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UNIVERSIDADE D COIMBRA

Daniela Craveiro da Costa

GROWTH, MATURATION, FUNCTIONAL BODY COMPOSITION, ISOKINETIC STRENGTH AND BONE HEALTH:

MULTILEVEL MODELLING OF DEVELOPMENTAL CHANGES IN MALE ADOLESCENT ATHLETES CONTRASTING IN MECHANICAL LOADING

PhD Thesis of the Doctorate Program in Sport Sciences, Branch of Sport Training, supervised by Professors Manuel J. Coelho-e-Silva, João Valente-dos-Santos, and Daniel Courteix, and submitted to the Faculty of Sport Sciences and Physical Education of the University of Coimbra.

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“*Out of clutter, find Simplicity.*

From discord, find Harmony.

In the middle of difficulty lies Opportunity.”

Albert Einstein

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Abstract

The present thesis aimed to examine the independent and combined effects of growth, maturation, and training using a mixed-longitudinal design, initially, on developmental changes related to muscle strength and, on parameters of bone tissue in male young athletes that are involved in sports characterized by different mechanical loading on the skeleton. The thesis was organized into six chapters. Firstly, chapter I introduced a brief literature review and the main concepts regarding growth and maturation, bone health, and muscle strength in youth male athletes. Chapter II presented a detailed description of the study design, participants, procedures, and analysis performed for the two studies presented in chapters IV and V. The specificity of the methods section for a systematic review was well described in chapter III, which presented a manuscript that identified and dissertated forty-four articles. This study merged different methods that were already used in the literature to describe and quantify bone content, density, bone biochemical markers, hormonal profile, bone geometry, and bone stiffness among adolescent male athletes. This systematic review highlighted new technologies and approaches to access bone that continue to differentiate adolescent athletes by sports. In order to better understand the existence of the learning effect on the protocol used to assess the isokinetic strength of the knee flexors and extensors on different contractions in the present thesis, chapter IV presented an analytical study on the strength assessment protocol. The study presented the gradient of best peak torque for 38 adolescent swimmers with higher values and an intra-class correlation coefficient for the knee extensors when compared with knee flexors that presented the lowest values for intra-class correlation coefficient on concentric contractions. The chapter V of the thesis presented a developmental study to examine the effect of sport over a 12-month period among male adolescent soccer players and swimmers on body size, body composition, and indicators of bone health. Swimmers showed greater training volume during the study period and higher values for lean soft tissue. Regarding areal bone mineral density, soccer players were characterized by significantly higher in the lower limbs. Of interest, mean values did not differ at upper limbs or trunk between groups but regarding proximal femur, soccer players showed consistently higher areal bone mineral density than swimmers. Finally, the last chapter discussed and summarized the main findings of each study and, suggested future research, practical implications, and, conclusions. The main findings and contributions of the present thesis were that: *i)* The adult skeleton reflects the background of each individual and, to predict and prevent future fragilities, variables such as the content, density, bone-specific biochemical markers, stiffness, and, geometry should be combined when studying youth population; *ii) i*sokinetic dynamometer protocols used to access the peak torque on concentric and eccentric actions in the pediatric population must include at least five repetitions per test, knowing that knee flexors and eccentric repetitions are not easy reliable, especially in young participants; *iii)* federated sports with higher mechanical loading produce greater results on areal bone mineral density (mostly at specific and relevant clinical sites) than the ones with lower mechanical loading during the crucial years of adolescence. Training variables such as the time spent on higher impact loading exercises per session may help technicians to control and help to improve athletes bone health parameters.

Keywords: bone health; muscle strength; developmental changes; adolescent athletes; youth training

Resumo

A presente tese teve como objetivo examinar os efeitos independentes e combinados do crescimento, maturação e treino recorrendo a um desenho longitudinal misto, inicialmente, dedicado ao desenvolvimento de parâmetros de tecido ósseo em adolescentes masculinos de modalidades desportivas contrastantes em termos de impacto mecânico. Após uma breve apresentação do problema apoiada na literatura pediátrica em ciências do desporto, atinente ao escopo da pesquisa, é apresentada de forma detalhada as opções metodológicas da investigação. Seguiu-se uma revisão sistemática da literatura dedicada aos jovens atletas pubertários, com especial atenção á compreensão dos marcadores bioquímicos e hormonais que concorrem para o desenvolvimento dos diversos parâmetros de saúde óssea em que se inclui o conteúdo, densidade, geometria e rigidez. A revisão sistemática abordou criticamente as tecnologias disponíveis para avaliação do tecido ósseo. Alinhado com estudos precedentes do mesmo projeto de investigação conexo à presente tese, foi desenvolvido um estudo analítico que pretendeu quantificar e discutir os efeitos de aprendizagem inerentes ao protocolo de avaliação força da musculatura do joelho por dinamometria isocinética, conforme opções do estudo longitudinal. Assim, com base em medidas repetidas de 38 nadadores, foi possível reportar a variação intra-individual para cada uma das formas de manifestação (concêntrica e excêntrica) dos momento de força máximo dos extensores e flexores do joelho. Finalmente, foi examinada a variabilidade inter-individual e das trajetórias intra-individuais por um período de 12 meses em dois grupos de pubertários masculinos envolvidos na prática de futebol e natação, tendo como variáveis dependentes medidas de composição corporal e densidade mineral no corpo todo e em regiões de interesse. Os futebolistas, apesar de treinarem menos, apresentaram consistentemente valores significativamente superiores nas medidas de densidade mineral extraídas dos membros inferiores, incluindo região proximal do fémur, não tendo sido observadas diferenças nem para os membros superiores nem para o tronco. Por fim, o último capítulo discutiu e resumiu os principais resultados de cada estudo, sugerindo pistas para futuras investigações, implicações práticas e conclusivas, a saber: o esqueleto reflete as opções de estilo de vida e, de forma a prever e prevenir fragilidades na população de idade avançada, torna-se fundamental, prosseguir com estudos de maior duração, abordando um conjunto mais vasto de variáveis, como por exemplo marcadores bioquímicos específicos do osso que deve ser progressivamente avaliado com base em medidas de rigidez e geometrianuma perspectiva desenvolvimentista, isto é, longitudinal; o protocolo de dinamometria isocinética utilizados para avaliar o momento máximo de força da musculatura do joelho, nas contrações concêntricas e excêntricas, pelo menos na população pediátrica, recomendam não menos de cinco repetições, tendo-se evidenciado menor estabilidade dos desempenhos intra-individuais nos flexores de joelho, especialmente na contração excêntrica; para além de benefícios de formação geral, o desporto federado em modalidades com maior carga mecânica concorre para maximizar os efeitos do salto de crescimento pubertário no que diz respeito ao incremento da densidade óssea, sendo necessário explorar o contributo de parâmetros de treino e competição, em minutos, sessões, intensidade, conjuntamente com medidas de atividade física habitual, nutrição, sono para desenhar programas de desporto infanto-juvenil dirigido para atletas em percurso com expectativas de alta competição, ou simplesmente de participação desportiva num contexto organizado, competitivo e supervisionado.

Palavras-chave: *saúde óssea; força muscular; alterações do desenvolvimento; atletas adolescentes; treino infanto-juvenil*

Resumé

La présente thèse visait à examiner les effets indépendants et combinés de la croissance, de la maturation et de l'entraînement en utilisant une technique d'analyse longitudinale mixte, sur les changements développementaux liés à la force musculaire et sur les paramètres du tissu osseux chez les jeunes athlètes masculins impliqués dans des sports. Produisant des sollicitations mécaniques différentes sur le squelette. La thèse était organisée en six chapitres. Le chapitre I a présenté une brève revue de la littérature et les principaux concepts concernant la croissance et la maturation, la santé osseuse et la force musculaire chez les jeunes athlètes de sexe masculins. Le chapitre II a présenté une description détaillée de la conception de l'étude, des participants, des procédures et de l'analyse effectuée pour les deux études présentées dans les chapitres IV et V. La spécificité des méthodes pour une revue systématique a été décrite dans le chapitre III, qui a présenté une analyse basée sur quarante-quatre articles. Cette étude a fusionné différentes méthodes déjà utilisées dans la littérature pour décrire et quantifier le contenu osseux, la densité, les marqueurs biochimiques osseux, le profil hormonal, la géométrie osseuse et la rigidité osseuse chez les athlètes masculins adolescents. Cette revue systématique a mis en évidence de nouvelles technologies et approches pour accéder à l'os qui continuent de différencier les athlètes adolescents par sport. Afin de mieux comprendre l'effet de l'apprentissage sur le protocole utilisé pour évaluer la force isocinétique des fléchisseurs et extenseurs du genou sur différentes contractions, le chapitre IV a présenté une étude analytique sur le protocole d'évaluation de la force. Le gradient du meilleur couple de pointe chez 38 nageurs adolescents avait des valeurs plus élevées et un coefficient de corrélation intra-classe pour les extenseurs du genou par rapport aux fléchisseurs du genou qui présentaient les valeurs les plus basses comparées aux contractions concentriques. Le chapitre V de la thèse a présenté une étude longitudinale pour examiner l'effet du sport sur une période de 12 mois chez des joueurs de football et des nageurs adolescents masculins sur la taille corporelle, la composition corporelle et les indicateurs de la santé osseuse. Les nageurs ont montré un plus grand volume d'entraînement au cours de la période d'étude et des valeurs plus élevées pour les tissus maigres. En ce qui concerne la densité minérale osseuse, les footballeurs se caractérisaient par une densité minérale osseuse significativement plus élevée au niveau des membres inférieurs. Fait intéressant, les valeurs moyennes ne différaient pas au niveau des membres supérieurs ou du tronc entre les groupes, mais en ce qui concerne le fémur proximal, les joueurs de football présentaient une densité minérale osseuse surfacique systématiquement plus élevée que les nageurs.

Enfin, le dernier chapitre a discuté et résumé les principaux résultats de chaque étude et a suggéré des recherches futures, des implications pratiques et des conclusions. Les principales conclusions et contributions de la présente thèse étaient que : i) Le squelette adulte reflète le contexte de chaque individu et, pour prédire et prévenir les fragilités futures, les variables osseuses doivent être combinées lors de l'étude d'une population jeune ; ii) les protocoles de dynamomètre isocinétique utilisés pour accéder au couple maximal sur les actions concentriques et excentriques dans la population pédiatrique doivent inclure au moins cinq répétitions par test, sachant que les fléchisseurs du genou et les répétitions excentriques ne sont pas facilement fiables, en particulier chez les jeunes participants; iii) les sports avec une charge mécanique plus élevée produisent de meilleurs résultats sur la densité minérale osseuse (principalement sur des sites cliniques spécifiques et pertinents) que ceux avec une charge mécanique plus faible *pendant les années cruciales de l'adolescence. Les variables d'entraînement telles que le temps passé sur des exercices à impact plus élevé par session peuvent aider les techniciens à contrôler et à améliorer les paramètres de santé osseuse des athlètes.*

Mots clés: *santé osseuse; force musculaire; changements développementaux; athlètes adolescents; entraînement des jeunes*

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List of Abbreviations

aBMD	areal bone mineral density
ADP	air-displacement plethysmography
ANOVA	analysis of variance
APHV	age at peak height velocity
BAP	bone-specific alkaline phosphatase
BIA	bioimpedance
BMC	bone mineral content
BMD	bone mineral density
BMDCS	bone mineral density in childhood study
CA	chronological age
cc	Concentric
CRF	cardiorespiratory fitness
CSA	cross-sectional area
CSMI	cross-sectional moment of inertia
CTX-1	C-terminal propeptide of type 1 procollagen
CTX-MMP	carboxyterminal cross-linked telopeptide of type 1 collagen
CV	coefficient of variation
DPD	deoxypyridinoline
DPD/Cr	deoxypyridinoline crosslinks to creatine ratio
DXA	dual-energy x-ray absorptiometry
ecc	eccentric
FFM-BIA	fat-free mass retrieved from bioimpedance
FFQ	food frequency questionnaire
FM-BIA	fat mass retrieved from bioimpedance
GH	growth hormone
HELP	collagen I alpha 1 helicoidal peptide
HSA	hip structural analysis
Hyp	hydroxyproline
ICC	intra-class correlation coefficient
IGF-1	insulin-like growth factor 1
IGFBP-3	insulin-like growth factor binding protein-3

KE	knee extensors
KEcc	knee extensors concentric
KEecc	knee extensors eccentric
KF	knee flexors
KFcc	knee flexors concentric
KFecc	knee flexors eccentric
LS	lumbar spine
LST	lean soft tissue
MRI	magnetic resonance imaging
MVPA	moderate to vigorous physical activity
NHLBI	National Heart, Lung, and Blood Institute
NTX-1	aminoterminal cross-linked telopeptide of type 1 collagen
OC	osteocalcin
P1NP	N-terminal propeptide of type 1 procollagen
PF	proximal femur
pQCT	peripheral quantitative computed tomography
PT	peak torque
PYD	pyridinoline
QCT	quantitative computed tomography
QUS	quantitative ultrasound
SA	skeletal age
SEE	standard error of estimate
SOS	speed of sound
T	torque
TBS	trabecular bone score
TOYA	training of youth athletes
Z	section modulus
βCTx	β-crosslaps

Chapter I

INTRODUCTION AND BRIEF REVIEW OF THE LITERATURE

1. Introduction and brief review of the literature

1.1. Background and concepts

Sports is perhaps the main form of physical activity during youth. It positively affects the health of the athletes (Malina et al., 2004; Armstrong & van Mechelen, 2017). Nowadays, federated and organized sports are part of the daily life of most of the young worldwide, and every sport is characterized by different and specific modalities. This implies the need for a constant search to increase knowledge on each sports practice and the influence it could have on the participants.

Depending on their modalities, sports have different influences on body composition and functional capacities. And although cross-sectional studies present some facilities on the procedures, such as the time for collecting data and participant recruitment, only a longitudinal study measuring the same participants through a period in different time measurements can provide precise information on the changes that occur during growth in an individual (Baxter-Jones, 2017). This can be obtained through longitudinal or mixed-longitudinal studies (Malina et al., 2004) that can observe these changes or differences using repeated observations of the same participants over time.

1.2. Growth and maturation among young male athletes

Regarding the age groups in the postnatal period, three to four groups can be defined: infancy, early childhood, middle childhood, and adolescence. The first year of life represents infancy, with the rapid growth of the human systems and dimensions. Childhood begins on the first birthday and can be divided into early childhood until the 4-5 years of age and middle childhood from there to the beginning of adolescence, this latter period is characterized by a decreased rate of growth and maturation. Adolescence presents a more difficult timing to define but usually, in males, occurs from 10 years and can continue until 22 years of age (Malina et al., 2004). This period is widely described in the literature once is a determinant of individual adult life characteristics.

During this age group changes, three main processes occur (growth, maturation, and development). Though growth and maturation are strictly related, they are two separated concepts. Growth refers to the size (dimensions), proportions, physique, composition, and systemic. Maturation refers to timing and tempo, a process that occurs during growth that can be assessed through different parameters such as: skeletal, sexual, somatic, dental, or neuromuscular (Malina et al., 2004). Timing represents the moment in the chronological age when a specific maturation event occurs (Malina, 2017). Tempo represents the time of transitions from one stage to another and its rate (Malina, 2017). Body mass (kg) and stature (cm) are the two most common variables for assessing growth status. On the other hand, maturity status assessment is more complex and it can be assessed by different methods, such as age at peak height velocity (APHV), age at menarche, sexual maturation, and skeletal age (Armstrong, 2019). Development divides into two pathways, biological and behavioral. Biological development refers to cells, tissues, organs, and functional unit development since the prenatal period and continues during postnatal life. The second pathway is influenced by the environment where the individual grows (Malina et al., 2004).

Regular and specific practice with variance in intensity and duration refers to training, which is not the same as physical activity (Malina et al., 2004). A mixed longitudinal study aimed to examine the effects of growth, maturation, and training during circumpubertal years among athletes from four different sports: tennis, soccer, gymnasts, and swimming (Baxter-Jones et al., 1995). The study sample included 231 male athletes that were assessed for 3 consecutive years. Participants were assessed by five cohorts in different stages (ages ranging from 8 to 19 years) allowing the authors to study an 11-year window in growth and maturation. This study concluded that participants from sports that demand larger physical components are usually early matured (i.e. swimming and tennis) when compared with the ones that did not (i.e. gymnastics). Nonetheless, the authors did not conclude that these differences were caused by training and suggested that they can be derived from sports specificities in recruitment. This study was part of the training of youth athletes (TOYA) project, which assessed different sports to discuss their practice influence on growth (Baxter-Jones et al., 1995), injuries (Maffulli et al., 2005), aerobic performance (Baxter-Jones et al., 1993)., and muscle strength (Maffulli et al., 1994).

More recently, the Coimbra Longitudinal Study assessed young male soccer players (10-18 years). By using skeletal age to control their maturity status, the same research group observed the intra-individual development trajectories on field tests (Valente-dos-Santos et al., 2012a). This study allowed the creation of models for developmental changes as a scientific output. Developmental models were obtained for repeated sprint ability (Valente-dos-Santos et al., 2012b; Valente-dos-Santos, 2012c), agility, and dribbling speed (Valente-dos-Santos et al., 2014a; Valente-dos-Santos et al., 2014b) and aerobic endurance given by a 20-m multistage protocol (Valente-dos-Santos et al., 2012d).

1.3. Bone development in young male athletes

Bone is a dynamic connective, vital and active tissue that modifies in shape, content, and architecture and that is continuously passing for a biological process called remodelling in which bone formation and resorption occur (Kemper & Fernandes, 2017; Weaver & Peacook, 2019). The skeleton suffers constant changes across the life span with functions that comprise mechanical support and protection, mineral homeostasis hematopoiesis, and endocrine (Burr, 2019).

During youth, the bone changes through three different processes: growth, modelling, and remodelling (Faulkner & Bailey, 2007). Bone growth starts during the prenatal period and, for most bones, continues until the late teens and early twenties. Bone modelling can occur on different preexisting bone surfaces, independently, and at different skeletal sites, having as aim major changes in bone geometry (Kenkre & Bassett, 2018; Allen & Burr, 2019). This process refers to the activation or inhibition of bone cells: osteoblast for bone formation, and osteoclast for bone resorption. Bone remodelling differentiates from modelling because it requires a mediated cell activation (Allen & Burr, 2019) where these two processes by osteoclast and osteoblast are highly connected in space and tempo, allowing the bone volume and structure to maintain (Kenkre & Bassett, 2018).

In 1870, Julius Wolff hypothesized that based on the mechanical stress implied on the skeleton, it should be possible to use mathematical rules, to predict the bone structure (Wolff, 1986). Wolff's Law refers to a negative feedback system that debuts when stress is implied, activating the bone remodeling process to adapt to the strain, thusly increasing or decreasing bone mass (Robling et al., 2019). Meantime, during the late 1980s, the Mechanostat theory was designed by Frost, declaring that skeletal responses are 'error driven' and depend on strain magnitude. Four different adaptation windows were defined by bone cell activation or inhibition. Later, Duncan & Turner (1995) defined mechanotransduction as the cellular response to a biophysical force that occurs in a living organism. When this process occurs on bone, it starts with the reception of the mechanical signal by a sensor cell. Then, it turns into a biochemical signal that allows the transmission to the effector cell, endings with a final tissue response (Duncan & Turner, 1995). Based on these well-described processes between the moment when the mechanical loading occurs and the bone response, it is stated that weight-bearing activities during childhood and adolescence can increase bone mass and mineral accrual and that these age groups are crucial to maximizing peak bone mass (Robling et al., 2019).

Osteoporosis, even nowadays, continues to show an important rate of morbidity and mortality (Kralick & Zemel, 2020). Childhood and adolescence are well described in the literature as key periods to predict and delay the inception of this disease in adult life (Sherar & Cumming, 2017). The reason lays on the fact that it is during growth and development that bone achieves higher rates of bone acquisition in mass, resistance, and dimensions (Karlick & Zemel, 2020). These two first decades of life show a great window for bone mass accrual completing 85-90% of the adult bone mass (Johnell & Kanis, 2006).

Dual-energy x-ray absorptiometry (DXA) is the most common method to assess bone health and control the risk for fractures or osteoporosis later in life (Bonjour, Chevalley et al., 2012). In the pediatric population, when a bone mineral density (BMD) T-score is < -2.0 standard deviations and exists at least one fracture history, osteoporosis can be assumed (Guss, McAllister, & Gordon, 2020). BMD and bone mineral content (BMC) are the two most reported variables when using DXA to assess bone mass and resistance during childhood and adolescence, mainly because it shows low exposure to radiation, facilities in using the equipment, and the duration of an assessment, plus its reproducibility in different skeletal sites (Bonjour et al., 2012). This BMD is not a volumetric parameter, it represents the quantity of mineral and bone dimensions (g/cm^2), and when measured in children is affected by bone area that is rapidly changing during growth (Shaw, 2008). Quantified by the ratio between BMC and bone scanned area, this variable is referred in the literature as areal bone mineral density (aBMD) (Ammann and Rizzoli, 2003).

One of the most common sites that suffer a fracture during adult life is the proximal portion of the femur. The first studies focusing on this skeleton site included the femoral head, neck, and trochanteric region as portions of the proximal femur to take into account (Theintz et al., 1992; Cooper & Aihie, 1995). Theintz et al. (1992) had study the longitudinal bone mass gain, by age group, from 9-11 yrs to 18-20 yrs at the lumbar spine, femoral neck, and femoral shaft. More recently, it has been declared that in adolescent males, when analyzing the proximal femur (PF), the major incrementation during growth occurs from 13 to 17 years old (Bonjour et al., 2012).

Physical activity during youth increases bone mineral mass accrual and it is known that growing bones show more response to mechanical loading than adult ones (Bonjour et al., 2012). Adolescent athletes of sports with higher mechanical loading/impact (i.e. soccer, basketball, gymnastics, tennis) are described in the literature as better in bone health parameters than the ones that practice activities with lower mechanical loading (i.e. swimming, cycling, water polo) (Courteix et al., 1998; Lima et al., 2001; Narciso et al., 2020). Participation in soccer, characterized as a weight-bearing activity, showed positive effects on bone mineral content at the load sites (Zouch et al., 2014; Vlachopoulos et al., 2017), on the other hand, swimming practice, classified as non-weight-bearing activity, presented negative effect on bone parameters (Gomez-Bruton et al., 2018).

1.4. Youth athletes muscle strength

Skeletal muscle is the main responsible for allowing the body to move and practice sports, representing high plasticity and being the tissue that consumes more energy on the human body (Malina et al., 2004). Muscle strength represents the skeletal muscles capability to exert force, to resist or move against external loads or gravity (Armstrong & McManus, 2017).

The motor neurons located in the peripheral nerve conduct the message from the central nervous system until the skeletal muscle, resulting in muscle contraction. In the absence of neural information, the muscle fibers relax (Muscolino, 2017). Muscle activation shows different types of contractions. Isometric when the internal and external load is the same (Armstrong, 2019), a force production with no differences in muscle length (Davis et al., 2019). Concentric action occurs when the origin and insertion of the muscle group become closer. Eccentric contraction occurs when the muscle group lengthens (Armstrong, 2019). Although this type of contraction has been reported to cause more damage to the muscle than the concentric (Davis et al., 2019) a recent paper demonstrated that eccentric exercise seems to be more efficient than concentric in bone density, content, and geometry (Julian et al., 2020).

Muscle strength shows a constant increase from childhood until approximately 13-15 years, from there continues to slowly increase in males and shows a plateau in females (Armstrong & McManus, 2017). The period one year after the peak height velocity occurs matches the beginning of the adolescent strength spurt (Rowland, 2004). The high concentrations of androgenic hormones during puberty enhance the rate of growth in males and, consequently, strength improves (Rowland, 2004).

The isokinetic dynamometer has been reported as a reliable and easy method to assess muscle strength in young populations (Weir, 2000). When using this apparatus, the term torque is feasible to express strength (De Ste Croix, 2017). Torque (T) refers to the rotatory effect that is generated when a force is applied and can be measured as the product of force and the perpendicular distance (N.m) (Hall, 2015). When measuring muscle strength using an isokinetic dynamometer the joint torque depends on different components that are selected for the protocol, such as the range of motion, angular velocity, test position, feedback during the test, number of repetitions, and rest intervals between sets (Davies, Heiderscheit & Brinks, 2000).

