Universidade de Lisboa

Faculdade de Motricidade Humana

PHYSICAL FITNESS:

IDENTIFIERS OF SPORT PARTICIPATION

AND

BONE HEALTH IN YOUTH

DUARTE MIGUEL HENRIQUES NETO

Orientador: Doutor Luís Fernando Cordeiro Bettencourt Sardinha

Tese elaborada com vista à obtenção do Grau de Doutor em

Motricidade Humana na especialidade de Atividade Física e Saúde

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2021

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Concluir um programa de doutoramento resulta da realização de um longo desenvolvimento académico e pessoal. A realização do meu projeto de doutoramento dependeu, fundamentalmente, de todas as pessoas que me acompanharam durante esta longa jornada. Foram avaliados cerca de 2800 participantes, com idades entre os 10 e os 18 anos, distribuídos por um total de cerca 15.000 quilómetros de estradas nacionais num período de 15 meses. Todos estes momentos de trabalho apenas foram realizados graças à amizade, ao espírito de entreajuda, da elevada competência presente no Laboratório de Exercício e Saúde, de uma orientação distinta, de amigos excecionais, de uma família única e do sentido de compromisso de todos os participantes deste projeto. Não seria esquecimento, mas seria uma ingratidão ao terminar esta etapa e não demonstrar a minha amizade e gratidão às pessoas que contribuíram para a minha felicidade.

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ABBREVIATIONS

ACE	Angiotensin-converting enzyme
ACTN3	Alpha-actinin skeletal muscle isoform 3
AMPD1	Adenosine monophosphate deaminase 1
AMPK	5 adenosine monophophate-actived protein kinase
ANOVA	Analysis of variance
APHV	Age of peak height velocity
AUC	Area under the curve
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
CI	Coefficient interval
CRF	Cardiorespiratory fitness
DNA	Deoxyribonucleic acid
DXA	Dual-energy radiograph absorptiometry
H2	Heritability
HG	Handgrip strength
HJ	Horizontal jump test
ICC	Interclass correlation coefficient
IGF-1	Insulin-like growth factor-I
IOC	International Olympic Committee
IPDJ	Portuguese National Institute of Sport and Youth
LIPA	Light intensity of physical activity
LTAD	Long Term Athlete Development
MF	Muscular fitness

MRI	Magnetic resonance imaging
mTOR	Mechanistic target of rapamycin
MVPA	Moderate-vigorous of physical activity
N	Newton
PA	Physical activity
PACER	Progressive aerobic cardiovascular endurance Run
PBM	Peak bone mass
PF	Physical fitness
PHV	Peak height velocity
pQCT	Peripheral quantitative computed tomography
QCT	Quantitative computed tomography
QUS	Quantitative bone ultrasonometry
ROC	Receiver operating characteristic curve
R-SoS	Distal third radius speed of sound
SD	Standard deviation
SHTR	4x10m shuttle run test
SoS	Speed of sound
SP	Sports participation
ST	Sedentary time
T-SoS	Midshaft tibia speed of sound
VJ	Vertical jump test
$VO_{2\,max}$.	Maximal oxygen consumption
VPA	Vigorous physical activity
WC	Waist circumference
WHO	World Health Organization

ABSTRACT

Physical fitness (PF) assessment is a key tool in monitoring sports performance and health indicators in young people, mainly because PF is related to indicators of both sporting potential and performance, used in signalization, recruitment and surveillance, and bone health. Therefore, this tool may be considered of valuable use to both sport and health professionals. However, there is not a single standardised protocol able to assess the several attributes of PF among young athletes from different sport backgrounds and non-athletes. In this context, this dissertation presents four investigations developed in two major research areas in the evaluation of PF: sports performance and bone health. Regarding sports performance, the reliability of the PF tests, in young athletes from different competitive levels and sports backgrounds were tested. The battery of the FITescola® program proved to be applicable in young athletes of both sexes, constituting a reliable instrument of simple application in the field of sports performance. The discriminatory power of each FITescola® test for signalling young people with sport potential, in consolidated sport organization models, according to gender and age was also analysed. The results showed that PF tests are a valid instrument for signalling young people with sport potential, and that the discriminatory power of the tests varied according to gender and age. In the area of bone health, one investigation was conducted to analyze the relationship between different PF tests and speed of sound (SoS) values in the radius and tibia of young athletes and non-athletes. Different degrees of association were observed between the several PF tests and the radial and tibial SoS values. The degrees of association varied according to gender and athlete status of the participants. The results of this investigation indicate that, for the purpose of monitoring bone health, different PF tests should be applied depending on whether or not young people were athletes. Another analyzed, for the first time, the relationship between vigorous physical activity and SoS in the radius and tibia and whether this relationship was mediated by different PF tests and fatness. The results suggested that some of the effects of vigorous physical activity on radial and tibial SoS were mediated by cardiorespiratory fitness in girls, while the upper and lower muscle strength, speed of displacement and fat mass (%) were found to be mediators in boys. The mediating effect of a PF tests and fat mass (%) on the association of vigorous physical activity with bone health outcomes differed between boys and girls. In summary, this dissertation provides observational scientific evidence on the importance and applicability of PF assessment in the context of competitive sport and, simultaneously, for monitoring bone health among young athletes and non-athletes. These findings may be relevant for present and future interventions in competitive sport as well as in public health.

Keywords: physical fitness, youth, athletes, sports potential, bone health.

RESUMO

A avaliação da aptidão física é um instrumento fundamental para a monitorização do rendimento desportiva e da saúde, principalmente devido ao facto da aptidão física estar associada a indicadores de potencial desportiva, utilizados no processo de sinalização e recrutamento de jovens, bem como a indicadores de saúde óssea. Desta forma, este instrumento pode ser considerado como uma mais-valia para profissionais da área do desporto e saúde. Todavia, não existe um único protocolo estandardizado capaz de avaliar os vários atributos da aptidão física entre jovens atletas de diferentes modalidades e não atletas. No âmbito do rendimento desportivo, foi testada a reprodutibilidade e a fiabilidade dos testes de aptidão física em jovens atletas de diferentes níveis competitivos. A bateria do programa FITescola® demonstrou ser aplicável em jovens atletas de ambos os sexos, constituindo um instrumento fiável de aplicação simples no âmbito do rendimento desportivo. Adicionalmente, foi analizado o poder discriminativo de cada teste do FITescola® para sinalizar jovens com potencial desportivo, em função do sexo e da idade. Os resultados demonstraram que os testes de aptidão física são um instrumento válido para a sinalização de jovens com potencial desportivo e que o poder discriminatório dos testes variou de acordo com o sexo e a idade. Na área da saúde óssea, foi conduzido uma investigação para analisar a relação entre os diferentes testes de aptidão física e os valores da velocidade do som no rádio e na tíbia, em jovens atletas e não atletas. Os graus de associação variaram em função do sexo e da condição de atleta dos participantes. Os resultados desta investigação indicam que, para efeitos de monitorização da saúde óssea, diferentes testes de aptidão física devem ser aplicados, em função da condição de atleta. Outra investigação analisou, pela primeira vez, a relação entre a atividade física vigorosa e a velocidade do som no rádio e na tíbia, e se esta relação era mediada por diferentes testes de aptidão física e pela massa gorda (%). Os resultados sugerem que nas raparigas, uma parte dos efeitos da atividade física vigorosa na velocidade do som no rádio e na tíbia ere mediada pela aptidão cardiorrespiratória, enquanto, nos rapazes, a força muscular superior e inferior, a velocidade de deslocamento e a massa gorda (%) foram identificados como mediadores desta relação. A presente dissertação fornece evidência científica observacional sobre a importância e aplicabilidade da avaliação da aptidão física tanto no contexto competitivo, como na monitorização da saúde óssea em jovens atletas e não atletas. Estes resultados podem ser relevantes para intervenções presentes e futuras no âmbito do desporto de competição e da saúde pública.

Palavras-chave: aptidão física, jovens, atletas, potencial desportivo, saúde óssea.

CHAPTER 1

Introduction to the dissertation

1.1 - DISSERTATION STRUCTURE

The investigation of physical fitness (PF) in adolescents has played an essential role in public policies to promote sports participation and healthy lifestyle. Sports participation during youth has shown to be an essential strategy to increase physical activity (PA). Motivated by the importance of sports participation (SP) for promoting healthy lifestyles and the increasing incidence of bone fractures in adolescents, especially in athletes, this dissertation entitled "*Physical Fitness: Identifiers of Sport Participation and Bone Health in Youth*" was developed. The main purpose was to investigate the role of PF on the signalising process of potential athletes in the school context and the specific relationship between several PF tests, body composition, PA measures, and bone health outcomes on the radius and tibia. Adolescence is a unique period of life, during this phase 90 per cent of future peak bone mass is acquired, and PA plays a vital role in its potentiation. To achieve the main purpose, the present dissertation incorporates a collection of four research articles submitted or in press in peer-reviewed journals with an established ISI Impact Factor. To clarify the framework of these investigations, this dissertation is organised as follows:

Chapter 2 includes a literature review, highlighting the current scientific evidence regarding the biologic (e.g. sex, maturation, genetics) and environmental factors (e.g. PA participation) influencing PF and the role of PF on sport performance and health. In addition, we reviewed the role of PA on bone health during childhood and adolescence, looking in detail to the promotion of youth sports participation as a strategy to fight against physical inactivity and to improve bone health.

A specific and detailed review of the methodology used in this dissertation is shown in **Chapter 3**. In this chapter, we will provide detailed information regarding the methods used to build the research project and the methods used trough the investigations of this dissertation.

Chapters 4 to 7 correspond to the four investigations that were conducted to answer the research goals described in chapter 2.

Chapter 8 corresponds to a general discussion which provides a summary and the main findings obtained within the four investigations of this dissertation, highlighting the most relevant aspects of the overall research, as well as reflections for further investigations.

The bibliographic references are presented at the end of each section using the American Psychological Association style.

In the end, the annexes section includes supplementary material that is mentioned across the dissertation and may be viewed as essential within its broader scope.

1.2 - LIST OF ARTICLES AND CONFERENCE ABSTRACTS AS THE FIRST AUTHOR

The investigation carried out as part of the present doctoral research program resulted in the following publications and communications (oral/poster) as the first author:

1.2.1 - Peer-Reviewed articles as first author published or submitted that are related to the dissertation:

Henriques-Neto, D., Minderico, C., Peralta, M., Marques, A., & Sardinha, L. B. (2020). Test–retest reliability of physical fitness tests among young athletes: The FITescola®battery. *Clinical Physiology and Functional Imaging, 40*(3), 173-182. doi: 10.1111/cpf.12624

Henriques-Neto D, Hetherington-Rauth M, Magalhães JP, Júdice PB, Sardinha LB. (*Submited*). Predicting sports potential in youth through physical fitness tests.

Henriques-Neto, D., Magalhães, J. P., Hetherington-Rauth, M., Santos, D. A., Baptista, F., & Sardinha, L. B. (2020). Physical Fitness and Bone Health in Young Athletes and Nonathletes. *Sports Health: A Multidisciplinary Approach, 12*(5), 441-448. doi: 10.1177/1941738120931755

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CHAPTER 2

Literature review

2.1 - PHYSICAL FITNESS COMPONENTS AND INFLUENCING FACTORS

Physical Fitness (PF) is a multidimensional construct defined as a *"set of attributes that are either health or skill-related. The degree to which people have these attributes can be measured with specific tests"* (Caspersen, Powell, & Christenson, 1985).

The individual PF level is determined by biologic factors or "nature" (e.g., genetic characteristics, sex, maturity) and environmental factors or "nurture" (e.g., level of habitual physical activity [PA]) (C. Bouchard, Blair, & Haskell, 2012; Yan, Papadimitriou, Lidor, & Eynon, 2016). The most common attributes of PF studied in the scientific literature are: cardiorespiratory fitness (CRF), explosive strength, endurance strength, speed of movement, agility, coordination and balance. Additionally, some authors, include measures of body composition, such as the body mass index (BMI) and waist circumference (WC) as PF attributes (Ortega, Ruiz, & Castillo, 2013).

2.1.1 – Cardiorespiratory fitness

The maximal aerobic power or CRF is one of the most studied and well-documented attributes of PF in exercise physiology. It is characterized by the capacity of both the cardiovascular and respiratory systems to deliver metabolic energy during extended duration exercises at high intensity (Taylor, Buskirk, & Henschel, 1955). The CRF is usually quantified by the maximal oxygen consumption (VO_{2max}) and can be estimated by direct and indirect methodologies (Wilder et al., 2006). The gold standard measurement for CRF is using direct gas-exchange analysis in the laboratory environment. However, in the ecologic context and for epidemiologic studies, the most common tests used to assess CRF in young people are the 1 mile run test and the 20-m shuttle run test, and the Yo-Yo intermittent test for young athletes (Bangsbo, Iaia, & Krustrup, 2008; Zemková & Hamar, 2018). The protocols of these tests are sensitive, reliable and low-cost, and their measures are well-studied indicators of health in youth (F. B. Ortega, J. R. Ruiz, M. J. Castillo, & M. Sjostrom, 2008).

2.1.2 – Muscular fitness

Muscular fitness (MF) is the capacity of a muscle or a group of muscle to produce tension against a resistance or submaximal workloads. The size and number of the muscle and the coordination of the muscle fibers are the main factors that influence MF. The maximal strength that a group of muscle fibers can produce is usually quantified in N or Kg (Radák, 2018a; Wilder et al., 2006). The kinetic ability of the body to move, lift loads or do daily activities has been used to characterize an individual's integrated status of muscular strength and muscular endurance. The most studied attributes of MF related to sports performance and health are: explosive strength and endurance strength (Garcia-Hermoso, Ramirez-Campillo, & Izquierdo, 2019; Suchomel, Nimphius, & Stone, 2016). Endurance strength is the ability of a group of muscles to perform repeated contractions over a specific period of time that is sufficient to cause fatigue. On the other hand, explosive strength is defined by an all-out muscular effort in a relatively short time period (Bazyler, Abbott, Bellon, Taber, & Stone, 2015). Besides this two attributes of MF, strength can also be isometric and dynamic. Dynamic strength is related to the movement of the body against an external load, while isometric strength is the muscle tension produced against a motionless external load (Suchomel, Nimphius, Bellon, & Stone, 2018). There are several test protocols by which different attributes of MF can be assessed. Tests such as, the push-up test, the vertical jump test and the standing broad jump or horizontal jump (HJ) have been widely used for assessing explosive strength and endurance strength among young people (Beunen, Peeters, & Malina, 2010; Petrigna et al., 2019; Jonatan R. Ruiz et al., 2006).

2.1.3 – Speed of movement

The speed of movement is the ability to move quickly across the ground. It is dependent on one's acceleration, i.e., how quickly the speed is increased from a stationary position, maximal speed of movement, i.e., the fastest speed an individual can reach, and speed maintenance, i.e., the ability to minimize deceleration. Speed of movement is related to muscle strength, but also to body weight. In addition to the recruitment of muscle fibers, it is also essential to have efficient mechanics of movement in order to optimize the muscles for the most economical movement technique (Seitz, Reyes, Tran, de Villarreal, & Haff, 2014; Suchomel et al., 2016). This attribute of PF is notably associated with performance in sports characterised by movements of high-intensity and short duration (e.g. football, volleyball, basketball) (Beato, Bianchi, Coratella, Merlini, & Drust, 2018; Zemková & Hamar, 2018). Considering the specificity of each sport, there are several field tests to assess the speed of movement. However, the most common and generic speed of movement tests are the 20m run and the 40m run (Young et al., 2008; Zemková & Hamar, 2018). These tests are usually performed in the school and youth sports contexts. were developed for specific sports (Radák, 2018b).

2.1.4 – Agility

Agility is the ability to quickly accelerate, decelerate and change course while controlling the direction and position of the body. This attribute of PF is strongly related to strength, coordination and balance, but also to the skill level, and can be improved by developing the appropriate level of strength and conditioning for the individual's age. It is considered an important PF attribute in most ball games and in combat sports (Radák, 2018b). There are several tests to assess agility and many of them were developed for specific sports (Zemková & Hamar, 2018).

2.1.5 – Flexibility

Flexibility is determined by the range of motion around a joint, and the assessments of this attribute may be used to evaluate general health and sports performance (Fukuda, 2018). In the sport context, flexibility is highly individualized and should be interpreted within a specific context, i.e. gymnastic or football (Fukuda, 2018). The sit and reach test is the most widely used test to assess flexibility in the ecologic context and on large populations of young people (J. R. Ruiz et al., 2009). Improving flexibility based on careful and progressive training programs is crucial for young people performance and health. Flexibility should be reflected on acceptable levels of function of specific body part (e.g. knee, ankle), and extreme laxity or stiffness in the specific biologic structures (e.g. muscle tissue, joints) may be reflect a potential injury (Fukuda, 2018).

2.1.6 – Biologic and physiologic factors influencing physical fitness

Sex is one of the most important determinants of PF and maturity is a major factor influencing the development of PF throughout adolescence. Genetic characteristics have different impact weights in the several components of PF, for example, around 80% on height, between 15% and 90% on muscle strength, 50% on peak oxygen uptake, and 46% to 84% on anaerobic power (Tucker & Collins, 2012). Furthermore, the genetic predisposition is known to be a contributing factor to both health and sport performance, that should be taken into account. (Joyner, 2019; Roth, 2007; Schutte, Nederend, Hudziak, de Geus, & Bartels, 2015; Suppiah, Low, & Chia, 2015).

2.1.6.1 – Sex, maturity and physical fitness

The PF performance varies with sex and according to chronological aging and biologic maturation (Neil Armstrong, van Mechelen, Armstrong, & McManus, 2017). Anatomic and physiologic differences between male and female elite athletes are so significant, that they rarely compete against each other (Tucker & Collins, 2012). Sports where strength, acceleration and speed have an essential role on sport performance and thus the final result, are generally dominated by males athletes (N. Armstrong & McManus, 2011; Neil Armstrong, van Mechelen, Armstrong, & McManus, 2017).

