to explain the inter-individual variability on Left Ventricular Mass in adolescent female soccer players.

Studies demonstrating FFM as the best predictor of cardiac dimensions are consistent (Whalley et al., 2004; Pressler et al., 2012; D'Ascenzi 2015). In a recent report it was verified that lean body mass provides a "size-free" expression for cardiac dimensions in college athletes (Giraldeau et al., 2015). Another study based in longitudinal data demonstrated that LVM increased in relation with FFM in 15-year-old female competitive distance runners (Kinoshita, Katsukawa, & Yamazaki, 2015).

Furthermore, it was reported that lean body mass explained 75% of the variance of LVM in non-athlete boys and girls aged 11.7 years (Daniels et al., 1995) meanwhile in male elite endurance athletes, FFM explained 30% (Whalley et al., 2004). Results of the current study agree with literature indicating FFM as the primary body size determinant of LVM. Female soccer players in our study showed higher mean values of Fat Free Mass Index (FFM/height^2) than a sample of female non-athletes (n=1010) of similar age (14.3±2.9 years) (Park et al., 2015). The higher values of FFM relative to stature, of our soccer players (15.8 kg/m^2 vs 13.6 kg/m^2, soccer and non-athletes, respectively) could be attributed to the stimulus that training induces specially in lower limbs muscle mass. In a recent study (Suarez-Arrones et al., 2019) the changes of body composition were monitored during the in-season training period in 18 male professional young soccer players (16.1 ± 0.8 years of age). The subjects increased 6% their Leg Fat Free Mass.

The association of more specific anthropometric variables (as thigh muscle cross-sectional area) with LVM must be analyzed in future research in order to better explain variance of LVM in female soccer players.

Contribution of biological maturation to adjust LVM has been analyzed in previous studies showing opposite results. Some concluded that maturation does not have any effect on the LVM (Daniels et al., 1995; Goble, Mosteller, Moskowitz, & Schieken, 1992; Janz, Dawson, & Mahoney, 2000), whereas others demonstrated its relevance (Valente-dos-Santos et al., 2013; Valente-Dos-Santos et al., 2015). Discrepancies between these studies are attributed to differences in the characteristics of the sample of each study, and the methods used to determine biological maturation. In a study including adolescent male athletes in which was used proportional allometric modelling it was demonstrated that skeletal age increased the impact of the prediction of LVM when it was combined with different size descriptors (Valente-dos-Santos et al., 2013).

Sitting stature is an indicator of upper body length of easily measuring that could better represent the adjusting of LVM than overall stature. However, results of the present study showed a moderate correlation between sitting height and LVM. This contrasted with data of adolescent male roller hockey players revealing sitting height as the strongest determinant of LVM (Valente-dos-Santos et al., 2013). Peak growth of leg length and trunk occurs in different periods in boys and girls causing an accentuated difference in sitting height during childhood and adolescence (Zivicnjak et al., 2003). On the other hand, the robust relationship between sitting height and LVM could be an attribute exclusively of the sample analyzed in the study referred.

Long-term training is associated with cardiac remodeling in soccer players (T. Olm, K. Baskin, 2017). Nevertheless, the influence of the period of training exposure on specific cardiac adaptations is currently being explored in adolescent athletes. A recent report following a longitudinal design showed that during a period of three years adolescent female distance runners increased its LVM and showed an enlargement on LVEDD. However biological maturation was not considered in the mentioned study, besides, there was not included a control group. Hence, the particular effect of training remained uncertain (Kinoshita et al., 2015).

It has been reported that body mass is a robust predictor of LVM (Goble et al., 1992) and it has been extensively recommended to normalize LVM in non-athlete populations (Brumback et al., 2010; Garner et al., 2000). Results of the present study support the use of body mass as a valid alternative to adjust LVM in adolescent female soccer players, especially when FFM is not available to properly normalize LVM.

A moderately chamber dilatation of left ventricle and wall thickening have been observed in adult soccer players (T. Olm, K. Baskin, 2017). A recent study including male adolescent athletes from different sport modalities reported an eccentric remodeling (Castanheira et al., 2017). Data from adolescent female athletes are rather limited and studies regarding elite female athletes indicate a LVEDD enlargement without concentric hypertrophy. Values for echocardiographic variables of the female soccer players of the present study were located between the normal ranges of reference (Lang et al., 2005). A study including highly trained female athletes indicated that LV wall thickness and chamber size did not differ from the limits of normal reference (Finocchiaro et al., 2017). Moreover, recently it was reported that 3-hour per week of training plus one match did not stimulate any increase of LVM in adolescent athletes (Valente-Dos-Santos et al., 2015). According to our results and data from literature, it can be concluded that cardiac adaptations in adolescent females, provoked by soccer training, do not exceed the range of normal values.