The literature has been testing the knee flexors and extensors strength at concentric and eccentric modes the most (Carvalho et al., 2012; Rochcongar et al., 1988). Testing eccentric contractions in youth populations has been described as more difficult because it demands a high muscle force production that can increase injury risk (De Ste Croix et al., 2003). For this reason, reactive strength testing can be used. Reactive strength can be well defined as the capacity of an individual to change rapidly from eccentric to concentric muscular action (Young, 2006). Reactive strength testing can provide additional information regarding how an individual achieves a certain standard of dynamic/functional performance (Suchomel et al., 2016). Previous research has determined that reactive strength assessment is reliable (Lloyd et al., 2009), allows to distinguish between field athletes with higher or lower acceleration abilities (Lockie et al., 2011), can be used to monitor neuromuscular fatigue and as an indicator of the current training conditions (Suchomel et al., 2016).

1.5. Objectives and structure of the thesis

The present thesis aimed to examine the independent and combined effects of growth, maturation, and training using a mixed-longitudinal design, initially, on developmental changes related to muscle strength and, on parameters of bone tissue in young male athletes that are involved in sports characterized by different mechanical loading on the skeleton.

Considering the previous background, the hypothesis is that sports practice during youth has an impact on bone and muscle function. A second hypothesis is that the practice of sports with different mechanical loading during this period show different results when testing muscle strength and assessing bone health parameters.

The length of the thesis will be constructed by six chapters. The main project design, participants, materials, methodologies, procedures, and analysis will be described in chapter II. Chapters III, IV, and V will present three manuscripts as scientific outputs of the present thesis, with the following objectives:

(1) To summarize a systematic understanding of bone development during childhood and adolescence, particularly of the youth athlete, considering the biochemical mechanisms associated to variation in bone mineral outputs (content, density, strength, geometry) (Chapter III);

(2) To describe the learning effect in the laboratory protocol used to assess the isokinetic muscle strength of the knee flexors and extensors on different contractions among adolescent athletes (Chapter IV);

(3) To examine inter-and intra-individual variability between youth athletes participating in sports contrasting in mechanical loading (soccer vs swimming) on developmental changes in bone mineral density in young athletes (Chapter V);

Regarding the hypothesis, chapter VI combines and discusses the main findings of each study, together with the thesis conclusions.

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Chapter II

Methods

2. Methods

2.1. Procedures

The project was granted by the Portuguese Foundation for Science and Technology (SFRH/BD/136193/2018). It is linked to the strategic plan of the Research Unit for Sport and Physical Activity of the Faculty of Sport Sciences and Physical Education, University of Coimbra (uid/dtp/04213). Procedures were approved by the local Ethics Committee for Sports Sciences (CE/FCDEF-UC/00182016) and data collection was conducted in accordance with ethical standards for sports medicine (Harriss and Atkinson, 2017) that fits the Declaration of Helsinki for human studies by the General Assembly of the World Medical Association (2014).

2.2. Participants

Young athletes from two sports that contrast in mechanical loading (soccer as a weight-bearing activity and swimming as a non-weight-bearing activity) were assessed in a total of four-time moments over two years (six-months intervals), with repeated assessments over the time taken on cohorts (entry age 11-13 years). Participants were recruited from local clubs that signed a protocol. Parents or legal guardians had signed and provided written informed consent. Inclusion criteria were: (1) participation in at least two federated seasons; (2) ≥ 2 training sessions per week; (3) approval of medical exams (4) without bone fractures or musculoskeletal injuries in the lower limbs in the previous year. Table 1 describes the study design, participants, sport, and study variables that after are developed in chapters IV and V.

Table 1 - Study design, sample, sport, and variables of chapters IV and V

Chapter	Study design	Sample (Sport)	Variables
IV	Mixed-longitudinal	n=38 (swimmers)	Chronological age, skeletal age anthropometry, dual-energy x-ray absorptiometry (DXA), isokinetic strength of the knee flexors and extensors
V	Mixed-longitudinal	n=40 (n=20 soccer players; n=20 swimmers)	Chronological age, biological (somatic) age, anthropometry, training variables, dual-energy x-ray absorptiometry (DXA), food frequency questionnaire (FFQ)

2.3. Variables

Chronological and skeletal ages

Chronological age (CA) was calculated to the nearest 0.1 years. The Fels method was used to estimate the skeletal age (SA) (Roche et al., 1988). This 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). Posterior-anterior radiographs of the left hand-wrist were taken: the participant was with the hand in pronation on a transverse plane and with the fingers in maximum extension and maximum distance between each other. The third finger was in the prolongation of the antebrachial segment. After, ratings and ratios were entered into the Fels software program (Felshw 1.0 Software, Lifespan Health Research Center, Departments of Community Health and Pediatrics, Boonshoft School of Medicine, Wright State University, Dayton, Ohio) by the same observer that computed the skeletal age and related standard error (Roche et al., 1988). 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).

Biological maturation

Maturity offset was obtained from an algorithm requiring CA, body mass, stature, sitting height, and estimated leg length. It refers to the distance (in years) to age peak height (Mirwald et al., 2002) as presented in Equation 1. The standard error of estimate (SEE) of the equation was reported by the authors as 0.592 (95% confidence intervals: 1.18 years). Maturity offset minus chronological age determines the age at peak height velocity (APHV). After, participants were classified as pre- or post-APHV.

Equation 1

Maturity offset (years): -9.236 + (0.0002708 * (leg length * sitting height)) + (-0.001663 * (CA * leg length)) + (0.007216 * (CA * sitting height)) + (0.02292 * ([body mass / stature] * 100))

Anthropometry

A single observer measured stature, body mass, and sitting height following standardized protocols (Lohman et al., 1988).

Participants were weighed barefoot and dressed only in thigh shorts. Stature and sitting height were measured to the nearest 0.1 cm using a portable stadiometer (Harpenden model 98.603, Holtain LTD, Crosswell, UK) and sitting height table (Harpenden model 98.607, Holtain LTD, Crosswell, UK). The observer told the participants to take the anthropometric position of reference, and ensure the orthogonality of the *Frankfort* reference line relative to the scale. Leg length was calculated as the difference between stature and sitting height. Body mass was measured to the nearest 0.1 kg using a portable balance (SECA model 770, Hanover, MD, USA).

Dual-energy x-ray absorptiometry (DXA)

Body composition was examined as the sum of fat tissue, lean soft tissue, and bone mineral content (BMC) using DXA (Lunar DPX-MD+ (Software: enCORE version 4.00.145, GE Lunar Corporation, 726 Heartland Trail, Madison, WI 53717-1915 USA). A single certified technician extracted the data following the guidelines published by the manufacturer (DXA Quality Assurance Center, San Francisco Coordinating Center, 2013). Participants were positioned on the table in a supine position with the body aligned along with the central axis. This scan permits the calculations of BMC, bone area, and areal bone mineral density (aBMD) in addition to fat tissue and lean soft tissue. These data were repeated for the whole body, trunk, and upper, and lower limbs. The analysis of the proximal femur required a second scan to assess aBMD of the femoral neck, Ward triangle, trochanter, and shaft.

Isokinetic dynamometer of the knee extensors and flexors

This assessment was carried out by Biodex System 3 dynamometer (Shirley, NY, USA) at $60°.s^{-1}$, $180°.s^{-1,}$ and $300°.s^{-1}$. The test was carried out with a 5 minutes warm-up in a cycle ergometer (814E Monark, Varberg, Sweden) with a resistance value corresponding to 2% of the body mass of the subject (Brown, 2000) cycling between 50 and 60 rpm, followed by three exercises of static stretching of the quadriceps, hamstrings, and adductors, with a duration of 20 seconds each.

The subjects sat on the chair, the lever arm was aligned with the lateral condyle of the knee and the fixing strip to the tibial tarsal joint was placed aligned with the medial malleolus of the tibia. According to manufacturing guidelines, it was measured a standardized 85° hip flexion from the anatomical reference position. It was asked the subject to undertake voluntary maximum extension member and considered the value 0°, then 5 degrees were subtracted to account for the 10% initial shaft motion of the torque button setting. Range of motion during testing was defined using 85° degrees from knee extension 5° to 90° of knee flexion. Before the subjects performed the test, a correction of the gravity effect of the lower limb and the severity of the lever arm was made by weighing the relaxed member at 30° of knee flexion. The subject was also instructed to, during the test, have their arms crossed on his chest with the hands-on his shoulders (Brown, 2000; De Ste Croix, Deighan & Armstrong, 2003).

The test started with three continuous repetitions for familiarization with the equipment, motion, and strength, and then five maximal repetitions with 60 seconds intervals between each (De Ste Croix et al., 2002). The moments of maximum force in flexion and extension were expressed in Newton per meter (N.m), and then the peak torque (PT) extracted in both movements, the angle at which it occurred, and the torque at 30º, 45º, and 60º of motion in the five repetitions using the program Acknowledge, version 4.1 (Biopac Systems, Inc.). The peak torque was obtained from the highest point of the isokinetic torque curves (Perrin, 1993).

Food frequency questionnaire (FFQ)

A semi-quantitative food frequency questionnaire (FFQ) was used to estimate dietary intake validated for the Portuguese population (Lopes et al., 1994; Lopes et al., 2007). The questionnaire was structured based on the model of the FFQ of Willet et al. (1998) and was developed by the Department of Public Health of the Faculty of Medicine, University of Alicante (Vioque & Gonzalez, 1991). Composed of 86 items covering the intake of solid and liquid food during the 12 months before filling it in. The FFQ included food groups, beverage categories, and frequency intake with nine qualitative options (from "never or less than once a month" to "6 or more times per day"). Calories, macronutrient intake, cholesterol, fiber, and calcium were estimated using the software Food Processor SQL (ESHA Research Inc., Salem, OR, USA) and, subsequently, were retained for the analysis. The questionnaire is used to estimate calories (kcal), protein (%), carbohydrate (%), total body fat (%), saturated fat (%), monounsaturated fat (%), polyunsaturated fat (%) cholesterol (mg), dietary fiber (g), ethanol (g) and calcium (mg).

2.4. Analysis

The doctoral thesis is part of an ongoing longitudinal devoted to growth, maturation, body composition, bone strength, and parameter of bone health. The data collection will be concluded in 2022/2023. As the initial output of the research project, it was decided to produce a systematic review of bone health in growing children who participate in sports (chapter III), an analytical study to inform about the data quality of the functional protocol (chapter IV), and a developmental study to interpret the longitudinal change of bone mineral density at different sites considering sport-specific variability given by comparisons between swimmers and soccer players (chapter V). In fact, the research proposal is now aiming to include a control group (school boys).

To examine the significance of intra and inter-individual variance in the isokinetic strength protocol the scores of five repetitions within a single day were tested among 38 swimmers. The preceding was performed using multilevel modeling (level 1: time; level 2: participants). It was clear that youth participants fluctuate their performance values. Because intra-class correlation coefficients for the different knee muscle actions were not impressive, it supported the decision to retain the best peak torque for future analysis as often adopted in the sports sciences literature.

Afterward, following the procedure that retains the best score from the five strength curves for future analysis, data quality was diagnosed based on technical error of measurement and respective coefficient of variance (in %). The previous was completed using data of a subsample of 17 participants who visited the lab for a second occasion. It was also possible to determine mean differences using paired t-test to report fluctuation of strength performance during a short period. After examining intra and inter-individual variance of the total sample to conclude that intra-class correlation coefficients do not suggest nearly perfect stability, after examining mean differences over time, the individual error was inspected using Bland-Altman procedures, that is, by combining individual differences (time moment 2 minus time moment 1) to individual mean (average of the repeated scores). The technique is used to check the potential association of individual errors on youth contrasting in strength values.

The study examining the 12-month effect of participation in sports considered two groups and consequently required an analysis of variance 2x2 (two groups, two time-moments). The analysis of variance for repeated measurements may be used as a scientific alternative to multilevel modeling which would have required more than two-time moments for obtaining intra-individual trajectories. Meantime, since the two groups (swimmers and soccer players) differed in body size given by stature, the repeated measurements of 2x2 analysis of variance (ANOVA) were confirmed using analysis of covariance (controlling for stature at baseline) following the same design (2x2, that is, two groups over two-time moments).

Statistical analysis was performed using SPSS version 20.0 (SPSS Inc., IBM Company, N.Y., USA), using MLwiN v2.26 (Center for Multilevel Modelling, University of Bristol, Bristol, UK) for the study on chapter IV, and Graphpad Prism (version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com). Statistical significance was accepted when $p < 0.05$.

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Chapter III

Study 1

Systematic review on bone development among youth athletes

3. Sports practice and bone changes during adolescence in male athletes: a systematic review

3.1. Abstract

Background: Participation in sports during adolescence represents a crucial role in the adult skeleton. The aim of the present study was to search and summarize the existing knowledge that concerns the study of bone changes regarding content, density, biochemical markers, geometry, and stiffness taking into account the practice of sports contrasting in mechanical loading and different methods used to assess bone.

Methods: PubMed, Scopus, Web of Science, and, SPORTDiscus were searched for studies until 15th of July of 2021. The search target population included healthy, male, and, adolescent athletes (10-18 years), studies assessing bone in adolescent athletes and describing its quantitative parameters, and comparisons that included different groups of athletes and/or a control group. Outcomes such as bone content or density, bone stiffness, bone geometry, and biochemical markers, and, finally cross-sectional or longitudinal assessments. Studies were excluded if they were not written in English, involved injuries or pathologies, and if outcomes of interest were not reported (i.e. did not include bone). The risk of bias for each study was assessed with the Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies. Results were synthesized in a table for study characteristics and in the discussion. The study was registered in the International Prospective Register of Systematic Reviews database.

Results: Initially, 1830 records were identified by the search equation. After the removal of duplicates and exclusion of articles by title and abstract, 135 full-text articles were assessed and, 44 met the eligibility criteria. Four main outcomes emerged for discussion: bone mineral content and bone mineral density, biochemical markers and hormonal profile, bone geometry, and bone stiffness.

Conclusion: It is clear that adolescent athletes that are involved on sports with mechanical loading show different and positive benefits on bone content, density, geometry, biochemical markers, hormonal profile and bone stiffness. Sports with lower mechanical loading need to have longitudinal follow-ups to try to find training tools and exercises that help minimize the differences that were presented and well described in this systematic review.

Keywords: Biochemical markers; adolescence athletes; bone health; bone geometry; bone stiffness;

3.2. Introduction

Bone is a dynamic tissue that changes in composition and geometry as a response to physical activity (Tendorde & Fredericson, 2011). Through the years, the literature has been assuming that the longer time to achieve the peak bone mass the greater the delay in bone mass loss during adult life. Peak bone mass represents the higher amount of bone mass acquired until the process of resorption starts to be higher than formation (Zemel, 2013).

The two years that surround the peak height velocity are critical in bone mineral accrual. It is estimated that 40% of the adult skeleton bone mineral content (BMC) is acquired during this period (Bailey et al., 1999). Participation in sports during the crucial years for growth and maturation results in a higher peak bone density (Tendorde & Fredericson, 2011). BMC and bone mineral density (BMD) measurements can be assessed in childhood and adolescence as predictors for fracture risk (Kalkwarf et al., 2010). Agostinete et al. (2020a) studied adolescent athletes from ten different sports according to their areal bone mineral density (aBMD). From the whole body aBMD it was possible to conclude that in sports with higher mechanical loading, such as soccer, gymnastics, judo, volleyball, and, track & field participants showed higher aBMD than both non-athletes, and the ones involved insports with lower mechanical loading such as karate, baseball, kung-fu, and swimming. The study also looked for specific regions of the skeleton with minor differences in the categories. Thereby, BMC and aBMD show great relevance and were already dissertated in previous literature. For the International Society for Clinical Densitometry (ISCD), dual-energy x-ray absorptiometry (DXA) is the preferred method to assess these two variables (Guss et al., 2021).

The process responsible for the balance between bone resorption and formation is called bone remodelling. Bone biochemical markers apply great relevance to this process having specific functions on the metabolic processes of bone turnover (Seibel, 2005; Swaminathan, 2001). During childhood and adolescence the amount of markers secreted depends on growth and maturity status (Szulc et al., 2000). Serum analysis allows researchers to distinguish bone formation markers: Bone-specific alkaline phosphatase

(BAP), osteocalcin (OC), and N-terminal propeptide of type 1 procollagen (P1NP). Meantime, in order to assess markers of bone resorption, urine and serum are used. The most commonly gauged markers, are related to collagen: Hydroxyproline, total and dialysable (Hyp), hydroxylysine-glycosides, pyridinoline (PYD), deoxypyridinoline (DPD), carboxyterminal cross-linked telopeptide of type 1 collagen (CTX-MMP), C-terminal propeptide of type 1 procollagen (CTX-1), aminoterminal cross-linked telopeptide of type 1 collagen (NTX-1), and Collagen I alpha 1 helicoidal peptide (HELP) (Seibel, 2005). Also, the increase concentration of growth hormone (GH), insulin-like growth factor 1 (IGF-1), and insulin-like growth factor binding protein-3 (IGFBP-3) can lead to circadian variations of markers synthesize by bone formation and resorption (Szulc et al., 2000).

Bone geometry or microarchitecture represents the assessment of the cortical bone, and can be also used as a factor to predict bone strength and fracture risk (Hans et al., 2011). This analysis can be acomplished by different methods, such as, the (peripheral) quantitative computed tomography (pQCT, QCT) (Gomez-Bruton et al., 2017), magnetic resonance imaging (MRI) (Ducher et al., 2009), trabecular bone score (TBS) that can be obtained from DXA (Pothuaud et al., 2008), and hip structural analysis (HSA) also derived from a DXA evaluation (Vlachopoulos et al., 2017a).

Finally, bone stiffness has been assessed by quantitative ultrasound (QUS), i.e. by measuring the speed of sound (SOS) in a specific bone, such as, tibia or radius (Falk et al., 2007). This measurement reflects the time that passes since the pulse transmission until the first reception of signal (Falk et al., 2007).

Therefore, the aim of the present study was to search and summarize the existing knowledge that concerns the study of bone changes regarding content, density, biochemical markers, geometry, and stiffness taking into account the practice of sports contrasting in mechanical loading and different methods used to assess bone.

3.3. Methods

Eligibility criteria

Studies were included meeting the PICOS search tool criteria (Methley et al., 2014). The search target population (P) included healthy, male, and, adolescent athletes (10-18 years), interventions (I) that included assessing bone in adolescent athletes and describing its quantitative parameters, and comparison (C), studies that included different groups of athletes and/or a control group, outcomes (O) such as bone content or density, bone stiffness, bone geometry, and bone biochemical markers and hormonal profile, and, finally a study design (S) of cross-sectional or longitudinal assessment of adolescent athletes. Exclusion criteria were the following: (1) studies not written in English; (2) studies that involved injuries or pathologies; (3) if outcomes of measurement or interest were not reported (i.e. did not include bone).

Information sources & search strategy

All searches were conducted on the same day, 15th of July 2021, in four different electronic databases: PubMed, Scopus, Web of Science, and, SPORTDiscus. A general search equation was performed using keywords: (“young athlete*" OR "youth athlete*" OR "adolescen* athlete*") AND ("Mechanical loading" OR osteogenic OR weight-bearing OR bone OR "bone health" OR skelet* OR maturation OR "growth hormone" OR testosterone OR "muscle tissue" OR "muscle mass"). The specific search on each database is presented in table 2.

Table 2 - Search strategy described by database, including filters and limits.

Database	Line search strategy
PubMed	("young athlete*"[All Fields] OR "youth athlete*"[All Fields] OR ("adolescen*"[All Fields] AND "athlete*"[All Fields])) AND ("Mechanical loading"[All Fields] OR ("osteogene"[All Fields] OR "osteogeneic"[All Fields] OR "osteogenic"[All Fields] OR "osteogenically"[All Fields] OR "osteogenicity"[All Fields]) OR ("weight bearing"[MeSH Terms] OR "weight bearing"[All Fields] OR ("weight"[All Fields] AND "bearing"[All Fields]) OR "weight bearing"[All Fields]) OR ("bone and bones"[MeSH Terms] OR ("bone"[All Fields] AND "bones"[All Fields]) OR "bone and bones"[All Fields] OR "bone"[All Fields]) OR "bone health"[All Fields] OR "skelet*"[All Fields] OR ("maturate"[All Fields] OR "maturated"[All Fields] OR "maturating"[All Fields] OR "maturation"[All Fields] OR "maturational"[All Fields] OR "maturations"[All Fields] OR "maturative"[All Fields] OR "mature"[All Fields] OR "matured"[All Fields] OR "maturer"[All Fields] OR "maturers"[All Fields] OR "matures"[All Fields] OR "maturing"[All Fields] OR "maturities"[All Fields] OR "maturity"[All Fields]) OR "growth hormone"[All Fields] OR ("testosterone"[MeSH Terms] OR "testosterone"[All Fields] OR "testosteron"[All Fields] OR "testosterones"[All Fields] OR "testosterone s"[All Fields]) OR "muscle tissue"[All Fields] OR "muscle mass"[All Fields])
Scopus	(TITLE-ABS-KEY ("young athlete*" OR "youth athlete*" OR "adolescen* athlete*") AND TITLE-ABS-KEY ("Mechanical loading" OR osteogenic OR weight-bearing OR bone OR "bone health" OR skelet* OR maturation OR "growth hormone" OR testosterone OR "muscle tissue" OR "muscle mass"))
Web of Science	"young athlete*" OR "youth athlete*" OR "adolescen* athlete*" (All Fields) and "Mechanical loading" OR osteogenic OR weight-bearing OR bone OR "bone health" OR skelet* OR maturation OR "growth hormone" OR testosterone OR "muscle tissue" OR "muscle mass" (All Fields)
SPORTSDiscus	"young athlete*" OR "youth athlete*" OR "adolescen* athlete*" Abstract AND "Mechanical loading" OR osteogenic OR weight-bearing OR bone OR "bone health" OR skelet* Abstract OR maturation OR "growth hormone" OR testosterone OR "muscle tissue" OR "muscle mass" Abstract

Selection, data collection processes, and data items

Two independent reviewers (DC, HS) screened titles and abstracts to identify manuscripts that could fit the inclusion criteria. Duplicates were excluded and, after, full-text articles were retrieved and independently screened to select the ones that meet the criteria. If the two first reviewers discord at any point, a participation of a third reviewer was required (MJCS). The data collection process occurred using a standardized extraction spreadsheet, where the main information for each manuscript was included. Mendeley Desktop Software (Version 1.19.8) was used to export and organize all manuscripts. The flow diagram of the identification, screening, eligibility, and inclusion process is provided in Fig. 3.1. Outcomes were categorized as follows: (1) BMC and BMD; (2) bone biochemical markers and hormonal profile; (3) bone geometry; (4) bone stiffness. In the present review, data was collected on the manuscript (authors, year, and journal), the study design (cross-sectional or longitudinal), the study sample characteristics and methods, and the study main findings

Study risk of bias

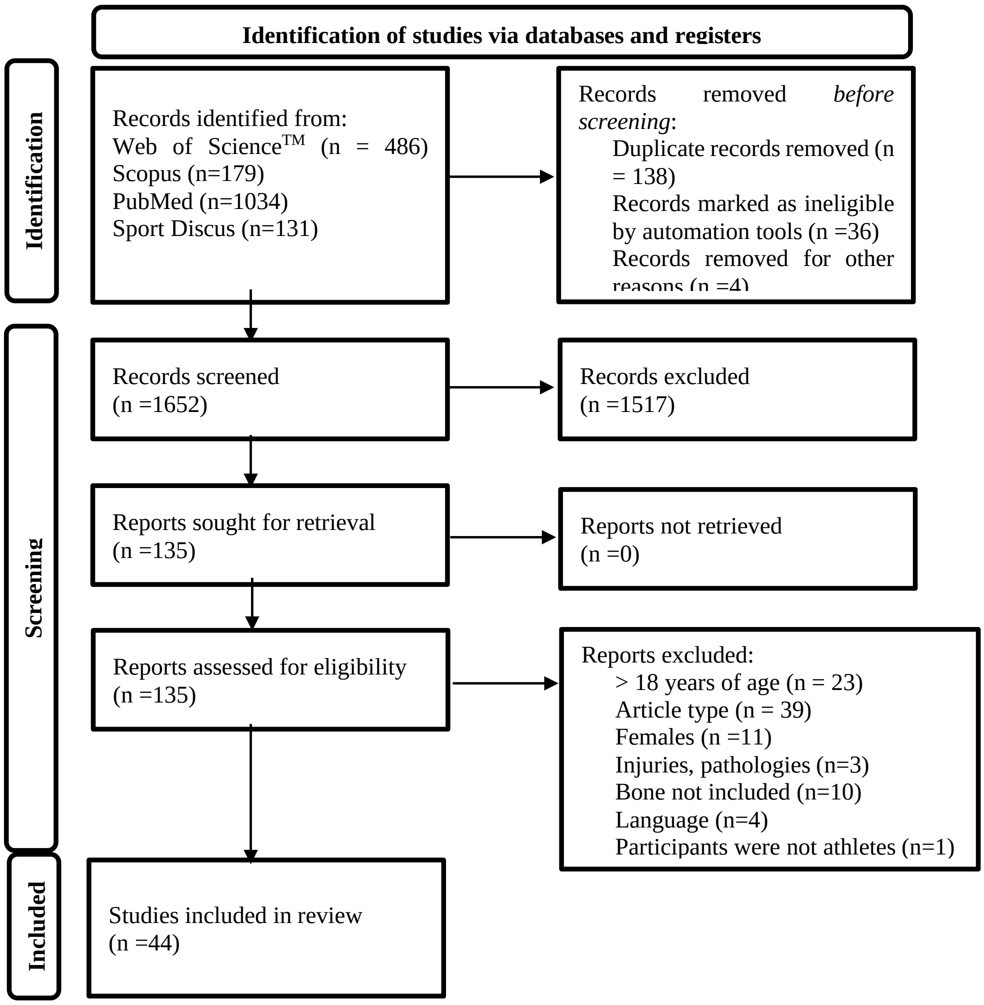
Two review authors assessed the risk of bias for each study using the Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies developed by the National Heart, Lung, and Blood Institute (NHLBI) and Research Triangle Institute International (National Institution of Health, 2013). Disagreements were solved by a third reviewer (MJCS). All studies were divided as cross-sectional studies (n=28) (Supplementary material – Table 3) and longitudinal studies (n=16) that fit the questions for observational cohort studies of the tool.