Generally, men have larger and longer bones and higher ratio of muscle mass to fat mass. These characteristics provide an advantage, compared to women, promoting a higher leverage, as well as a wider frame to support muscle tissue (Heyward, Johannes-Ellis, & Romer, 1986; Mascherini, 2017). Additionally, the main reason why strength levels are greatly different between male and female athletes is essentially because of the difference in the dimension of the cross-sectional area of the muscle, which is highly related with strength production (M. Jones et al., 2016; A. E. J. Miller, MacDougall, Tarnopolsky, & Sale, 1993). The aforementioned may explain why the records on swimming and running are lower in female competitions than male competitions (Thibault et al., 2010).

The VO_{2max} is related with size, body composition and the blood biochemical composition. The male body is larger and has more capacity to transport the oxygen to the muscle cells. Besides the body size, other reason why female athletes reach lower maximum oxygen consumption than male athletes, is probably because women present around 12% lower concentrations of hemoglobin than men (W. G. Murphy, 2014). Notwithstanding, on long duration sports, the glycogen is an essential bioenergetics source. The endurance depends of the body capacity to convert calories into metabolic energy (Heydenreich, Kayser, Schutz, & Melzer, 2017). In this regard, men seem to be less efficient in converting glycogen into energy than women, because of that the differences between men's and women's performance in long duration sports are smaller, than those seen in sports where strength and muscle mass have a more prominent role (Neil Armstrong & Barker, 2012; Devries, 2016).

On the same line, male and female young athletes have anatomic and physiologic differences between them (N. Armstrong & McManus, 2011; McManus & Armstrong, 2010). However, contrary to adulthood, during youth two essential and different biologic processes occur, growth and development. During the prepubertal period very few and small differences, between boys and girls, are observed in the rates of growth and development, as well as, in strength, speed, power, endurance and motor coordination. Physiologic differences between boys and girls are more evident during maturation, which occurs in different chronologic ages between boys and girls (Lloyd & Oliver, 2012).

Biologic and physiologic differentiation starts in the embryonic development. The SRY and SOX9 genes are the main genes involved on determining sex. SRY activates the sex-determining region Y protein, which is involved in male sexual development (Gilbert, 2000; Tucker & Collins, 2012). In boys, testosterone secretion starts near to 3 months in the foetus life, while in girls this process is absent in this period of life. The non-secretion of testosterone in girls foetus life, allows the development of female reproductive organs, and accounts for subtle differences in cardiac function and body composition at birth (Wells, 2007). Growth, muscle mass development, bone mineralization, and metabolic adaptations during youth depends essentially of the complex interaction between growth hormone, insulin-like growth factor with anabolic hormones such as testosterone (boys) and estrogen (girls) (McManus & Armstrong, 2010).

The growth and development processes are not linear and do not happen simultaneously within young people. In fact, young people of the same age have different maturity status which influences the functioning of several biologic systems (e.g., endocrine system and nervous system). Boys usually begin pubertal growth after girls and have slower growth rates, however the magnitude of the growth spurt is greater in boys (Lloyd & Oliver, 2012). The maturity status is often assessed by the peak height velocity (PHV) which can occur between 13 and 15 years old in boys, and between 11 and 14 years old in girls (Robert M. Malina, 2011).

This is an important issue as maturity highly impacts PF. Muscle strength development is reliant on a combination of muscular, neural and structural factors (Bergeron et al., 2015). After PHV, muscular strength increases much faster in boys than in girls, when up until then it has been increasing fairly linearly in both sexes (R. M. B. Malina, Claude; Bar-Or, Oded, 2004). Additionally, at late puberty, boys can have up to 50% more muscular strength than girls. Similarly, maturation also has a positive impact on CRF. The peak VO_{2max} during maturation increases around 70% in boys and 25% in girls aged 12-17 years old (Neil Armstrong & Welsman, 2001). Even considering young people of the same sex, maturity plays and important role in sport performance. While in most sports, boys and girls with greater maturity levels tend to be more successful, in gymnastics and driving sports those with later maturation have advantage (Neil Armstrong, van Mechelen, Armstrong, & McManus, 2017). This is a results of the specificity of each sport. Understanding the growth and development processes, such as maturity, is essential to develop adequate tools to monitor PF in youth, and specially in young athletes (Neil Armstrong, van Mechelen, Armstrong, & McManus, 2017). Sport success during youth is highly related with physical and physiologic variables, which develop according to sex, chronologic and biologic age (McManus & Armstrong, 2010).

The physiologic bases of young sport science are well documented, and it is acknowledged that all components of PF are improved with the appropriate training (McManus & Armstrong, 2010). In this period of life several biologic changes occur, and these changes occur in different chronologic ages between sexes, but also within sexes. Therefore, tools to asses PF should reflect sex and age differences.

2.1.6.2 – Genetics and physical fitness

The genetic heritage has an essential role on the individual PF performance, as well as on the range of trainability of each PF component such as, body composition, CRF, strength, speed, flexibility, agility, and metabolic (Ahmetov & Fedotovskaya, 2012; Neil Armstrong, van Mechelen, Armstrong, & McManus, 2017; Neil Armstrong, van Mechelen, Schutte, Bartels, & de Geus, 2017; C. Bouchard, Hoffman, Eric P., 2011; Joyner & Lundby, 2018). As aforementioned, sex and maturity play an essential role on PF, but also do the family heritable characteristics.

The variation within the PF components during youth, depend on the differentiated contribution that both the genetic and environment factors have on each PF component (Ahmetov, Egorova, Gabdrakhmanova, & Fedotovskaya, 2016; Neil Armstrong, van Mechelen, Schutte, et al., 2017; Yan et al., 2016). Genetic assessment and analysis in the sports began in the early 2000s after the interpretation of deoxyribonucleic acid structure. Several studies found the first associations between genetic markers and PF attributes such as angiotensin-converting enzyme and adenosine monophosphate deaminase 1 gene variations with CRF, alpha-actinin skeletal muscle isoform 3 (ACTN3) with speed, injury risk and post-exercise recovery, and insulin-like growth factor-I (IGF-1) with quadriceps muscle strength gains (Goh et al., 2009; Kostek et al., 2005; Pickering & Kiely, 2017).

Variability on gains or losses in PF performance, when stimulated to comparable training programmes, depend, in part, of the individual genetic characteristics (Joyner & Lundby, 2018). Heritability (H^2) is estimated from the division of the genotype variation by the phenotype variation (H^2= genotype variation / phenotype variation) (Puthucheary et al., 2011). Overall, between 0% and 93% of the variability of PF components are due to genetic factors, where the relative contributions of genetic and non-genetic factors to the total PF phenotype are estimated. Results closer to 1 (100%) reflect a bigger genetic contribution, whereas results closer to 0 (0%) reflect a greater environment contribution. Family and twin studies (e.g. monozygotic [±100 % genetically identical]; dizygotic [±50% identical of genetic characteristics]) provide knowledge on the genetic and environment contributions to the PF phenotype (Neil Armstrong, van Mechelen, Schutte, et al., 2017).

Cross-sectional investigations have demonstrated that genetic influences several components of PF, however the magnitude of the effect varies widely (Neil Armstrong, van Mechelen, Schutte, et al., 2017). Even within each PF component a broad variability in the percentage explained by genetic factors is reported. A recent meta-analysis of investigations in young adults found H^2 estimates of 72%

for VO_{2max}. However, studies performed among adolescents do not allow to estimate the extent to which genes contribute to the variance in this PF component. A study among 10 year-olds reports that in girls 85% of the VO_2 max is heritable, while another one, with participants of the same age, observed an explained variance of 95% in both sexes (Neil Armstrong, van Mechelen, Schutte, et al., 2017; Maes et al., 1996). Among older adolescents, 15 and 17 year-olds, another two studies show estimates of 35-60% (Lortie et al., 1982; Schutte, Nederend, Hudziak, Bartels, & de Geus, 2016). Regarding muscular fitness, the lower limbs strength, assessed by the vertical jump, and upper limbs strength, assessed by the HG, were estimated to be 71% and between 44% and 58% attributable to genetic, respectively (Chatterjee & Das, 1995; R. M. Malina & Mueller, 1981). A meta-analysis summarizing evidence on this component estimated that genetic factors explained most of the variance in the vertical jump (62%), HG strength (63%) and flexibility (50%) (Schutte et al., 2016).

Very few longitudinal studies investigated the contribution of genetic factors to adolescents' PF. A longitudinal family study showed that changes over time in the trunk flexibility, push-ups, sit-ups and HG strength were 48%, 52%, 41% and 32% heritable, respectively. Similarly, a twin investigation has demonstrated that genetic factors explain between 47% and 92% of the variance in power and between 44% and 83% of the variance in isometric strength (Beunen et al., 2010).

An experimental reference investigation in this field, the HERITAGE Family Study, aimed to study the role of the genotype in the cardiovascular and metabolic responses to aerobic exercise training. This investigation indicated that

20 weeks of aerobic exercise training promote changes on CRF, hemodynamic characteristics, and other indicators of health, and the differences responses to the training may be explained genetic characteristics (An et al., 2003; C. Bouchard et al., 1999; C. Bouchard et al., 1995; Hong, Rice, & Gagnon, 2000).

The variability of the impact from H^2 into PF phenotypes in young people may be due to several methodologic factors such as, type of population investigated (e.g. children, adolescents), maturation status, and characteristics of exercise (e.g. type, volume, and intensity) (Neil Armstrong, van Mechelen, Schutte, et al., 2017; Joyner & Lundby, 2018). Additionally, the lack of standardization of genetic tests, the lack of knowledge of the specificity of which genes are being tested, the small effect of genes on associations with non-communicable diseases and the recognized influence of environmental factors in sport and health status reinforce the need to look and interpret these results with caution. Despite the impacts of genetic on PF, from applicative clinical and technical perspective, large investigations to explaining in detail the complex associations between genetic, environment with physiologic phenotypes are necessary (Joyner, 2019; Zehsaz et al., 2019). However, so far the methodology and the process to assess the genetic information is still very invasive and it is not the most advisable to use pertaining this knowledge in the sport and the general public (Pickering & Kiely, 2017).

2.1.7 – Environmental factors influencing physical fitness

Even though H^2 explains some of the PF phenotype (Ahmetov et al., 2016), other factors, external to the individual, also have some influence (André F. Seabra, Mendonça, Göring, Thomis, & Maia, 2008). Thus, young people should not be exposed only to genetic testing to predict PF performance (Joyner & Paneth, 2019; Webborn et al., 2015). All PF attributes are susceptible to trainability. The concept of trainability describes the range or magnitude of the physiologic response to a physical stimulus (e.g. sports training) (Joyner & Lundby, 2018). Environmental factors, such as sleep habits, nutrition education, and PA participation or a single physical exercise also impact PF.

2.1.7.1 – Sleep, nutrition and physical fitness

Sleep is an essential biologic state that boost and repairs all human systems in response to hormonal and circadian regulation changes promoted by several stress stimulus (Sheldon, 2014). After suffering different "stress" throughout the day, sleeping time is when several repair or restore processes occur within the body (e.g. body tissues, brain), which allow to answer efficiently to the new demands in short and long term (e.g. training) (Bergeron et al., 2015; Bruce, Lunt, & McDonagh, 2017).

During sleep, the lymphocyte activity is improved (e.g. IL-1 and IL-2) in order to promote an efficient inflammatory response, also the endogenous anabolic steroids function is improved (e.g. prolactin, testosterone, cortisol, hormone luteinizing) (Sheldon, 2014). Some investigations have showed that, in young people, during sleep some somatic repair processes occur, such as muscle tissue regeneration, peak levels of growth hormone, increased catabolic steroid concentration (during the first hours of sleep), and the rate of bone growth (Sheldon, 2014). The immune and endocrine systems are excellent indicators of the human biologic systems function in response to stress, especially in response to PA participation or training (Cadegiani & Kater, 2019).

Across adolescence, social demands, such as school-start times, screen-time at night and academic demands promote conflict with the biological sleeping process, which can result in an insufficient period of recovery or regeneration of the different biological systems (Bergeron et al., 2015; Knufinke et al., 2018). For an optimal period of sleep, during adolescence, it is recommended 8 to 11 hours (e.g. 6-13 years old between 9-11 hours and 14-17 years old between 8-10 hours) between falling asleep and waking-up (Crowley, Tarokh, & Carskadon, 2014; Hirshkowitz et al., 2015).

General health, injuries and lower sport performance are associated with sleep patterns, thus being important a "regenerative sleep" (Bergeron et al., 2015). Although the literature regarding sleep and PF among adolescents is scarce, an investigation among Portuguese girls demonstrated that poor sleep quality was associated with lower CRF, while no associations were found with BMI (Mota & Vale, 2009). Another investigation, among Spanish school-aged children, have found that sleep-related anxiety problems predicted being overweight or obese in both sexes, and that sleep quality problems predicted being overweight or obese in girls (García-Hermoso, Aguilar, Vergara, Velásquez, & Marina, 2015). Furthermore, the authors suggest that sleep restriction over time could diminish the positive impact of resistance training on metabolic health (Fobian, Elliott, & Louie, 2018; Knowles, Drinkwater, Urwin, Lamon, & Aisbett, 2018). Another investigation, among Chinese university students aged 16-30 years old, have found that a better sleep quality was associated with greater muscle strength, and that shorter bouts of sleep duration could be a risk for decreased muscle strength (Chen, Cui, Chen, & Wu, 2017).

Besides sleep, nutrition is also an important factor influencing PF in adolescents. Nutritional strategies, before-, during- and post-exercise are crucial to maximize the adaptations promoted by the physical effort. For optimal PF, nutrient intake should be adequate based on the individual characteristics (e.g. age, sex) of each practitioner and the characteristics of the exercise (e.g. volume, intensity, continuous, interval, lower impact, high impact) (Bergeron et al., 2015; Desbrow et al., 2014).

Recognising the large number of factors which influence the necessary energy requirements in young athletes, it is not suitable to draw a single diet plan that fits all. Notwithstanding, it is important to know the main principles of nutrition that apply to those practicing in regular competitive sports (Desbrow et al., 2014). The periodization of protein, carbohydrate, fat, calcium, vitamin D and iron intake according to the guidelines, allows a faster recovery, improves performance and prevents injuries in youth athletes (Desbrow et al., 2014; Meng et al., 2018).

Overall, nutritional education for young athletes and all personal involved in their sport development process (e.g. parents, coaches, medical staff, federations, clubs) should highlight eating behaviours as important strategies to improve long-term health and sport performance (Bergeron et al., 2015; Slater et al., 2019).

2.1.7.2 – Physical activity, exercise and physical fitness

When engaging in PA and physical exercise almost all biologic systems are activated to support muscle contraction and energy production (C. Bouchard et al., 2012). Adolescents should engage in at least 60 minutes of mostly aerobic PA with additional muscle-strengthening exercises per day (Committee, 2018). Engaging in adequate levels of PA increases muscular strength and the cardiorespiratory capacity, which are determinants of success in many sports and health (Ross et al., 2016; Suchomel et al., 2016).

Biologic and physiologic adaptations to PA are expressed on the PF phenotype. The cardiovascular and respiratory systems immediately respond to PA by increasing the availability of oxygen for energy production in the muscle (Gastin, 2001). Also, depending specifically on the type of exercise, muscles when regularly stimulated improve their ability to function optimally (McPherron, Guo, Bond, & Gavrilova, 2014). These biologic and physiologic adaptations stimulated by regular PA or isolated bouts of exercises promotes the development of PF in youth. Young athletes specifically benefit from aerobic and resistance training, during their sports participation, for long-term development of sport-specific athletic performance (Gäbler, Prieske, Hortobágyi, & Granacher, 2018). Understanding the effects of training programmes at a molecular level, may be an essential factor to continue to develop future training strategies aiming to improve sports performance and health.

Aerobic and strength exercises are the major signalling pathways for the promotion of muscular tissue remodelling, through DNA methylation, protein translation and mitochondrial biogenesis (Pilegaard, Saltin, & Neufer, 2003;

Robinson et al., 2017; Simoes & Vogiatzis, 2018). The mRNA or DNA transcription occurs through phosphorylation of several substrates in the nucleus and cytoplasm, where biologic process such as hypertrophy, inflammation and gene expression are included (B. F. Miller, Konopka, & Hamilton, 2016). Energetic unbalance at the cellular level, promoted by exercise (Thomson, 2018), activates the AMP-actived protein kinase (AMPK). The AMPK, then, regulates the energetic status and initiates the process of phosphorylating and activating PGC-1α (Richter & Ruderman, 2009). This signalling process, initiated by PA, promotes changes on mitochondrial oxidative enzymes capacity and consequently the CRF (Robinson et al., 2017; Thomson, 2018) (Figure 2.1). An example of this is the HERITAGE family study, where after 20 weeks of endurance exercise, sedentary adults presented a 15-25% positive change in CRF (C. Bouchard et al., 2011).

Hypertrophy and muscle regeneration are adaptive responses of the muscle tissue to exercise, through increased protein metabolism and fusion of satellite cells to the existent myofiber. Resistance training activates FAK which leads to the inhibition of TSC, thus allowing activation of mTOR; simultaneously IGF-1 activation is promoted and mTOR is activated. This initiates the signalling pathway that leads to protein translation and hypertrophy (Simoes & Vogiatzis, 2018) (Figure 2.1).

According to the concept of training specificity (Behm, 1995; Hakkinen, Mero, & Kauhanen, 1989), resistance training improves muscular strength while aerobic training improves CRF. Among adults, concurrent resistance and aerobic training can interfere with each other, and this interference can lead to inferior gains on muscular or CRF in comparison to isolated training (Docherty & Sporer, 2000;

Wilson et al., 2012). This interference occurs when resistance and aerobic exercise stimuli both target different peripheral adaptations, such as hypertrophy and muscle capillarisation (Docherty & Sporer, 2000). On the other hand, a recent systematic review performed among children and adolescents concluded that concurrent training has a better effect on PF that isolated training, whether resistance or aerobic training (Gäbler et al., 2018). For example, concurrent training was more effective than endurance training in improving athletic performance in children and particularly adolescents. This may be because, children and adolescents have different anthropometric, physiological, and biomechanical characteristics than adults that can affect their response to training, specifically regarding the adaptation capacity (Gäbler et al., 2018).