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Chapter V

5. Bone health among female adolescent athletes

5.1. Abstract

The aim of the study was to compare the amount of bone mass in adolescent female athletes involved in sports with different weight-bearing impact. Thirty-three soccer players (12.79 ± 1.28 years of age) and 36 swimmers (12.30±1.05 years of age) composed the sample. The Fels method was used to obtain skeletal maturity (Roche, Chumlea, & Thissen, 1988). Bone mineral density (BMD), bone mineral content (BMC), lean mass, and fat mass were measured using dual-energy X-ray absorptiometry (DEXA). Soccer players presented higher values of BMC and BMD in the pelvis and lower limbs, compared to swimmers. Skeletal age was similar between soccer players and swimmers. No significant differences were observed in lean soft tissue and fat mass between the two groups. Soccer, a weight-bearing activity with high impact load stimulus, demonstrated positive effects on BMC and BMD, potentially maximizing bone mineral mass. On the other hand, swimming, a non-weight-bearing sport, did not show significant effects on BMC and BMD.

keywords: Bone health, body composition, BMD, adolescent female soccer players, adolescent female swimmers

5.2. Introduction

Acquisition of bone mineral mass during childhood and adolescence is considered a preventive strategy for osteoporosis in later life (Ho & Kung, 2005; Rizzoli, Bianchi, Garabédian, McKay, & Moreno, 2010). Peak bone mass (PBM) refers to the age in which occurs the highest amount of bone tissue (Bonjour, Theintz, Law, Slosman, & Rizzoli, 1994). About 75% of hip fractures related to osteoporosis occur in women. About 25 percent of the total bone mass is obtained between the two years of peak bone mass (Robin M Daly & Petit, 2007). Females have a higher risk of osteoporosis than men is because they get a lower bone mass in youth (Armstrong & Mechelen, 2017).

Among females, peak bone mass tends to occur around 12 years of age (McKay, Bailey, Mirwald, Davison, & Faulkner, 1998). Therefore, the period between 10 and 14 years of age is considered critical to bone mass accrual. Although it has been established that genetic can explain about 80% of the variance in bone mass (Davies, Evans, & Gregory, 2005), physical activity is a controllable factor that can maximize bone strength (Morseth, Emaus, & Jørgensen, 2011). It is well recognized that activities that implicate high intensity loading forces effectively increase bone mass in children and adolescent (Kohrt, Bloomfield, Little, Nelson, & Yingling, 2004). Soccer is a weight-bearing activity that involves prompts of high impact load stimulus and muscular power actions (Bangsbo, Mohr, & Krustrup, 2006) that stimulate osteogenetic (Düppe, Gärdsell, Johnell, & Ornstein, 1996; Minett, Binkley, Weidauer, & Specker, 2017). Its positive effect on bone mineral density (BMD) has been considerably demonstrated (Alfredson, Nordström, & Lorentzon, 1996; Minett et al., 2017; Mohr et al., 2015). Practice of soccer during childhood and adolescence may contribute to maximize bone mineral mass and reduce the bone mass decrement that occurs in late adulthood and consequently reduce the risk fracture in dangerous sites. In contrast to soccer, swimming is a non-impact sport. Some studies show that swimming training has neither a positive nor a negative effect on BMD (Courteix et al., 1998; Hind, Gannon, Whatley, Cooke, & Truscott, 2012; Maïmoun et al., 2013), additional evidences suggests that a high level of swimming training is associated to a decrease in bone mass (Magkos et al., 2007). Moreover, some studies have demonstrated an increase of BMD on upper extremities as a response of the force applied by muscles implied on swimming action (Shaw & Stock, 2009a, 2009b). The objective of the present study was to compare the amount of bone mass in adolescent female athletes involved in sports with different weight-bearing impact (soccer and swimming)

5.3. Methods

Thirty-three soccer players (12.79 ± 1.28 years of age) and 36 swimmers (12.30±1.05 years of age) composed the sample of the current study. Participants were involved in formal training and competition for at least one year in their respective sport. All subjects self-identified as caucasian. Athletes were subjected to a radiograph exam of the left hand-wrist in order to assessing skeletal maturity, and then were submitted to a DXA exam for the measurement of bone mass and body composition. The same trained operator performed all the DXA examinations, and a certified technician was the responsible for obtaining the radiographs.

Biological maturation

The Fels method was used to obtain skeletal maturity (Roche, Chumlea, & Thissen, 1988). It requires a posterior-anterior hand-wrist radiograph; it was reading and interpreted the specific indicators for the 22 bones of hand and wrist and the ratios of the measurements of epiphyseal and metaphysical widths considered in the method. Once obtained, all the ratings and ratios were processed and computed using the Felshw 1.0 software in order to obtain the skeletal age and the standard error of estimate. The same trained observer analyzed all the radiographs.