This tool was composed by fourteen questions in which a direct answer was attributed (i.e. Yes, No, or NA (not applicable)), concerning the aim, and mainly the methods of each study. After that, a general classification based on the aforementioned was determined (i.e. good, fair, or poor). For the sixteen longitudinal studies (Supplementary material – Table 4) the same tool was employed once the questions fit and it additionally included more than one measurement over time and also the terms baseline and follow-up.

3.4. Results

Study selection

The line search strategy for each database, as represented in table 2, resulted in the identification of 1830 records. Before the screening, 178 records were removed automatically and manually; then through a title and abstract analysis, 1517 studies were excluded. Consequently, 135 full-text articles were reviewed. Of those, 91 did not follow the eligible criteria: (1) the mean age of the sample was above 18 years old (n=23); (2) other reviews, systematic reviews, case reports, conference papers, and short reports (n=39); (3) studies that only included females (n=11); (4) manuscripts that assessed injuries or pathologies (n=3); (5) studies where bone were not included in the results or discussion (n=10); (6) studies that were not in English (n=4); (7) and one study where the sample did not include athletes. All in all, 44 full-text articles were taken into account for this systematic review.



From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71

Figure 1 - PRISMA 2020 flow diagram for new systematic reviews which included searches of data bases and registers only

Study characteristics

A resume of the characteristics for each study is reported in supplementary material (table 5). The detailed description of each study included the aim, and sample characteristics, and explained how the study assessed bone, outcomes, and main findings. When needed, additional observations were made concerning the analysis and what was retrieved for each study.

Studies were divided into four categories of outcomes. The first one included the two main variables described in the literature, BMC and BMD, present in studies measured by the same method, DXA. The second, a less explored field during adolescence, bone biochemical markers, and also the hormonal profile of participants from different loading activities. These indicators were normally studied through venous blood or urinary samples. The third regarded bone geometry studied by different methods, such as MRI (Ducher et al., 2009), HSA by DXA (Vlachopoulos et al., 2017a; Vlachopoulos et al., 2017b) and TBS equally obtained by DXA (Vlachopoulos et al., 2018a), pQCT (Gomez-Bruton et al., 2017). Lastly, bone stiffness that was obtained through QUS using SOS as quantitative variable (Falk et al., 2010; Vlachopoulos et al., 2018b).

Risk of bias in the study

The Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies was used to assess the risk of bias in each study and divided into cross-sectional studies (supplementary material – table 3.2) and longitudinal studies (supplementary material – table 3.3). Most of the studies included one or more groups of different sports and compared different bone parameters. After answering the fourteen questions for each study, a quality rating was determined most of the studies present good or fair quality with only one study being classified as poor.

3.5. Discussion

Bone mineral content and bone mineral density

BMC and BMD were assessed in most of the studies by comparing different levels of mechanical loading. Some studies did comparisons between one sport and age-matched control group (Virvidakis et al., 1990; Rico et al., 1993; Conroy et al., 1993), others combined sports with higher mechanical loading, sports with low mechanical loading and a age-matched control group (Lima et al., 2001; Vlachopoulos et al., 2017a).

In 1990 a study assessed BMC of 59 male adolescent competitive weightlifters using single photon absorptiometry (Virvidakis et al., 1990). Measuring athletes and age-matched controls at the non-dominant forearm it was found that athletes showed BMC mean values two standard deviations above age-matched controls. Three years later, a study compared total BMC and leg BMC of 22 postpubertal male cyclists and 27 sedentary age-matched controls (16.9 ± 0.3 yrs) but this time with DXA technology (Rico et al., 1993). The authors concluded that the groups only differed at the leg BMC, with cyclists showing lower values than the control group.

A longitudinal study assessed prepubescent and pubescent soccer players and a control group to determine the effects of soccer on these two stages. At baseline, prepubescent soccer players showed higher BMC than controls at the whole-body, total hip, and both legs. This pattern was still the same one year after, at the second time measurement. One of the main findings of this study was that pubescent soccer players presented greater gains at the femoral neck, lumbar spine and supporting leg than soccer athletes who were still prepubescent, and then pubescent controls (Zouch et al., 2014).

When comparing longitudinally (1-year) the BMC from sports with different mechanical loading (swimming, n=37; soccer players, n=37; cyclists, n=28; and a control group, n=14), regarding the lumbar spine (LS) and proximal femur (PF) as regions of interest, soccer players presented a significant higher amount than swimmers and cyclists (Vlachopoulos et al., 2017a). These two regions have been studied as specific loading sites of interest. Another research aimed to compare values for BMD at the spine and proximal femur between junior Olympic weightlifters, age-matched controls, and reference values for the normal adult population. Conroy et al. (1993) used DXA and concluded that the athletes group had greater BMD values than the other two groups. These results reinforce the idea that higher mechanical loading explains higher BMD values. The proximal femur represents a region where the mechanical loading from different sports has different accrual on BMD. The effect of combat sports on BMD among adolescent participants was also investigated by Nasri et al. (2015). The whole-body, whole spine, lumbar spine (L2-L4), legs, and arms were assessed and were significant ($p<0.001$) at all sites for combat athletes when compared with a sedentary age-matched group. The biggest difference between groups was found at the whole-body and lumbar spine. Silva et al. (2011) assessed soccer players (n=10, 14.7 ± 0.82 yrs), tennis players (n=10, 14.1 ± 1.61 yrs), swimmers (n=12, 13.8 ± 2.45 yrs), and controls (n=14, 13.4 ± 2.02 yrs). They concluded that proximal femur of soccer players presented significantly higher BMD than swimmers and controls.

Meantime, Lima et al. (2001) compared a control group (n=24), an impact load group (n=18, gymnasts, athletics, basketball and tennis players), and an active load group (n=27, swimmers and water polo players). The sample aged 12 to 18 years old. Regarding BMD comparisons the impact load group presented higher values not only for the total body (n=18, 1.153 ± 0.143 g.cm-2) but also for lumbar spine (n=18, 1.041 ± 0.171 g.cm-2) and femoral neck (n=18, 1.024 ± 0.155 g.cm-2). The same study also reported that active load participants showed similar lumbar spine (n=26, 0.915 ± 0.108 g.cm-2) and femoral neck BMD values (n=28, 0.945 ± 0.108 g.cm-2). Similar research assessed male adolescents, with a 9-month follow-up, from five different sports (basketball, karate, soccer, judo, and swimming) and a control group to analyze the effect on BMD acquisition (Agostinete et al., 2016). The regions assessed included the whole body, upper limbs, lower limbs, and spine. Basketball participants had significant higher acquisition on BMD than other groups. Results showed similarities between swimmers and controls except for the upper limbs where swimmers had higher accrual.

Other body segments have already been described in literature, such as total hip, arms and humerus that were assessed in a longitudinal study of 43 ice hockey players (16.7 ± 0.6 yrs) and n=25 controls (16.8 ± 0.3 yrs). The present review only observed the first-time moment because in the second the sample surpassed the age of eighteen. The ice hockey group was divided into former and active. When comparing groups, the authors concluded that at 16 years of age groups did not differ on BMD in the non-dominant humerus and total hip (Nordström et al., 2005). Also, in ice hockey and badminton players, sports that also have impact load on the upper limbs, at the baseline of a longitudinal study (17.3 ± 1.8 yrs), athletes showed higher BMD at humerus and most sites than age-matched controls (Tervo et al., 2009).

Adolescent Tunisian soccer players BMC and BMD values were compared with age matched controls. Different body regions were taken into account, whole-body, lumbar spine, femoral neck, lower limbs and pelvis. When dividing the sample into pubertal groups, early and late soccer players presented greater values than controls (Nebigh et al., 2009). More recently, Quiterio et al. (2011) compared this two variables at the whole-body, upper limbs, lumbar spine and lower limbs on a high-impact group (n=34, gymnasts, basketball and handball players), a control group (n=26), and a non-impact group (n=20, swimmers). The group of athletes engaged in high-impact loading sports showed higher values at all sites. The same was found by Seabra et al. (2017), when comparing adolescent futsal players (n=28), swimmers (n=20) and controls (n=22).

Agostinete et al., (2017a) compared adolescent basketball athletes (n=18), swimmers (n=15) and controls (n=20) by quantifying BMC and BMD. After adjusting for potential confounders (chronological age, maturity offset, vitamin D and training volume) results were similar between controls and athletes. Nonetheless basketball athletes presented greater BMC and BMD than swimmers on the lower limbs.

The lower mechanical loading of swimming has a great impact on literature in the field of study for bone parameters. To study the specificity of this modality and the longitudinal gains on BMD in adolescents involved in national competitions, Ribeiro-Dos-Santos et al. (2016) assessed a control group (n=13) and a group of swimmers (n=16) on the whole body, upper limbs, lower limbs and spine. BMD accrual over 9-months was higher in the control group than in swimmers, yet only with significant differences at the lower limbs when adjusted by chronological age, vitamin D score, peak height velocity, engagement in resistance training and previous practice of swimming in months. Authors also identified a negative relationship between time engaged in swimming and the BMD gains during adolescence (Ribeiro-dos-Santos et al., 2016). Another study equally found higher values for BMD in controls than swimmers (Agostinete et al., 2017b).

Cycling, similar to swimming, is a modality with low mechanical loading. A study hypothesized that during adolescence healthy participants would show higher bone mass accrual than cyclists (Olmedillas et al., 2011). Results on the younger age group (15-16 yrs) corroborated this hypothesis, with cyclists showing lower values of BMC and BMD than the healthy age-matched group. BMD was greater ($p<0.01$) in controls than cyclists at legs, pelvis, total hip, trochanter and inter-trochanteric subregion.

Bone biochemical markers and hormonal profile

The first research found on this systematic review for bone biochemical markers and hormonal profile with young male population aimed to assess the effects of load on BMD, body composition, sexual hormones, and bone biochemical markers (Lima et al., 2001). Bone-specific alkaline phosphatase (BAP) concentration, as a formation marker, was significantly higher in the active group (swimming and water polo) when compared with the impact group (gymnastics, athletics, basketball and tennis) and controls. The same group also had greater urinary deoxypyridinoline crosslinks to creatinine ratio (DPD/Cr) values but only significant compared with controls. DPD is a bone resorption marker that is measuring and corrected for the urinary concentration of creatinine (Conti et al., 1998). On the other hand, testosterone concentrations were significantly higher in the impact load participants (n=17, 567.82 ± 224.05 ng/dl) than in the active load group (n=27, 402.52 ± 155.37 ng/dl).

A group of 91 soccer players (13.4 ± 0.3 yrs) and 61 controls (13.3 ± 0.5 yrs) were assessed to measure the concentration of the growth hormone (GH), insulin-like growth factor-1 (IGF-1), insulin-like growth factor binding protein 3 (IGFBP-3), and testosterone (Nebigh et al., 2009). IGF-1 and IGFBP-3 concentrations were significantly greater in soccer players than controls, and on late puberty GH and testosterone were also significantly higher. Nebigh et al. (2009) have discussed and related these results with the impact load that is associated with soccer practice.

To investigate the correlation between bone parameters and hormonal parameters Nasri et al. (2013) assessed 50 combat athletes (17.08 ± 0.2 yrs) and 30 controls (17.1 ±

0.4 yrs) and collected bone samples to analyze GH and testosterone concentration. Authors found a correlation between GH concentration and BMD for the whole body ($r=0.3$; $p<0.05$) and spine ($r=0.28$; $p<0.05$). Testosterone concentrations showed no significant differences across groups. More recently, these authors assessed alkaline phosphate (AP) activity, propeptide N-terminal Procollagene type 1 (P1NP), plasma β-crosslaps (βCTx), and osteocalcin (OC) with the same population as in 2013 (50 combat athletes, and 30 sedentary controls) (Nasri et al., 2015). The sedentary group (17.1 ± 0.4) had greater ($p<0.05$) concentrations of AP and P1NP than combat athletes. These two biomarkers represent bone formation markers (Gifre et al., 2013).

Recently, a study assessed during a 1-year period (two measurements), bone biochemical markers on swimmers (n=37), soccer players (n=37), cyclists (n=28), and controls (n=14) (Vlachopoulos et al., 2017a). P1NP, CTX-1, 25(OH)D and total calcium were analyzed. The one-year differences by sport indicated that P1NP (bone formation) was greater in soccer players than swimmers, and cyclists. Soccer players also had significantly higher 25(OH)D than swimmers and controls. The same authors with a 9-month follow-up and the same sports represented in the sample measured P1NP as a bone formation marker, and CTX-1 as bone resorption marker (Vlachopoulos et al., 2018a). The study observed that the markers did not vary in the sports groups, but when comparing with controls, differences were found on CTX-1 between the control group of swimmers and cyclists.

Bone geometry

Greene et al., (2006) used magnetic resonance imaging (MRI) to assess distal third tibia geometry of 20 adolescent male middle distance runners and 20 age-matched controls. There were no differences between groups. Another previous study aimed to determine differences in bone geometry among adolescent tennis players from three different pubertal stages (Ducher et al., 2009). Bone dimensions at the humerus were measured using MRI. The playing and non-playing mid and distal humerus surfaces of cortical bone were measured at the pre-puberty, peri-puberty and post-puberty groups. Results reported that the non-playing humerus was approximately 60% larger in post-pubertal than pre-pubertal participants. The cortical area was greater in the peripubertal than prepubertal group and the periosteal area was larger in post-puberty than in peri-puberty (Ducher et al., 2009). The differences on the playing and non-playing humerus showed that tennis impact loading on this specific region seem to increase the bone size.

Hip structural analysis (HSA-DXA) was assessed to characterize the hip geometry estimates of the femoral neck in participants from activities with different mechanical loading (Vlachopoulos et al., 2017a). Three main variables that describe geometry can be retrieved from this method. The cross-sectional area (CSA, mm2) represents the total bone surface area of the hip, less the soft tissue area and trabecular bone, the cross-sectional moment of inertia (CSMI, mm4) characterizes the mass distribution in the center of a structural element, giving an index of structural rigidity, at last, the section modulus (Z, mm3) can be obtained as a determinant of the maximal bending strength in a cross section (Khoo et al., 2005). Vlachopoulos et al. (2017a) compared soccer players, cyclist, swimmers, and a control group in these components. The study concluded that, with two measurements (12-month follow up) soccer players presented greater CSA (7.1%), CSMI (10.2%), and Z (8.9%) than cyclists, however similar to the control group. Swimmers and cyclist presented similarities the studied variables. The same group of authors did comparisons on the same sample of adolescents athletes (swimmers, n=41; soccer players, n=37; cyclists, n=29; controls, n=14) at baseline. Results showed that swimmers and cyclists did not show any contribution on bone geometry, and soccer players presented higher values for hip structural geometry than the other groups (Vlachopoulos et al., 2017b; (Vlachopoulos, Barker, Williams, et al., 2017c)).

Trabecular bone score (TBS) can be obtained by DXA analysis and, is quantified by scores. The higher the score the higher should be the strength and fracture resistance of the bone (Hans et al., 2011). Vlachopoulos et al. (2017a) measured TBS in the same sample that was previously described (adolescent soccer players, swimmers, cyclists and controls) and found that soccer players presented significantly higher improvements within a 12-month period than the same age group for athletes enrolled in sports with lower mechanical loading (i.e. swimming and cyclists).

HSA and TBS at the lumbar spine were also analyzed on 3 different sport groups and respective controls (soccer players, swimmers and cyclists) to characterize bone geometry.

The athletes group on swimming and cycling had greater CSMI than the matched-controls (Vlachopoulos et al., 2018a).

Finally, pQCT is also used as a methodology to assess bone geometry, being able to obtain total area, cortical area and also the cortical thickness of a cross sectional bone area. Radius and tibia had previously been studied among swimmers (14-16 yrs) within a 8-month period. Yet again, results established no significant differences on the pQCT studied variables (Gomez-Bruton et al., 2017)

Bone stiffness

Three groups of children and adolescents from different loading activities (soccer, aquatic sports, and controls) were studied using a quantitative ultrasound method (QUS) (Falk et al., 2007). The speed of sound (SOS) is obtained measuring the time between pulse transmission and the first reception of a signal (Falk et al., 2007). The distal one third of the radius and mid-shaft of the tibia were compared between groups. In summary, no differences were found among the aquatic athletes and soccer players between the age groups. Also, no differences were found regarding SOS (m/sec) in the children and adolescents groups.

More recently, the same authors published a study with the same sample characteristics but without the aquatic sports group, aiming to compare bone properties in soccer and hockey players, and also active controls (Falk et al., 2010). This study also used QUS to assess SOS on the same regions as previously reported. They concluded that, when compared with controls, soccer players show higher bone strength at the lower limbs and, on the other hand, hockey players show in the upper limbs, bringing to discussion that these characteristics are related with the sport-specificity loading patterns.

Adjusted bone stiffness valuers were measured by QUS on the dominant foot of four adolescent groups (swimmers, n=41; soccer players, n=37; cyclists, n=29; controls, n=14). Soccer players, that practice a sport with high mechanical loading, showed greater bone stiffness on the dominant foot than the other groups. Cyclists had more 2.7% at the dominant and 4.4% at the nondominant foot than swimmers (Vlachopoulos et al., 2017c).

3.6. Conclusion

By merging all the studies and main findings it is possible to conclude that it is crucial and relevant to assess different methods and factors that influence bone growth. It is clear that adolescent athletes who are involved in sports with mechanical loading show different and positive benefits on bone content, density, geometry, biochemical markers, hormonal profile and bone stiffness. Sports with lower mechanical loading need to have longitudinal follow-ups to find training tools and exercises that help to minimize the differences that are presented and well described in this systematic review.

The study presents some limitations, such as, the search equation and keywords. The search results represented a larger main field that concerns bone and involves different topics among adolescents. This major lack of studies can become heavy to discuss all in the same manuscript summarize all the ideas in each one of the four topics.

In summary, the present systematic review brings novelty into the subject area and allows readers to better understand the major role that sports practice during growth plays in the adult life of each individual. Hopefully the combination of all these variables will bring to the subject what is missing in the literature and pinpoint a starting point for further research on adolescent athletes.

3.7 Other information

The present systematic review followed PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) (Page et at., 2021) and was registered in the International Prospective Register of Systematic Reviews database (PROSPERO, number CRD42022318977).

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3.8. Supplementary material

Supplementary Material 1.

Table 3 - Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies to examine the risk of bias for each manuscript with only one measurement time.

Supplementary Material 2.

Table 4 - Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies to examine the risk of bias for each manuscript with more than one measurement moment.

Supplementary Material 3.

Table 5 - Study characteristics (sport, age, body size, body composition), outcomes, and main findings.

Table 3 - Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies to examine the risk of bias for each manuscript with only one measurement time.

Study	Virvidakis et al. (1990)	Conroy et al. (1993)	Rico et al. (1993)	Lima et al. (2001)	Greene et al. (2006)
1. Was the research question or objective in this paper clearly stated?	Yes	Yes	Yes	Yes	Yes
2. Was the study population clearly specified and defined?	Yes	Yes	Yes	Yes	Yes
3. Was the participation rate of eligible persons at least 50%?	NA	NA	NA	NA	NA
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	Yes	No	Yes	No	Yes
5. Was a sample size justification, power description, or variance and effect estimates provided?	No	No	No	No	No
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	NA	NA	NA	NA	NA
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	NA	NA	NA	NA	NA
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	NA	NA	NA	Yes	NA
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes	Yes
10. Was the exposure(s) assessed more than once over time?	No	No	No	No	No
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes	Yes
12. Were the outcome assessors blinded to the exposure status of participants?	NA	NA	NA	NA	NA
13. Was loss to follow-up after baseline 20% or less?	NA	NA	NA	NA	NA
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	Yes	Yes	No	No	No
Quality Rating (good, fair, poor)	Fair	Fair	Fair	Fair	Fair

Table 3 - Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies to examine the risk of bias for each manuscript with only one measurement time.

Study	Falk et al. (2007)	Ducher et al. (2009)	Nebigh et al. (2009)	Falk et al. (2010)	Silva et al. (2011)
1. Was the research question or objective in this paper clearly stated?	Yes	Yes	Yes	Yes	Yes
2. Was the study population clearly specified and defined?	Yes	Yes	Yes	Yes	Yes
3. Was the participation rate of eligible persons at least 50%?	NA	NA	NA	NA	NA
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	Yes	Yes	Yes	Yes	Yes
5. Was a sample size justification, power description, or variance and effect estimates provided?	No	No	Yes	No	No
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	NA	NA	NA	NA	NA
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	NA	NA	NA	NA	NA
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	Yes	Yes	Yes	Yes	Yes
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes	Yes
10. Was the exposure(s) assessed more than once over time?	No	No	No	No	No
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes	Yes
12. Were the outcome assessors blinded to the exposure status of participants?	NA	NA	NA	NA	NA
13. Was loss to follow-up after baseline 20% or less?	NA	NA	NA	NA	NA
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	Yes	No	No	Yes	No
Quality Rating (good, fair, poor)	Good	Fair	Fair	Good	Fair

Table 3 - Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies to examine the risk of bias for each manuscript with only one measurement time.