Sports participation has profound and unequivocal positive effects on health, through several stimuli which increase muscle mass, improve CRF and other PF attributes in youth at short- and long-term (Smith et al., 2019). Regular participation in PA promotes the adaptation of several systems to the activation of the muscular contraction and energy production by increasing their capacity or efficiency (McPherron et al., 2014). Because of its relationship with several biologic systems, PF is recognised as one of the most important biomarkers of sports performance and health (F. B. Ortega, J. R. Ruiz, et al., 2008). This increasing capacity and more efficient response of the biological systems, arising from regular PA participation, improves one's PF levels. On the light of this facts, it is possible to understand that PF is, in part, determined by the heritage and genetic characteristics and, in part, by the environment factors, such as sport participation, training or physical activity levels.

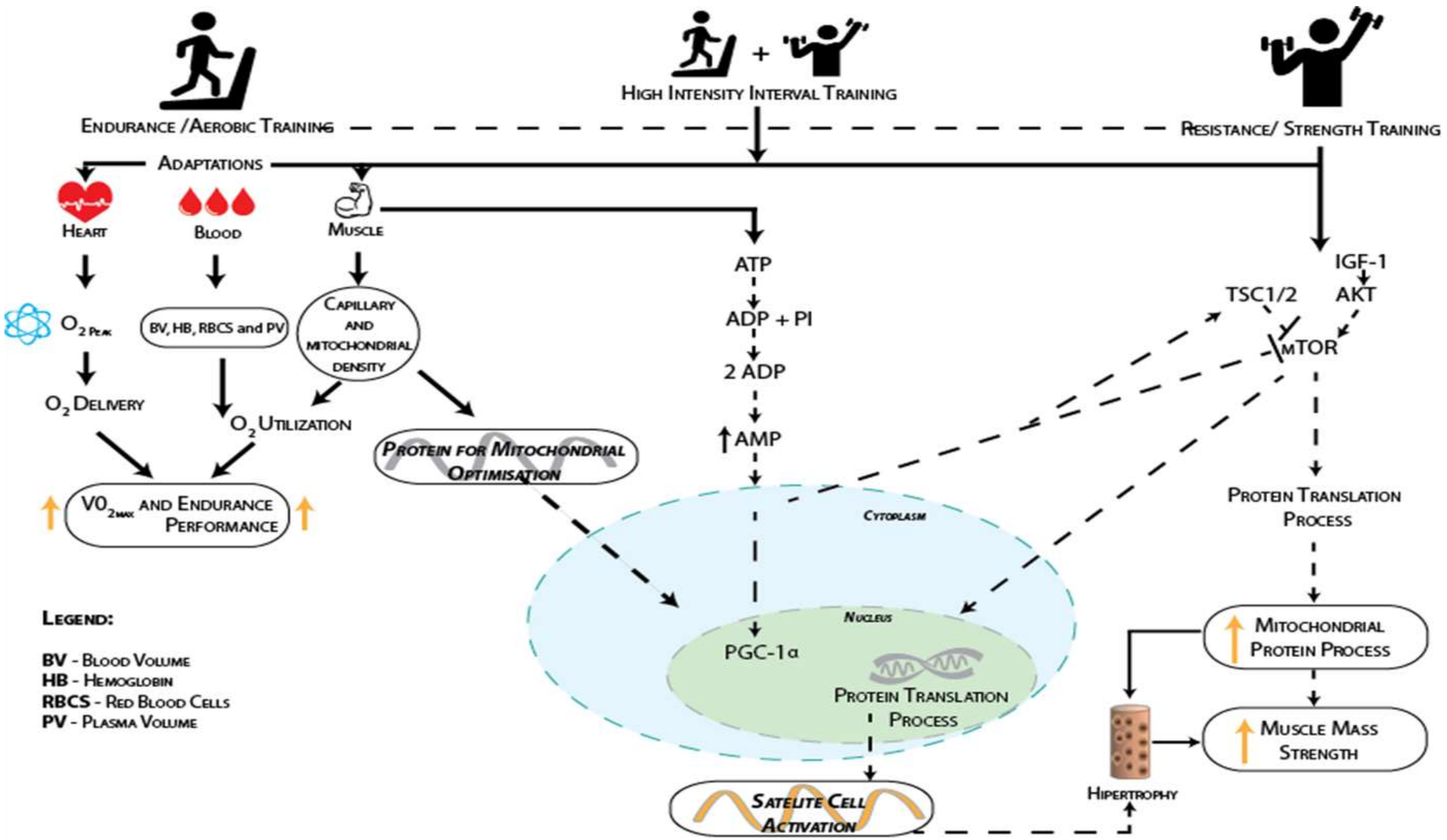

Figure 2.1 – Pathways involved in muscle hypertrophy and mitochondrial biogenesis in response to exercise.

Adapted from Simões, D. and Vogiatzis – Can muscle protein metabolism be specifically targeted by exercise training in COPD – 10 – S1367-S1376 – 2018, and Kmuiman, P et al. – Protein and adaptive response with endurance training: wishful thinking or a competitive edge? – 9 – 598 – 2018.

2.2 - Physical fitness as a smart tool for assessing sport performance and health in youth

Assessing PF may play an important role on identifying the grade of trainability (slow or fast), either with the objective of monitoring and improving health, or when aiming to monitor and improve sports performance (Neil Armstrong, van Mechelen, Schutte, et al., 2017; C. Bouchard, Hoffman, Eric P., 2011; Joyner & Lundby, 2018). Measuring the components of PF allows to monitor the functional status of most, if not all, human biologic systems, and to identify the individual capacity to perform physical exercises at a specific range of physiologic and psychologic capacities (Neil Armstrong, van Mechelen, Armstrong, & Barker, 2017; F. B. Ortega, J. R. Ruiz, et al., 2008).

2.2.1 – Physical Fitness and Sport Performance

Performance-related fitness regarding one's athletic skills or a set of athletic skills is a good indicator of an individual's athletic performance in an athletic competition (C. Bouchard et al., 2012; Campbell, Jesus, & Prapavessis, 2013). As seen before, the young athlete performance is the product of the interaction between genetic and environmental factors (C. Bouchard, Hoffman, Eric P., 2011; Tucker & Collins, 2012). Part (66%) of the athlete status is genetic, composed of hereditable characteristics (De Moor et al., 2012). Genetics characteristics have a significant contribution to the predisposition to practice with success in certain sports and to the overall components of sport performance for each athlete, such as body composition, neuromuscular function, power, strength, endurance, flexibility and other phenotypic characteristics (Ahmetov & Fedotovskaya, 2012).

It is clear that PF components such as cardiovascular capacity, strength, agility and flexibility are excellent and useful biomarkers (Palacios et al., 2015). Assessing PF attributes can determine the current fitness level of athletes, as well as non-athletes, identify individual differences, evaluate the training process, and the sporting potential of youth, thus guiding athletes to the proper event or sport (Calleja-González et al., 2016; Fukuda, 2018; T. Kramer, Huijgen, Elferink-Gemser, & Visscher, 2017; Mujika, Santisteban, Impellizzeri, & Castagna, 2009; Sharhey, 1986). However, it is essential to know and understand how each PF attribute differentially impacts sports performance, and why one attribute may be more relevant to assess over others depending on the sport. For example, in basketball, football and handball, lower limbs strength, agility and speed and cardiovascular capacity are crucial to differentiate the competition level of performance (Haycraft, Kovalchik, Pyne, & Robertson, 2017; Hoare, 2000; Lidor et al., 2005; Mujika et al., 2009). In gymnastics, performance is more determined by handgrip strength (HG) and lower limbs strength (Boullosa et al., 2019), whereas in rugby the essential PF attributes to achieve success are the total muscular power and strength (Chiwaridzo et al., 2019). Having this knowledge can help in identifying children and adolescents with the potential for higher success in various sports.

Assessing the different components of PF in children and adolescents can be performed in the laboratory setting. Although these methodologies are effective, most of these PF tests require qualified technicians, specific equipment, have a high logistic cost, may not be feasible in large groups and misrepresent the natural environment context (Artero et al., 2010; Bergeron et al., 2015; J. R. Ruiz et al., 2010). An alternative to these laboratory PF test are PF tests batteries, which unlike the laboratory PF test, require minimal equipment and are able to be performed in an ecologic context (J. R. Ruiz et al., 2010).

One of the biggest reasons why the PF tests have not been implemented in many countries in the past is the overabundance and lack of uniformity in the tests used to assess physical performance between athletes from several sports. Thus, there is a need for a large generic PF test battery that can assess different sports and sexes (Neil Armstrong & McManus, 2010; McManus & Armstrong, 2010; Johan Pion et al., 2014).

2.2.2 – Physical Fitness and Health

Health-related fitness is generally related to the health status and is associated positively or negatively by one's habitual physical activity habits (Campbell et al., 2013), it is also considered a good indicator of health (C. Bouchard et al., 2012). Among young people, the several attributes of PF are associated to obesity, cardiovascular health, bone health, metabolic health and mental health (Braam et al., 2016; F. B. Ortega, J. R. Ruiz, et al., 2008). Figure 2.2 presents a summary of the associations between PF attributes and health in youth.

The most studied PF attribute in the heath literature is CRF (C. Bouchard et al., 2012; F. B. Ortega, J. R. Ruiz, et al., 2008). This attribute of PF is known to be related to cardiovascular health, cholesterol and blood lipids, obesity and mental health (Janssen & LeBlanc, 2010; J. R. Ruiz et al., 2009). Children and adolescents with higher levels of cardiorespiratory fitness are known to have healthier cardiovascular and metabolic profiles (F. B. Ortega, J. R. Ruiz, et al., 2008).

Furthermore, CRF is inversely related with total adiposity and, having high levels CRF can counteract the harmful consequences attributed to having a higher inflammatory profile (F. B. Ortega, J. R. Ruiz, et al., 2008; J. R. Ruiz et al., 2009). Also, CRF is related to brain structure and functions, such as neuroelectric indices of attention, working memory, response speed and cognitive processing speed (Hillman, Castelli, & Buck, 2005; Marques, Santos, Hillman, & Sardinha, 2018).

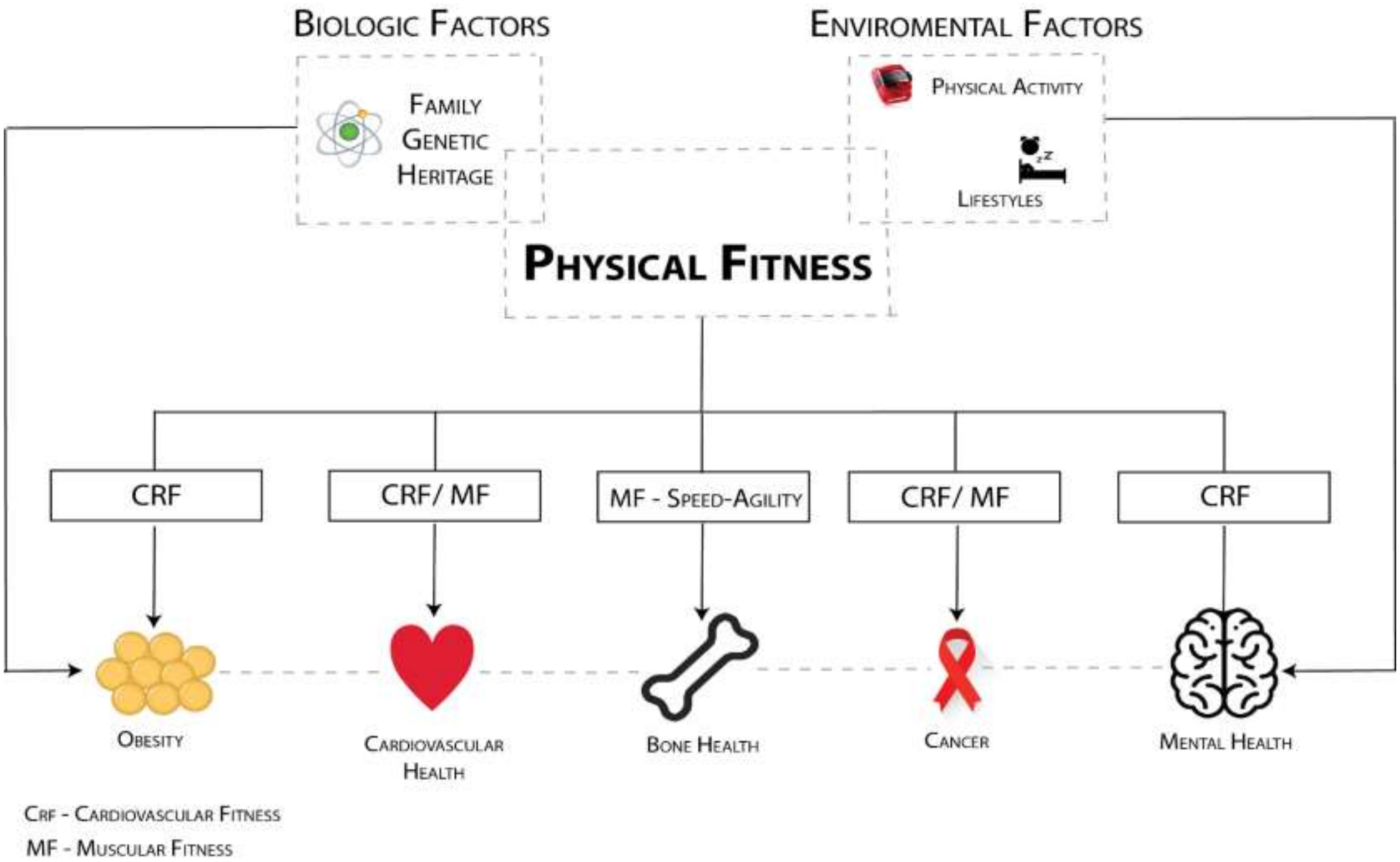

Figure 2.2 – Associations between PF and health

Adapted from FB. Ortega, JR Ruiz, MJ Castillo and M Sjostrom – Physical fitness in childhood and adolescence: a powerful marker of health, 32, 1-11, 2008

Evidence indicates that improving MF has beneficial effects on several health outcomes in youth, such as body composition, blood lipid profiles and insulin sensitivity, bone mineral density and bone geometry (Baptista, Mil-Homens, Carita, Janz, & Sardinha, 2016; Daly, Saxon, Turner, Robling, & Bass, 2004; Garcia-Hermoso et al., 2019) and mental health (Smith et al., 2014). Also, MF is inversely associated with cardiovascular disease risk (Grøntved et al., 2015) Muscle hypertrophy increases lean mass and contributes to improving the resting metabolic rate (McPherron et al., 2014). Positive association between total and site-specific bone mineral status and MF have been found among adolescents (Baptista et al., 2016). Thus, the bone mineral content of the whole body seems to be directly associated with MF and mediated by the association between fitness and lean mass (Vicente-Rodríguez et al., 2008). Prospective investigations, revealed that the enhancement of bone mass was associated with improvements in the speed of movement and explosive strength (Vicente-Rodriguez et al., 2004). Furthermore, adolescents with higher MF have a decreased risk of fracture when practicing PA (Clark et al., 2011). The relationship between PF and bone health will be presented with more detail posteriorly.

In youth, PF attributes are important indicators of performance and health. Therefore, PF test batteries may be a valuable tool to use in order to simultaneously assess the training progress and sports performance, as well as monitoring health (Neil Armstrong & McManus, 2010; Gracia-Marco, Vicente-Rodríguez, et al., 2011). Additionally, assessing and interpreting the main factors influencing the PF status, contribute to the promotion of healthy sport participation in youth (Bergeron et al., 2015).

2.3 - Sports participation in youth: an integrated strategy to improve physical activity and health

Insufficient PA has been identified as one of the leading risk factors for all deaths, representing to 1.9 million deaths per year (Ding et al., 2016). Therefore, the World Health Organization (WHO) has made it one of their global initiatives to increase the populations' PA levels and reduce by 10% the prevalence of insufficient PA by 2025, in order to promote health outcomes (Guthold, Stevens, Riley, & Bull, 2018; WHO, 2015). Promoting the life-long practice of PA is a significant challenge since 27.7% of adults, and 80.3% of adolescents worldwide are either sedentary or physically inactive (Guthold et al., 2018). In particular, Portuguese adolescents spend higher amounts of time sedentary and have low compliance with the recommended PA levels (Mota, Coelho-e-Silva, Raimundo, & Sardinha, 2016). Given the negative health consequences associated with inactivity, such as obesity, cardiovascular diseases, metabolic syndrome, lower bone strength, all adolescents should engage in at least 60 minutes/day in moderate to vigorous PA intensity, and, on the other hand decrease the time spend in sedentary behaviour (Committee, 2018). Three days a week they should engage in vigorous-intensity activities, including those that strengthen muscle and bone (Onerup et al., 2019). A significant emphasis has been devoted to the investigation of several strategies to increase the PA levels and improve lifestyle habits among young populations in order to meet the recommended PA requirements for overall health.

Children and adolescents can be active in different contexts, such as domestic, occupational, active commuting and leisure-time physical activity (Eime et al., 2015). Since 2010 the WHO and the International Olympic Committee have been working together to encourage SP during childhood and adolescence in order to promote positive health outcomes (WHO, 2010). Increasing SP during childhood and adolescence is an investment that works to promote PA (Stewart G. Trost, Blair, & Khan, 2014). It has been found that children and adolescents who participate in organized sports have a healthier lifestyle than non-participants, and this effect is not only exclusive in youth, but can also have positive repercussions later in life (Grima & Thalassinos, 2017; Schmidt, Tittlbach, Bös, & Woll, 2017; Rohan M. Telford et al., 2016).