Bone mineral density

Bone mineral density (BMD; g/cm^2), bone mineral content (BMC; g), lean mass (LST; g), and fat mass (FM; g), were obtained by dual-energy X-ray absorptiometry (DXA; Hologic QDR 4500A, Hologic Inc., Waltham, MA, USA). Bone area (cm^2), BMC (g/cm2) and BMC (g) were obtained for the Whole Body, Left and Right Arm, Left and Right Ribs, Thoracic Spine, Lumbar Spine Pelvis, Left and Right Leg. Calibrations of the equipment were performed following instructions and practical considerations of the manufacturer (Wilson, 2011). The same trained evaluator performed all the scanning exams.

Food frequency questionnaire

Daily calorie and calcium intake of soccer players and swimmers were calculated using a semi-quantitative food frequency questionnaire (Lopes 2000; Lopes et al. 2007). The questionnaire was composed food items and nine close categories of consume frequency ranging from "never or once a month" to "six or more times by day"

Analysis

Characteristics of soccer players and swimmers (chronological age, height, body mass, skeletal age) were determined using descriptive statistics (means, standard deviations). The differences in body composition and bone mass between sport groups were analyzed using pairwise comparisons including the determination of mean differences, t-test and effect size given by the ratio between mean differences and combined standard deviation. For comparisons the magnitude of the effect was interpreted as follows (Hopkins et al. 2009).: trivial (<0.200), small (0.200 to 0.599), moderate (0.600 to 1.199), large (1.200 to 1.999) and very large (2.000 to 3.999).

5.4. Results

Table 5.1 summarizes the mean values for chronological age, skeletal age, training experience, anthropometry, and food intake, comparing swimmers and soccer players. The findings indicate significant differences between the two groups in terms of age at which they started participating in their respective sports and their training experience, with soccer players commencing their training later. Furthermore, there were moderate disparities in protein and cholesterol intake, with swimmers consuming higher quantities of both nutrients. However, the remaining variables exhibited either minor or negligible differences between the groups.

Table 5.2 presents the mean values and standard deviations for body composition in swimmers and soccer players. No significant disparities were observed in lean soft mass and fat mass between the two groups.

In Table 5.3, the mean values and standard deviations of bone mineral content (BMC) and bone mineral density (BMD) in swimmers and soccer players are presented. The results demonstrate a notable discrepancy in BMC between the two groups, with soccer players exhibiting higher BMC in the pelvis and lower limbs. Additionally, a significant variation in BMD was found, with soccer players displaying greater BMD in the subhead, trunk, and lower limbs. These findings suggest that the choice of sport may influence bone health, as soccer players exhibit higher BMC and BMD in specific regions of the body compared to swimmers.

Table 5.1. Means and standard deviation by sport and test of mean differences to examine variation on training experience, chronological age, anthropometry and food intake

Dependent Variable	Unit	Swimmers (n=36)		Soccer players (n=33)		Mean difference	Comparison		Effect size	
		Mean	SD	Mean	SD		t	p	d	(qualitative)
Chronological age	years	12.3	1.05	12.79	1.28	-0.49	-1.660	0.102	-0.43	Small
Skeletal age	years	13.56	1.85	13.90	2.36	-0.34	-0.657	0.502	-0.16	Trivial
Age of sport initiation	years	6.6	2.2	8.7	2.8	-2.1	-3.539	0.001	-0.85	Large
Training experience	years	4.4	2.2	2.3	1.5	2.1	4.711	0.000	1.12	Large
Stature	cm	152.3	8.2	151.5	7.8	0.8	0.397	0.693	0.10	Trivial
Sitting height	cm	79.8	4.8	79.9	5.0	-0.1	-0.42	0.967	-0.02	Trivial
Body mass	kg	44.9	9.1	48.2	11.8	-3.3	-1.322	0.191	-0.32	Small
Daily calorie intake	kcal	2314	1203	2015	922	299	1.047	0.299	0.28	Small
Protein intake	%	20.2	3.2	17.9	3.5	2.3	2.641	0.011	0.70	Moderate
Carbohydrate intake	%	49.2	7.0	52.0	7.8	-2.8	-1.472	0.146	-0.38	Moderate
Fat intake	%	32.6	7.3	32.1	5.2	0.5	0.278	0.782	0.08	Trivial
Saturated fat	%	9.2	1.6	9.2	2.2	0	-0.092	0.927	0.00	Trivial
Monounsaturated fat	%	13.6	4.2	13.5	3.5	0.1	0.089	0.929	0.03	Trivial
Polyunsaturated fat	%	6.0	2.0	5.0	1.0	1.0	0.250	0.803	0.63	Moderate
Cholesterol intake	mg	408	260	281	121	127	2.265	0.027	0.63	Moderate
Fibre intake	g	33	25	26	16	7.0	1.319	0.192	0.34	Small
Ethanol intake	g	3.0	4.0	2.0	3.0	1.0	0.508	0.613	0.29	Small
Calcium intake	g	1069	639	840	419	229	1.688	0.097	0.43	Small