Study	Quiterio et al. (2011)	Oldemillas et al. (2011)	Nasri et al. (2013)	Nasri et al. (2015)	Akgül et al. (2015)
1. Was the research question or objective in this paper clearly stated?	Yes	Yes	Yes	Yes	Yes
2. Was the study population clearly specified and defined?	Yes	Yes	Yes	Yes	Yes
3. Was the participation rate of eligible persons at least 50%?	NA	NA	NA	NA	NA
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	Yes	Yes	Yes	Yes	Yes
5. Was a sample size justification, power description, or variance and effect estimates provided?	No	No	No	No	No
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	NA	NA	NA	NA	NA
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	NA	NA	NA	NA	NA
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	Yes	No	No	No	No
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes	Yes
10. Was the exposure(s) assessed more than once over time?	No	No	No	No	No
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes	Yes
12. Were the outcome assessors blinded to the exposure status of participants?	NA	NA	NA	NA	NA
13. Was loss to follow-up after baseline 20% or less?	NA	NA	NA	NA	NA
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	Yes	Yes	Yes	Yes	No
Quality Rating (good, fair, poor)	Good	Fair	Fair	Fair	Poor

Table 3 - Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies to examine the risk of bias for each manuscript with only one measurement time.

Study	Tenforde et al. (2015)	Agostinete et al. (2017a)	Vlachopoulos et al. (2017b)	Seabra et al. (2017)
1. Was the research question or objective in this paper clearly stated?	Yes	Yes	Yes	Yes
2. Was the study population clearly specified and defined?	Yes	Yes	Yes	Yes
3. Was the participation rate of eligible persons at least 50%?	No	No	NA	No
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	Yes	Yes	Yes	Yes
5. Was a sample size justification, power description, or variance and effect estimates provided?	Yes	No	No	No
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	NA	NA	NA	NA
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	NA	NA	NA	NA
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	No	Yes	Yes	Yes
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes
10. Was the exposure(s) assessed more than once over time?	No	No	No	No
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes
12. Were the outcome assessors blinded to the exposure status of participants?	NA	NA	NA	NA
13. Was loss to follow-up after baseline 20% or less?	NA	NA	NA	NA
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	Yes	Yes	Yes	No
Quality Rating (good, fair, poor)	Fair	Fair	Good	Fair

Table 3 - Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies to examine the risk of bias for each manuscript with only one measurement time.

Study	Vlachopoulos et al. (2017c)	Agostinete et al. (2017b)	Fautino-da-Silva et al. (2018)	Gummus et al. (2019)
1. Was the research question or objective in this paper clearly stated?	Yes	Yes	Yes	Yes
2. Was the study population clearly specified and defined?	Yes	Yes	Yes	Yes
3. Was the participation rate of eligible persons at least 50%?	No	NA	NA	NA
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	Yes	Yes	Yes	Yes
5. Was a sample size justification, power description, or variance and effect estimates provided?	No	Yes	No	No
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	NA	NA	NA	NA
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	NA	NA	NA	NA
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	Yes	Yes	No	Yes
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes
10. Was the exposure(s) assessed more than once over time?	No	No	No	No
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes
12. Were the outcome assessors blinded to the exposure status of participants?	NA	NA	NA	NA
13. Was loss to follow-up after baseline 20% or less?	NA	NA	NA	NA
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	Yes	Yes	Yes	Yes
Quality Rating (good, fair, poor)	Good	Good	Fair	Fair

Table 3 - Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies to examine the risk of bias for each manuscript with only one measurement time.

Study	Kalabiska et al. (2020)	Maillane-Vanegas et al. (2020)	Agostinete et al. (2020a)	Narciso et al. (2020)	Agostinete et al. (2021)
1. Was the research question or objective in this paper clearly stated?	Yes	Yes	Yes	Yes	Yes
2. Was the study population clearly specified and defined?	Yes	Yes	Yes	Yes	Yes
3. Was the participation rate of eligible persons at least 50%?	NA	NA	NA	NA	NA
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	Yes	Yes	Yes	Yes	Yes
5. Was a sample size justification, power description, or variance and effect estimates provided?	No	Yes	Yes	Yes	Yes
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	NA	NA	NA	NA	NA
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	NA	NA	NA	NA	NA
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	Yes	Yes	Yes	Yes	Yes
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes	Yes
10. Was the exposure(s) assessed more than once over time?	No	No	No	No	No
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes	Yes
12. Were the outcome assessors blinded to the exposure status of participants?	NA	NA	NA	NA	NA
13. Was loss to follow-up after baseline 20% or less?	NA	NA	NA	NA	NA
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	No	Yes	Yes	Yes	Yes
Quality Rating (good, fair, poor)	Fair	Good	Good	Good	Good

Table 4 - Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies to examine the risk of bias for each manuscript with more than one measurement moment.

Study	Nordstrom et al. (2005)	Tervo et al. (2009)	Zouch et al. (2014)	Agostinete e al. (2016)
1. Was the research question or objective in this paper clearly stated?	Yes	Yes	Yes	Yes
2. Was the study population clearly specified and defined?	Yes	Yes	Yes	Yes
3. Was the participation rate of eligible persons at least 50%?	NA	NA	NA	NA
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	Yes	Yes	Yes	Yes
5. Was a sample size justification, power description, or variance and effect estimates provided?	No	No	Yes	Yes
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	Yes	Yes	Yes	Yes
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	Yes	Yes	Yes	Yes
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	No	Yes	Yes	Yes
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes
10. Was the exposure(s) assessed more than once over time?	Yes	Yes	Yes	Yes
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes
12. Were the outcome assessors blinded to the exposure status of participants?	NA	NA	NA	NA
13. Was loss to follow-up after baseline 20% or less?	No	No	No	No
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	No	Yes	No	Yes
Quality Rating (good, fair, poor)	Fair	Fair	Fair	Good

Table 4 - Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies to examine the risk of bias for each manuscript with more than one measurement moment.

Study	Ribeiro-dos-Santos et al. (2016)	Vlachopoulos et al. (2017a)	Gomez-Bruton et al. (2017)	Vlachopoulos et al. (2018a)
1. Was the research question or objective in this paper clearly stated?	Yes	Yes	Yes	Yes
2. Was the study population clearly specified and defined?	Yes	Yes	Yes	Yes
3. Was the participation rate of eligible persons at least 50%?	NA	NA	NA	NA
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	Yes	Yes	Yes	Yes
5. Was a sample size justification, power description, or variance and effect estimates provided?	Yes	No	Yes	Yes
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	Yes	Yes	Yes	Yes
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	Yes	Yes	Yes	Yes
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	No	Yes	Yes	Yes
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes
10. Was the exposure(s) assessed more than once over time?	Yes	Yes	Yes	Yes
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes
12. Were the outcome assessors blinded to the exposure status of participants?	NA	NA	NA	Yes
13. Was loss to follow-up after baseline 20% or less?	No	No	No	No
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	Yes	Yes	Yes	Yes
Quality Rating (good, fair, poor)	Good	Fair	Good	Fair

Table 4 - Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies to examine the risk of bias for each manuscript with more than one measurement moment.

Study	Vlachopoulos et al. (2018b)	Ubado-Guisado et al. (2018a)	Vlachopoulos et al. (2018c)	Ubado-Guisado et al. (2019)
1. Was the research question or objective in this paper clearly stated?	Yes	Yes	Yes	Yes
2. Was the study population clearly specified and defined?	Yes	Yes	Yes	Yes
3. Was the participation rate of eligible persons at least 50%?	NA	NA	NA	NA
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	Yes	Yes	Yes	Yes
5. Was a sample size justification, power description, or variance and effect estimates provided?	No	No	No	Yes
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	Yes	Yes	Yes	Yes
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	Yes	Yes	Yes	Yes
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	Yes	Yes	Yes	No
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes
10. Was the exposure(s) assessed more than once over time?	Yes	Yes	Yes	Yes
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes
12. Were the outcome assessors blinded to the exposure status of participants?	NA	NA	NA	NA
13. Was loss to follow-up after baseline 20% or less?	No	No	No	No
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	Yes	Yes	Yes	Yes
Quality Rating (good, fair, poor)	Good	Fair	Fair	Good

Table 4 - Quality Assessment Tool for Observational Cohort and Cross-Sectional Studies to examine the risk of bias for each manuscript with more than one measurement moment.

Study	Rapún-Lopez et al. (2019)	Luiz-de-Marco et al. (2019)	Luiz-de-Marco et al. (2020)	Agostinete et al. (2020b)
1. Was the research question or objective in this paper clearly stated?	Yes	Yes	Yes	Yes
2. Was the study population clearly specified and defined?	Yes	Yes	Yes	Yes
3. Was the participation rate of eligible persons at least 50%?	NA	NA	NA	NA
4. Were all the subjects selected or recruited from the same or similar populations (including the same time period)? Were inclusion and exclusion criteria for being in the study prespecified and applied uniformly to all participants?	Yes	Yes	Yes	Yes
5. Was a sample size justification, power description, or variance and effect estimates provided?	No	Yes	No	Yes
6. For the analyses in this paper, were the exposure(s) of interest measured prior to the outcome(s) being measured?	Yes	Yes	Yes	Yes
7. Was the timeframe sufficient so that one could reasonably expect to see an association between exposure and outcome if it existed?	Yes	Yes	Yes	Yes
8. For exposures that can vary in amount or level, did the study examine different levels of the exposure as related to the outcome (e.g., categories of exposure, or exposure measured as continuous variable)?	No	No	Yes	Yes
9. Were the exposure measures (independent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes
10. Was the exposure(s) assessed more than once over time?	Yes	Yes	Yes	Yes
11. Were the outcome measures (dependent variables) clearly defined, valid, reliable, and implemented consistently across all study participants?	Yes	Yes	Yes	Yes
12. Were the outcome assessors blinded to the exposure status of participants?	NA	NA	NA	NA
13. Was loss to follow-up after baseline 20% or less?	No	No	No	No
14. Were key potential confounding variables measured and adjusted statistically for their impact on the relationship between exposure(s) and outcome(s)?	No	Yes	Yes	Yes
Quality Rating (good, fair, poor)	Fair	Fair	Fair	Good

Table 5 - Study characteristics (sport, age, body size, body composition), outcomes, and main findings.

Study	Aim	Sample	Age (yrs)	To access bone	Outcomes	Main findings	Obs.
Virvidakis et al. (1990)	Measure BMC of competitive weightlifters.	n=59 weightlifters n=91 control group	15-20	Single photon absorptiometry	BMC	Weightlifters had > BMC than controls at the forearm.	
Conroy et al. (1993)	1) Compare values for BMD of the spine and proximal femur (junior weightlifters vs controls vs reference adult population) 2) Examine possible relationships between muscle strength, BM, FFM, and BMD of the spine and proximal femur.	n=25 weightlifters n=11 control group	17.4±1.4 16.9±1.1	DXA	BMD	A strong relationship between force production (sport-specific strength) and BMD of PF and lumbar spine. Junior weightlifter > controls > adult reference values.	
Rico et al. (1993)	Assess total bone mineral content (TBBM) and leg bone mineral content (LBMC) in cyclists (15-19 yrs) and compare with age-matched controls.	n=22 cyclists n=27 control group	16.2±0.7 16.9±0.3	DXA	BMC	Controls > calcium; Controls > leg BMC; When normalized for body weight the differences between groups disappear.	

DXA (dual-energy x-ray absorptiometry); BMC (bone mineral content); BMD (bone mineral density); PF (proximal femur), BM (body mass); FFM (fat free mass)

Table 5 - (continued)

Study	Aim	Sample	Age (yrs)	To access bone	Outcomes	Main findings	Obs.

Lima et al. (2001)	1) Elucidate on the effects of loading patterns on BMD, body composition, sexual hormones, and bone biochemical markers of male adolescent athletes whose physical activity was considered to involve impact loading with those athletes whose physical activity was considered to involve active loading;
	
	2) Examine the contributing effects that body composition constituents have on BMD in this group.
		n=18 impact group (gymnastics, athletics, basketball, and tennis);
		
		n=27 active load group (swimming and water polo);
		
		n=24 control group;
			12.0-18.0	DXA
				
				Blood sample
				
				Urine sample
					Biochemical markers
					
					Hormonal profile
					
					BMD
						Male adolescents who practice impact load sports showed > BMD at all sites ($p>0.05$) compared with controls and aquatic athletes. BMD on the lumbar spine and femoral neck were similar between the impact group and controls. BAP was sig. > in the active than impact and controls. Testosterone levels were sig higher in the impact compared with active.	

Nordstrom et al. (2005)	Investigate the effect of training and detraining on BMD of weight-bearing and non-weight-bearing bone.	n=43 ice hockey players	16.7±0.6 16.8±0.3	DXA	BMD	Ice hockey > BMD than controls at total hip and humerus non-dominant but not in the other assessed regions (humerus dominant, arms, pelvis, femur, and femoral neck);	This study was analyzed only at baseline because in the follow-up participants were > 18yrs;

DXA (dual-energy x-ray absorptiometry); BMD (bone mineral density); BAP (bone-specific alkaline phosphatase)

Table 5 - (continued)

Study	Aim	Sample	Age (yrs)	To access bone	Outcomes	Main findings	Obs.
Greene et al. (2006)	Explore differences in HSA at the femoral neck and BSI at the distal tibia between elite, male and female adolescent middle-distance runners and non-active, age- and gender-matched controls.	n=20 male adolescent middle-distance runners n=20 controls	16.8±0.6 16.4±0.7	MRI DXA DXA-HSA Blood sample	Bone geometry BMC Hormonal profile	No differences between groups in calcium intake, testosterone, BMC, and bone properties at the distal tibia and femoral neck. Male athletes do not seem to show greater outcomes than controls at bone strength at the distal tibia or femoral neck.	Only males were taken into account for the present study.
Falk et al. (2007)	Assess differences in bone properties, using quantitative ultrasound in males involved in weight-bearing sports, non-weight-bearing sports, and controls.	Children (n=34 soccer players, n=34 swimmers and water polo players; n=25 controls); Adolescents (n=32 soccer players, n=31 swimmers and water polo players; n=31 controls)	10.9±0.9 11.4±0.8	QUS	Bone stiffness	Children and adolescents did not differ in SOS. A possibly higher BMD in younger soccer players may not be reflected at SOS. Calcium intake was lower than the recommended value in all groups.	The study presented a group of adults who were not included in the analysis of the present review.
Ducher et al. (2009)	Determine whether exercise-induced changes in bone mass and geometry were site-specific and varied with maturational status in young boys.	Tennis players: n=9 prepubertal n=26 peripubertal n=8 postpubertal	 11.3±0.3 13.9±0.2 17.1±0.4	MRI DXA	Bone geometry BMD BMC	Regarding geometry, the nonplaying humerus was larger in post than prepubertal boys. The mechanical loading perceived by prepubertal tennis players results in a larger bone size and cortical cross-sectional area. Also, at this age, loading shows greater accrual in the periosteal surface than controls.	

DXA (dual-energy x-ray absorptiometry); BMD (bone mineral density); BMC (bone mineral content); HSA (hip structural analysis); BSI (bone strength index); SOS (speed of sound); QUS (quantitative ultrasound)

Table 5 - (continued)

Study	Aim	Sample	Age (yrs)	To access bone	Outcomes	Main findings	Obs.
Nebigh et al. (2009)	Examine the effects of soccer in relation to the hormonal concentration, on the bone mass of young Tunisian players at different pubertal stages.	n=91 soccer players; n=61 controls	13.4±0.3 13.3±0.5	DXA Blood sample	BMC BMD Hormonal profile Bone biochemical markers	Young soccer players > bone mass and hormonal concentration than controls; BMD and BMC were > in soccer players for the whole body and regions (lumbar spine, femoral neck, pelvis, and lower limbs); IGFBP-3 and IGF-1 were correlated with BMD;	

Tervo et al. (2009)	Investigate the association between BMD loss and reduced physical activity at trabecular and cortical bone sites in males.	Ice hockey and badminton
		n=86 athletes approx. 9 h/wk;
		
		n=51 former athletes;
		
		n=25 controls with approx. 2h/wk.
			17.3±1.8
			
			17.2±1.3
			
			16.9±0.6
				DXA	BMD	Athletes had > BMD than controls, more specifically at the humerus, leg, and femur;
						There were no differences between groups in head BMD.
							This study was analyzed only at baseline because in the next follow-up participants were > 18yrs;

Falk et al. (2010)	Compare bone properties in competitive male child and adolescent athletes and, their minimally active (<2x/week) age- and maturity-matched counterparts, using QUS technology.	Children: n=34 controls n=26 soccer n=30 hockey Adolescents: n=31 controls n=30 soccer n=31 hockey	 11.1±0.7 11.1±0.5 11.2±0.8 15.2±0.7 15.2±0.7 15.3±0.9	QUS	Bone stiffness	Hockey players showed higher radial SOS and soccer players higher tibial SOS compared with controls. Results refute that mechanical loading is associated with the increment of bone strength in sports-specific sites.	

DXA (dual-energy x-ray absorptiometry); BMD (bone mineral density); BMC (bone mineral content); QUS (quantitative ultrasound); SOS (speed of sound)

Table 5 - (continued)

Study	Aim	Sample	Age (yrs)	To access bone	Outcomes	Main findings	Obs.
Silva et al. (2011)	Investigate the impact of different sports on total and regional bone mineral density in adolescent Brazilian athletes.	n= 14 controls n= 12 swimmers n=10 tennis n=10 soccer	13.4±2.02 13.8±2.45 14.1±1.61 14.7±0.82	DXA	BMD	Proximal femur BMD was higher in soccer and tennis players when compared with swimmers and controls.	
Quiterio et al. (2011)	Compare total and regional DXA-measured bone mineral parameters and fat-free body mass between two different categories of impact-loading sports in adolescent male athletes, having as reference an age-matched control group.	High-impact n=34 gymnastic, basketball, and handball Non-impact n=20 swimming Controls n=26	15.7±1.6 16.4±2.5 15.9±2.8	DXA	BMC BMD	After potential confounding variables were measured, athletes from the high-impact group presented greater levels of BMC and BMD at whole-body, lumbar spine, and appendicular skeleton.	
Oldemillas et al. (2011)	1) Describe the bone status in adolescent cyclists compared to a healthy age-matched group. 2) Analyse the effect of years of cycling practice on the acquisition of bone mass.	Younger group n=24: 11 cyclists 13 controls Older group n=20 11 cyclists 9 controls	15.5±0.9 15.2±1.2 18.4±1.4 18.7±1.2	DXA	BMC BMD	Cyclists showed lower values for BMD at the legs, pelvis, total hip, trochanter, and inter-trochanteric subregion than controls (p<0.01).	
Nasri et al. (2013)	Investigate the correlation between bone parameters and grip strength in hands, explosive legs power, and hormonal parameters and to identify the most determinant variables of bone density.	Combat athletes n=50 Sedentary group n=30	17.1±0.2 17.1±0.4	DXA Blood sample	BMD Hormonal profile	Combat athletes > BMD in the lumbar spine, at lower and upper limbs than sedentary participants. Grip strength was a good predictor of BMD variance. There were no differences in growth hormone between groups.	

DXA (dual-energy x-ray absorptiometry); BMD (bone mineral density); BMC (bone mineral content);

Table 5 - (continued)

Study	Aim	Sample	Age (yrs)	To access bone	Outcomes	Main findings	Obs.
Zouch et al. (2014)	Determine whether soccer could have different bone benefits in prepubescent and pubescent boys.	Prepubescent n=35 n=22 soccer n=13 control Pubescent n=41 n=26 soccer n=26 control	T0 Prepubescent Soccer 11.93±0.81 Control 11.69±0.61 T0 Pubescent Soccer 12.86±0.79 Control 12.52±0.59 T1 Prepubescent Soccer 12.93±0.81 Control 12.62±0.57 T1 Pubescent Soccer 13.84±0.80 Control 13.46±0.61	DXA	BMC	In control participants, BMC gain was significantly higher in boys who became pubescent than in boys who remained prepubescent in the whole body, total hip, dominant and non-dominant arm. This study found that pubescent soccer players have significantly > BMC gain in the femoral neck, lumbar spine, and supporting leg versus soccer-playing boys who remained prepubescent and then pubescent controls.	
Nasri et al. (2015)	1) Determine the impact of combat sports practice on BMD. 2) Analyze the relationship between bone parameters and anthropometric measurements, bone markers, and activity index. 3) Detect the most important, determinant of BMD in the adolescent period among male athletes engaging in combat sports.	Combat sports n=50 10 judoists 10 karatekas 10 karatekas kyokushinkai 10 kung fu 10 boxers. Sedentary n= 30	17.08±0.2 17.1±0.4	DXA Blood sample	BMD Bone biochemical markers	All athletes presented > BMD than the sedentary group. The major difference was found in the whole body and lunar spine (L1-L2) BMD. Regarding biomarkers, the sedentary group showed higher ($p<0.05$) AP and P1NP concentrations than athletes but the same was not observed in OC and βCTx.	

DXA (dual-energy x-ray absorptiometry); BMD (bone mineral density); BMC (bone mineral content); AP (Alkaline phosphate); P1NP (Propeptide N-terminal Procollagenetype 1); OC (osteocalcin); βCTx (plasma β-CrossLaps)

Table 5 - (continued)

Study	Aim	Sample	Age (yrs)	To access bone	Outcomes	Main findings	Obs.
Akgül et al. (2015)	Determine the effect of swimming on bone metabolism during adolescence in competitive adolescent swimmers and evaluate the effect of different strokes.	Swimmers n= 41 male n= 38 females	Mean 12.1 Range (10-21)	DXA Blood sample	BMD Bone biochemical markers	There was no statistically significant effect of type of stroke on BMD. The study reinforces the idea that high-impact exercise outside the pool is important for bone structure maintenance.	
Tenforde et al. (2015)	Characterize sex-specific risk factors for low BMD in adolescent runners.	n=42 boys n=91 girls	16.3±1.3	DXA	BMD (z-score)	Male adolescent runners showed a risk for suboptimal bone mass.	The study included also females but only results for males were analysed.
Agostinete et al. (2016)	Analyse the effect of different sports on bone mineral density (BMD) accrual among male adolescents during a 9-month follow-up.	n=13 controls n=14 basketball n=9 karate n=18 soccer n=12 judo n=16 swimming	11.9±2.2 13.4±1.2 13.1±1.8 12.4±1.9 13.1±1.5 13.5±1.5	DXA	BMD	Basketball showed higher gains than controls and every sport, but similar results to judo at the upper limbs BMD. Swimming showed no additional BMD improvement compared to the control group.	
Ribeiro-dos-Santos et al. (2016)	Analyse the effect of swimming on BMD gain among Brazilian adolescents engaged in national-level competitions.	Swimmers n=55 n=29 boys n=26 girls Controls n=26 n=16 boys n=10 girls	Boys 12.7±2.1 Girls 13.1±2.1	DXA	BMD	The groups were assessed 9-months apart and were found that gains in BMD-Spine were higher among swimmers than controls. Longer involvement in swimming during adolescence and childhood negatively affected BMD gains,	Results for girls were not taken into account.

DXA (dual-energy x-ray absorptiometry); BMD (bone mineral density)

Table 5 - (continued)

Study	Aim	Sample	Age (yrs)	To access bone	Outcomes	Main findings	Obs.
Agostinete et al. (2017a)	To compare whole-body and regional indicators of bone tissue, in parallel to adolescent athletes contrasting in mechanical load (basketball and swimming measurements of blood lipid and inflammatory profiles in athletes) and non-athletes counterparts.	n=20 controls n=18 basketball n=15 swimmers	13.0±1.3 14.5±0.9 15.9±2.1	DXA	BMC BMD	Basketballers players showed > BMD and BMC, specifically at the lower limbs than swimmers. On the other hand, metabolic parameters (lipids) were more likely to be affected by swimming practice.	
Vlachopoulos et al. (2017a)	To determine the longitudinal (1-year) differences in clinically relevant DXA-measured BMC sites, hip geometry estimates, TBS, and bone turnover and nutritional markers in adolescent male athletes engaged in football, swimming, and cycling and active controls aged 12 to 14 years at baseline.	Baseline n=37 swimmers n=37 footballers n=28 cyclists n=14 controls	Baseline 13.5±1.0 12.9±0.9 13.2±1.0 12.3±0.5 Follow-up 14.6±1.0 13.9±0.9 14.2±1.0 13.2±0.5	DXA DXA-HSA TBS Blood sample	BMC Bone geometry Bone biochemical markers	During a one-year period, footballers demonstrated higher accrual than swimmers and cyclists related to BMC, HSA, and, TBS. This last group has similar results to controls. Regarding bone biochemical markers, football players showed higher bone formation than the other groups.	Football was used as the British English to soccer.