Cross-sectional, longitudinal studies, and systematic reviews have indicated that young sports participants tend to have higher PA levels, better mental health, and lower risk of chronic physical conditions during youth and adulthood when compared with non-participants or recreational physically active participants (Lee, Pope, & Gao, 2016; M. H. Murphy, Rowe, & Woods, 2016; Palomäki et al., 2018; Schmidt et al., 2017; Silva et al., 2013; Rohan M. Telford et al., 2016). Trends over recent years have shown increased youth SP in different sports categories as well as increased PA levels during childhood and early adolescence (AIS, 2017; Caine, Walch, & Sabato, 2016; K. Green, Thurston, Vaage, & Roberts, 2013; Townsend N, 2015). On the other hand, this phenomenon has not been observed at later ages (17 to 24 years), where people have lower PA levels and there is less youth sports participating (AIS, 2017; Townsend N, 2015; WHO, 2011). These findings inspired policymakers from different countries to develop a consistent model to organise, promote and improve SP during the youth, with an integrated, multisectoral, sustainable approach between the several national systems (i.e. national sports federations, public health system, and educational government entities) (AIS, 2017; Canada, 2018; The English Sport Council, 2016).

In Europe, 29 countries had a policy document to health promotion and only seven countries to sports promotion officially promoted sports (Daugbjerg et al., 2009). Additionally, the last data analysis from 2015 in several European countries, the health-enhancing physical activity is not organised by the same governmental entities across Europe. In Italy and Portugal, the health-enhancing physical activity is linked to the health sector. On the other hand, only in Slovenia and Switzerland are linked to the sports sector. However, only the Netherlands and Switzerland have policies combined with sport and education (Bull et al., 2015).

School is the place where children and adolescents spend most of their time during the day, and, thus, it is an appropriate environment to influence a healthy lifestyle on the young population (O'Brien, 2019). In particular, physical education classes provide the opportunity for structured PA, that is organised in a safe environment by professionals specialised in PA (i.e. physical educations teachers). These classes support the acquisition of fundamental motor skills, which form the foundation for all later PA and competitive sports (Gallahue DL, 1998).

A framework considering health, physical education, and sports has shown to have beneficial effects on the development of physical fitness/technical skills and the provision of PA during childhood and adolescence (Gould, 2010; Lovell, Fransen, Bocking, & Coutts, 2019; A. F. Seabra, Mendonca, Thomis, Malina, & Maia, 2007).

This ecosystem promoted by physical education classes can be understood in terms of young's development in 5 areas: physical, lifestyle, affective; social, cognitive (R. Bailey, 2006). Physical education-sport programmes or after-school activities (e.g. School Sport and Club Links [2002 at 2008]; Physical Education and Sport Strategy for Young People [2008-2011]; School Games [2011-2014] the Australian Curriculum: Health and Physical Education [2017]) have been implemented in many countries worldwide. These programs have produced excellent results, such as increasing the health of youth, fighting social exclusion, providing same opportunities for the development of valued individual attributes and offering an opportunity for youth to integrate into a sports team or a club (see example Figure 2.3) (AIS, 2017; R. Bailey, 2006; Wallhead & O'Sullivan, 2005).

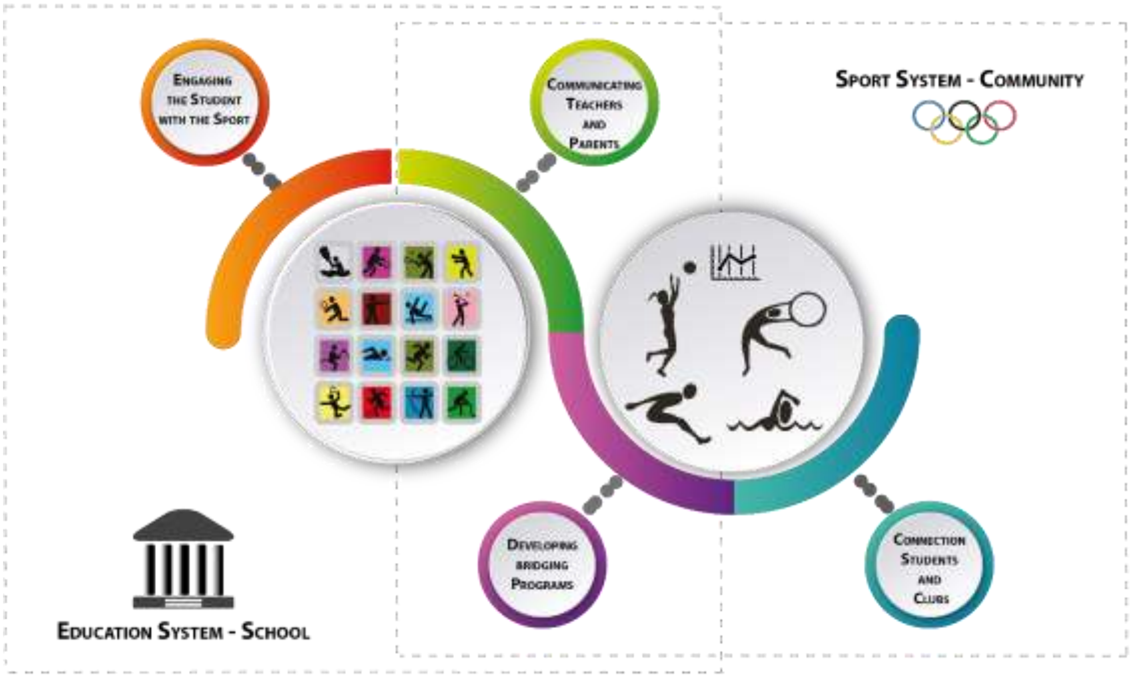

Figure 2.3 - Transitioning students to ongoing sports participation

Adapted from Australian Government – Australian Sports Commission 2017

2.4 - A FRAMEWORK TO IMPROVING SPORTS PARTICIPATION IN YOUTH

Interaction between entities from different areas, maximise the impact of public and private investment in the public health system, education and high sports performance, the long term athlete development (LTAD) (I. Balyi, & Hamilton, A., 2003). Thus, have been demonstrated a useful model for teachers, professional of sports and most sports federations, to promote and develop healthy young athletes (Ford et al., 2011; Granacher & Borde, 2017; Lloyd et al., 2016).

LTAD has been developed in Canada, based on the research of Canadian Sports Centers LTAD expert group, and has been applied in various countries (i.e. Australia (adapted), Qatar and the UK) (Academy, 2019; Bailey RP, 2010; UKA, 2010). Portuguese National Institute of Sports and Youth, Portuguese Olympic Committee, and Portuguese Football Federation have been follow the LTAD philosophy and guidelines (COP, 2019; FPF, 2018; IPDJ, 2009). This model is not exclusive for young athlete, the LTAD provide across all stages of development opportunities for all children and adolescents to grow integrated into self-assured, healthy and active adults (i.e. 1- Active Start; 2 - Fundamental; 3 - Learning to Train; 4 - Training to Train; 5 - Learning to Compete; 6 - Training to Compete; 7 - Learning to Win; 8 - Winning for a Living; 9 - Active for Life) (Figure 2.3).

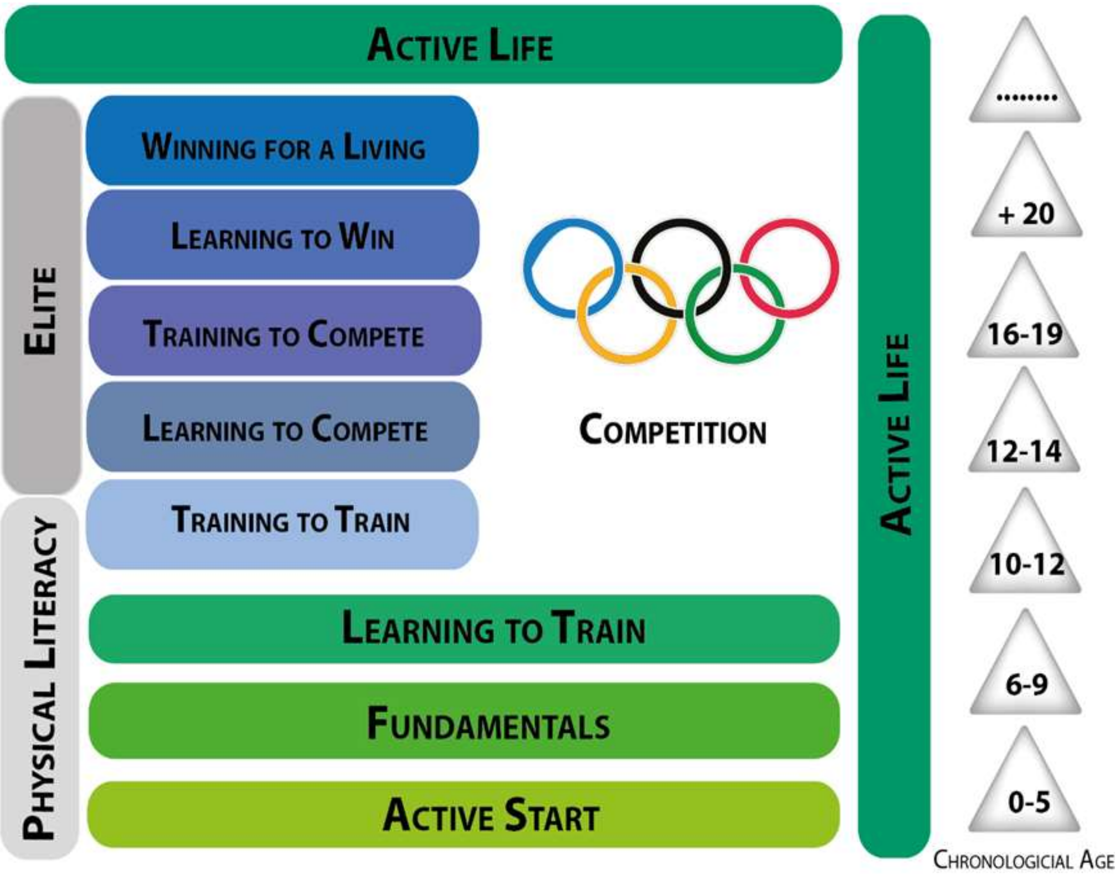

Figure 2.4 - Stages of the long term athlete development and integrate active life.

Adapted from Balyi, Gramantik, Gmitroski, Kaye and Way. Athletics Canada – Long Term Athlete Development. Canadian Heritage, 21, 1-22, 2006.

LTAD model has been developed basis in scientific knowledge, and successfully training methods, with propose to adapting the process training across the process of growth and development during childhood and adolescence ages (Ford et al., 2011). This model is structured and organised progressively to achieve youth athleticism and to engage in lifelong, physical activity, as well as healthy-enhancing (see example Figure 2.5) (I. Balyi, Way, R., and Higgs, C., 2013).

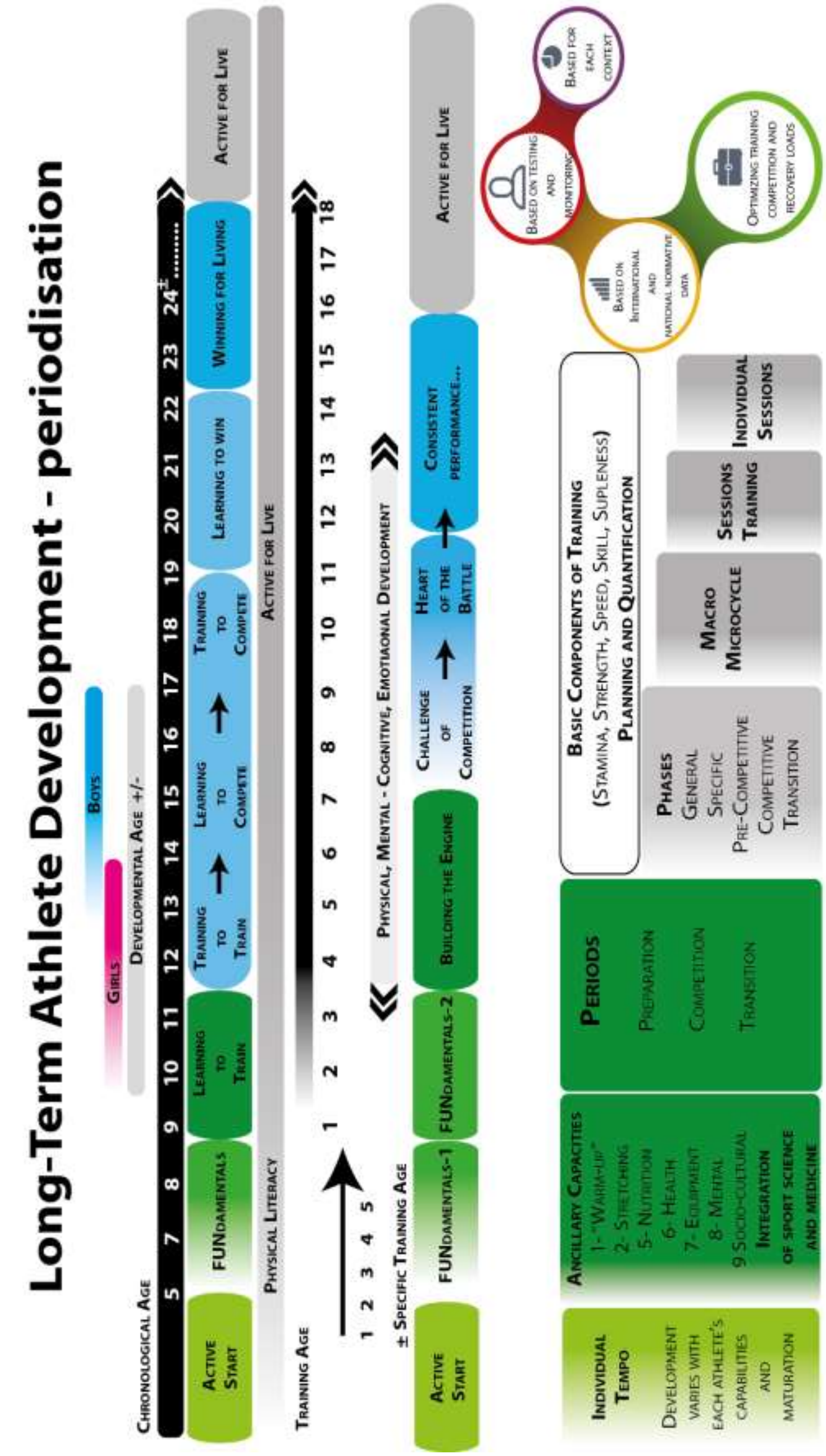

Figure 2.5 - Stages of the long term athlete- LTAD - periodisation

2.5 - Recruitment, Retention and Transition: Three Fundamental Stages for a Sport Development Model

With the importance of sports recognised in all areas of the society, governments, international federations and national federations have put forth two goals: 1) to increase the number of participants actively engaged in sports activities and 2) to improve performances in sport (B. C. Green, 2005). These goals have formed the basis for sport policies and have set the framework for sports development. According to the dynamic pyramid model of sport development, there are three crucial stages (1- Mass-participation; 2- Competitive Sport; 3 – High-performance), where athlete recruitment, retention, and transition are based according to individual performances (B. C. Green, 2005; K. Green et al., 2013).

Figure 2.6 - Dynamic pyramid model of sport development

Adapted from Grenn, B.C. (2005) – Building Sport Programs to Optimize Athlete Recruitment, Retention and Transition: Toward a Normative Theory of Sport Development. 19, 233-253.

The biggest challenges to researchers and entities who are promoting SP are potential performance and continued participation across later life.

Recruitment refers to the recognition of potential performers who are not yet engaging in the sport. Retention refers to the maintenance of young athletes in the sport system for as long as possible (e.g. monitoring training process according individual characteristics) (B. C. Green, 2005). To achieve these goals, it is necessary to understand all factors (biologic and environmental) which influence youth athletes (Figure 2.7).

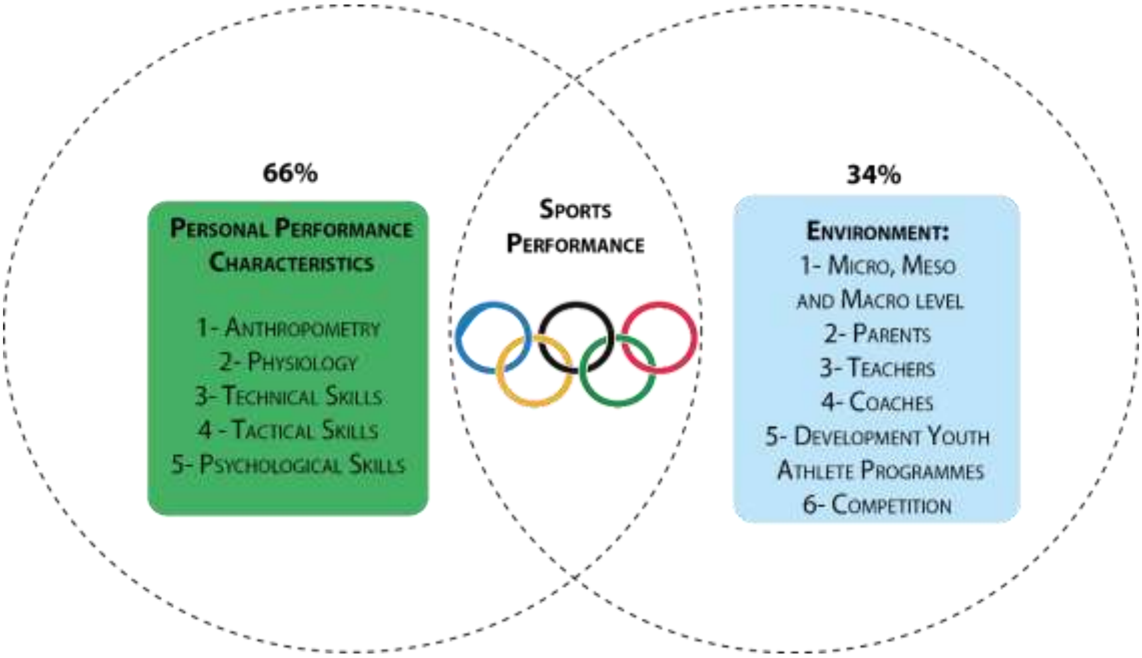

Figure 2.7 - The dynamic model of young athlete characteristics.