Table 5.2. Means and standard deviation by sport and test of mean differences to examine variation on body composition outputs

Dependent Variable	Unit	Swimmers (n=36)		Soccer players (n=33)		Mean difference	Comparison		Effect size	
		Mean	SD	Mean	SD		t	p	d	(qualitative)
Lean soft tissue										
Whole body	g	30726	5381	31101	5380	-375	-0.289	0.773	-0.07	Trivial
Trunk	g	14501	3198	14893	2991	-392	-0.525	0.602	-0.13	Trivial
Left upper limb	g	1526	373	1422	232	104	1.412	0.163	-0.34	Small
Right upper limb	g	1645	414	1559	275	86	1.007	0.318	0.25	Small
Left lower limb	g	5084	963	5219	1283	-135	-0.496	0.621	-0.19	Trivial
Right lower limb	g	4960	924	5291	957	-331	-1.460	0.149	-0.36	Small
Fat mass										
Whole body	g	12506	4154	14246	6592	-1740	-1.324	0.190	-0.32	Small
Trunk	g	4624	1740	5485	3293	-861	-1.373	0.174	-0.34	Small
Left upper limb	g	676	293	757	416	-81	-0.931	0.355	-0.23	Small
Right upper limb	g	705	306	782	436	-77	-0.858	0.394	-0.21	Small
Left lower limb	g	2757	906	3150	1264	-393	-1.496	0.139	-0.37	Small
Right lower limb	g	2708	928	3107	1295	-399	-1.478	0.144	-0.36	Small
Android	g	695	307	846	502	-151	-1.489	0.143	-0.37	Small
Gynoid	g	2280	801	2684	1267	-404	-1.597	0.115	-0.39	Small
Total	%	27.5	4.9	29.2	6.3	-1.7	-1.243	0.218	-0.31	Small

Table 5.3. Means and standard deviation by sport and test of mean differences to examine variation on bone mineral content (BMC) and bone mineral density (BMD)

Dependent Variable	Unit	Swimmers (n=36)		Soccer players (n=33)		Mean difference	Comparison		Effect size	
		Mean	SD	Mean	SD		t	p	d	(qualitative)
Bone mineral content										
Whole body	g	1430	320	1524	341	-94	-1.180	0.242	-0.29	Small
Subhead	g	1057	263	1161	290	-104	-1.561	0.123	-0.38	Small
Trunk	g	377	103	414	119	-37	-1.356	0.180	-0.34	Small
Pelvis	g	155	49	187	56	-32	-2.512	0.014	-0.62	Moderate
Upper limb										
Left	g	82	23	79	21	3	0.753	0.454	-0.14	Trivial
Right	g	90	24	88	23	2	0.494	0.623	0.09	Trivial
Lower limb										
Left	g	255	57	294	70	-39	-2.495	0.015	-0.62	Moderate
Right	g	249	59	285	68	-36	-2.390	0.020	-0.58	Moderate
Bone mineral density										
Whole body	g/cm^2	0.916	0.102	0.950	0.111	-0.034	-1.348	0.182	-0.32	Small
Subhead	g/cm^2	0.788	0.091	0.837	0.101	-0.049	-2.119	0.038	-0.52	Moderate
Trunk	g/cm^2	1.086	0.168	1.186	0.171	-0.1	-2.436	0.018	-0.60	Moderate
Upper limb										
Left	g/cm^2	0.613	0.065	0.591	0.061	0.022	0.145	0.885	0.35	Small
Right	g/cm^2	0.614	0.064	0.601	0.061	0.013	0.907	0.368	0.21	Small
Lower limb										
Left	g/cm^2	0.921	0.096	1.005	0.122	-0.084	-3.190	0.002	-0.78	Moderate
Right	g/cm^2	0.926	0.105	1.001	0.120	-0.075	-2.790	0.007	-0.80	Large

5.5. Discussion

Most studies that have examined bone mass in female soccer players and swimmers have been carried out in adults. The present study analyzed the potential influence of sports contrasting in mechanical impact (soccer and swimming) on the development of bone mineral density among female adolescent athletes. It was found that soccer practice offers benefits on bone tissue in specific sites while swimming training does not modify the acquisition of bone mineral content.

Several actions performed in soccer, during match and training, stimulate bone mineral accrual in particular sites (Ferry et al., 2011; Hind, Gannon, Whatley, & Cooke, 2012; Mohr et al., 2015). The overload on bone imposed by gravity acting on body mass is accentuated by impact efforts as jumps, explosive sprints and abrupt changes of direction. These activities correspond to an osteogenic effect mainly in pelvis, hip and lower limbs (Alfredson, Nordström, & Lorentzon, 1996; Minett, Binkley, Weidauer, & Specker, 2017). The current results corroborated that soccer drives a specific improve on bone strength in adolescent females. When soccer players and swimmers were compared, the former presented higher values of BMD and BMC only in pelvis and lower limbs but not in upper limbs neither spine.