DXA (dual-energy x-ray absorptiometry), BMD (bone mineral density), BMC (bone mineral content), HSA (hip structural analysis), TBS (trabecular bone score)

Table 5 - (continued)

Study	Aim	Sample	Age (yrs)	To access bone	Outcomes	Main findings	Obs.
Vlachopoulos et al. (2017b)	Provide novel insight into the contribution of the independent predictors of sports participation (football, swimming, and cycling), stature, region-specific lean and fat mass, serum calcium and vitamin D, MVPA, muscular fitness, and CRF (all adjusted by each other) on aBMD and hip geometry estimates in adolescent male athletes.	n=41 swimmers n=37 soccer players n=29 cyclists n=14 controls	13.5±1.0 12.9±0.9 13.2±1.0 12.3±0.5	DXA DXA-HSA	BMD Bone geometry	When comparing different sports with controls, soccer practice was the only one that was a significant predictor for aBMD and hip geometry estimates. In contrast, swimming and cycling showed no positive contribution to aBMD at any skeleton site and on geometrical parameters.	
Gomez-Bruton et al. (2017)	1) Evaluate swimmers' aBMD with DXA and bone mass, bone strength, and structure with pQCT during a swimming season and compare them to those of normo-active controls. 2) Evaluate whether practicing an extra weight-bearing sport in addition to swimming might confer any aBMD, bone mass, strength or structure benefits.	Baseline n=11 swimming sport n=23 swimming pure n=28 controls Follow-up n=11 swimming sport n=23 swimming pure n=28 controls	Baseline 15.1±2.8 15.0±2.2 14.1±2.3 Follow-up 15.8±2.8 15.7±2.2 14.9±2.3	pQCT DXA	Bone geometry BMC aBMD	Swimming practice showed no effects on radius aBMD, BMC, strength, and structure and minor effects on tibia bone strength, structure, and BMC. Swimmers with more training hours (Swimming sport) showed differences on arm aBMD at baseline and follow-up.	

CRF (cardiorespiratory fitness), MVPA (moderate to vigorous physical activity), aBMD (areal bone mineral density), DXA (dual-energy x-ray absorptiometry), pQCT (peripheral quantitative computed tomography), HSA (hip structural analysis)

Table 5 - (continued)

Study	Aim	Sample	Age (yrs)	To access bone	Outcomes	Main findings	Obs.
Seabra et al. (2017)	Investigate the bone mass of adolescent male futsal players, comparing it with swimmers (a non-weight-bearing and low-impact sport) and a control group (subjects of the same gender and age).	n= 28 futsal n=20 swimmers n=22 controls	13.5±1.2 13.2±1.2 13.4±0.9	DXA	aBMC aBMD	The authors confirmed the hypothesis that adolescent participants who practice futsal had higher bone mass than swimmers and controls. There were no differences in calcium intake.	
Vlachopoulos et al. (2017c)	Investigate whether bone properties, assessed by DXA, HSA, and QUS, differ between 12- and 14-yr-old boys who perform osteogenic (soccer) and non-osteogenic (swimming, cycling) sports in comparison to a control group after controlling for a comprehensive set of confounders.	n=41 swimmers n=37 soccer players n=29 cyclists n=14 controls	13.4±1.0 12.8±0.9 13.2±1.0 12.3±0.5	DXA-HSA QUS DXA	BMD BMC Bone geometry Bone stiffness	Cyclists presented > BMD than controls only at trochanter, > BMC and BA only at arms compared with controls. Football players showed greater adjusted BMD and BMC and hip structural geometry compared with other sports. Cyclists and swimmers presented similar bone mass, geometry, and bone stiffness.	
Agostinete et al. (2017b)	1) To compare bone mineral variables in adolescents according to different sports; 2) To identify whether the potential differences in bone mineral variables attributed to sports participation are dependent on somatic maturation status.	n=42 controls n=26 soccer and basketball n= 25 swimming	13.1±1.1 12.8±0.9 13.2±1.0 12.3±0.5	DXA	BMC BMD	Swimmers showed < BMD than controls and impact sports (soccer and basketball). After adding confounders variables, swimmers showed a higher percentage of overall BMD in the skull and upper limbs than the impact group and controls.	Both sexes equally.

DXA (dual-energy x-ray absorptiometry), (a)BMD ((areal)bone mineral density), (a)BMC ((areal)bone mineral content), QUS (quantitative ultrasound), HSA (hip structural analysis); BA (bone area)

Table 5 - (continued)

Study	Aim	Sample	Age (yrs)	To access bone	Outcomes	Main findings	Obs.
Vlachopoulos et al. (2018a)	Examine the effects of a 9-month progressive jumping intervention program on BMC, hip geometry estimates at the femoral neck, TBS at the lumbar spine, and bone turnover markers in adolescent male swimmers, soccer players, and cyclists.	Swimmers n=19 intervention n=18 control Soccer players n=15 intervention n=15 control Cyclists n=14 intervention n=12 control	Pre-intervention Swimmers 12.3±0.5 14.7±1.1 Soccer players 13.8±1.0 13.7±0.8 Cyclists 14.1±1.1 14.1±0.9 Pos-intervention Swimmers 15.3±0.9 15.4±1.1 Soccer players 14.6±1.0 14.5±0.8 Cyclists 14.9±1.1 14.9±0.9	DXA DXA-HSA Blood sample TBS	BMC Bone geometry Bone biochemical markers	Non-weight-bearing sports showed significant improvements in BMC, HSA, and TBS bone after a 9-month progressive jumping intervention program at specific sites of the skeleton such as the lumbar spine and femoral neck. The same protocol did not show significant improvements in weight-bearing sports. Regarding biochemical markers, bone resorption significantly decreased in the control group of swimming and cyclist and did not vary in intervention groups.	
Ubado-Guisado (2019)	Identify the determinants of 12-month changes on bone outcomes (aBMD, hip geometry estimates and TBS) in adolescent male athletes.	n=39 swimmers n=37 footballers n=28 cyclists	Baseline 13.5±1.0 12.9±0.9 13.3±1.1 Follow-up 14.6±1.0 13.9±1.0 14.2±1.0	DXA Blood sample DXA-HSA TBS	aBMD Bone geometry Bone biochemical markers	Region-specific lean mass was the most explanatory variable of differences in aBMD, also the practice of swimming and cycling were negative predictors of aBMD, hip geometry estimates (only cycling), and TBS.	

DXA (dual-energy x-ray absorptiometry), aBMD (areal bone mineral density), HSA (hip structural analysis), TBS (trabecular bone score)

Table 5 - (continued)

Study	Aim	Sample	Age (yrs)	To access bone	Outcomes	Main findings	Obs.
Vlachopoulos et al. (2018b)	Examine the effects of a high-impact 9-month jumping intervention on bone mass, bone stiffness, and fitness parameters in male adolescents involved in swimming, football, and cycling.	Swimmers n=19 intervention n=18 control Football players n=15 intervention n=15 control Cyclists n=14 intervention n=12 control	Pre-intervention Swimmers 14.5±0.9 14.7±1.1 Football players 13.8±1.0 13.7±0.8 Cyclists 14.1±1.1 14.1±0.9 Pos-intervention Swimmers 15.3±0.9 15.4±1.1 Footballers 14.6±1.0 14.5±0.8 Cyclists 14.9±1.1 14.9±0.9	DXA QUS	BMC Bone stiffness	The high-impact jumping intervention showed significant improvements in BMC at TBLH, leg and bone stiffness. In swimming and cycling but not in football.	Football was used as the British English to soccer.
Faustino-da-Silva et al. (2018)	Compare BMD in different body segments among adolescents engaged and not engaged in track and field, using a pilot sample of the (ABCD - Growth Study).	17 controls n=17 track and field	16.0±1.13 16.0±1.08	DXA	BMD BMC	Athletes > BMD in lower limbs at the whole body. Athletes > BMC in lower limbs and right arm than controls. Authors relate these results also with the impact exerted by LST.	Both genres(22 males, 12 females).

DXA (dual-energy x-ray absorptiometry), BMD (bone mineral density), BMC (bone mineral content), HSA (hip structural analysis), TBS (trabecular bone score), LST (lean soft tissue)

Table 5 - (continued)

Study	Aim	Sample	Age (yrs)	To access bone	Outcomes	Main findings	Obs.
Vlachopoulos et al. (2018c)	Investigate the effect of 12-month participation on BMC and bone stiffness in osteogenic (football) and those non-osteogenic sports (swimming and cycling) compared to an active control group after adjusting for baseline bone outcomes, age, height, lean mass and MVPA.	n=37 swimmers n=37 football players n=28 cyclists n=14 active controls	Baseline 13.5±1.0 12.9±0.9 13.2±1.0 12.3±0.5 Follow-up 14.6±1.0 13.9±0.9 14.2±1.0 13.2±0.5	DXA QUS	BMC Bone stiffness	The study allowed a perception of changes in BMC on relevant skeletal sites during a one-year period. Football players showed significant accrual in BMC and bone stiffness than swimmers and cyclists but non-significant when compared with active controls. Swimmers and cyclists present similar out-comes in this period with no significant differences in BMC and bone stiffness compared to controls.	Football was used as the British English to soccer.
Ubado-Guisado et al. (2018)	To investigate differences in bone outcomes according to age from PHV in young male athletes participating in OS (football) or NOS (swimming or cycling)	n=23 osteogenic sport n=38 non-osteogenic sport	At PHV: 13.7±0.4 13.6±0.4	DXA DXA-HSA QUS	BMC Bone geometry Bone stiffness	Osteogenic sports showed higher values in all bone outcomes compared with non-osteogenic sports. When adjusted with confounding variables, the osteogenic group continued to have significant higher TBLH BMC between the PHV and +2 years from PHV. This group also had grater HSA estimates, TBS and stiffness index. These differences seemed to increase with biological age.	

MVPA (moderate to vigorous physical activity), PHV (peak height velocity), OS (osteogenic sport), NOS (non-osteogenic sport), DXA (dual-energy x-ray absorptiometry), QUS (quantitative ultrasound), BMC (bone mineral content), HSA (hip structural analysis)

Table 5 - (continued)

Study	Aim	Sample	Age (yrs)	To access bone	Outcomes	Main findings	Obs.
Rapún-López et al. (2019)	Measure the effect of cycling on bone metabolism in adolescent cyclists and to compare it to active age-matched peers.	n=7 cyclists n=8 controls	Pre 16.3±0.9 15.8±1.5 Post 17.6±1.2 16.9±1.5	Blood sample	Bone biochemical markers	Cyclists and controls showed similar concentration of markers. Cyclists showed decreased vitamin D in this one-year period.	
Gumus et al. (2019)	Evaluate the influence of different sports with different mechanical loading properties (weight bearing, non-weight bearing and combinations of both types of loading patterns) on BMD among young athletes.	n=50 swimming n=18 pentathlon n=7 figure skating	11.8±1.6 13.0±2.5 14.3±2.1	DXA	BMD BMC	The lumbar spine showed no differences between groups for BMC and BMD. The same occurred with BMC of the left proximal femur. Serum levels of osteocalcin, a specific bone formation marker, were significantly higher in the swimming group (p<0.05). Swimmers had the lowest BMD and BMC in the lumbar spine and femoral neck compared with other sports.	The sample included both genres. Results did not distinguish sexes.
Luiz-de-Marco et al. (2019)	Identify determinants of sex-related BMD differences between male and female adolescents, and to identify the role of sports participation in this phenomenon	n=24 boys n=24 girls	12.0±1.6 12.1±1.6	DXA	BMD	LST was the most relevant and explanatory variable for BMD.	Participants were matched according to sex (1:1). The main finding was equal for both sexes. Comparisons between sexes were ignored.

DXA (dual-energy x-ray absorptiometry), BMD (bone mineral density), BMC (bone mineral content), LST (lean soft tissue)

Table 5 - (continued)

Study	Aim	Sample	Age (yrs)	To access bone	Outcomes	Main findings	Obs.
Luiz-de-Marco et al. (2020)	Analyze the impact of changes in lean soft tissue (LST) and fat mass on areal BMD accrual among adolescents engaged in sports with different weight-bearing levels.	Baseline n=104 controls n=34 swimmers n=147 WB 12-months after n=66 controls n=25 swimmers n=100 WB	15.78±2.11 14.25±2.42 14.05±1.86	DXA Blood sample	aBMD Bone biochemical markers	Osteocalcin did not differ between groups. In the adjusted model, changes in LST were positively related to areal BMD gains in all groups analyzed, explaining 38.9%, 56.1%, and 50.6% of all gains for the control, swimming, and weight-bearing sports groups, respectively.	The sample included both sexes. Results did not distinguish the sexes. Age is represented as the baseline of the participants that remain in the study for the follow-up.
Kalabiska et al. (2020)	Develop reference values for BMC, BMD, and TBLH BMD based on DEXA in a sample of Hungarian youth athletes of both sexes.	Categories by age group 11 yrs (n=25, 11.56 yrs); 12 yrs (n=28, 12.63 yrs); 13 yrs (n=63, 13.55 yrs); 14 yrs (n=98, 14.57 yrs); 15 yrs (n=200, 15.50 yrs); 16 yrs (n=174, 16.48 yrs); 17 yrs (n=174, 17.49 yrs); 18 yrs (n=123, 18.43 yrs); 19 yrs (n=42, 19.53 yrs); 20 yrs (n=29, 20.34 yrs)		DXA	BMD BMC	Comparing study values with reference by age, BMC and BMD of youth athletes showed increases. Intensive physical activity was associated with increased BMC and BMD.	1385 athletes, 1019 males, and 366 females. Groups were made by age and only males are here described.
Maillane-Vanegas et al. (2020)	Identify differences in BMD according to different sports among adolescents and to identify assessments of relevant mediators.	n=111 non-athletes n=42 swimming n=106 soccer n=33 karate n=53 judo n=49 kung fu n=35 basketball	13.1±1.6 13.6±1.8 14.9±1.8 12.6±1.4 13.1±1.8 13.8±1.7 13.7±1.3	DXA	BMD	Soccer, karate, and basketball showed the highest BMD values when compared with non-sport group.	n=300 boys, n=129 girls. Only the male results were analyzed. Sample and age have both sexes represented.

DXA (dual-energy x-ray absorptiometry), BMD (bone mineral density), BMC (bone mineral content), LST (lean soft tissue), WB (weight-bearing)

Table 5 - (continued)

Study	Aim	Sample	Age (yrs)	To access bone	Outcomes	Main findings	Obs.
Agostinete et al. (2020a)	Categorize adolescent athletes from 10 different loading sports and a non-sport group according to bone and soft tissue profiles as well as to quantify the differences in bone density and soft tissues by controlling for important confounders.	n = 54 judo n=41 karate n=39 kung fu n=67 swimming n=55 basketball n=100 soccer n=32 volleyball n=27 track and field n=22 baseball n=16 gymnastics n=172 control group	13.8±1.3 15.0±1.8 13.2±1.8 15.0±1.1 13.1±1.8 13.1±1.8 13.4±1.7 12.1±1.6 13.6±1.7 15.6±1.8 14.5±2.3	DXA	aBMD BMAD	Whole-body aBMD, the highest value was observed in soccer (1.082 g·cm−2) and the lowest in swimming (0.959 g·cm−2). The highest BMAD value was observed in the gymnastics group (0.094 g·cm−3), and the lowest BMAD value was found in the swimming group (0.085 g·cm−3). Specifically at lumbar spine, judo showed the higher values and swimming the lowest.	Results did not distinguish sexes.
Agostinete et al. (2020b)	Investigate the impact and non-impact sports on bone mineral density (BMD) accrual in adolescents over a period of 18 months.	<u>Baseline n=184</u> n=69 impact n=30 non-impact n=85 controls <u>1st follow-up</u> n=69 impact n=30 non-impact n=85 controls <u>2nd follow-up</u> n=69 impact n=30 non-impact n=85 controls	<u>Baseline</u> 12.7±1.3 12.7±1.2 12.6±2.6	DXA	aBMD BMAD	The main findings of the present study indicate that children and adolescents involved in impact sports, such as basketball and marital arts, over a period of 18 months have significantly higher adjusted aBMD and BMAD accrual compared with non-impact sports, such as swimming.	Sample was composed by males and females. Results did not distinguish sexes.

DXA (dual-energy x-ray absorptiometry), (a)BMD ((areal) bone mineral density), BMAD (bone mineral apparent density),

Table 5 - (continued)

Study	Aim	Sample	Age (yrs)	To access bone	Outcomes	Main findings	Obs.
Narciso et al. (2020)	Examine the mediating role of LST on the relationship between somatic maturation and aBMD in adolescents considering sex and sport participation.	n=63 basketball n=100 soccer n=2 volleyball n=19 karate n=35 judo n=41 kung-fu n=13 baseball n=15 tennis n=11 track and field	All sample: 14.1±1.8	DXA	aBMD	LST mediated the association between somatic maturation and aBMD at all skeletal sites (upper limbs, lower limbs, spine and TBLH) in male and female adolescents regardless of sport participation. Furthermore, the direct association between somatic maturation and aBMD remained significant after accounting for LST as a mediating variable, except for lower limbs and TBLH in the non-sport group.	
Agostinete et al. (2021)	Identify the influential role of body LST in the association between training volume and aBMD in different body segments in male adolescents engaged in different impact sports.	n=156 controls n=53 basketball n=101 soccer n=58 swimming n=33 volleyball n=36 karate n=48 judo n=30 kung-fu n=12 baseball n=10 gymnastics n=6 tennis n=51 track and field	All sample: 14.1±2.0	DXA	aBMD	Higher training volume is associated with higher aBMD in different body segments. These associations are influenced by LST, mainly in the upper limbs. In addition, the influence of LST on aBMD seems to be lower where the body segment is more exposed to mechanical loading during sports practice.	Sample was composed by males and females. Results did not distinguish sexes.

aBMD (areal bone mineral density), DXA (dual-energy x-ray absorptiometry), LST (lean soft tissue)

Chapter IV

Study 2

Analytical study on strength assessment protocol

4. Learning effect on an isokinetic knee strength test protocol among male adolescent athletes

4.1. Abstract

The learning effect occurs when the best performance does not occur at the earliest repetitions of repeated motor task. The present study aimed to examine intra-individual variation in an isokinetic strength protocol composed of five reciprocal concentric and eccentric contractions of knee extensors (KE) and knee flexors (KF) among male adolescent swimmers aged 10-13 years. The sample included 38 participants aged 10.06-13.26 years. Isokinetic dynamometry was used to assess concentric and eccentric contractions of KE and KF at an angular velocity of 60°.s^{-1}. The protocol included three preliminary repetitions that were not retained for analysis, a 60-second interval, and five reciprocal maximal concentric contractions (cc). The preceding sequence was repeated for eccentric contractions (ecc). Multilevel regression confirmed intra-individual and inter-individual levels as significant sources of variance in peak torque (PT) values. Intra-class correlation (ICC) fluctuated between 0.580 and 0.754 and, in general, a substantial percentage of participants need more than three repetitions to attain their highest PT: KEccc (36.8%), KEeccc (23.7%), KFcc (39.5%), KFecc (18.4%). Based on a subsample of 17 swimmers who completed a second visit to the laboratory, intra-individual mean differences of the best PT were trivial or small. In summary, this study does not recommend the adoption of protocols of less than 5 repetitions to obtain the best PT, particularly if participants did not experience a preliminary session for familiarization.

KEYWORDS: Youth sports; Dynamometry; Quadriceps and hamstrings; Concentric and eccentric contractions; Consistency; Reproducibility

4.2. Introduction

Assessment of muscle strength has a large spectrum of applications. It is routinely used in rehabilitation (Pierce et al., 2006), sports training (De Ste Croix et al., 2017), and exercise sciences (Gavanda et al., 2020). Meantime, isokinetic dynamometry is consensually considered a valid protocol to assess dynamic strength (Kellis et al., 2022). It corresponds to a dynamic muscular contraction maintaining the velocity of movement constant (Batzopoulos & Brodie, 1989). Several parameters are used in the context of isokinetic strength, peak torque (PT) refers to the maximum value obtained from the isokinetic curve and is often retained as the best single measure of muscle strength in dynamic conditions.

Measurement protocols demand cooperation from participants. Habituation is considered critical for pediatric strength testing since the learning period facilitates a phase in which the specific movements, neuromuscular patterns, and demands of the dynamometer become familiar. The number of repetitions required to obtain the peak torque is not consistent in the literature. Among 20 male college athletes, the peak torque was determined by performing three repetitions, after five submaximal contractions as a warm-up (Appen & Duncan, 1986). More recently, adult male athletes completed a protocol composed of three repetitions for familiarization before five repetitions for analysis (Duarte et al., 2018). Meantime, four trials of complete extension and flexion of the knee joint were allowed to familiarize 241 participants aged 18-28 years on an isokinetic dynamometer (Dibrezzo et al., 1985): two trials at the submaximal effort and two at maximal effort; prior to four consecutive trials. The preceding studies include familiarization as part of the protocol in the assessment of adult participants. In fact, another study (Carvalho et al., 2012) adopted a familiarization session one week before male adolescent basketball players completed the testing protocol to examine the relationships between knee joint isokinetic strength, biological maturation, and body size.

When several repetitions are completed at short intervals, participants often evidence improvements in attained strength performance. This phenomenon is known as the learning effect and is present when the best performance is registered after the earliest repetitions. By inference, poor reliability should be assumed when a testing procedure only considers one or very few repetitions (Lund et al., 2005). The previous is critical in longitudinal studies and may affect individual developmental trajectories. Taking into account the preceding, the current study aimed to examine the learning effect during an isokinetic strength protocol composed of five reciprocal concentric contractions of knee extensors (KE) and knee flexors (KF) in addition to a set of five repetitions of the eccentric contractions of the cited knee muscles among male adolescent swimmers aged 10-13 years.

4.3. Materials and methods

Procedures

The present study followed the ethical standards established for human studies (Harriss et al., 2019) and was previously approved by the Ethics Committee for Sports Sciences of the University of Coimbra (CE/FCDEF-UC/00182016). Participants were recruited from clubs in Portugal. Informed consent was signed by parents or legal guardians and, subsequently, swimmers were informed about the objectives, methods, benefits, and risks and also that they could withdraw from the experiment at any time. Data was collected under standardized conditions using the same equipment and by a single observer.

Sample

The sample included 38 male adolescent swimmers aged 10.06-13.26 years. Chronological age (CA) was obtained as the difference between the date of laboratory visit and the date of birth. Inclusion criteria were: [i] registration in the Portuguese Swimming Federation for at least two years, [ii] participants who did not report lower limb injuries in the last six months. Training experience, expressed in years, was obtained by interview.

Anthropometry

A single observer measured body size following the protocols described by Lohman et al. (1998). Stature was measured to the nearest 0.1 cm using a portable stadiometer (Harpenden model 98.603, Holtain LTD, Crosswell, UK). Body mass required a portable scale (Seca model 770, Hanover, MD, USA), and measurements were completed to the nearest 0.1 kg.