Adapted from Elferink-Gemser & Visscher (2005) and Dee Mor et al. (2012)

Young athlete recruitment and the development process are multidimensional and challenging to assess (Bergeron et al., 2015; Elferink-Gemser M.T., 2012). Growth and development are marked by individual differences during childhood and the adolescent period, and in these specific periods of life, the sport system should encourage further participation in various sports categories and provide more training opportunities to meet these differences (B. C. Green, 2005; Wicker, Breuer, & Pawlowski, 2009). The promotion of activities in the school (e.g. standardised protocol of PF tests) could aid in the early recruitment of youth athletes by allowing teachers the ability to identify specific sports characteristics.

Promoting recruitment programs during youth in a school context, encourage sport participation, reinforce the number of young athletes and increase the national sports performance level (B. C. Green, 2005; K. Green et al., 2013).

Childhood and adolescence are dynamic periods characterised by the growth and development of different organic systems (e.g. bone tissue, muscle tissue), which rarely progress at the same time or in a linear fashion. It is a misconception that the sport's success of young athletes can be predicted based on the same characteristics that distinguish top adult athletes, as these characteristics in adults may not be observed during adolescence (J. Baker, Schorer, & Wattie, 2017; Johnston, Wattie, Schorer, & Baker, 2017; B. Jones et al., 2018; Li, De Bosscher, Pion, Weissensteiner, & Vertonghen, 2018).

During the initial stages of growth, some individual characteristics may be successfully recognised simply based on hereditary factors. Despite the 34% of athletic ability being based on environmental factors, 66% are genetic, such as those that determine strength, power, endurance, muscle fibre size and composition, flexibility and neuromuscular coordination as well as other phenotypes (De Moor et al., 2012).

Effective tools for assessing sports performance and health in the sports and educational systems are needed to increase the success of recruitment and development programs, the national sport level and healthy SP (Bergeron et al., 2015; Canada, 2018; Johnston et al., 2017). Although sports provide a positive environment that may enhance physical growth and development, they are also very competitive in nature, even amongst different levels of youth sports competition (Caine et al., 2016; Mountjoy et al., 2008).

It is recommended that researchers, sports professionals, and sports authorities (e.g. national olympic committees, national sports federations, and clubs) determine the potential of talent identification and development systems, to promote health, by also evaluating the positive health of the athlete rather than solely focusing on performance outcomes (Rongen, McKenna, Cobley, & Till, 2018).

Tools for assessing sports potential and performance should also be designed to identify the characteristics of the youth and training process at these ages, to ensure a balance between sports performance and health (Mountjoy et al., 2008).

In particular, factors such as overloaded competition, excessive travels, inadequate nutrition, and unadjusted high intensity and volume of training increase the vulnerability of youth to stress fractures are some of the principal contributors to bone injuries and diseases in the young population (Caine et al., 2016; Faulkner, Davison, Bailey, Mirwald, & Baxter-Jones, 2006; Mountjoy et al., 2008).

2.6 - BONE HEALTH IN YOUTH

Although osteoporosis symptoms usually do not manifest until later in life, the disease is thought to have its roots at the beginning of childhood.

Osteoporosis and bone fractures are considered a significant public health matter worldwide (Aziziyeh et al., 2019; Svedbom et al., 2013). Osteopenia and osteoporosis are usually defined as having a bone density -1.5 and -2.5 SD, respectively, below that of a young healthy adult (Compston, McClung, & Leslie, 2019). Since children and adolescents are growing and bones are still not fully developed the International Society for Clinical Densitometry defined the expression "lower bone mass for age" in children and adolescents when the bone mineral density (BMD) Z-score is -2.0 or lower (Gordon, Leonard, & Zemel, 2014). Children who present -1 or lower SoS Z-score on radius are considered a risk group (Chong, 2015). Also, the youth who present more than one vertebral fracture or lower BMD for age and clinical history of fractures (considering: two or more large bone fractures before the age of ten years, or three or more fractures before nineteen years old) are considered a risk group (Bachrach & Gordon, 2016).

Bone is a hard tissue which provides structure and support for the body and is continually being broken down and regenerated throughout life in the process of modelling and/or remodelling (Clarke, 2008). This process is the biologic mechanism responsible for determining the longitudinal and cross-sectional size and shape of the bone (Frost, 2003). During adolescence, bone mainly undergoes the process of modelling, whereas, in adults, remodelling predominates. Not all bones are being modelled/remodelled at the same time nor in a parallel way. For instance, while the longitudinal growth of bone takes place until early adulthood, radial growth occurs throughout life. Longitudinal growth of bone is characterised by the formation of new bone tissue at the growth plates in the metaphyseal and epiphyseal areas of long bones (Figure 2.8), and the radial growth or subperiosteal appositional growth is the process responsible for increasing the periosteal and endocortical diameters (Figure 2.8) (Clarke, 2008; Duan, Beck, Wang, & Seeman, 2003).

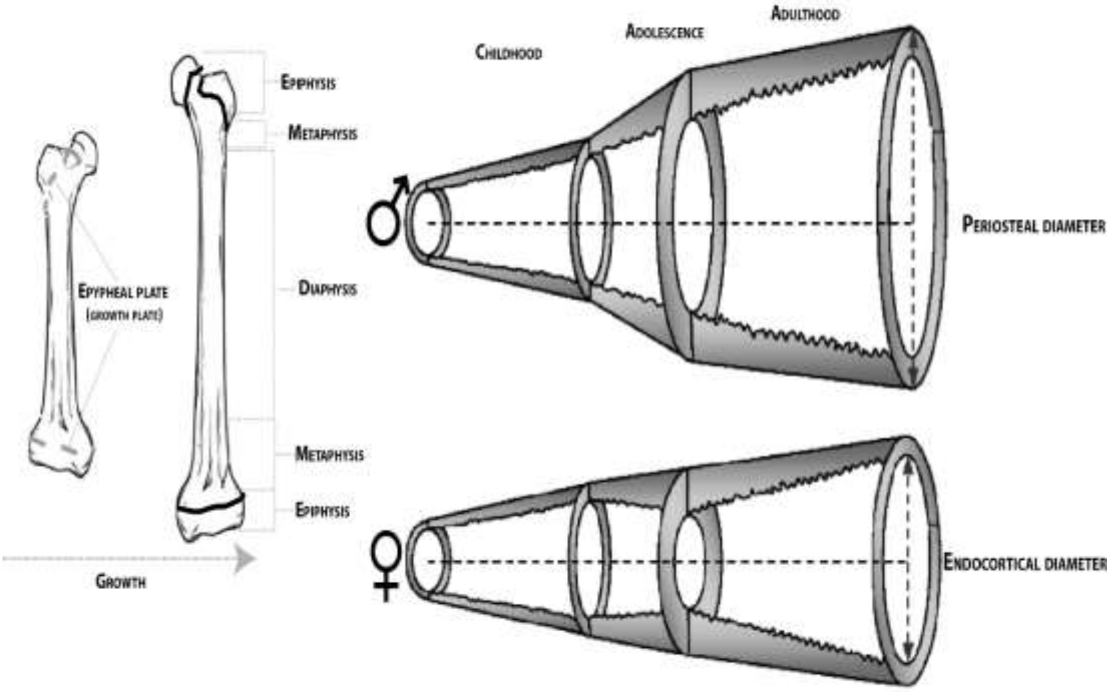

Figure 2.8 - Schematic representation of the longitudinal and radial growth across life in males and females.

Adapted from Duan et al. (2003)

Bone tissue development starts in intrauterine life and continues relatively slowly through childhood until adolescence when the peak of development occurs. At the onset of puberty, children reach what is called peak height velocity (PHV), which corresponds to the period when the rate of longitudinal growth and bone mineral accretion is at its highest (Gordon et al., 2014).

During the time of PHV bone mineral accrual is at its highest such that by the end of adolescence approx. 80-90% of peak bone mass is achieved. Peak bone mass (PBM), which is the maximum amount of bone mass one is to have in life is achieved by approximately age 30. (Figure 2.9) (D. A. Bailey, McKay, Mirwald, Crocker, & Faulkner, 1999). During four years surrounding the PBM of 39% of total body, the mineral is acquired and four years after the PBM 95% of adult bone has achieved. Notwithstanding, in this specific period of life, the BMD is lower between children's and adolescents, when compared with adults, particularly in boys.

Changes in bone density and bone strength are continuous until the thirty years old (Weaver et al., 2016). Hence, childhood, and specifically adolescence is an opportune time for bone disease prevention as this is a time characterised by a fast-growing skeleton (Buchs, Slosman, Theintz, Bonjour, & Rizzoli, 1991), when the majority of the adult bone mineral is being laid down (Rizzoli, Bianchi, Garabédian, McKay, & Moreno, 2010). Maximising bone mineral mass during this time will have a significant impact on bone values later in adulthood and older ages (Plaza-Carmona et al., 2016).

Bone mass and strength are characteristics of bone tissue and are determined fundamentally by two factors: the non-modificable factors (e.g. sex, age, and maturity) and the modifiable factors (e.g. tobacco use, contraceptives, nutrition and PA) (Weaver et al., 2016). Although between 60-80% of bone characteristics are genetically determined, environmental factors during youth can have a profound influence on adult bone mass and strength (McDevitt, McGowan, & Ahmed, 2014). Among the modifiable factors, PA is known to play a role in augmenting and maximising bone mass and strength in youth, and, thus, can be an effective strategy to improve the current and future bone health of this population (Weaver et al., 2016).

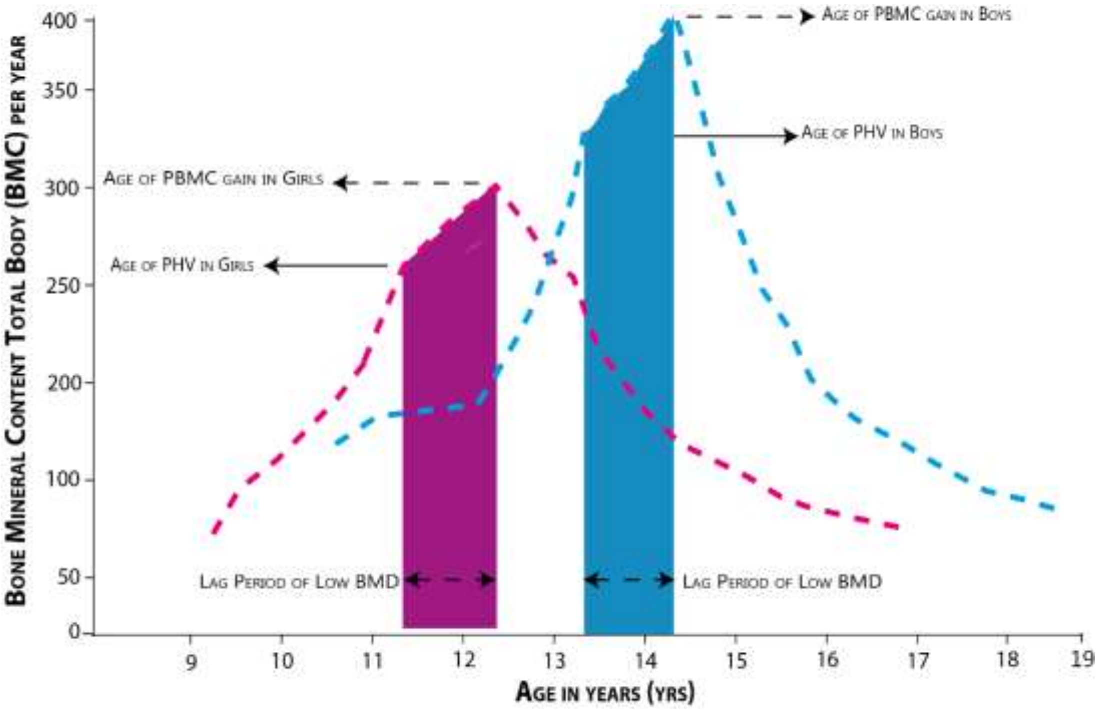

Figure 2.9 - Peak height velocity (PHV) and peak bone mineral content velocity (PBMCV) in boys and girls.

Adapted from Bailey et al. (1999).

On the other hand, when there is an unbalance between PA levels, recovery periods and nutrition, not only are sports performance and athletic health compromised, but bone stress injuries and early osteoporosis can occur as a result. These phenomena can lead to downtime and wasted training and competition time for up to 20 weeks. (Goolsby & Boniquit, 2016; Jenkins, Keating, & Simpson, 2010). Thus, adequate type and intensity of PA, and frequent monitoring of training loads and bone health status are essential to preventing injuries and long-term bone diseases (Goolsby & Boniquit, 2016; Lloyd et al., 2016).

2.7 - PHYSICAL ACTIVITY AND BONE HEALTH IN YOUTH

Gravitational loading and muscle loading are the main mechanical loads applied to the skeleton, which activate the mechanotransduction process in the bone (Kohrt, Barry, & Schwartz, 2009). These mechanisms were described previously in Frost's mechanostat theory. This theory states that mechanical loads of different magnitudes and/or directions stimulate biochemical signals to make recipient bone cells (i.e. osteocytes, osteoclasts and osteoblasts) adapt to new challenges (Frost, 1996). Additionally, the mechanotransduction is a process by which mechanical forces are converted and integrated into a cellular response directed towards bone repair and regeneration, which result in increasing the bone strength by changes in the bone's architecture, mass, and density (Frost, 2003; Huang & Ogawa, 2010). Modelling and remodelling are an integrated biologic cell process of the osteocytes, osteoclasts, and osteoblast, all of which act together to modify the bone in response to these physiologic or mechanical forces (Frost, 2003). The osteocytes serve as sensory cells and the osteoclasts and osteoblasts as the effector cells.

This process starts when the mechanical load creates a fluid shear stress within the bone canicular network, activating the osteocytes, which in turn signals the molecules that regulate osteoclast-mediated bone resorption and osteoblast-mediated bone formation (Figure 2.10).

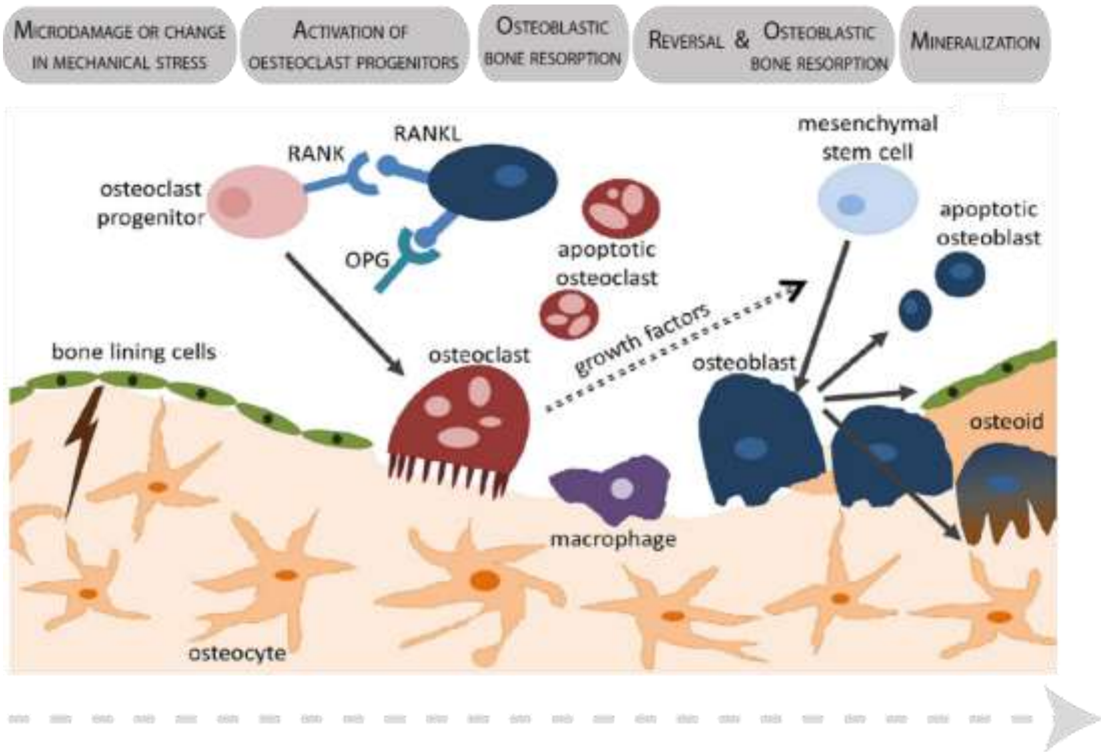

Figure 2.10 - Bone remodelling cycle.

Adapted from Wittkowske, C. et al. (2016).

The biologic balance between bone cells results in an adequate bone remodelling process (Huang & Ogawa, 2010). The bone tissue needs a balance between a “time in” and “time off” from mechanical loading to complete all the osteogenesis process within a specified safety range. Consequently, the practice of PA should be organised into the exercise load sessions separated by recovery periods to promote an overall positive effect on bone health in a PA type-specific loading pattern (Huang & Ogawa, 2010). Several studies have demonstrated the PA effects on bone health outcomes with several types of PA, intensity and volume (D. A. Bailey et al., 1999; Dias Quiterio, Carnero, Baptista, & Sardinha, 2011; Gomez-Bruton, Matute-Llorente, Gonzalez-Aguero, Casajus, & Vicente-Rodriguez, 2017; Gracia-Marco, Moreno, et al., 2011; Piasecki et al., 2018; Vuori & Heinonen, 2000).