Studies in female adults have reported similar results (Ferry et al., 2011; Mohr et al., 2015) with better bone health parameters by athletes from impact sports compared to non-impact sports. However, differences between adult swimmers and soccer players reported in literature were consistently more pronounced than differences found between soccer players and swimmers of the present study. The higher bone mineral accrual related with age (Weaver, 2002), the longer exposure to soccer practice and the higher-level training might explain the larger differences in adults.

Swimming is a non-weight-bearing sport that, even its positive effect on the increasing of muscle mass, apparently, does not generate improvements in bone strength. A meta-analysis and a review have been recently published (Gómez-Bruton, Gónzalez-Agüero, Gómez-Cabello, Casajús, & Vicente-Rodríguez, 2013; Lee & Kim, 2015). The studies consistently concluded that young adult swimmers present lower values of BMD than weight-bearing sports (handball, soccer, basketball and distance running) and similar values when compared with non-athletes. The data of the current study agrees with the results that suggest swimmers with lower values in whole BMD in pelvis and lower limbs than soccer players.

The only site in which swimmers showed greater mean values of BMD than soccer players were upper limbs, however the magnitude effect of this difference was small (Cohen d=0.35), in the present sample.

A study including pre-pubertal female swimmers, gymnasts and controls did not found higher values of BMC in upper limbs for swimmers, and showed similar values that controls in all sites measured (whole body, upper limbs and hip) (Courteix et al., 1998). Actually, studies that have reported greater arm BMD in swimmers when compare with other athletes have been conducted in highly trained male groups (Gómez-Bruton et al., 2013). Whether the higher arm BMD found in those swimmers was consequence of the muscle force produce during swimming action or by alternative means of training performed out of the swimming pool it was not reported.

Although adaptations in bone strength depend on the type of activity, a minimum period of exposure to training is necessary. For example, a group of 25 healthy sedentary adolescent females followed a plyometric program for 7 months. At the end of the program, BMD was compared with controls and significant differences were uniquely found in greater trochanter, failing to find higher values in other sites: femoral neck, and femoral shaft as was expected (Witzke & Snow, 1999). Another study including post-menarchal highly trained soccer players and swimmers (aged 16.2 ± 0.7 and 15.9 ± 2.0, respectively) followed an intensive training in their respective sports during eight months. It was possible to report improvements of BMD only in the soccer group (2.93% in whole BMD) (Ferry et al., 2011).

According to present results, adolescent females can obtain benefits on bone strength after about 2.3 years of soccer training exposure. In contrast, 4.4 years of swimming training does not seem to induce improvements in bone mineral density. Since our data are cross-sectional it is not possible to affirm whether higher values of BMD in soccer players is the result of a progressive increase of bone mineral in response to soccer training or whether it has been attained a plateau that has been maintained. Further research based on longitudinal data is necessary to understand the comportment of gain of mineral content and its relationship with training.

Puberty is characterized by a high rate of bone mineral accrual (Rizzoli, Bianchi, Garabédian, McKay, & Moreno, 2010; Weaver, 2002). During this period, it could be more difficult to differentiate the gain of bone mineral obtained by physical exercise from that inherent in the natural process of biological maturation. Jackowski et al. (2011) examined longitudinal data aged 15 years at a baseline and found a strong relationship between maturity status and BMD. Late mature girls presented a lower rate of bone mineral accrual than their on-time and early mature counterparts. Skeletal age of soccer players in our study was greater than chronological age (SA-CA= 1.2) suggesting a trend to be advanced in biological maturation. The specific impact that maturation had on BMC and BMD of the soccer players of the present study was not analyzed. However, since swimmers presented a similar advance in skeletal age, the higher values of the soccer players can be attributed to training. Further research is necessary to elucidate the precise contribution of biological maturation on BMD in female soccer players and swimmers.

Results of the current study confirm that soccer has a positive influence to BMD, especially in pelvis and in lower limbs. These findings agree with previous data in literature comparing adult athletes from swimming and soccer. Future studies may need to examine maturity-associated variation in bone health parameters within each particular sports or controlling for differences due to type of sports.

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Part III

GENERAL DISCUSSION AND CONCLUSIONS

Chapter VI

6. General discussion and conclusions

This section presents a summary of the main findings and practical applications derived from three studies in this thesis. It investigates growth and maturation among female soccer players and tested the hypothesis of maturity-associated selection in adolescent female soccer. Additionally, it explores the feasibility of establishing women's soccer categories using skeletal age as a criterion. Furthermore, it analyzes body composition in adolescent female soccer players and emphasizes the significance of considering biological maturation, body mass, fat-free mass by employing proportional allometric models when evaluating exercise-induced cardiac remodeling in young female soccer players.