Isokinetic dynamometry

Isokinetic strength of the knee extensors and flexors was assessed in the preferred lower limb using a Biodex System 3 dynamometer (Shirley, NY, USA) adopting an angular velocity of 60°.s-1. The protocol included a 5-minute warm-up performed on a cycle ergometer (814E Monark, Varberg, Sweden) with a resistance corresponding to 2% of the body mass (Brown, 2000) and a cadence of 50-60 rpm. Afterwards, participants were seated in the isokinetic dynamometer. The lever arm was aligned to fit the lateral condyle of the knee. Strips were adjusted in the trunk in addition to the proximal thigh and slightly above the medial malleolus of the tibia. Range of motion was defined for 85 degrees (5º to 90º). Swimmers were asked to perform a voluntary maximal knee extension corresponding to the position of 0 degrees. The initial 5 degrees of the flexion were suppressed in order to allow the participant to exert at least 10% of the assigned torque limit. Correction for the gravity effect was done by weighing the relaxed member at the angular position of 30°. Swimmers were instructed to keep their upper arms crossed and their hands on the opposite shoulder during the test as previously published (Duarte et al., 2018a; Brown, 2000; De Ste Croix et al., 2003). The test started with three continuous reciprocal concentric contractions of KE and KF for familiarization. After a 60-second interval, five reciprocal maximal concentric (cc) contractions of KE and KF were performed (Duarte et al., 2018; De Ste Croix et al., 1999). Then, participants rested for 60 seconds and the previously described sequence was repeated for the reciprocal eccentric (ecc) contractions of KF and KE. For each set (KEcc, KFcc, KFecc, KEecc), five isokinetic curves were examined using specific software (Acknowledge - version 4.1, Biopac Systems, Inc.). The preceding permitted the extraction of peak torque (PT) values and the angle at which it occurred, in addition to verification of the angular velocity. PT was expressed in N.m and refers to the highest value obtained from an isokinetic curve (Perrin, 1993). Atypical isokinetic curves were registered as invalid data.

Analysis

Descriptive statistics were calculated for the total sample. The significance of intra-individual (5-repetitions) and inter-individual variances on PT values were tested using multilevel regression models. The preceding was done separately for KE, and KF according to the type of contractions, that is concentric and eccentric. Repetitions were the fixed factor at level 1 and participants interpreted as level 2 (between individuals). In parallel, the intra-class correlation coefficient (ICC) was determined. Subsequently, the frequencies of the highest PT across the five repetitions of the protocol adopted to assess isokinetic strength were calculated in order to examine the distribution of the highest intra-individual value per repetition. Based on a subsample of 17 swimmers who visited the laboratory in a different day, intra-individual mean differences were determined in addition to technical error of measurement and coefficient of variation for the highest PT from the 5-repetition protocol. Magnitude effect was given by d-value (Cohen, 1997). It was interpreted was follows (Hopkins et al., 2009): $d<0.20$ (trivial), $0.20<d<0.59$ (small), $0.60<d<1.19$ (moderate), $1.20<d<1.99$ (large), $2.00<d<3.90$ (very large). Finally, Bland-Altman procedure (Bland & Altman, 1986) was used to examine the relationship between intra-individual differences and the average of the repeated measures. Analyses were completed using MLwiN v2.26 (Center for Multilevel Modelling, University of Bristol, Bristol, UK), SPSS version 20.0 (SPSS Inc., IBM Company, N.Y., USA) and Graphpad Prism (version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com). The significance level was kept at 5%.

4.4. Results

Thirty-eight male adolescent swimmers aged 12.03±0.89 years were examined and descriptive statistics on chronological age, training experience, and body size, given by stature and body mass are summarized in Table 6. The descriptive statistics also considered PT mean and standard deviation separately for each of the five repetitions derived from the isokinetic strength protocol. The gradient for PT values was KEecc > KEcc > KFecc > KFcc. Meantime, multilevel regression models confirmed intra-individual and inter-individual variability as significant sources of variance. ICC fluctuated between 0.580 (KFcc) and 0.754 (KEcc). According to Table 7, the highest PT tends to be extracted within the first three repetitions (KEccc: 63.1% of the cases; KEeccc: 76.3%; KFcc: 60.5% KFecc: 81.6%) but a substantial number of participants attained their highest PT on subsequent repetitions. In other words, if the protocol was uniquely composed of three repetitions, a substantial number of individuals would have attained a poorer score. As illustrated in Figure 2, the fluctuation of two consecutive repetitions tended to be trivial or small with the exception of KEcc. The preceding evidenced a moderate increment between the first and second repetition.

Table 6 - Descriptive statistics on chronological age, training experience, body size, and isokinetic strength outputs of 5-repetition protocol derived from the assessment of knee muscle groups on concentric and eccentric contractions $60^0 .s^{-1}$ in addition to multilevel regression models to explain intra-individual and inter-individual peak torque variances (middle) and intra-class correlation coefficient (right) among male adolescent swimmers (n=38)

Variable	rep	units	n	Descriptive statistics	Multilevel regression models			ICC	
				mean±SD	Constant	e_{ij}	u_j	value	(95% CL)
Chronological age		years	38	12.03±0.89					
Training experience		years	38	3.8±1.6					
Stature		cm	38	151.4±8.8					
Body mass		kg	38	41.7±8.1					
Knee extensors, concentric					67.844(3.498)	76.601(8.845)	385.758(92.072)	0.754	(0.572; 0.865)
	1	N.m	37	67.7 ± 22.4					
	2	N.m	37	80.6 ± 20.2					
	3	N.m	38	82.5 ± 22.3					
	4	N.m	38	81.3 ± 22.4					
	5	N.m	38	80.9 ± 20.0					
Knee extensors, eccentric					91.044(4.822)	226.293(26.396)	649.598(159.895)	0.724	(0.526; 0.848)
	1	N.m	37	90.7 ± 29.1					
	2	N.m	38	86.9 ± 29.7					
	3	N.m	38	89.6 ± 33.4					
	4	N.m	36	89.5 ± 27.8					
	5	N.m	36	80.5 ± 28.9					
Knee flexors, concentric					41.718(2.200)	76.903(8.910)	107.100(28.233)	0.580	(0.320; 0.759)
	1	N.m	38	41.7 ± 14.3					
	2	N.m	36	42.0 ± 11.5					
	3	N.m	37	40.9 ± 11.3					
	4	N.m	38	41.2 ± 12.3					
	5	N.m	38	42.9 ± 18.2					
Knee flexors, eccentric					61.216(2.604)	68.323(8.256)	189.433(47.036)	0.716	(0.514; 0.843)
	1	N.m	38	61.2 ± 16.7					
	2	N.m	34	59.2 ± 17.9					
	3	N.m	35	60.7 ± 17.9					
	4	N.m	35	57.6 ± 13.6					
	5	N.m	33	55.8 ± 14.1					

Table 7 - Absolute frequencies of the best score for peak torque derived from five repetitions separately by knee muscle group and type of contraction among male adolescent swimmers (n=38).

Muscle	contraction	repetition	best peak torque	
			f	%
Knee extensors	concentric	1	3	07.9%
		2	14	36.8%
		3	7	18.4%
		4	7	18.4%
		5	7	18.4%
Knee extensors	eccentric	1	16	42.1%
		2	7	18.4%
		3	6	15.8%
		4	8	21.1%
		5	1	02.6%
Knee flexors	concentric	1	11	28.9%
		2	6	15.8%
		3	6	15.8%
		4	6	15.8%
		5	9	23.7%
Knee flexors	eccentric	1	12	31.6%
		2	12	31.6%
		3	7	18.4%
		4	4	10.5%
		5	3	07.9%

f (absolute frequencies)

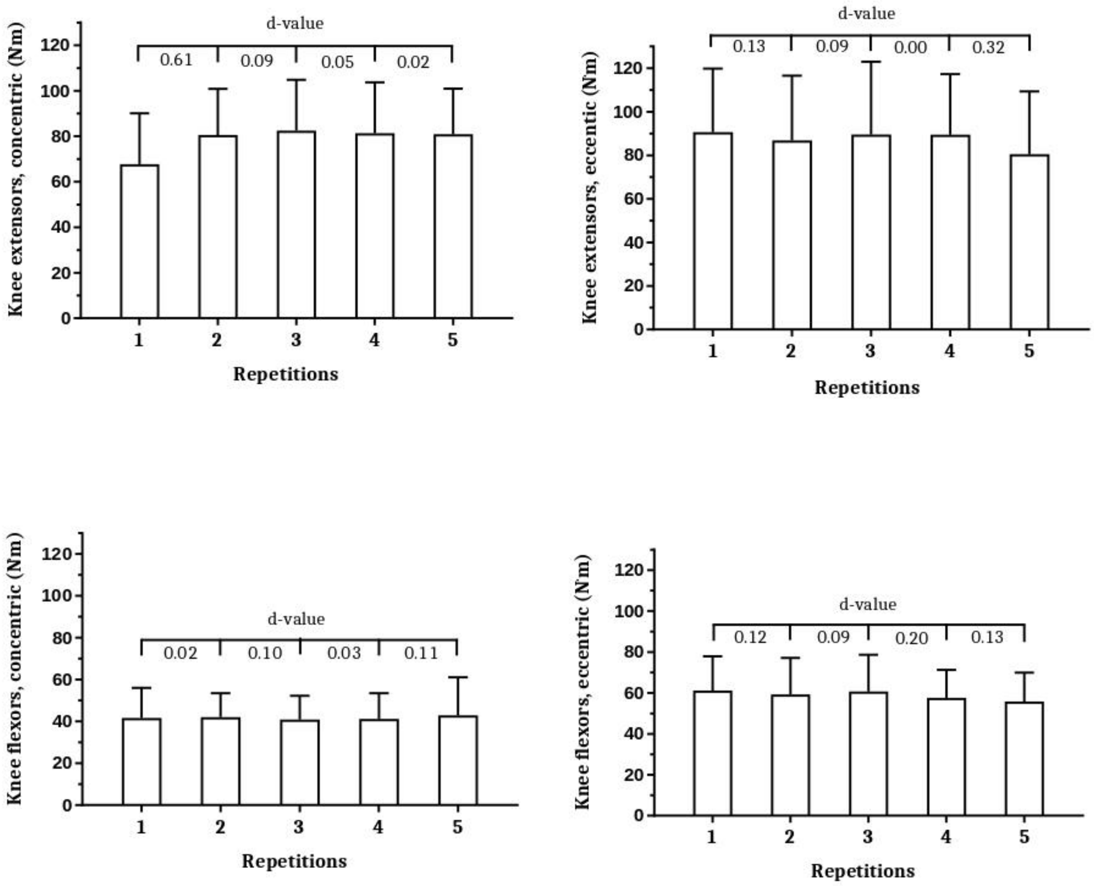

Figure 2 - The magnitude of differences for mean peak torque by the number of repetitions for each muscle group and contraction, among male adolescent swimmers.

The study added information on the reproducibility of isokinetic strength assessments determined in two independent testing sessions as presented in Table 8. In fact, 17 male adolescent swimmers completed a second visit to the laboratory and intra-individual mean differences were positive, i.e. mean values tended to increase, although the magnitude was trivial or small. As illustrated in Figure 3 , Bland-Altman confirmed that, despite the trend for improvement in mean values from session 1 to session 2, the BIAS was apparently modest (KEcc: +3.0 N.m; KEecc: +3.2 N.m; KFcc: +1.8 N.m; KEcc: +2.2 N.m;) and several participants registered a decrement compared to the first visit.

Table 8 - Descriptive statistics for time-moments 1-2, intra-individual mean differences between time-moments, technical error of measurement, and magnitude effect between repeated measurements among male adolescent swimmers (n=17).

Muscle group	contractions		Mean ± standard deviation		Intra-individual differences		TEM	%CV	Magnitude effect	
			TM1	TM2	mean	(95% CL)			*d*	(qualitative)
KE	cc	N.m	81.9±15.8	90.2±17.5	8.3	(2.0;14.6)	10.3	11.9	0.51	(small)
	ecc	N.m	101.8±22.2	110.2±19.3	8.3	(1.5;15.2)	10.9	10.3	0.42	(small)
KF	cc	N.m	45.9±9.8	49.7±11.7	3.8	(-0.0;7.7)	5.8	12.2	0.36	(small)
	ecc	N.m	68.1±12.6	69.4±11.1	1.3	(-3.3;5.9)	6.2	9.0	0.11	(trivial)

KE (knee extensors); KF (knee flexors); cc (concentric contractions), ecc (eccentric contractions)

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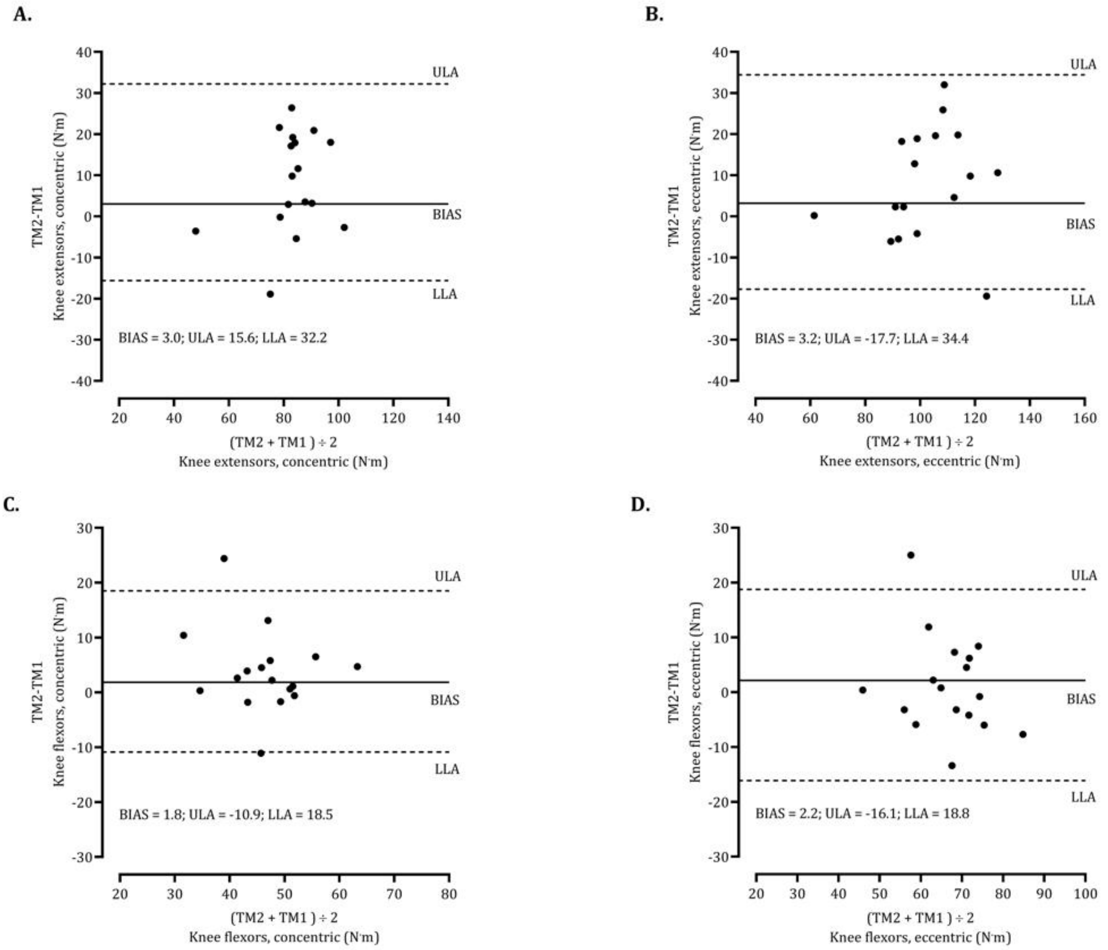

Figure 3 - Agreement between repeated measures for peak torque by muscle group and contraction. Mean differences between TM2 and TM1 and the corresponding mean [95% confidence intervals (95% CI)]. Dashed lines represent 95% limits of agreement (±1.96 SD); Bias, lower limits of agreement (LLA), and upper limits of agreement (ULA) are also represented.

4.5. Discussion

The current study initially examined the learning effect of a 5-repetition isokinetic strength protocol designed to assess reciprocal concentric contractions of KE and KF in addition to a similar set of eccentric contractions among male adolescent swimmers aged 11-12 years. Although the highest PT tended to occur during the earliest three repetitions, a substantial number of participants obtained the best peak torque while performing the fourth and fifth repetitions. Consequently, it seems adequate to maintain five repetitions for each knee muscle group and type of contraction, despite the possible induction of fatigue that would be particularly relevant if the protocol includes several angular velocities and tests both legs. In fact, both intra and inter-individual variance were diagnosed as significant sources of variability of peak torque values among male adolescent swimmers. Not surprisingly, ICC derived from the five repetitions were modest. By inference, a protocol with less than five repetitions would have lost relevant information. Meantime, repeated measurements of the highest PT obtained from the 5-repetition protocol performed on two independent occasions corresponded to a trivial or small learning effect. The gradient of isokinetic strength assessment of knee muscle actions was identical on repeated occasions: KEecc > KEcc > KFecc > KFcc.

The relationships between isokinetic strength assessment of knee joint muscle actions, indicators of somatic maturation, and body size were examined in male basketball players aged 14-16 years (Carvalho et al., 2012). It was concluded that predicted time from age at peak height velocity contributed to explain inter-individual variance in isokinetic strength outputs. The literature consistently indicated that maximal force generated by the skeletal muscle is primarily a function of muscle size (Armstrong & McManus, 2011). Meantime, a recent study modeled longitudinal changes in isokinetic strength of the KE and the KF in Belgian male adolescent soccer players (Duarte et al., 2018a). The preceding study assessed 67 soccer players aged 11.0–13.9 years at baseline who were followed over a five-year period and concluded that isokinetic strength of the knee muscle groups may reasonably be predicted from chronological age, stature, and estimated fat-free mass with age per se contributing to an annual increment of 5.4 N.m in KE and 2.7 N.m in KF (Duarte et al., 2018a). Longitudinal studies claim for information about data quality preferably per occasion (time moment).

Research in youth sports may need to integrate the familiarization as an independent fluctuation in relation to developmental changes. Familiarization corresponds to an erroneous variation of the data that is expected to be more accentuated in early laboratory experiences of children and youth participating in longitudinal studies. The literature (De Ste Croix, 2017) suggested KE as more reliable compared to KF and, additionally, concentric contractions as more likely to be reliable than eccentric contractions.

To observe the day-to-day reproducibility of five reciprocal contractions, 27 male adolescent basketball players were assessed twice on knee extension and knee flexion at 60°.s^{-1} in both concentric and eccentric contractions (Carvalho et al., 2011). The coefficient of variation and associated intra-class correlation for the KE were: CV=8.1% (ICC=0.89) for concentric contraction; CV=16.5% (ICC=0.78) for eccentric contraction. Respective values for KFcc and KFecc were: CV=15.1% (ICC=0.74) and CV=17.4% (ICC=0.72). Recently, resistance-trained male adults aged 27.9±5.3 years (n=32) were assessed on three occasions within 8 days to determine the variability of the PT by adopting a protocol of five repetitions of the KEcc at the same angular velocity of the present study, that is, 60°.s^{-1} (Van Tittelboom et al., 2022). A modest intra-individual variability of the highest PT was evidenced between occasions (CV ranged 6.4-15.3%). Previously (Duarte et al., 2018b), 26 male athletes aged 24.0±0.7 years from different sports completed two laboratory visits within a single week to determine the intra-individual variation of knee muscle actions assessed by isokinetic strength at the angular velocity of 60°.s-1. Coefficient of variation was lower than 5.2%, which is less than the respective values obtained by male swimmers aged 10-13 years in the current study who were exposed to the same protocol (warm-up and 5-repetitions of KE and KF both performing concentric and eccentric contractions). The preceding suggested intra-individual fluctuation in isokinetic strength as more problematic in youth than in trained adults.

Meantime, the reliability of the isokinetic dynamometer in concentric passive mode was tested among 21 children (10 girls, 11 boys) aged 5-12 years who were tested twice within eight days (Santos et al., 2013). They performed five consecutive repetitions of KE and KF at 60°.s^{-1} adopting the passive mode and uniquely the concentric contraction. The ICC values were 0.84 and 0.81 respectively for KE and KF. The preceding parameters were slightly higher than the respective coefficients calculated in the present study. Future research in youth sports needs to combine repeated measurements of passive and reactive modes.

The current study has limitations that need to be recognized. The sample is limited to male adolescent swimmers selected by convenience and only 17 from the initial sample re-visited the laboratory for a second time moment. Therefore, generalization needs caution. The protocol only considered the angular velocity of $60°.s^{-1}$. It should be hypothesized that familiarization would be distinct while adopting other angular velocities. Additionally, eccentric contraction was assessed following a reactive mode that is supposed to be different from the passive mode regarding learning effect. The reactive protocol in the current study implied a reduction of the range of motion to avoid the initial five degrees (zero degrees corresponds to full extension). Finally, future research may consider a larger sample for the two independent visits. Probably the number of repetitions should be continued until improvements would not be observed. Future studies may also test familiarization on dominant and non-dominant legs adopting different angular velocities.

4.6. Conclusions

According to the available literature, the number of repetitions of an isokinetic strength protocol to determine the highest PT of KE and KF tends to fluctuate between 3 and 5 contractions. Studies also vary regarding warm-up. The present study confirms KF as more problematic than KE and does not recommend the adoption of protocols based on less than 5 repetitions to derive the highest PT, particularly if participants do not have the chance to experience the protocol prior to the testing session.

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Chapter V

Study 3

Developmental longitudinal changes of bone mineral density among adolescent athletes from sports contrasting in mechanical loading

5. Growth, body composition and bone mineral density among pubertal male athletes: intra-individual 12-month changes and comparisons between soccer players and swimmers

5.1. Abstract

Background: Puberty is a period of intense changes in human body and, additionally, participation in sports is viewed as a prominent form of physical activity among male adolescent athletes. The current study aimed to examine the intra-individual changes in body composition and bone tissue during the years of maximal growth and the effect of 12-month participation in sports contrasting in mechanical impact.

Methods: The sample included 40 male adolescent athletes (soccer: n = 20; swimming: n = 20) aged 12.57 ± 0.37 years who were followed for 12 months. Stature and body mass were measured, and bone mineral content (BMC), areal bone mineral density (aBMD), lean soft and fat tissues assessed using DXA. Food intake was estimated using a questionnaire and training sessions individually monitored. Repeated measures ANOVA tested the differences between sports and 12-month intra-individual variation (time moments: TM1, TM2). The analyses on aBMD for total body and total body less head were repeated controlling for variation in stature at baseline**.**

Results: Soccer players completed 63 ± 31 sessions (95 ± 47 h). Respective values for swimmers were 248 ± 28 sessions and 390 ± 56 h. In general, the analysis of aBMD as dependent variable evidenced significant effect of sport-associated variation (F = 5.254, p < 0.01; η2 = 0.35) and 12-month increments, particularly at lower limbs (F = 97.238, p < 0.01; η2 = 0.85). Respective mean values for aBMD were SCCTM1 = 0.885 g.cm-2, SWMTM1 = 0.847 g.cm-2, SCCTM2 = 0.939 g.cm-2, SWMTM2 = 0.880. Regarding the lean soft tissue, the magnitude of effects was very large for intra-individual variation (F = 223.043, p < 0.01; η2 = 0.92) and moderate between sports (F = 7.850, p < 0.01; η2 = 0.41): SCCTM1 = 30.6 kg, SWMTM1 = 34.9 kg, SCCTM2 = 35.8 kg, SWMTM2 = 40.5 kg). Finally, d-cohen values reporting percentage of intra-individual changes in aBMD between soccer players ad swimmers were large for the trochanter (d = 1.2; annual increments: SCC = 8.1%, SWM = 3.6%).

Conclusion: Puberty appeared as a period of significant intra-individual changes in lean soft tissue and bone mineral density. With increased accumulated training experience, mean difference between sports contrasting in mechanical impact tended to me more pronounced in particular at the lower limbs.

Keywords: Body composition; Bone health; DXA; Mechanical loading; Youth sport.

5.2. Background

It is estimated that 85-90% of the adult bone mass is gained during the first two decades of life (Johnell & Kanis, 2006; Kempe & Fernandes, 2017). During the years of maximal growth velocity, physical activity is directly associated with gains in peak bone mass (Baxter-Jones et al., 2011) and afterwards, during adulthood, weight-bearing activities are supposed to improve bone strength and maintain bone mass (Faienza et al., 2020). The skeleton is sensitive to transverse and torsional forces directly applied on bones in addition to mechanical stimulus derived from muscle contractions. The preceding are essential characteristics of sports which is probably the main form of physical activity in young people involving jumps, sprints, changes of directions and plenty physical contact (Tenforde & Fredericson, 2011; Narciso et al., 2020). By inference, during circumpubertal years, sport participation is hypothesized to assume an important influence on bone health. The type of sport may be a source of variation in developmental changes of the bone tissue.