Engagement in regular PA is a recommended strategy for promoting bone health during adolescence and over adulthood (Gunter, Almstedt, & Janz, 2012; Rizzoli et al., 2010). It has been suggested that, in order to guarantee optimal bone health, adolescents should practice at least 20 minutes/day of vigorous PA (VPA) or 45 minutes/day of moderate-vigorous PA (MVPA). It is well-recognised that bone health is more dependent of intensity (i.e. light, moderate and vigorous) and the type of exercise (i.e. high-impact loading, variable impact loading and non-impact) than the volume (Figure 2.11) (Gracia-Marco, Moreno, et al., 2011). Additionally, participation in sports that stimulate compressive, shear, bending, tensile and torsion forces, adequate body composition, and having a balanced diet (i.e. containing adequate calcium and vitamin D) can elicit mechanostat-related processes during growth (Abrams, 2011; Dias Quiterio et al., 2011; Faustino-da-Silva et al., 2018; Gracia-Marco, Moreno, et al., 2011; McDevitt et al., 2014; Varley, Hughes, Greeves, Fraser, & Sale, 2017). On the other hand, some studies in special populations with higher PA levels (e.g. athletes and military), the levels of bone formation and turnover were not elevated or predictive of subsequent changes in bone characteristics, suggesting that the bone response to mechanical loading depends fundamentally upon the bone site and the management of loads

(Goldring, 2015; Izard, Fraser, Negus, Sale, & Greeves, 2016; Lozano-Berges et al., 2018; Varley, Greeves, & Sale, 2018).

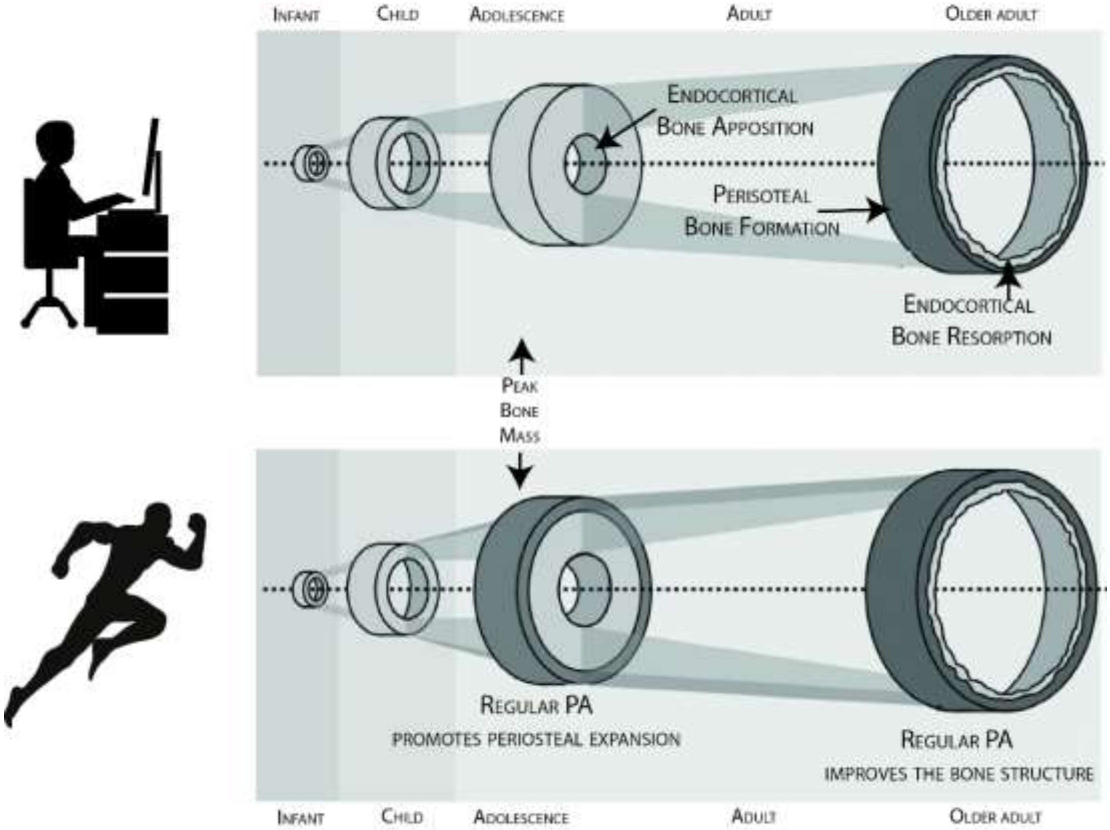

Figure 2.11 - Structural changes of bone tissue throughout the lifespan with regular PA.
Adapted from Gunter, K. et al. (2012).

For instance, when the bone is under a constant mechanical load without rest and recovery, it becomes desensitised to the loading stimulus. Thus, managing load and allowing for breaks in between loads is important.

In recent years, the incidence of bone fractures in youth has been increasing (Kirkwood, Hughes, & Pollock, 2018). Tibial shaft and distal radius fractures are the most common fractures during this period, particularly between 10 and 20 years old (Larsen et al., 2015). Adolescence is one of the periods of life when the skeleton is porous, presenting several fragile points at the physes and metaphyses. Factors such as inactivity, adiposity and a deficit of vitamin D are the main reasons behind radial fractures (Ryan et al., 2010). However, tibial fractures are a result of specific anatomy (brittle in narrower), excessive localised loads (e.g. torsional, shear stress and compression), poor management of training loads, and eating disorders or poor nutrition (Izard et al., 2016; Mounasamy, Patel, Horstman, Kuester, & Sambandam, 2018; Tommasini, Nasser, Schaffler, & Jepsen, 2005).

Young athletes are exposed to several high-intensity training/competition hours during the week, with low recovery time, over many years, which potentiates a higher risk of fractures (Hedström, Svensson, Bergström, & Michno, 2010; Larsen et al., 2015).

It remains unclear whether exposure to sport participation during adolescence confers advantages on tibial and radial strength. There is a lack of knowledge regarding the independent associations between different types of PA (i.e. recreational and competitive) with peripheral bone health outcomes, and the interaction of these associations with other relevant factors such as body composition and the several components of PF (Committee, 2018; Cray et al., 2015).

2.8 - PHYSICAL FITNESS AND BONE HEALTH IN YOUTH

PF level is strongly associated with PA level, body composition and several health outcomes. Thus, monitoring youth PF levels has been gaining recent attention in epidemiological studies (J. R. Ruiz et al., 2010). An individuals' PF level is linked to their PA level and physiologic responses to exercise (i.e. acute and chronic). Assessing each attribute of PF, through specific tests, provides an integrated spectrum of several biologic systems, including the musculoskeletal system. In this regard, PF has been suggested as an excellent biomarker of bone health in young populations and used worldwide by sports and health researchers (Baptista et al., 2016; Dias Quiterio et al., 2011; Gracia-Marco, Vicente-Rodríguez, et al., 2011; Ortega et al., 2013; Ramos-Sepúlveda, Ramírez-Vélez, Correa-Bautista, Izquierdo, & García-Hermoso, 2016).

The influence of several components of PF (i.e. CRF, MF and body composition) on bone health in youth has been reported (Baptista et al., 2016; Fonseca, de Franca, & Van Praagh, 2008; Garcia-Hermoso et al., 2019; Gracia-Marco, Vicente-Rodríguez, et al., 2011; Smith et al., 2014; Torres-Costoso et al., 2015), and most studies suggest a positive association between PF and bone health. However, some studies showed controversial associations between specific PF components and bone health outcomes.

CRF, one of the PF components, is strongly associated with several indicators of health in youth, such as obesity or metabolic syndrome (F. B. Ortega, J. R. Ruiz, et al., 2008). CRF is inversely associated with adiposity, which in turn is related to bone health (Vicente-Rodríguez et al., 2008). Thus, it is reasonable that CRF is positively associated with bone health; however, some studies have presented mixed results regarding this relationship (Clark, Tobias, Murray, & Boreham, 2011; Cray et al., 2015; Fonseca et al., 2008). For instance, Gracia-Marco et al. (2011) found that, while inactive adolescent and active boys with worse CRF had lower whole-body bone mineral content (BMC), active girls with worse CRF had higher whole-body BMC. Another investigation with Brazilian adolescents showed that, for boys, CRF was not associated with total body BMD, whereas for girls, it was inversely associated (Fonseca et al., 2008). These results suggest that other factors, such as body composition (e.g. lean mass, body mass) or sample characteristics (e.g. active, inactive, athletes, non-athletes), could be confounding the relationship between CRF and bone health (Cray et al., 2015).

On the other hand, MF has been consistently associated with bone health (Smith et al., 2014; Vicente-Rodríguez et al., 2008). Several studies showed that children and adolescents with a better HG, speed, agility, horizontal jump (HJ) and vertical jump (VJ), also had better bone health (Baptista et al., 2016; Clark et al., 2011; Gracia-Marco, Vicente-Rodríguez, et al., 2011; Vicente-Rodriguez et al., 2004).

High levels of MF are a consequence of increased muscle mass, which in turn allows for the generation of greater loading forces on the bone. These forces on the bone promote the osteogenic process and, thus, supporting the positive associations between MF and bone health, as well as, its preventive role against bone injuries and diseases (Smith et al., 2014).

The majority of the studies associating PF with bone outcomes uses whole-body or body parts (e.g. upper limbs, lower limbs) measures to assess bone health. However, bone fractures are frequently in specific body regions; for instance, the tibial shaft and the distal radius are the two regions that present the highest incidence of fractures (Larsen et al., 2015; Mounasamy et al., 2018; Ryan et al., 2010). Therefore, it is important to understand if the associations seen when using whole-body and body parts (e.g. trunk, upper and lower limbs) are also significant when assessing specific regions of the bone, in this case for the tibial shaft and the distal radius.

From a public health perspective, a better understanding of these associations is key to comprehend the role of PF as a bone health indicator and its potential role for monitoring and promoting bone health in youth. Furthermore, associations between PF and bone health outcomes may be mediated by the effect of training load, PA levels and body composition (i.e. muscle mass, fat mass) (Cray et al., 2015; Gracia-Marco, Vicente-Rodríguez, et al., 2011). Due to the differences between young athletes and non-athletes, at those levels, it is interesting to investigate if the association between PF and peripheral bone outcomes are also different in these two groups.

2.9 - METHODOLOGIES TO ASSESS BONE HEALTH IN YOUTH.

Bone health in youth can be assessed using dual-energy radiograph absorptiometry (DXA), quantitative computed tomography (QCT), peripheral QCT (pQCT), high-resolution peripheral QC (HR-pQCT), quantitative bone ultrasonometry (QUS), magnetic resonance imaging (MRI) or plain films (radiogrammetry). Three-dimensional densitometry methodologies (QCT, pQCT and HR-pQCT and MRI) are the gold standards because they are able to measure volumetric BMD as well as aspets of bone architecture. However, assessments using these techniques in pediatric populations are difficult to interpret due to lack of standardised scanning protocols and normative data. Therefore, DXA is the method preferred for clinical assessments of bone density in youth, because it is reproducible, is not time-consuming, has lower radiation exposure, and there are robust pediatric reference data available (Bachrach & Gordon, 2016). Notwithstanding, DXA concerns are the higher radiation and the potential misinterpretation of DXA pediatric data which generate restrictions on physical activity or unnecessary use of drugs, while the QCT method has high costs associated (Maggioli & Stagi, 2017). Despite its advantages over the other imaging techniques, DXA's measure of BMD is influenced by changes in bone sizes during the growth. Due to its two-dimentional nature, DXA tends to underestimate the BMD in small individuals and overestimate it in large individuals. (Binkley, Berry, & Specker, 2008).

The principle behind QUS, is that the speed at which ultrasound waves pass through or along the bone, is proportional to both the density and the structural properties of bone. Ultrasound is emitted by the generating transducers, are transmitted along the bone and then are received at the detectors. Proprietary techniques subtract the time during which the signal travels through soft tissue, and the results reflect the strength of bone (Figure 2.12).

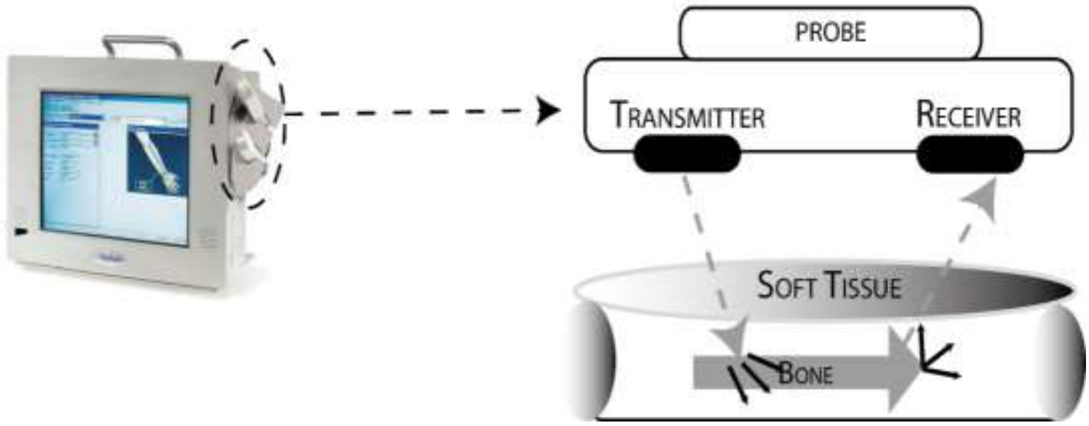

Figure 2.12 - The ultrasound equipment used in this study.

QUS is highly correlated with several bone characteristics assessed by DXA (Sievanen, Cheng, Ollikainen, & Uusi-Rasi, 2001) and reflect a range of bone status factors, such as elasticity, structure, microstructure, and cortical thickness, which are essential aspects to overall bone strength (Baroncelli, 2008). SoS values are positively associated with cortical thickness and inversely associated with fracture risk factors (Clark, Ness, Bishop, & Tobias, 2006).

QUS is considering an excellent method to assess the bone health status in youth, due to it is portability, use of no radiation, and it is ability to take into consideration factors such as the variation of the physical distribution of trabecular or cortical bone at the region of interest (i.e. distal 1/3 radius [R-SoS] and mid-shaft tibia [T-SoS]), sex, age, maturity, and body composition, when measuring bone values (Maggioli & Stagi, 2017).

2.10 - THE AIM OF THIS INVESTIGATION

The overall aim of this thesis was to understand the relationships between PA, PF and values of QUS in young athletes and non-athletes. Moreover, assess how testing of PF in youth may be a strategy for the promotion of young sport participation as well as a way to assess bone health in youth.

The present dissertation presents four research works under the framework of PF, young SP and bone health.

Investigation 1 (in chapter 4) was conducted to analyse the reliability of FITescola® tests in young athletes from a different sports background and competition levels. This program of fitness tests was previously validated for the general population, where athletes were not differentiated from non-athletes. The reliability of FITescola® in young athletes is important to standardise PF tests in different populations with several types and intensities of PA to assess sports performance.

Investigation 2 (in chapter 5) assessed the specificity and sensibility of each PF test, as well as their cut-off based on sex and age for being able to identify potential young athletes in a school context according to the sex, chronologic age and individual performance.

Investigation 3 (in chapter 6) was conducted to understand the associations of several tests from FITescola® with R-SoS and T-SoS in young athletes and non-athletes. Consequently, the aim of this investigation was to fill a gap in knowledge regarding the relationship between PF and bone health.

PA plays an essential role in bone health during the period of adolescence; however, other indirect effects such as sex, maturity, body composition, nutrition or PF level can affect this relationship. Therefore the aim of **investigation 4** (in chapter 7) was to investigate the indirect effect of VPA, mediated by PF and/or fat mass (%), on R-SoS and T-SoS in adolescents.

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CHAPTER 3

Methodology

A brief description of the investigation design and statistical sample are presented in this chapter. However, specific details concerning the methodologies are presented separately in each investigation (i.e. within chapters 4 to7).

3.1 - INVESTIGATION DESIGN AND SAMPLING

The Faculty of Human Kinetics University of Lisbon Ethics Committee according to the Oviedo *Convention from 1997 (Organs, 1997) and* Declaration of Helsinki from 2013 (WMA, 2013), approved all investigations included in the present thesis (CEFMH – Status: Approved; N.º 19/2018) (Anexo I). A grant supported the PhD candidate by Comité Olímpico de Portugal (COP) and University of Lisbon, entitled *"Physical Fitness: Identifiers of Sport Participation and Bone Health in Youth".* The corresponding investigations presented in this dissertation result from the observational investigation for 15 months, including the assessment of body composition, PF tests, PA levels, QUS characteristics, and nutrition frequency. Investigation 1 result from an observational and Test and Re-test analysis using young athletes from different sports entities (Lisbon and Porto). For the other investigations, cross-sectional data were collected from 12 schools in 3 different districts (Lisbon, Porto, Santarém and Setúbal). All participants gave their assent, and informed consent was obtained from their legal guardians (Anexo II).

3.1.1 - The Sample Power

The sample size was estimated using the G*Power software (v3.1.9.4, Düsseldorf, Germany), following specific statics (Beck, 2013) and considering the main investigation characteristics:

1. Population range age – 10 at 18 years old.

2. Progressive Aerobic Cardiovascular Endurance Run (PACER) laps. The cardiorespiratory fitness (CRF) is the PF component with the highest number of associations with health (J. R. Ruiz et al., 2016) and sport performance outcomes from several types of sports (Schaun, 2017).

3. Reference CRF data to Portuguese population with 14 years (Santos et al., 2014) (reference age to initialised participation in organised sports) (R. S. Lloyd et al., 2015).

4. An official number of young athletes under and with 18 years old (Male - 422.046 and Female – 168.622) (Juventude, 2018).

5. Difference between two independent means (two groups) (*T-test Student*)

The calculations generated a total size of 360 (36 each age group for both sexes), based on predicted of a mean number of laps in PACER (male- 48.9±21.6 and female – 29.1±12.5), with effect size 1.12. The error type I was controlled in 5%, with statistical power 95%. A total sample was defined in 720 participants (athletes and non-athletes) with range 10-18 years old.

In Table 3.1 are summarised the sampling and design characteristics of each investigation: sampling, design and statistical analysis.