6.1. Biological maturation bias in the selection of female soccer players

It has been observed that in male soccer players, the similarity in skeletal age is notable among players aged 10 to 12 years. However, starting from the ages of 13-14, a higher proportion of players are classified as "early maturers" (Bidaurrazaga-Letona et al., 2019; Malina et al., 2010). This indicates a tendency to select players who are more advanced in biological maturation after the age of 13-14 (Lovell et al., 2015). In contrast, Study 1 of the present thesis revealed a significant number of female soccer players classified as "early mature" or "already mature" in all age groups, starting from the age of 12-13. This suggests that the bias of selecting early-maturing players is present at an earlier age in female soccer players.

The evidence presented in the conducted studies emphasizes the need for a more rigorous recruitment of female soccer players. The results reveal a potential bias based on biological maturation in the selection process, which can have negative consequences on both athletic performance and the incidence of sports injuries (Johnson et al., 2009). Moreover, this bias affects the promotion of equal opportunities in sports. Therefore, it is crucial to implement specific strategies that improve the selection process of female soccer players and foster a fair and equitable selection based on their sporting abilities, rather than their biological maturity. To rectify this situation, it is essential to establish clear and objective criteria for assessing sporting skills and physical capabilities in the selection process, including the evaluation of biological maturation, to ensure a fair and equitable selection. It is crucial to recognize that early biological maturation does not necessarily reflect a player's talent or potential and that all players deserve equal opportunities, regardless of their biological maturity status (Cumming, Brown, et al., 2018; Cumming et al., 2017; Malina et al., 2015).

6.2. Categorization in female soccer based on skeletal maturation

The results of the first study in this thesis demonstrated that the majority of female football players achieved skeletal maturity at the age of 16 years, with all players reaching it by the age of 17 years. These findings provide a scientific basis for considering a possible revision of the current regulations for women's football in Portugal regarding the structuring of categories. At present, due to a low participation, in women's football, there are only two age-categories ("junior" and "senior"). This situation compels players to decide to incorporate in training and competitions of men's football, where various age-based categories such as "youth," "under-15," and "under-17" are established. As an alternative, some female players request an early promotion to the "senior" category to remain in women's competition. However, in light of the results of the present study on skeletal maturation, it is possible to consider that young female footballers could be eligible to play in the "senior" category at the age of 17 years instead of the current age of 19 years. Enabling early access to the "senior" category would offer young players the opportunity to continue competing in a suitable environment, alongside more experienced players. This, in turn, can facilitate their skill development (Cumming, Searle, et al., 2018) and potentially reduce the likelihood of player dropouts.

During the growth spurt, which occurs around the age of 12 years in girls and 14 years in boys, notable changes in body composition occur, highlighting distinct patterns between genders. Boys tend to experience an increment in muscle mass, while girls tend to exhibit an increase in body fat (Malina & Bouchard, 2004). Related to disparities in body composition, variations in physical capacities such as strength, speed, and aerobic endurance influence soccer performance (Toro-Román et al., 2023a). In the study 1 of the present thesis, the majority of female soccer players in all three age categories, including the youngest, were classified as "early maturing" or "already matured"; the skeletal age of the youngest group (12-13 years) was 14.92 ± 1.40 years. These findings emphasize that disparities in physical characteristics between male and female soccer players become apparent from an early stage of their involvement in the sport. They lend support to the recommendation of introducing female categories, such as "youth" and "junior," at younger ages in women's soccer to address the biological disparities that arise during puberty between boys and girls.

6.3. The Influence of Biological Maturation on Position Allocation in Youth Female Soccer

The results of the study 1 of the present thesis suggest that biological maturation influences position allocation differently in female soccer players compared to male players. Although in female players of the present study skeletal age was similar across positions in all age groups, a high proportion of players were classified as early or advanced in maturation, suggesting a general bias in player selection. It is important to note that in the present study, central and lateral defenders positions were included in the same group as defenders. Previous research has found that in young male soccer players, the bias related to biological maturation is greater in central defenders especially in the early categories (Sweeney et al., 2023; Towlson et al., 2017a). This is because more advanced players in their maturation tend to have greater height and physique, which gives them advantages in key defensive situations such as aerial ball disputes and strength in body-to-body contact (Slimani & Nikolaidis, 2019). However, as categories progress, differences in biological maturation appear to decrease between playing positions; in general, there is a tendency for all positions to be classified as "early maturers." The increase in the proportion of "early maturers" in the more advanced categories can be explained by two main factors. On the one hand, it is possible that there is a "catch-up" by players who had presented a less advanced state of maturation in previous categories. And on the other hand, the selection process that tends to favor biologically more mature players translates into a greater preference for them in all positions as they advance in category (Towlson et al., 2017b). Considering that the findings from study 1 of this thesis indicate a significant proportion of early maturing female soccer players from the age of 12 years, it is imperative to recognize the potential biases in position allocation during the early stages of sports participation. Specifically, avoiding the common practice of assigning biologically more mature players to defensive roles, as observed in male players. In future studies, it will be important to analyze whether this bias occurs in early stages of development in female soccer players, and to distinguish between central and lateral defender positions.