Dual energy x-ray absorptiometry (DXA) is probably the most used instrument to obtain indicators of bone tissue. Further, this technology is also being used in the assessment of total and regional body composition (Ackland et al., 2012). In addition, DXA allows gathering information about the proximal femur which is considered a region of interest to prevent osteoporotic fractures (Warriner et al., 2011; Siris et al., 2014). Recent research on adolescent athletes considered the above cited region (Ward et al., 2020; Agostinete et al., 2020). Nevertheless, the ambivalent characteristics of DXA, studies did not systematically consider the interrelationship among bone tissue and variation in body composition. During circumpubertal years, variation in body composition is essentially attributable to growth, maturation (Malina et al., 2004), plus, in the case of youth athletes, type, frequency and duration of training sessions are also plausible longitudinal predictors.

In Portugal, as in many other countries (Hulteen et al., 2017), soccer and swimming consistently merge among the most popular sports. Swimming is classified as hypogravity and requests propulsive forces mostly produced by the upper body (Crowley et al., 2017). In parallel, soccer is characterized by high-intensity intermittent efforts demanding repeated actions of the lower limbs: jumps, sprints, changes of direction, and tackles (Bangsbo et al., 2006; Zouch et al., 2014). Recent research (Agostinete et al., 2020) evidenced weight bearing sports such as soccer, basketball or volleyball had a positive osteogenic effect given by increasing levels of bone mineral content (BMC) and areal bone mineral density (aBMD). Meantime, another study concluded that participation in soccer induced significantly greater improvements in BMC and bone stiffness over 12 months compared to cycling and swimming (Vlachopoulos et al., 2017) . The preceding was derived from a 12-month study conducted in 116 adolescent males engaged in soccer, swimming and cycling who were compared with an active control group. Another study reporting young athletes from non-weight bearing sports such as swimming and cycling did not present any substantial osteogenic effect (Narciso et al., 2020).

Research investigating the independent and combined effects of sports and 12-month training on bone development in adolescent males is still scarce, particularly considering adjustment for stature and including parameters of body composition. Of relevance, soccer and swimming correspond to distinct training routines regarding the number of training sessions and weekly volume. This is supposed to have impact on body composition. Unfortunately, the literature did not systematically collect data about training experience. Taking into account the previous, this study aimed to examine the effect of sport over a 12-month season among male adolescent soccer players and swimmers on body size, body composition and indicators of bone health. It was hypothesized that adolescent athletes from sports contrasting in mechanical impact differ in terms of inter- and intra-individual bone aBMD with intra-individual gains more pronounced at the proximal femur.

5.3. Methods

Study design and procedures

The current research was approved by the Ethics Committee for Sports Sciences of the University of Coimbra (CE/FCDEF-UC/00182016) following the Declaration of Helsinki for human studies (World Medical Association, 2013). Participants were male adolescent athletes recruited from Portuguese clubs. Signed informed consents were obtained from parents or legal guardians. Participants were informed about the objectives, procedures, benefits, risks and also that they could withdraw from the study at any time. All data was collected within a 12-month period (baseline or TM1; 12-month follow-up or TM2) under standardized conditions at the same laboratory by the same observers and instruments.

Participants

This project contacted 12 clubs that allow participation in soccer and swimming for 262 male adolescents. Eighty-one male adolescents visited the laboratory. Exclusion criteria were: [i] registered in competitive sports at respective Portuguese federation for less than two complete seasons at baseline: 10 soccer and 6 swimmers were excluded; [ii] failed to be classified as pre-peak height velocity (PHV) based on estimated PHV, i.e., positive predicted maturity-offset values at baseline excluded 9 soccer players and 8 swimmers; [iii] presence of fractures, chronic diseases, eating disorders or medication that could have affected bone metabolism: any exclusion, [iv] eight participants failed the second visit for repeated measurements: 3 soccer players and 5 swimmers. After considering the inclusion criteria, the sample was composed of 40 participants (soccer players: n=20; swimmers: n=20). By using G*Power software (v3.1.9.2, University of Kiel, Germany), two groups (soccer vs. swimming), measured twice (baseline vs. after 12 months) and including a covariate correspond to a power sample equal to 87%.

Chronological age, skeletal age and training experience

Training experience was obtained at the TM1 and expressed in years. Information regarding the number of training sessions and accumulated minutes of training were individually collected by contacting coaches and assistant coaches on a regular basis.

Chronological age (CA) was calculated to the nearest 0.1 year. The Fels method was used to estimate the skeletal age (SA) (Roche et al., 1988). This method provides an estimate of skeletal age in addition to standard error associated with each assessment, granting a greater degree of confidence regarding the predicted values (Malina et al., 2004). Posterior-anterior radiographs of the left hand-wrist were taken. The participant had the hand in pronation on a transverse plane, with the fingers in maximum extension and maximum distance between each other. The third finger was in the prolongation of the antebrachial segment. After, ratings and ratios were entered into the Fels software program (Felshw 1.0 Software, Lifespan Health Research Center, Departments of Community Health and Pediatrics, Boonshoft School of Medicine, Wright State University, Dayton, Ohio) by the same observer that computed the skeletal age and related standard error (Roche et al., 1988). 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).

Anthropometry

A single observer measured stature, body mass and sitting height following standardized protocols (Lohman et al., 1988). Stature and sitting height were measured to the nearest 0.1 cm using a portable stadiometer (Harpenden model 98.603, Holtain LTD, Crosswell, UK) and sitting height table (Harpenden model 98.607, Holtain LTD, Crosswell, UK). Leg length was calculated as the difference of stature and sitting height. Body mass was measured to the nearest 0.1 kg using a portable scale (SECA model 770, Hanover, MD, USA). Body mass index was calculated.

Biological maturation

A non-invasive indicator of biological maturation was used to confirm that all participants were pre-PHV at the baseline. Maturity offset was obtained from an algorithm requiring CA, body mass, stature, sitting height and estimated leg length. It refers to the distance (in years) to age peak height (Mirwald et al., 2002) as presented in Equation 1.

Equation 1:

Maturity offset (years): -9.236 + (0.0002708 * (leg length * sitting height)) + (-0.001663 * (CA * leg length)) + (0.007216 * (CA * sitting height)) + (0.02292 * ([body mass / stature] * 100))

Food intake

A semi-quantitative food frequency questionnaire (FFQ) was used to estimate dietary intake validated for Portuguese population (Lopes et al., 1004; Lopes et al., 2007). The FFQ included food groups, beverage categories and frequency intake with nine qualitative options (from “never or less than once a month” to “6 or more times per day”). Calories, macronutrients intake, cholesterol, fiber and calcium were estimated using the software Food Processor SQL (ESHA Research Inc., Salem, OR, USA) and, subsequently, were retained for the analysis.

Dual-Energy X-Ray Absorptiometry

Body composition was examined as the sum of fat tissue, LST, BMC using DXA (Hologic QDR-4500 scanner, version 9.10, Hologic Inc., Bedford, Massachusetts, USA). A single certified technician extracted the data following the guidelines published by the manufacturer (DXA Quality Assurance Center, 2013). Participants were positioned on the table in supine position with the body aligned along with the central axis. This scan permits the calculations of BMC, bone area, and aBMD in addition to fat tissue and LST. The data collection was repeated for the whole body, trunk, upper limbs and lower limbs. The analysis of the proximal femur required a second scan in the non-dominant leg in order to assess aBMD for the femoral neck, ward triangle, and trochanter.

Analysis

Means and standard deviations by sport separately for the baseline and 12-month follow-up were calculated for body size, body composition in addition to aBMD. The subsamples presented identical characteristics for CA at baseline (soccer: 12.4±0.3 years; swimmers: 12.7±0.4 years) and also for maturity-offset (soccer: -1.6±0.5 years; swimmers: -1.2±0.6 years). Consequently, repeated measures analysis of variance (ANOVA) was used to test the effect of sports, intra-individual 12-month changes and interaction term sport*12-month. Meantime, swimmers were slightly taller than soccer players and the repeated measures ANCOVA (controlling for stature at baseline) was performed for two aBMD outputs: total body and subhead, i.e., total body less head. The preceding was recommended for children and adolescents (Crabtree et al., 2014). The effect size for each factor (12-month participation, type of sports, interaction) was given by eta squared that was interpreted as follows (Hopkins et al., 2009): η2<0.1 (trivial), 0.1<η2<0.3 (small), 0.3<η2<0.5 (moderate), 0.5<η2<0.7 (large), 0.7<η2<0.9 (very large), 0.9<η2 (nearly perfect). Intra-individual differences were individually calculated and expressed as percentage of baseline values. These were based on means and standard deviation of intra-individual changes for soccer players and swimmers Cohen d-value were calculated (Cohen, 1997) and interpreted as follows (Hopkins et al., 2009): d<0.2 (trivial), 0.2<d<0.6 (small), 0.6<d<1.2 (moderate), 1.2<d<2.0 (large), 2.0<d<4.0 (very large) and d≥4.0 (nearly perfect). Finally, descriptive statistics were calculated by sport for body mass index and indicators derived from the food questionnaire in addition to comparisons between groups using student t-test. Statistical significance was set at 5%. All analyses were performed using SPSS version 20.0 (SPSS Inc., IBM Company, N.Y., USA) and Graphpad Prism (version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com).

5.4. Results

Table 9 summarizes descriptive statistics separately for the two groups. Soccer players and swimmers significantly differed on lean soft tissue at all sites with swimmers always presenting higher mean values: total body (F=7.850, p<0.01, η2=0.41), trunk (F=8.186, p<0.01, η2=0.42), upper limbs (F=11.598, p<0.01, η2=0.48) and lower limbs (F=4.371, p<0.05, η2=0.32). During the follow-up period, on average, soccer players completed 63±31 training sessions corresponding to 5696±2808 minutes. Respective values for swimmers were 248±28 training sessions and 23378±3368 minutes. Significant differences were also noted between soccer players and swimmers for aBMD on total body (F=5.545, p<0.05, η2=0.36) and lower limbs (F=9.146, p<0.01, η2=0.44). In the preceding sites, aBMD means of soccer players exceeded the means of the swimmers. Regarding the parameters of the proximal femur, the effect of sports was always significant: femoral neck (F=5.052, p<0.05, η2=0.34), ward triangle (F=8.129, p<0.01, η2=0.42) and trochanter

($F=7.718$, $p<0.01$, $\eta 2=0.41$). Again, soccer players were characterized by higher mean values in aBMD compared to swimmers.

Intra-individual mean differences were always significant for body size, body composition and aBMD except for the upper limbs as summarized in Table 10 that also included significant interaction terms for all aBMD sites with the exception of upper limbs. Figure 4 illustrates the intra-individual changes (time moment 2 – baseline) expressed as percentage of baseline values, separately for each sport. The effect size between groups for lean soft tissue was small for the lower limbs ($d=0.25$). When the groups were compared on annual gains in lean soft tissue on total body, trunk and upper limbs, the differences were negligible. Finally, Figure 5 evidences a large effect size between soccer players and swimmers ($d=1.2$) when the groups were compared in aBMD at the trochanter.

Finally, table 11. reports descriptive statistics for BMI and indicators obtained from FFQ, at baseline, separately for swimmers and soccer players. The two groups did not differ.

Table 9 - Descriptive statistics (mean ± standard deviation) by sports and time moment in addition to estimated means for aBMD of the total and subhead (standard error) controlling for variation in stature among male adolescent athletes (soccer players, n=20; swimmers, n=20).

Dependent variable	Groups			
	Soccer (n=20)		Swimming (n=20)	
	Baseline	12-month	Baseline	12-month
	mean ± standard deviation			
Training minutes		5696±2808		23378±3368
Training sessions		63±31		248±28
Stature, cm	149.8±5.9	157.8±7.1	154.8±7.5	161.2±8.2
Body mass, kg	41.6±7.2	46.8±8.6	44.1±7.4	49.0±7.1
Fat tissue, %	18.0±7.6	17.1±7.9	14.0±8.5	11.6±7.8
LST: total body, kg	30.6±3.8	35.8±5.6	34.9±5.2	40.5±6.2
LST: trunk, kg	13.75±1.76	16.20±2.72	15.83±2.63	18.68±3.09
LST: upper limbs, kg	2.91±0.43	3.47±0.59	3.53±0.63	4.16±0.80
LST: lower limbs, kg	10.81±1.58	12.84±2.25	12.13±1.93	14.16±2.37
aBMD: total body, $g.cm^{-2}$	0.993±0.041	1.027±0.055	0.956±0.065	0.976±0.074
	1.001 (0.012)†	1.037 (0.014)†	0.948 (0.012)†	0.966 (0.014)†
aBMD: subhead, $g.cm^{-2}$	0.885±0.050	0.939±0.069	0.847±0.073	0.880±0.077
	0.896 (0.013)†	0.952 (0.015)†	0.636 (0.013)†	0.867 (0.015)†
aBMD: trunk, $g.cm^{-2}$	0.806±0.041	0.857±0.061	0.799±0.072	0.843±0.077
aBMD: upper limbs, $g.cm^{-2}$	0.684±0.110	0.698±0.053	0.667±0.046	0.688±0.061
aBMD: lower limbs, $g.cm^{-2}$	1.056±0.077	1.122±0.094	0.987±0.091	1.016±0.106
aBMD: PF-neck, $g.cm^{-2}$	0.950±0.084	1.013±0.104	0.895±0.111	0.923±0.115
aBMD: PF-ward, $g.cm^{-2}$	0.941±0.105	1.003±0.128	0.849±0.139	0.866±0.139
aBMD: PF-trochanter, $g.cm^{-2}$	0.822±0.078	0.889±0.099	0.762±0.099	0.788±0.097

LST (lean soft tissue); aBMD (areal bone mineral density); PF (proximal femur); † (Repeated measures ANCOVA controlling for stature at baseline; centered stature=152.3 cm; estimated means and associated standard error)

Table 10 - Results of repeated measures ANOVA to examine the effects of sports (soccer vs. swimming), 12-month follow-up (time-moment 1 vs. time-moment 2) and interaction factor (sport * 12-month) among male adolescent athletes (soccer players, n=20; swimmers, n=20).

Dependent variable	Effects								
	Between sports			12-month			Interaction (sport * 12-month)		
	F	p	η^2	F	p	η^2	F	p	η^2
	Factorial ANOVA								
Stature, cm	3.472	0.070	0.29‡	319.355	<0.001	0.95¶	4.211	0.047	0.32 #
Body mass, kg	0.989	0.326	0.16‡	170.417	<0.001	0.90¶	0.193	0.663	0.07
Fat tissue, %	3.733	0.061	0.30#	9.281	0.004	0.44#	1.067	0.192	0.21‡
LST: total body, kg	7.850	0.008	0.41#	223.043	<0.001	0.92¶	0.393	0.535	0.10‡
LST: trunk, kg	8.186	0.007	0.42#	177.569	<0.001	0.91¶	1.011	0.391	0.16‡
LST: upper limbs, kg	11.598	0.002	0.48#	130.695	<0.001	0.88$	0.472	0.496	0.11‡
LST: lower limbs, kg	4.371	0.043	0.32#	207.302	<0.001	0.92¶	0.001	0.989	0.01
aBMD: total body, $g.cm^{-2}$	5.545	0.024	0.36#	66.908	<0.001	0.80$	4.847	0.034	0.34#
†	11.088	0.002	0.48#	2.087	0.157	0.23‡	7.627	0.009	0.41#
aBMD: subhead, $g.cm^{-2}$	5.254	0.028	0.35#	90.383	<0.001	0.84$	5.195	0.028	0.35#
†	13.223	0.001	0.51*	0.564	0.457	0.12‡	6.521	0.015	0.39#
aBMD: trunk, $g.cm^{-2}$	0.248	0.621	0.08	92.522	<0.001	0.84$	0.459	0.502	0.11‡
aBMD: upper limbs, $g.cm^{-2}$	0.569	0.455	0.12‡	1.725	0.197	0.21‡	0.111	0.741	0.05
aBMD: lower limbs, $g.cm^{-2}$	9.146	0.004	0.44#	97.238	<0.001	0.85$	14.294	0.001	0.52*
aBMD: PF-neck, $g.cm^{-2}$	5.052	0.030	0.34#	40.643	<0.001	0.72$	6.015	0.019	0.37#
aBMD: PF-ward, $g.cm^{-2}$	8.129	0.007	0.42#	28.575	<0.001	0.66*	9.401	0.004	0.45#
aBMD: PF-trochanter, $g.cm^{-2}$	7.718	0.008	0.41#	56.514	<0.001	0.77$	11.106	0.002	0.48#

LST (lean soft tissue); aBMD (areal bone mineral density); PF (proximal femur); F (F-value); p (significance value);

† (Repeated measures ANCOVA controlling for stature at baseline; centered stature=152.3 cm)

Magnitude effect: ‡ (small); # (moderate); * (large); $ (very large); ¶ (nearly perfect)

Table 11 - Mean (95% confidence limits of the mean) by sports (soccer vs. swimming) and comparisons between groups on training experience, body mass index and indicators obtained from food frequency questionnaire.

Dependent variable	units	Soccer		Swimming		t-test	
		Mean	95% CL	Mean	95% CL	t-value	p
Training experience	years	5.2	(4.4; 6.0)	4.4	(3.6; 5.2)	1.411	0.166
Body mass index	$kg.m^{-2}$	18.43	(17.30; 19.57)	18.35	(17.17; 19.52)	0.109	0.914
Age at peak height velocity	years	14.02	(13.84; 14.20)	13.92	(13.73; 14.12)	0.750	0.458
Calories	kcal	3282	(2298; 4265)	2628	(1892; 3364)	1.113	0.273
Proteins	%	23.7	(20.6; 26.8)	21.6	(19.5; 23.7)	1.169	0.250
Carbohydrates	%	60.6	(55.5; 65.7)	59.6	(56.3; 62.8)	0.364	0.718
Fat	%	15.7	(13.4; 18.0)	18.8	(16.1; 21.6)	-1.821	0.076
Cholesterol	mg	488	(317; 660)	357	(279; 436)	1.456	0.154
Fibres	g	36.9	(17.1; 56.7)	30.6	(21.9; 39.2)	0.615	0.545
Calcium	mg	1822	(1241; 2403)	1146	(701; 1590)	1.935	0.060

95% CL (95% confidence limits).

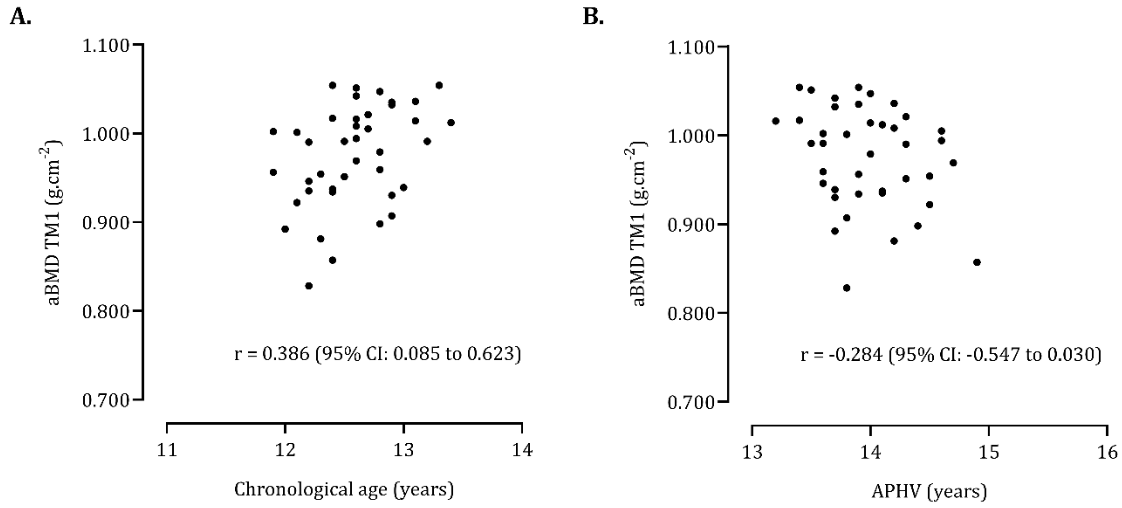

Figure 4 - Association between aBMD and chronological age (years) and aBMD and APHV (years) at baseline among both groups, adolescent soccer players (n=20) and swimmers (n=20).

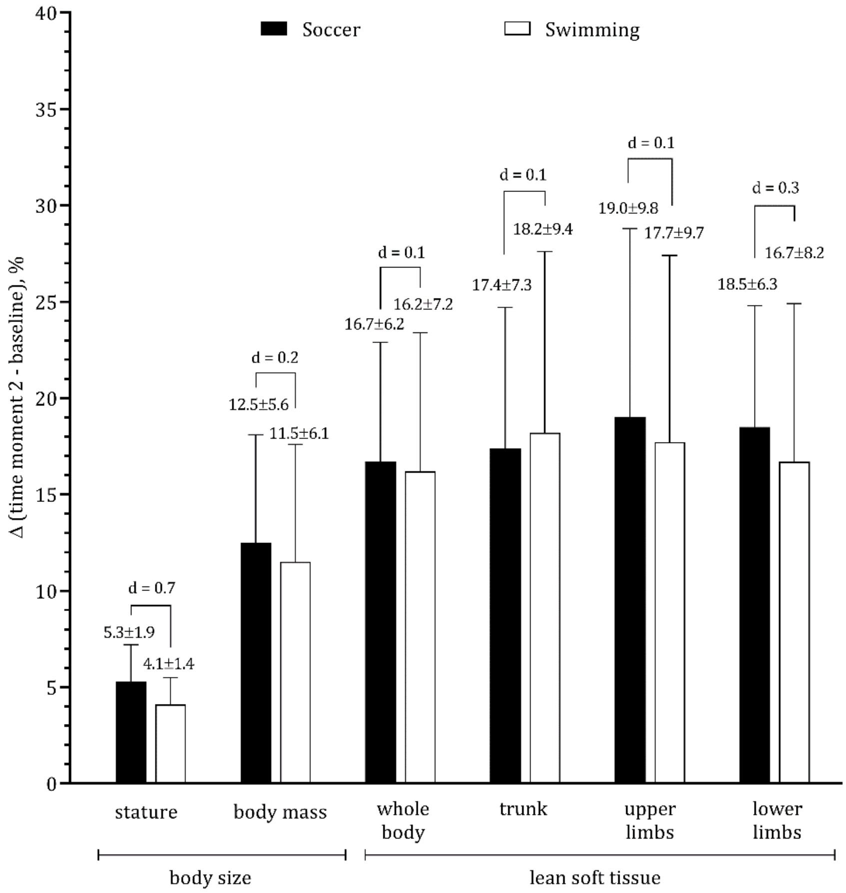

Figure 5 - Descriptive statistics (mean ± standard deviation) in the baseline and after 12-months plus magnitude of differences for different tissues, separately for soccer players and swimmers: fat tissue (panel a), lean soft tissue (panel b) and bone mineral content (panel c).