Investigation	Sample	Sex /Age range	Design	Statistical analysis
1	Athletes (n=138)	M-92 & F-46 10-18 yrs	Test-Re-Test	Descriptive, ICC, Bland & Altman
2	Athletes (n=595) Non-athletes (n=1236)	M-401 & F-194 M-514 & F-722 10-18 yrs	Cross-sectional	Descriptive, ROC Curve, Youden's Index
3	Athletes (n=230) Non-athletes (n=366)	M-156 & F-74 M-129 & F-237 10-18 yrs	Cross-sectional	Descriptive, multiple linear regression analyses
4	-	M-191 & F-221 10-18 yrs	Cross-sectional	Descriptive, mediation analyses
Abbreviations: F, Female; M, males; yrs, years				

Table 3.1 The sampling and design characteristics of each investigation

3.2 - BODY COMPOSITION MEASUREMENTS

3.2.1 - Anthropometry

Weight and height

Weight and height were assessed across all the investigations presented in this dissertation (see chapters 4 to 7). All participants were weighed to nearest 0.01 kg on an electronic scale (model 799 SECA, Hamburg, Germany) while wearing shorts and a t-shirt and without shoes. Height was measured to the nearest 0.1 cm with a stadiometer (model 220 SECA, Hamburg, Germany) (Lohman TG, 1998). BMI was calculated as body mass (kg)/height^2 (m).

Waist circumference

Waist circumference was taken at superior border of iliac crest. The measurements were taken 3 times and recorded to the nearest 0.1 cm with tape. Average of three circumferences was used in analysis ((NHANES), 2009).

Calf circumference

The participants with the feet about 20 cm apart and weight distributed equally on both feet. An inelastic tape measure was positioned parallel on the ground and horizontally around the right calf, and moved up and down to locate the maximum circumference in a plane perpendicular to the long axis of the calf. The level was signalized so that the calf skinfold could be measured at the same level (Lohman TG, 1998).

Skinfolds

Skinfold thicknesses were assessed on the right side of the body to the nearest 0.1 cm with a flexible anthropometric tape (Seca, Hamburg, Germany).

The skinfolds measurements were conducted by the same anthropometrist to reduce the risk of bias. Two measurements were performed for each point, and if it was 2 cm different, a third measurement was performed. The measurements values recorded, corresponding at the average for the 3 measured values. All skinfold measurements was implemented in the investigation 3 (chapter 6) respecting to the procedures described by Lohman et al. (Lohman TG, 1998).

Triceps. The triceps skinfold was measured in the midline in the posterior face of the arm pendant, between the lateral projection of the acromion process of the scapula concerning the elbow, specifically on the inferior margin of the olecranon process of the ulna. All measurements were performed, with the participant standing with the right arm hanging loosely and comfortably (Lohman TG, 1998).

Media Calf. The medial calf skinfold was measured with the right foot (barefoot) on a bench so that knee and hip are flexed to performing an angle with 90º. The maximal calf circumference (the same technique to calf circumference) was previously marked in the medial face of the calf. The assessment starts with the anthropometrist in front of the participant, raising a calf sinkfold longitudinal axis of the leg (vertical) (Lohman TG, 1998).

Body Fatness

Values corresponding to body fatness in adolescent were estimated by specific mathematic equations (Slaughter et al., 1988).

The skinfold equations to predict the body fat in children between 8 and 18 years old are as follows:

For skinfolds triceps and calf

Male: 0.735 (triceps+calf) +1.0

Female: 0.610 (triceps+calf) +5.1

All participants who presented a sum of triceps and subscapular skinfold greater than 35mm, the following equation were applied:

All males: 0.783 (triceps+subscapular) +1.6

All females: 0.546 (triceps+subscapular) +9.7

Fat-free Mass.

The mathematical operation to calculate the fat-free mass (FFM) in children between 8 and 18 years old are as follows: FFM= Weight – Body Fatness.

3.3 - MATURITY

Maturity offset was estimated as the years from the age of peak height velocity (APHV), based on sex-specific prediction equations from Moore et al. investigation (Moore et al., 2015). APHV was defined as the age at maximum growth puberty during adolescence, and result by the difference between chronological age and maturity offset.

In boys the predictive equation used was: Maturity Offset= -7.999994+ (0.0036124*(age*height))): R^2= 0.896; SEE= 0.542

In girls, the predictive equation used was as follows: Maturity-Offset= -7.709133+ (0.0042232*(age*height))): R^2= 0.898; SEE= 0.528

3.4 - PHYSICAL FITNESS TESTS

All investigations that compose this PhD dissertation used the FITescola® program battery to assess PF. FITescola® is a program to promote and monitoring PA and PF knowledge and levels among Portuguese school-aged children and adolescents through an online platform. This platform allows to develop an ecosystem between the school, families and students and share knowledge and recommendations regarding PA, PF and healthy lifestyles. This program is supported by the Education Ministry and is present in all public schools with students aged from 10 to 18 years old. The PF tests that compose the FITescola® battery are scientifically validated and are performed regularly during physical education classes (FMH, 2015).

The specific test from the FITescola® program (FMH, 2015) used to assess each component of PF is described below. All tests were used to monitoring several health outcomes, validated by the previous investigations for the general youth population (F. B. Ortega, E. G. Artero, et al., 2008; Santos et al., 2014; Vanhelst, Beghin, Fardy, Ulmer, & Czaplicki, 2016).

Progressive Aerobic Cardiovascular Endurance Run (PACER). The PACER test was used to examine CRF. Participants attained the test, where the main aim was to perform a maximum number of laps with a defined cadence.

The audio signal helps the participants managing running speed during the test. The test starts with a cadence of 8.5km/h and increases progressively 0.5 km/h every minute.

Push-up test. Upper body MF was assessed through the push-up test. This test starts with the hands and feet touching the floor and the body in the plank position, with feet apart and the hands positioned below the shoulder line. The participants were required to lower the body until forming a 90º angle between the arm and the forearm then return to the starting position. This action was repeated with a previously defined cadence of 20 push-ups per minute. The number of times the participants completed this action correctly was recorded.

Horizontal and vertical jumps. The horizontal jump (HJ) and the vertical jump (VJ) tests measured lower body MF. The HJ test starts in the standing position with the feet slightly apart from each other and below the shoulder line, just immediately behind a line drawn on the floor. Then the participant jumps as far as possible landing with the feet together. The distance between the starting line and the heel of the backward foot is recorded, the best result of two trials is considered. The VJ test starts with the participant standing sideways to a wall and reaching up with the arm extended drawing a line with frame chalk. From a stationary position (knees slightly bend and feet slightly apart from each other below the shoulder line) the participant jumps as high as possible drawing a second line on the wall with frame chalk. The difference between both lines (the standing reach height and the jumping-reach height) is recorded, the best result of two attempts is considered.

Sit and reach test. The sit and reach test was used to evaluate the flexibility of the lower back and the hamstrings. The protocol of this test is explained in detail elsewhere (F. B. Ortega, E. G. Artero, et al., 2008). For this test, the mean value from the two legs was calculated and considered for the data analysis.

4x10 m shuttle run (SHTR). Agility was assessed by the 4x10m shuttle run test. In this test, the participant has to run as fast as possible between two parallel lines 10m apart while holding a sponge. Three sponges are previously placed behind the lines (about 50 cm off), one at the starting line and two at the other line. Participants start to run without a sponge and must pick-up or exchange the sponge every time they cross a line. The lines must be crossed with the two feet. The total time of the test in seconds is recorded using a stopwatch.

Speed test at 20m and 40m. The speed at 20m and 40m was recorded in seconds. Participants started in standing and comfortable position with one foot in the front of the other and immediately behind the starting line. Participants were instructed to start running on the words "on your marks, ready, GO" and only to start to decelerate after crossing the finish line. Time was recorded using a stopwatch.

Handgrip Strength. Handgrip (HG) strength was assessed using an adjustable handle analogue HG dynamometer (Lafayette Hand Dynamometer – Instrument Company, Lafayette, Indiana, United States of America). Participants were given a demonstration and verbal instructions for the test. Previous all individual tests, the HG was adjusted according to the adolescents' hand size and technical specifications by the manufacturer. HG was measured with a participant in a standing position with arms parallel at the body but not in contact. The shoulder would be adducted and neutrally rotated. The participants to grip the handle during 3-5 seconds, with verbal encouragement by researchers. Two trials were performed and record the best score for each limb.

3.5. - OBJECTIVE MEASURES OF PHYSICAL ACTIVITY

3.5.1 - Actigraph accelerometer measurements

In chapter 7, to assess the physical activity (PA) and sedentary time (ST) objectively were used an accelerometer (ActiGraph, GT3X model, Fort Walton Beach, FL). The ActiGraph-GT3X+ model is a proper small device to measure the acceleration of regular human movements. To avoid methodological issues, on physical activity assessments by the accelerometer, at all participants were asked to wear the device on the right hip, near to the iliac crest for 7 days followed (Penpraze et al., 2006; S. G. Trost, McIver, & Pate, 2005). Each device was activated on the first day after realised all PF tests from this investigation and data were recorded in 15 seconds epochs. Aquatic activities (i.e., swimming, aquatic sports, bathing), sleep, and periods of at least 60 consecutive minutes of zero activity intensity counts were considered as non-wear time (S. G. Trost et al., 2005). A valid-day was considered as having 10h or more (≤ 600 min.) to wear time (Penpraze et al., 2006). A valid-week was defined at a minimum of 3 days and having at least 2 weekdays and 1 weekend day (Cain, Sallis, Conway, Van Dyck, & Calhoon, 2013). The assessment of total activity accumulated per day in different intensities was expressed as minutes. The cutoff values used to define the intensity of PA and therefore to quantify the meantime in each intensity (moderate or vigorous, moderate and light) were as follows: vigorous: ≥ 4009 counts·min^{-1}; moderate: 2296-4008 counts·min-1 ; light: 100-2295 counts·min-1; sedentary: < 100 counts·min-1 (Evenson, Catellier, Gill, Ondrak, & McMurray, 2008). All accelerometer data was performed using the software Actilife (v.6.10.4).

3.6 - BONE HEALTH

3.6.1 - Quantitative ultrasound

In chapters 6 and 7 (investigation 3 and 4) the bone health status was assessed by QUS along the surface of the third distal radius and midshaft tibia based on an axial transmission technique, using the sonometer Omnisense 8000P (Sunlight Omnisense, BeamMed, Tel Aviv, Israel) to measure the SoS.

In clinical evaluation is essential to have the correct supplies for assessments, as illustrated in Figure 3.1.

To avoid errors during assessments was performed previous procedures in the following order:

1- Selection a room with a stabilized temperature. Outside of the range 15-30º C, the equipment not provided a reading.

2- Exposition the phantom at environment assessment to identify the temperature room.

3- Checking if the systems and the probes are working according to manufacturer recommendations.

4- Performing system quality verification at least once daily. The verification process was repeated for three cycles.

After performing the 4 steps cited, was identified the region to be measured (the radial distal third and tibial midshaft). All measurements were performed with the participant in seating and comfortable position as possible, and conducted on the non-dominant limbs. In the case of the previous fracture or impairment in non-dominant limb, was assessed the dominant limb in the bone site being evaluated.

Figure 3.1 - Ultrasound equipment after daily verification

Measuring SoS at the radius

The radius site being evaluated was calculated by the lenght between the third finger and the gouge platform. For sinalizing the zone to be analysed, was drawn a line exactly half the previously measured length with a skin marker. All parts of the arm to the elbow (supported on the desktop) was aligned as straight as posible. On the radius, the arm was pronated, and the probe realised a scanner in a semi-circumference fashion around the distal third of radius.

Measuring SoS at the tibia

To determining the zone at the tibia to be scanned, the participants were seated in a chair with the leg bent 90º at the knee and the heel placed firmly on the floor. The height of the leg rest should be equal to the height of the chair. The leg was measured, starting from the plantar surface of the hell at the tip of the knee, and was drawn a line in halfway point to the distance measured. The area scanned was delimitated by a line straight across the dorso-anterior surface of the tibia. The participants' leg was rest comfortably, with the knee slightly bent to avoid discomfort from the overstretching the leg. The leg support was used every time in the ankle zone, and never above. For all participants was repeated continuously 3-5 measurements cycles for each limb.

Ultrasound Statistical Measures

Results are meaningful in relation to a reference database of SoS values taken from a large representative population. SoS is plotted as function of sex and age. ***Z-score:*** The z-score for R-SoS and T-SoS were calculated based in age, weight, height, sex and caucasian database reference by *Omnisense 8000s*.

3.7 - DIETARY ASSESSMENT

In investigation 3 and 4 (chapter 6 to 7) were calculated the daily calcium intake from an interview by a Food Frequency Questionnaire semi-quantitative questionnaire assessing intake of a wide set of typical Portuguese foods in last month. This questionnaire was formally validated in similar investigations with Portuguese adolescents (Cardadeiro et al., 2014; Lopes et al., 2017).

3.8 - EXTERNAL TRAINING LOAD ASSESSMENT

In investigation 1 and 2 (chapter 4 to 5) the weekly training and competition load (time in minutes and frequency) for each participant were self-reported.

3.9 - COMPETITIVE LEVEL ASSESSMENT

In investigation 1 and 2 (chapter 4 to 5) the level of competition was categorized based on past individual performance (*Non-Elite:* junior or senior regional competition; *Junior-Elite:* junior national or international competition; *Elite:* senior international competition and *Super-Elite*: gold medallists at Olympics or world championships) (Rees et al., 2016).

3.10 - TYPE OF PHYSICAL EXERCISE

In investigation 3 and 4 (chapter 6 to 7) were categorised the type of physical exercise as follows: non-osteogenic (i.e. swimming, water polo, equestrian) and osteogenic (i.e. football, handball, volleyball). Those who did not regularly participate in any sport were classified as non-exercisers. This categorisation has been used previously in bone health investigations in adolescents (Hong & Kim, 2018; Vlachopoulos et al., 2018).

3.10 - STATISTICAL ANALYSIS

Data analysis was performed using the IBM SPSS Statistics (SPSS Inc., an IBM Company, Chicago, Illinois, USA) version 24.0 (chapters 4 at 7) and G*Power software, version 3.1.9.4, 2019 (Düsseldorf, Germany) (Chapter 3).

The statistical procedures common to all investigations are presented in this section (chapter 4 to 7), as follow:

- Descriptive statistical analysis including mean and standard deviations were performed for all outcome measurements.
- Normality analysis of the variables was performed using Q-Q plots (subjective method), and to confirm the conclusion from graphical methods were performed the Kolmogorov-Smirnov and Shapiro-Wilk (Yap & Sim, 2011).
- Difference between the expected frequencies in several categories was performed using Chi-square test (chapter 4, 5 and 7).
- Means comparisons for two groups were performed using independent sample T-test (chapter 4, 5 and 7), whereas comparisons for three or more groups were performed using One-way ANOVA (chapter 6).

In investigation 1 (chapter 4) specific procedures were used to test units strongly in the same group resemble each other from the PF tests battery although of the interclass correlation coefficient (ICC) and was performed a Bland-Altman plot to analysing the agreement between two different assays moments.

In investigation 2 (chapter 5) a receiver operating characteristic curve (ROC) was performed to the analysis of diagnostic of the potential youth athlete based in different PF tests. Youden's index was used to summarising the performance of the diagnostic test and define a criterion for selecting the optimum cut-off.

In investigation 3 (chapter 6) multiple linear regression analyses were performed to examine the associations of PF test continuous variables with bone health continuous variables (R-SoS and T-SoS) in youth non-athletes and athletes for both sexes. Additionally, a second model was performed to adjust for covariates, such as, type of physical exercise, number of minutes exercising per week, APHV, and calcium intake

In investigation 4 (chapter 7) a parallel mediation model was used to analyse whether each PF test and fat mass (%) mediated the relationship between VPA time with R-SoS and T-SoS. Parallel mediation analysis was performed using the PROCESS SPSS macro (v3.3 – model 4) developed by Preacher and Hayes (Preacher & Hayes, 2004). The PROCESS macro analyses the total and specific indirect effects using bootstrap procedures (resampling of 5000 bootstrap samples was used), which do not require assumptions of normality of the sampling (Hayes, 2017).

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CHAPTER 4

Test-retest reliability of physical fitness tests among young athletes: The FITescola® battery

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ORIGINAL ARTICLE

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Test–retest reliability of physical fitness tests among young athletes: The FITescola® battery

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Abstract
Monitoring physical fitness in young athletes is essential to improve physical performance, identify talents and develop injury prevention programs. The aim of this investigation was to analyse the reliability of the physical fitness tests from the FITescola® battery in young athletes with different sports backgrounds and competition levels. Participants comprised of 138 young athletes (boys $n = 92$) aged 9 and 18 years old. Eight physical fitness tests assessing six components of physical fitness were performed: cardiorespiratory fitness (PACER); upper body muscular fitness (push-up); flexibility (sit and reach); lower-body muscular fitness (horizontal and vertical jump); agility (4 × 10 shuttle run); and speed (sprint at 20 m and 40 m). Each test was performed twice, with a one-week interval (7 days) between duplicate tests. No differences between duplicate tests were found ($p > .05$), except for the speed at 20 m in boys ($p < .001$) and speed at 40 m in girls ($p = .006$). The battery of tests had good or excellent reliability and concordance of the ICC between the two trials (ICC ≥ 0.75) with exception for the 20 m speed run in girls that presented moderate reliability (ICC = 0.57). The Bland–Altman plots showed high reliability for all the fitness tests for both sexes. Hence, The FITescola® battery may be a novel tool to assess the physical fitness of large groups of young athletes from different sports backgrounds.

KEYWORDS
injuries prevention, performance, physical fitness, reliability, young athletes

1 | INTRODUCTION

In youth, physical fitness is not only related to sports performance but also used as a surrogate marker of health-related outcomes (Armstrong & McManus, 2011; McManus & Armstrong, 2011). In addition, information on physical fitness fosters knowledge on understanding the physiologic development of youth (Brown, Patel, & Darmawan, 2017), while supporting the identification and development of athletic talents. Generally, physical fitness is assessed using a combination of tests aimed at analysing specific physiological components, such as anthropometric measures (e.g., height, weight, waist circumference), cardiorespiratory fitness (CRF), upper and lower muscular fitness (MF), and agility and speed (Ortega, Ruiz, & Castillo, 2008; Vanhelst, Beghin, & Fardy, 2016). The physical fitness monitoring of adults athlete populations is well established, while in young athletes it is scarce (Armstrong & Barker, 2017). Measuring and monitoring these different components of physical fitness in young athletes can be achieved objectively in the laboratory setting. However, most of these tests require specific equipment, qualified technicians and have high logistic costs (Artero et al., 2011). Therefore, applying laboratory methods may not be feasible in large groups and misrepresents the natural sports environment (Ruiz et al., 2011).