6.4. Body Fat of Female Adolescent Soccer Players

In Study 1, it was found that female soccer players across all three age groups had average heights that were close to the 50th percentile reference for the United States (Center for Disease Control and Prevention, 2000). On the other hand, mean values of body mass were placed around the 75th percentile for the 12-13 and 14-15 age groups, and between the 50th and 75th percentiles for the 16-17 age group.

The high values of body mass could be attributed to increased adiposity among the players, which may be partly influenced by biological maturation. In girls, hormonal change, that occurs during puberty (especially in gonadal steroids), is associated with an increment in body fat mass. In the Study 1 of the present thesis, the youngest group (12-13 years) presented a skeletal age of 14.92 ± 1.40 years, indicating that the players were advanced in biological maturity and possibly they were already in a phase of body fat increment.

Results of study 1 of this thesis showed that body fat percentage, estimated using the equation proposed by Slaughter et al (1988), was approximately 25% across all three age groups (24.5%, 24%, and 26.9% for the 12-13, 14-15, and 16-17 age groups, respectively). Study 3 of this thesis, which involved a distinct sample of Portuguese female soccer players (n=33; chronological age=12.79±1.28 years; skeletal age=13.90±2.36), confirmed the presence of elevated body fat. Body composition was measured by DEXA and yielded a Percent Body Fat of 29.2%.

Additionally, it was observed that, on average, the players consumed 2,015 ± 922 kcal per day, approaching the upper recommended limit of 1,800-2,200 kcal for recreational soccer players of the same age and sex (Nhlbi, 2023). Results of this study underscore the importance of individualized analysis, as soccer players exhibited significant inter-variability in both body fat levels and daily calorie intake. The presence of high body fat levels can have detrimental effects on athletic performance by impairing agility, speed, and mobility (Stanković et al., 2023; Toro-Román et al., 2023b; Toselli et al., 2022). Moreover, the additional weight resulting from increased adiposity increases the susceptibility to injuries (Domaradzki & Koźlenia, 2022; Toomey et al., 2017). Therefore, it is crucial for future research to concentrate on developing strategies that foster healthy body composition and promote proper nutritional habits among adolescent female soccer players.

6.5. Considerations for Left Ventricular Mass Assessment and Adjustment in Female Soccer Players

During childhood and adolescence, the heart undergoes morphological changes as part of growth, including adaptations in size, increased muscle mass, and development of cardiac cavities (Pluim et al., 2000). Sport training has been confirmed to induce structural adaptations in the heart of adolescent athletes (Castanheira et al., 2014). Therefore, accurate interpretation of cardiac structure variables is crucial, considering body size and using appropriate adjustment methods to differentiate between training-induced changes and those associated with biological development in young athletes (Nevill et al., 2005). Results from the study 2 support the use of allometric models as an appropriate option for nullifying the influence of body size when interpreting left ventricular mass (LVM) in adolescent athletes.

Despite the common utilization of height for adjusting Left Ventricular Mass (LVM) in children and young individuals (De Simone et al., 1992), it is not the optimal variable for such adjustment, particularly in athletes. In study 2 of this thesis, a moderate correlation was observed between LVM and height among adolescent female soccer players ($r = 0.434$). These findings align with those of a study encompassing both young athletes and non-athletes which reported a moderate correlation between LVM and height (Castanheira et al., 2014).

The allometric analysis in study 2 of this thesis revealed a height exponent of 1.9, differing from the commonly recommended "universal" coefficient of 2.7 for LVM adjustment (De Simone et al., 1992). The exponent of 2.7 was derived from a diverse sample without cardiac pathologies, encompassing various ages, body mass index ranges, and ethnic groups (Foster et al., 2008, 2013). However, the notion of a "universal exponent" has faced criticism from multiple authors. Notably, it has been demonstrated that the influence of body size remains significant, as normalized LVM values exhibit a negative correlation with height (Krysztofiak et al., 2019). The exponent of 1.9 obtained in study 2 of this thesis aligns more closely with exponents derived from specific samples. For example, a study found an exponent of 2.3 in a group of 791 children and adolescents, which proved to be more useful for predicting cardiovascular risk compared to exponents derived from more general samples combining children and adults (De Simone et al., 1995). In another study, sex-specific allometric exponents were generated, resulting in an exponent of 1.7, which demonstrated a more consistent adjustment and eliminated the effect of body size (height) without inverting the relationship between adjusted values and height values, unlike the "universal exponent" of k=2.7 (Chirinos et al., 2010).