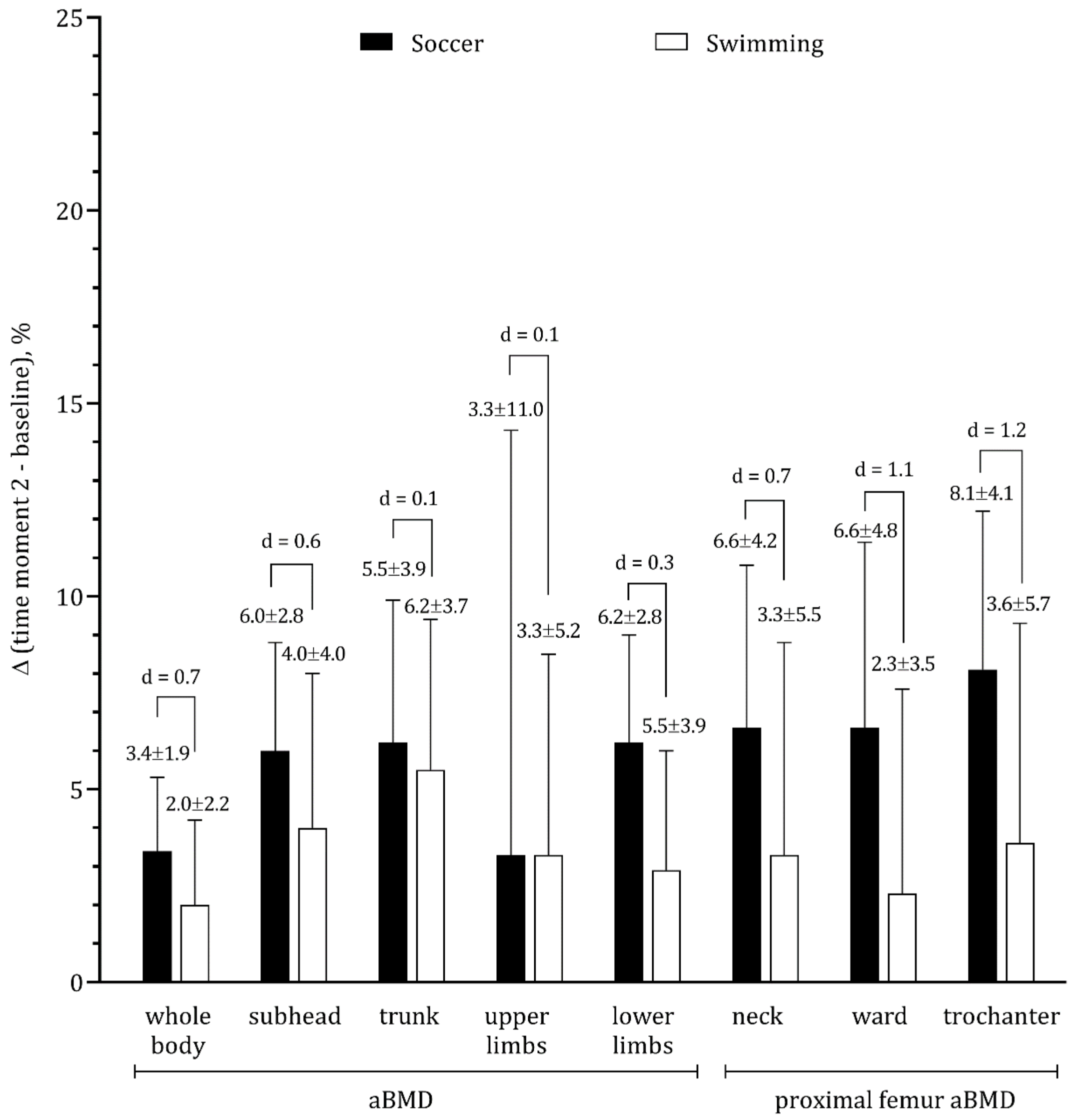

Figure 6 - Intra-individual mean changes (% of baseline) for male adolescent soccer players and swimmers on areal bone mineral density of whole body, subhead, trunk, upper limbs, lower limbs and proximal femur.

5.5. Discussion

The current sample of male adolescent athletes aged 11.9-13.4 years at baseline was characterized by negative values in the maturity offset. The previous confirmed all participants prior to estimated age at PHV that is consensually considered a crucial mark for bone accrual. Mean values of soccer players on stature-for-age and weight-for-age fluctuated between 25th and 50th percentiles of US reference (Kuczmarski et al., 2002). Although soccer players were, in general, 4.5 cm shorter and 2.3 kg lighter then swimmers, mean differences were not significant for body size descriptors. The mean stature of swimmers plotted above 50th percentile and mean body mass was at percentile 50th. The current study also assessed body composition given by predicted fat mass derived from DXA technology. The current sample (16.1% of fat mass) approached a previous study (Valente-dos-Santos et al., 2015) of 81 Portuguese male adolescent soccer players aged 14.61 years (17.3%).

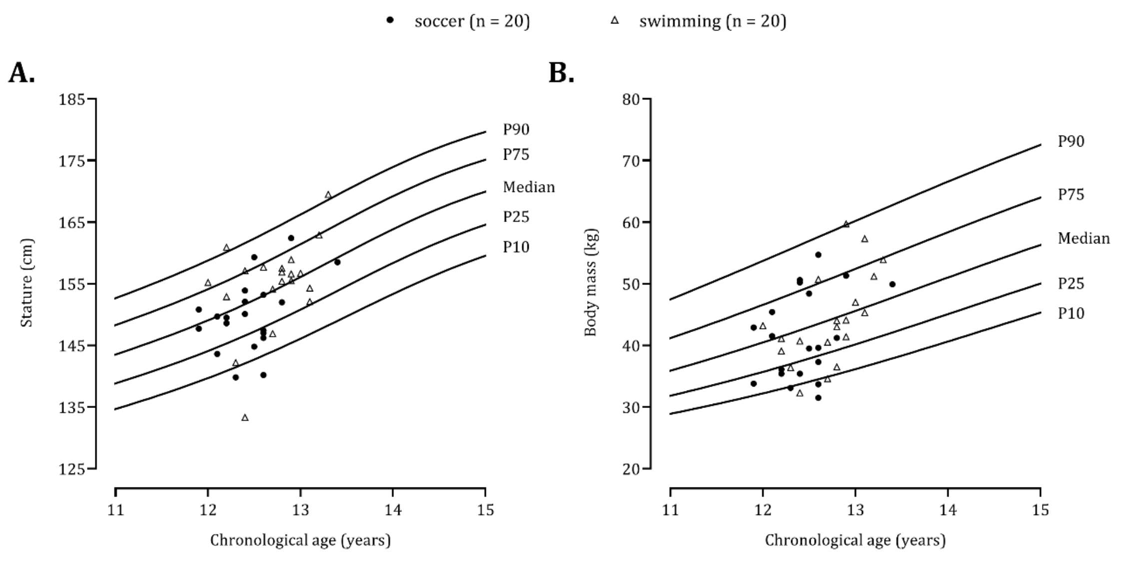

Figure 7 - Statures (panel A) and body masses (panel B) of individual male adolescent athletes plotted relative to U.S. percentiles (Kuczmarski et al. 2002).

During years of maximal height velocity, fat mass tends to plateau in boys (Malina et al., 2004). The average aBMD value in total sample was 0.975 g.cm-2 at the TM1 (age: 12.6±0.4 years) which is less than the 1.078 g.cm-2 reported for 28 Portuguese school boys aged 15.9±2.8 years (Dias Quiterio et al., 2011). In the current study, adolescents grouped by sports (soccer versus swimming) were assessed twice: in the TM1 and after 12 months. The groups differed in terms of training parameters with substantial more minutes and training sessions completed by swimmers. Not surprisingly, swimmers gained +4.3 kg of LST with significant differences at all segments (upper limbs, trunk and lower limbs). Regardless of changes in body mass and LST, soccer players were characterized by significant higher aBMD values in the lower limbs. Of interest, aBMD mean values of the two groups did not differ at upper limbs nor trunk. Regarding proximal femur scan, aBMD was consistently higher among soccer players compared to swimmers.

As bones increase in length, they also increase in width, so bone formation and resorption were coordinated to preserve structural strength (Rauch, 2007). Peak bone mass refers to the amount of bone acquired at the end of skeletal development and is viewed as an important determinant of lifespan skeletal health. Inter-individual variance in bone mass, as other tissue outcomes, needs to be interpreted in relation to age and biological maturation. In fact, circumpubertal years (-2 to + 2 years of PHV) are considered the most decisive period for bone mass accumulation, in which approximately 33% and 39% of all BMC observed at adult state is acquired in the femur neck and whole body of boys, respectively (Baxter-Jones et al., 2011). The literature refers the timing of puberty as inversely related to peak bone mass with late-maturing individuals characterized by lower levels of bone mass in young adulthood (Zemel et al., 2010). In the Saskatchewan Pediatric Bone Mineral Study, BMC was annually measured on six occasions to determine BMC velocity using a cubic spline fit in addition to peak accretion rates (Bailey et al., 2000). The resulting peak bone mineral accrual rate was, on average, 407 $g.year^{-1}$ for boys which corresponded to a peak calcium accretion rate of 359 $mg.day^{-1}$ (assuming 32.2% as the fraction of calcium in bone mineral). The study of Canadian adolescents estimated 14.0 years as the mean age of peak calcium accretion. Even among children of similar age, those who are tall for age have greater BMC and aBMD than those who are average or short for age (Zemel et al., 2010). The groups of adolescent boys participating in soccer and swimming did not significantly differ on stature nor body mass and, consequently, increment rates over a 12-month period should be mainly explained by type and frequency of participation in sports.

The onset and progression through puberty growth spurt is accompanied by alterations in body composition, particularly an increment in fat-free mass among boys, contrasting to an increase in fat mass that is commonly reported for girls (Malina et al., 2004). The variance in body mass on bone accretion is of particular interest because of the beneficial effects of weight-loading on bone accretion. Additionally, muscle contractions generate forces which stimulate bones to adapt their shape and density (Schoenau et al., 2002; Chang et al., 2021). Prospective studies have shown that lean body mass accretion was the primary determinant of total body peak BMC accretion, explaining 50% of the variability in peak BMC accretion (Rauch, 2007). Actually, among healthy school boys aged 10–17 years (Arabi et al., 2004), the contribution of lean tissue to BMC ranged 5.7-12.3% depending on the skeletal site. It was also concluded that lean tissue was strongly associated to BMC of the femoral neck, whereas fat mass was particularly associated to BMC of the whole body and lumbar spine. Peak gain in the cross-sectional muscle area is an indicator of muscle mass which tend to occur one year after the peak gain in tibia length and earlier than cortical cross-sectional area, total BMC and cortical volumetric BMD assessed by quantitative computed tomography (Xu et al., 2009). Thus, quantitative and qualitative changes in bone tissue seem to establish a dynamic interrelationship to changes in body mass and its components (fat and muscle tissues). In the present study, swimmers and soccer players did not differ neither in body mass as previously mentioned nor in fat tissue in contrast with lean soft tissue that consistently presented higher mean values among swimmers. Thus, sport-associated variation in aBMD emerged as a consequence of sport participation.

Regarding the potential effect of participation in sports on bone health, the literature has been primarily focused on comparisons of aBMD values between athletes and the general population (Elloumi et al., 2006). In the preceding mentioned study, adult rugby players showed greater body mass, and greater total lean and fat masses than controls; in addition, the sample of 20 rugby players aged 23.6 years displayed consistently higher aBMD man values than counterparts: lower limbs (16%), upper limps (21%) and pelvis (17%). In the current study of adolescent participants, mean differences on aBMD at various sites was essentially noted in the lower limbs. In contrast to rugby, physical demands and mechanical impacts in soccer are essentially concentrated in the lower extremities. More recently, another study (Vlachopoulos et al., 2017) examined the differences on bone density, geometry, and strength between soccer and non-impact sport (swimming and cycling) among male adolescents. The cross-sectional data of the previously cited study presented soccer players as having higher aBMD in subhead, hip and legs compared to swimmers (differences ranged 6.9-13.9%) and cycling adolescent athletes (5.3-12.7%). Another recent study (Maillane-Vanegas et al., 2020) confirmed lower values of aBMD at lower limbs in male swimmers compared to counterparts participating in karate, judo, basketball and soccer. Finally, adolescent swimmers aged 13.8 years were compared to controls, tennis and soccer players of similar age (Silva et al., 2011). Although they trained significantly more hours per week, as in the sample of the current study, the differences between swimmers and other athletes were more pronounced in proximal femur.

The current study assessed the non-dominant side, which, might be a limitation. An alternative is given by performing dual hip scans to obtain mean of right and left aBMC (Vlachopoulos et al., 2018). To explore whether there are differences in aBMD at the hip between dominant and non-dominant sides in young adult athletes participating in low- and high-impact sports, measurements of both hips were recorded on 194 athletes of both sexes using dual-energy X-ray absorptiometry (van Santen et al., 2019). Of relevance, the previous study concluded that aBMD mean values in the dominant hip was higher compared to non-dominant among participants in low impact sports, but not among athletes participating in high impact sports. Meantime, the proximal femur region is commonly assessed by DXA in adults, however, it is considered more challenging to evaluate young people with this technology (Crabtree et al., 2014). Despite of that, data from the Bone Mineral Density in Childhood Study (BMDCS) deemed age-related precision of the total hip and femoral neck as comparable to the spine and total body less head (Shepherd et al., 2011).

The present study classified all participants as PHV according to a non-invasive protocol (Mirwald et al., 2002). The algorithm used to predict maturity offset values was derived from Canadian and Belgian longitudinal studies and its applicability has been recently discussed (Malina et al., 2004; Koziel et al., 2018; Malina et al., 2021). Indeed, the equation to predict age at PHV seemed dependent from CA and body size, and inter-individual variance in predicted age at PHV was narrower compared to observed age at PHV. Future research should include a control group to distinguish the impact of participation in sports on bone health. Although the limited sample size and absence of a control group, the current 12-month follow-up research consistently confirmed the osteogenic effect of participation in sport as previously suggested in cross-sectional studies (Narciso et al., 2020; Valente-dos-Santos et al., 2018). Additional research is needed including measurements of habitual physical activity among non-participants and participants in sports.

5.6. Conclusion

During the years of maximal growth, participation in sports is associated to gains in LST and seems to prevent increments in fat mass. Bone accrual were also notorious in aBMD both in swimmers and soccer players with the magnitude of intra-individual gains more pronounced among soccer participants, particularly in the lower limbs and at proximal femur sites. The present study confirmed pubertal growth spurt as a critical period for aBMD outcomes besides the type of sports. Of particular relevance, participation in sports is often viewed as an important form of physical activity and, additionally, add beneficial adaptations on bone health indicators, particularly in sports characterized by mechanical loading.

5.7. References

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Chapter VI

General discussion and conclusions

6. General discussion and conclusions

Bone formation

The supposed mechanisms behind the local mechanical forces were recently summarized (Kemper & Fernandes, 2017). Mechanical demands, occurring by overload, are sensed by osteocytes via flows of interstitial fluid that stimulates the osteoclasts in removing the damaged structures and, simultaneously, the osteoblasts repair the structure of the bone matrix. In the case where mechanical load corresponds to an intensity below the fracture state, remodelling activities are stimulated and result in bone hypertrophy. Remodelling of the bone tissue as a consequence of mechanical load by physical activities was unequivocal evidenced in experimental animal studies (Fonseca et al., 2021). Recent literature is focused on verifying the dose-response relationship, that is, to confirm that the beneficial effects are proportional to the intensity of the load.

The present thesis studied two sports that contrast in mechanical loading: soccer and swimming. Training variables were taking into account and it was possible to notice that during 12-months, swimmers trained more approximately four times more minutes than soccer players (23,378 ± 3368 *versus* 5696 ± 2808, respectively). Although, this training amount was visible in the body composition of the adolescent athletes (swimmers showed higher gains in LST than soccer players), it cannot be traduced on the aBMD values once swimming is practice on a non-weight bearing environment (Gomez-Bruton et al., 2018).

Developmental studies

Puberty is a relative short period that is extremely relevant for the development of bone mass. Several cross-sectional studies consistently reported increments of BMD among both girls and boys. However, the studies substantially ranged in the magnitude of the results which can be attributed to several factors. Firstly, differences in the classification of puberty. As for lack of controlling other factors such as nutritional and/or habitual physical activity that are culturally specific in different countries. In addition, variation due to maturity status (early or late maturing) in a period coinciding with exposition to sex-specific hormones, i.e. estrogen levels in girls and testosterone levels in boys seem to be crucial aspect related to studies devoted to bone mass development. Childhood and adolescence represent an open window. Firstly, to stimulate bone having as goal retard the timing when peak bone mass is achieved and, as consequence, delay the bone mass loss in the adult skeleton (Zemel, 2013). Secondly, represents a key period to predict and delay possible bone fragilities and diseases in the future (Sherar & Cumming, 2017). Finally, as mentioned long time ago (Bailey et al.1993) reports on BMD changes should be interpreted with caution because of the methods used.

Assessment of bone tissue

Quantitative computed tomography (QCT) systems are progressively being adapted for the assessment of bone geometry, allowing cortical bone to be separated from trabecular portion. The technique provides a true measure of total, cortical, or trabecular bone mineral volumetric density (mg.mm^{-3}). However, it is substantially more expensive, and exposes patients to larger radiation doses compared to equipment adopted in the current studies. The preceding is a problematic issue in pediatric research. Meantime, peripheral QCT (pQCT) system emerges as an alternative method to assess bone morphology and geometry, in which the scan time is fast and produces low radiation exposure to the patient. Nevertheless, it has been recently recognized the absence of standardized methods to the technique (Ma & Gordon, 2012).

Recently, as reported on chapter III of the present thesis, hip structural analysis (HSA) has been used to describe hip geometry. This procedure is easily achieved with a DXA assessment and distinguishes the cross-sectional area (CSA, mm2) that represents the hip total bone surface area, minus soft tissue area and trabecular bone, the cross-sectional moment of inertia (CSMI, mm4) that characterizes the mass distribution in the center of a structural element, giving an index of structural rigidity and, at last, the section modulus

(Z, mm3) which can be obtained as a determinant of the maximal bending strength in a cross section (Khoo et al., 2005). Participants from sports with higher mechanical loading present greater results on these parameters than lower mechanical loading participants and control groups (Vlachopoulos et al., 2017).

Bone health parameters in sports sciences

Many sports, particularly gymnastics, appeared associated with marked improvements in bone density for long time (Bass et al. 1998) and, more recently, on bone strength (Liang et al. 2005) that may be retained at least partly later in life (Eser et al. 2009; Pollock et al. 2006). Bone geometric properties are an additional relevant indicator of bone health and therefore fracture risk. In fact, the association between participation in sports and upper limb bone mass, geometry, and strength in addition to muscle size and function was the studied in young gymnasts (Burt et al. 2012). Participation in non-elite gymnastics emerged as linked to musculoskeletal benefits in upper limbs bone geometry, muscle strength and function. Despite the differences between high and low training athletes for arm lean soft tissue and muscle cross sectional area, bone strength did not substantially differ. Studies retrieved from the systematic review on bone (chapter III) compared BMC and BMD across different sport and found that swimming and cycling participants present similar results between them, lower than sports with higher impact (i.e., soccer, gymnastic, basketball, combat sports), and most of the times with similarities with the age-matched controls (Rico et al., 1993; Olmedillas et al., 2011; Ribeiro-dos-Santos et al., 2016).

For clinical assessments of osteoporosis diagnosis, more often than not the analysis of the proximal femur shows relevance since it is one of the sites most affected by osteoporotic fractures (Warriner et al., 2011), most effectively in the bone mineral density on the femoral neck (Siris et al., 2014). Thus, recent research on adolescent athletes has been analyzing this specific region of interest aiming to understand the impact of sport participation on bone acquisition during this life period and the possibility to predict osteoporosis in the future (Ward et al., 2020). Study 3 assessed adolescent soccer players (n=20) and swimmers (n=20) twice during a 1-year period (baseline and 12-months after). Whole body, trunk, upper limbs and lower limbs aBMD were distinguished and, more specifically the proximal femur region was assessed (aBMD for femoral neck, ward triangle, and trochanter). As it was hypothesized, soccer players showed higher mean values in aBMD compared to swimmers, but regarding the proximal femur regions these differences were larger in the specific site trochanter. Soccer players showed greater accrual in this region over 12-months than swimmers. These results reinforce the conclusions of previous studies than assessed the region of the proximal femur (Vlachopoulos et al., 2017). It is important to take into account that in study 3, both groups showed no differences at baseline regarding somatic maturation and, on training variables, swimmers showed more training volume and higher LST.

Structural Muscle Bone Unit

In former views hormones, calcium, vitamin D and other humoral and non-mechanical agents dominated the subject of postnatal bone health including in research regarding children and adolescents. However, an emergent paradigm of skeletal physiology revealed that muscle tissue probably causes the largest load and the largest bone strains. These strains are central biological mechanisms that determine whole-bone strength. In general, hormones and other non-mechanical agents that are supposed to affect bone strength can help or hinder the "bone strength-muscle strength” relationship but cannot replace it (Frost & Schönau, 2000). Bone and muscle are connected as tissues that work together as one unit on the human support and motion. Molecular and biochemical interactions occur to ensure the normal human body function (Bonetto & Bonewald, 2019). Some factors that exert a bone effect by acting directly on bone cells also affect muscle strength. In other words, they seem to affect bone strength indirectly (growth hormone, adrenal corticosteroid, androgens, calcium, genes, vitamin D and its metabolites, etc). Thus, bone and muscle form an operational unit and future research should examine how the "bone strength - muscle strength" relationship develops. The preceding needs adequate research design, instruments and assessments.

Bone response to eccentric versus concentric activities

Based on the need to include sports in health promotion, much research on physical activity has been conducted to identify the best modality. When young populations have to apply a maximal eccentric contraction, the sensation seems new (De Ste Croix, 2012) and the central nervous system needs to adapt different strategies to produce maximal strength (Enoka, 1996). In fact, studies have consistently demonstrated the beneficial effects of participating in sports and in other forms of exercise programs (Vlachopoulos et al., 2018). However, the effect of alternative methods (moderate-intensity continuous training; high-intensity interval training; concurrent training) directly on body composition (fat mass, lean soft tissue) and indirectly on bone adaptations remains uncertain and deserves future research to estimate effectiveness associated to each training method. Recent research assessed adolescents included into two training methods (concentric and eccentric cycling) in addition to a control group (Julian et al., 2020). It was suggested that bone fragility given by a compromised relationship between density, geometry and strength was more likely to be prevented when participants were exposed to eccentric efforts.

Limitations

The present thesis combined the growth and maturation in youth athletes with bone health and strength parameters. The studies presented on chapters III, IV and, V showed limitations that were declared in the end of each. At this point, limitations had to be considered once more so we can understand what existed before, what this thesis added to the literature and, finally, which are the future challenges on research regarding these topics.

According to the background concepts and previous literature presented on chapter I, it was possible to understand that if the aim of the research is to assess changes on participants during childhood and adolescence it is essential to consider growth, maturation and a longitudinal study design. Future studies must present larger samples, contain age- and sex-matched control groups, and control confounders such us maturity status and training variables.

Chapter III reviewed a large spectrum of different topics that were included on the searching equation. It brings to light that when assessing bone there are components that can easily be assessed and should be addressed on future research that focuses bone health parameters. Bone biochemical markers, geometry and stiffness ought to be included more often as indicators in studies that already project to measure density and content. Chapter V also integrated the study of bone among adolescent athletes from contrasting sports over a 12-month period. While still being at these age groups, studies need to be longer and participants should be measured twice per year in a way that allows categorizing each group by maturity status. Study 2, presented in chapter IV tested a specific protocol that concluded that a minimum of five repetitions are recommended to derive the best repetition of PT, when the participants do the test for the first time. However future studies need to test different groups, controlling confounders, with different interventions to better analyze the results over time. Also, it can be suggested to perform isolated testing sessions for the concentric and eccentric testing.

Conclusions

The present thesis had as main goal to examine the independent and combined effects of growth, maturation, and training using a mixed-longitudinal design, initially, on developmental changes related to muscle strength and, after, on parameters of bone tissue in young male athletes that are involved in sports characterized by different mechanical loading on the skeleton. Since the beginning of the data collection until the writing thesis phase, the time spent at the university, with the research group and the foreseen timeline had been affected by the worldwide pandemic status that lasted for almost two years. Nevertheless, the initial objectives were achieved and the main conclusions and practical applications are the following:

i) The adult skeleton is the reflection of the background of each individual and, to predict and prevent future fragilities, variables such as the content, density, bone specific biochemical markers, stiffness and, geometry should be combined when studying youth population.

ii) Isokinetic dynamometer protocols used to access the peak torque on concentric and eccentric actions in pediatric population must include at least five repetitions of test, knowing that knee flexors and eccentric repetitions are not completely reliable, especially in young participants.

iii) Federated sports with higher mechanical loading produce greater results on aBMD (mostly at specific and relevant clinical sites) than the ones with lower mechanical loading during the crucial years of adolescence. Training variables such as time spent on higher impact loading exercise per sessions may help technicians control and help improving athletes bone health parameters.

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