In addition to height, several other factors, such as duration of exposure to training, body composition, and biological maturation, can influence cardiac dimensions in adolescent athletes (D'Ascenzi et al., 2017; Manolas et al., 2001; Miličmilič Evic et al., 1997; Pelà et al., 2015). Incorporating Fat-Free Mass (FFM) into scaling methods to explain variability in Left Ventricular Mass (LVM) in young athletes is crucial. FFM represents highly metabolically active tissue, and during physical exercise, both skeletal muscles and myocardium are stimulated, resulting in corresponding adaptations between these tissues as training progresses. The study 2 in this thesis revealed a robust correlation between FFM and LVM ($r = 0.658$, $p<0,01$) These results are consistent with those reported in previous studies (Whalley et al., 2004; Pressler et al., 2012; D'Ascenzi 2015). For example, a recent report verified that lean body mass offers a "size-free" representation of cardiac dimensions in college athletes (Giraldeau et al., 2015). Additionally, a longitudinal study demonstrated that left ventricular mass (LVM) increased in relation to fat-free mass (FFM) in 15-year-old female competitive distance runners (Kinoshita, Katsukawa, & Yamazaki, 2015).

Relying only on a single body size variable for adjusting LVM may be insufficient in adolescent athletes. Multiplicative allometric models incorporate multiple variables, account for heterogeneity in variable distribution, and offer biological plausibility (Nevill et al., 2005; Welsman & Armstrong, 2007). In the study 2 of this thesis, multiplicative models were used to explore linear indicators (height, sitting height) and three-dimensional indicators (FFM, weight). The model including sitting height and FFM exhibited the highest explanatory power for LVM variance ($r^2=0.375$). Prior studies on non-athlete adolescent males (Valente-Dos-Santos et al., 2014) and male adolescent hockey players (Valente-dos-Santos et al., 2013) have similarly highlighted the superior predictive capacity of combining linear indicators (height, sitting height) with three-dimensional indicators (FFM, body mass) for LVM.

The appropriate analysis of left ventricular mass (LVM) in adolescent athletes requires the careful consideration of both biological maturation and training experience. During the period of puberty, growth and maturation have a profound impact on body mass and composition (Siervogel et al., 2003), subsequently affecting LVM (Dai et al., 2009; Sr et al., 1995). Furthermore, the physical training undertaken by athletes elicits cardiac remodeling responses, the extent of which depends on the duration of exposure (training experience) and exercise intensity (Weiner et al., 2015). Thus, the inclusion of biological maturation and training experience becomes essential for appropriately adjusting LVM. Future research must prioritize the integration of these factors to ensure the elimination of size-related confounders in the analysis of LVM of female soccer players.

6.6. Bone Mineral Density in Female Adolescent Soccer Players and Swimmers.

Study 3 of this thesis focused on analyzing bone mineral density (BMD) in adolescent athletes engaged in impact and non-impact sports. The objective was to compare the BMD in adolescent female soccer players and swimmers. The results revealed significant differences in BMD between the two groups, with soccer players showing higher values in the pelvis and lower extremities. The findings indicated that adolescent females can benefit from increased bone strength after approximately 2.3 years of soccer training exposure. In contrast, 4.4 years of swimming training does not appear to induce improvements in bone mineral density.

These findings are consistent with prior research indicating that impact sports, like soccer, have a positive effect on bone mineral density compared to non-impact sports (Gregov & Šalaj, n.d.; Lozano-Berges et al., 2018; Maillane-Vanegas et al., 2020; Naughton et al., 2017). The mechanical loading and muscular force exerted during soccer activities appear to stimulate bone formation, whereas activities like swimming do not have a substantial effect on bone mass in adolescent females.

6.7. Conclusions

Fairer recruitment and selection practices are needed in women's soccer to address biases related to biological maturation. Implementing strategies that prioritize sporting abilities over biological maturity is crucial for equitable selection and equal opportunities for all players. Furthermore, revising categorization regulations in women's soccer to consider skeletal maturation and granting early access to the "senior" category at the age of 17 instead of 19 may support player development and reduce dropouts.

The presence of high body fat levels among female soccer players emphasizes the importance of individualized analysis. Significant inter-variability in body fat levels and daily calorie intake highlights the need for personalized approaches to optimize health and performance, and avoid injuries.

Accurate interpretation of cardiac structure variables in adolescent female soccer players requires appropriate adjustment methods considering body size, including factors such as fat-free mass, training experience and biological maturation. Incorporating multiple variables and utilizing multiplicative allometric models provide a more comprehensive approach to scaling left ventricular mass in adolescent female soccer players.

Practicing soccer has a positive influence on BMD, especially in pelvis and lower limbs of female adolescent players.

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