FITescola® is a digital platform aimed to monitor fitness and promote health in youth. It provides a set of instruments, including field tests, for assessing health-related physical fitness in 10- to 18-year-old students. The validation of the tests that comprise the FITescola® battery and the reliability of some tests, such as the 20 m shuttle run, horizontal jump, 4 × 10 m shuttle run, push-up test and the sit and reach has been previously examined in youth (general population) (Artero et al., 2011; Baumgartner, Oh, Chung, & Hales, 2002; Ortega, Artero, et al., 2008; Ruiz et al., 2011; Vanhelst et al., 2016). Although some of the tests comprising the FITescola® battery (e.g., the speed test) were tested for reliability in athlete samples from specific sports (e.g., football) (Pojskic et al., 2018), the reliability of the FITescola® battery as a whole was never tested among young athletes.

Young athletes present a particular variability in their physiologic, anthropometric characteristics (e.g., weight), and highest physical fitness levels when compared to non-athletes (Armstrong & McManus, 2011; McManus & Armstrong, 2011; Smoll & Schutz, 1985). In addition, athletes are more susceptible to greater fluctuations in their fitness performance due to their higher training loads and periodization in their fitness levels based on their competition season compared to their non-athletic peers (Kellmann et al., 2018). Thus, although good reliability has been reported for the tests that comprise the FITescola® battery in the general population of youth (Ortega, Ruiz, et al., 2008), the reliability of these tests in athletic youth may vary given these physiologic and fitness fluctuation differences commonly seen in athletes versus. non-athletes.

The reliability of effective tools for assessing physical performance in sport and educational systems is important, especially when sports authorities, clubs, federations and professionals aim to find young sports talents for a variety of sports or when designing talent identification programs (Bergeron et al., 2015; Johnston, Wattie, & Schorer, 2017; Pion, Segers, et al., 2015). To the best of our knowledge, no other research has reported the reliability of a physical fitness battery among young athletes from different sports and different levels of competition. Therefore, the purpose of this investigation was to determine the test–retest reliability of the FITescola® battery among young athletes from different sports backgrounds and competitive levels.

2 | METHODS

2.1 | Participants and investigation design

One hundred and thirty-eight children and adolescents (92 boys and 46 girls) aged between 9 and 18 years old, with different sport backgrounds (basketball, fencing, football gymnastics, handball, hockey, judo, karate, kickboxing, tennis, swimming, volleyball, weightlifting and wrestling) from several official federative club teams within Lisbon and Porto district areas, participated in the investigation. Participants were considered athletes (Araújo & Scharhag, 2016) if (inclusion criteria) (a) were engaged in organized sports with regular participation in official competitions promoted by each national federation recognized by the Portuguese Olympic Committee (COP) and the Portuguese Institute of Sports and Youth (IPDJ) in the last 12 months and (b) were engaged in at least three training sessions per week. Each participant performed eight physical fitness tests of the FITescola® battery in two separate sessions, which were repeated in the following week (7-day interval). The first session assessed upper and lower MF and flexibility. Forty-eight hours after the first session, the second session took place, where agility, speed and CRF were assessed. Each session lasted a total of 60 min. Before performing the tests, the participants did a 10-min standardized warm-up conducted by the researchers. The warm-up protocol was divided in three parts: part 1—low speed running exercises (e.g., straight ahead, hip out and quick forwards and backwards) combined with active mobilization of the upper limbs (e.g., arm circles and upper back mobility); part 2—"Waspital game"; and part 3—active stretching (upper and lower limbs). In order to minimize the effects of circadian rhythm variability and the effect of training level, all tests were performed in the same order and at the same time of the day, during the competitive microcycle for each athlete.

The physical fitness tests manual and videos, including procedures and protocols, are available in the FITescola® online platform (http://fitescola.dge.mec.pt/HomeTestes.aspx). The same researchers assessed all the physical fitness tests. Anthropometric evaluations were performed only in the first session. All participants were informed about the aim of the project, and written consent was obtained from their legal guardians before participation in the investigation. The investigation was followed to update the Declaration of Helsinki according to the last assembly (the 64th WMA General Assembly, Fortaleza, Brazil, October 2013) (World Medical Association, 2013) and was approved by the Ethics Committee of Faculty of Human Kinetics, University of Lisbon (CEFMH-Approved; No 19/2018).

2.2 | Measures

2.2.1 | Body composition

All participants were weighed to nearest 0.01 kg on an electronic scale (model 799 SECA: Seca GmbH) while wearing shorts and a t-shirt and without shoes. Height was measured to the nearest 0.1 cm with a stadiometer (model 220 SECA: Seca GmbH) (Lohman, Roche, & Martorell, 1998). BMI was calculated as body mass (kg)/height^2 (m).

2.2.2 | Competitive level/Training frequency

Weekly training frequency was self-reported and was counted as the usual number of training session per week at the time of the investigation. Athlete competition level was determined based on past and present individual performance and was categorized into the following groups based on the recommendations of the UK Sports Committee for the development of young athletes: *Non-Elite* = junior or senior regional competition; *Junior-Elite* = junior national or international competition; *Elite* = senior international competition; and *Super-Elite* = gold medallists at Olympics or world championships (Rees et al., 2016).

2.2.3 | Physical fitness

The FITescola® battery was used to assess physical fitness. The specific tests from the FITescola® battery used to assess each component of physical fitness are described below.

Progressive Aerobic Cardiovascular Endurance Run (PACER)

The PACER test was used to examine CRF. The main aim of this test was to perform the maximum number of laps with a defined cadence. An audio signal was used to help the participants manage running speed during the test. The test starts with a cadence of 8.5 km/hr and increases progressively 0.5 km/hr every minute.

Push-up test

Upper body MF was assessed through the push-up test. The test starts with the participant's hands and feet touching the floor, and the body in a plank position, with feet apart and the hands positioned below the shoulder line. The participants were required to lower the body until forming a 90° angle between the arm and the forearm and then return to the starting position. This action was repeated with a previously defined cadence of 20 push-ups per minute. The number of times the participants completed this action correctly was recorded.

Horizontal and vertical jumps

The horizontal jump and the vertical jump tests measured lower-body MF. The horizontal jump test starts in the standing position with the feet slightly apart from each other and below the shoulder line, just immediately behind a line drawn on the floor. Then, the participant jumped as far as possible, landing with the feet together. The distance between the starting line and the heel of the backward foot was recorded. The best result of two trials was considered. The vertical jump test started with the participant standing sideways to a wall and reaching up with the arm extended, drawing a line with frame chalk. From a stationary position (knees slightly bent and feet slightly apart from each other below the shoulder line), the participant jumped as high as possible drawing a second line on the wall with frame chalk. The difference between both lines (the standing reach height and the jumping-reach height) was recorded. The best result of two attempts was considered.

Sit and reach test

The sit and reach test was used to evaluate the flexibility of the lower back and the hamstrings separately. Participants performed the forward flexion movement of the trunk with both arms extended, one leg bent at 90° and other leg extended touching with the sole of the foot on the measurement box. For this test, the mean value from the two legs was calculated and considered for the data analysis.

4 × 10 m shuttle run

Agility was assessed by the 4 × 10 m shuttle run test. In this test, the participant had to run as fast as possible between two parallel lines 10 metres apart while holding a sponge. Three sponges were previously placed behind the lines (about 50 cm off), one at the starting line and two at the other end. Participants started without a sponge and had to pick-up or exchanged the sponge every time they crossed the lines. The lines had to be crossed with both feet. The total time of the test in seconds was recorded using a stopwatch.

20 m and 40 m run

The speed at 20 m and 40 m was recorded in seconds. Participants started in a standing and comfortable position, with one foot in front of the other and immediately behind the starting line. Participants were instructed to start running on the words "on your marks, ready, GO" and only to start deaccelerating after crossing the finish line. Time was recorded using a stopwatch. The speed tests were performed separately, with a minimum of two minutes recovery time between tests.

2.3 | Statistical analyses

Descriptive statistics (including mean, standard deviation and percentages) were calculated for all variables for the total sample and each sex. Differences between sexes were assessed using Students' *t* test for the continuous variables and chi-square test for the non-continuous variables. Differences for each physical fitness test between trial one (T1) and trial two (T2) were assessed using a paired sample *t* test. A sex interaction effect on the reliability of the studied physical fitness tests was found. Thus, the analyses were performed separately for boys and girls. Reliability between test–retest was analysed using the intraclass correlation coefficient (ICC) model with two-way mixed effects. Level of reliability for each test, considering the whole sample and then divided by sex, was classified according to the following reference values: ICC < 0.50—poor reliability; 0.50 ≤ ICC < 0.75—moderate reliability; 0.75 ≤ ICC < 0.90—good reliability; and ICC ≥ 0.90—excellent reliability (Koo & Li, 2016). Assessment of agreement between two session trials was performed with the Bland–Altman approach. To analyse bias and to estimate the agreement interval within 95% of the mean differences between the second and the first trials, we used the Bland–Altman plot method with the limits of agreement of ±1.96 standard deviation of the mean differences. We further assessed whether the mean difference in the repeated values of each of the fitness test varied for elite (i.e., elite, junior-elite and super-elite) versus non-elite athletes using a Mann–Whitney test. Given that the differences in the repeated measures of the fitness tests did not significantly differ between the elite and non-elite athletes, with the exception of the speed test 40 m, we chose to present results for all athletes combined in order to maximize sample size. Data analyses were performed using IBM SPSS Statistics version 24.0 (SPSS Inc., an IBM Company). Statistical significance was set at $p \leq .05$.

3 | RESULTS

Descriptive data for the participants' characteristics and their distribution are presented separately for boys and girls in Table 1. Significant ($p < .05$) differences between sexes were only evident for height, BMI and competition level.

Reliability of the physical fitness tests, including mean values, standard deviation, inter-trial differences (T2–T1) and the intraclass correlation coefficient (ICC) for each test, are reported in Table 2. In boys, the inter-trial difference was significant only for the 20 m test ($p < .001$), whereas for girls, there was a significant inter-trial difference for the 40 m test ($p = .008$). Notwithstanding, the ICC concordance of the two trials in boys and girls was classified as good or excellent (ICC ≥ 0.75) demonstrating strong agreement, with exception for the 20 m speed run in girls that presented moderate reliability (ICC = 0.57).

The concordance of the ICC between trials for all participants was reinforced by the Bland and Altman method. Figures 1 and 2 show the reliability values in the function of systematic errors and random errors (95% limits of agreement) of the FITescola® physical fitness tests analysed. The systematic error was approximately zero. In boys (Figure 1), mean differences and most data points were within the limits of agreement for all physical fitness tests, and no trend was found between the differences and the means of the trials.

For girls (Figure 2), the Bland and Altman plots showed high-reliability patterns for all physical fitness tests, where the systematic error was nearly zero and mean differences within the limits of the agreement established.

4 | DISCUSSION

This investigation aimed to determine the test–retest reliability of the FITescola® battery among young athletes with different sports backgrounds and competition levels. Our results point out that the FITescola® battery demonstrates high reliability and therefore can be used as a physical fitness monitoring tool for young athletes. Findings showed that the battery had good or excellent reliability and concordance of the ICC between the two trials (ICC ≥ 0.75) with exception for the 20 m speed run in girls that presented moderate reliability (ICC = 0.57), which was reinforced by the Bland–Altman analysis, where all physical fitness tests presented a mean difference close to zero.

The PACER test is widely used to assess CRF in youth (Mayorga-Vega, Aguilar-Soto, & Viciana, 2015), and its validation and reliability were previously tested for the general population (Ortega, Ruiz, et al., 2008; Ramírez-Vélez, Rodrigues-Bezerra, & Correa-Bautista, 2015; Vanhelst et al., 2016), but not in young athletes from different sports. It is common from several coaches to use different tests to assess the CRF based on the specificity of each sport (Asker, Waldén, & Källberg, 2017; Grgic et al., 2019; Jemni, Prince, & Baker, 2018). The PACER test is suggested to be an excellent surrogate measure of CRF to be used across the athletic population and to be incorporated in the monitoring of performance and talent identification programs for a wide variety of sports. Furthermore, CRF has been associated with sports performance in several sports (Barker & Armstrong, 2011; Jemni et al., 2018; Winter, Jones, & Davison, 2007). In accordance with previous studies in adolescents (Ortega, Ruiz, et al., 2008; Ramirez-Velez et al., 2015; Vanhelst et al., 2016), we found the PACER test to have good or excellent ICC (ICC-boys: 0.79; ICC-girls: 0.90) among our sample of athletes from different sports backgrounds and competitive levels.

Agility is considered as one of the most critical talent identification tests in several sports (Ali, 2011; Mungovan, Peralta, & Gass, 2018; Spasic, Krolo, & Zenic, 2015). Previous studies assessing the reliability of the 4 × 10 m shuttle run in the general population have found strong agreement between trials (Ortega, Ruiz, et al., 2008; Ramirez-Velez et al., 2015). However, to the best of our knowledge, the reliability of this test has not been assessed yet in the athletic population. In this investigation, the 4 × 10 m shuttle run showed a good ICC among young athletes from different sports backgrounds, suggesting that this test may be an excellent option to assess agility in multi-sport talent identification programs and fitness batteries. Beyond the 4 × 10 shuttle run test, there are several other tests to assess the agility in young athletes (e.g. Illinois agility test (IAT), the agility *t* test, change of direction-COD), all of which are usually modified by coaches or researchers upon application (Kutlu, Yapici, & Yilmaz, 2017; Mountjoy et al., 2014; Nimphius, Callaghan, & Bezodis, 2018; Rouissi, Chtara, & Berriri, 2016). The differences between procedures among coaches or researches hamper the standardization of a protocol and consequently a uniform application on the entire population of young athletes. Our results show that the 4 × 10 shuttle run test can be used, instead of others that may be harder to apply (Zemková & Hamar, 2018).

Previous studies have shown no significant differences in test–retest values for the horizontal jump in the general population (Fernandez-Santos, Ruiz, & Cohen, 2015; Ortega, Ruiz, et al., 2008; Vanhelst et al., 2016). Additionally, the horizontal jump test has shown to be a reliable measure for the assessment of lower-body MF for both boys and girls (Artero et al., 2011; Espana-Romero et al., 2010; Ortega, Ruiz, et al., 2008). Similar to the general population, the horizontal jump test proved to be a reliable tool to assess lower-body MF in athletes. The vertical jump test is also commonly used to assess the strength of the lower limbs and is highly correlated with several MF laboratory tests used to assess health (Artero et al., 2011; Ortega, Ruiz, et al., 2008) and performance (Fernandez-Santos et al., 2015). However, its reliability among young athletes in an ecologic context has not been established yet. In our investigation, we found the vertical jump

TABLE 1 Physical characteristics and level sports competition of youth athlete participants

	Mean ± *SD* or %			
	All (*n* = 138)	Boys (*n* = 92)	Girls (*n* = 46)	*p*-value
Age (years)	15.6 ± 2.2	15.5 ± 2.3	15.6 ± 2.1	NS
Weight (kg)	58.9 ± 12.4	59.4 ± 13.0	57.8 ± 11.4	NS
Height (m)	1.66 ± 0.1	1.69 ± 0.1	1.61 ± 0.1	<.001
BMI (kg/m^2)	21.1 ± 3.1	20.5 ± 2.9	22.3 ± 3.3	.001
Week frequency training (times)	5.0 ± 1.5	5.0 ± 1.3	5.4 ± 1.8	NS
Level sports competition (%)				
Non-Elite	56.5	71.4	27.7	<.001
Junior-Elite	36.2	23.1	61.7	
Elite	2.9	2.2	4.3	
Super-Elite	4.3	3.3	6.4	

Note: p-value for the *t* test in continuous variables, and the chi-square test to compare differences in proportions between boys and girls.

Non-Elite: junior or senior regional competition; Junior-Elite: junior national or international competition; Elite: senior international competition; Super-Elite: gold medallists at Olympics or world championships.

Abbreviations: BMI, body mass index; NS, non-significant; *SD*, standard deviation.

test to have a high agreement between trials, although this measure was better among girls than among boys. Hypothetically, the differences in jump height between trials can be due to systematic bias, such as the athlete's emotional stress (Schaal et al., 2011) or fatigue (Watkins et al., 2017), and variation in jumping mechanics (Attia et al., 2017). However, our overall findings indicate that the horizontal jump test and the vertical jump test may be an appropriate ecologic alternative to laboratory tests examining lower limb strength.

In some sports, such as surfing and rugby, the push-up test is used to predict performance (Parsonage et al., 2017; Speranza, Gabbett, & Johnston, 2015). The push-up test can be used as a differentiator tool between young non-athletes and athletes. Previous studies in the general adolescent population have examined the validity and reliability of the push-up test (Baumgartner et al., 2002; Pate, Burgess, & Woods, 1993) and concluded that results are dependent on the relationship between upper body strength and body fat percentage (Castro-Pinero et al., 2009). In adult athletes, the push-up test sensibility, validity and reliability have been assessed using force plates (Gillen et al., 2018; Wang et al., 2017), but these characteristics have not been assessed in young athletes. Our investigation results further reinforce that the push-up test is an adequate test to assess upper body MF in young athletes.

The sit and reach test is commonly used in gymnastics to assess physical performance, and its validity and reliability have already been assessed among the general population as a measure of health (Ortega, Ruiz, et al., 2008; Pion, Lenoir, & Vandorpe, 2015). Along these lines, we too also found this test to have excellent test-retest reliability in athletes of different sports backgrounds and competition levels.

The 20 m run, for the overall sample and boys, and the 40 m run, for girls, were the only tests where significant differences were found between test-retest values. These results differ from those of previous studies conducted in the general population, where the 20 m and 40 m run tests were found to be reliable (Castro-Pinero et al., 2010; Vanhelst et al., 2016). Notwithstanding, the 20 m and 40 m run tests presented good or excellent ICC values and strong to the almost perfect agreement in our sample of young athletes, with exception for the 20 m speed run in girls that presented moderate reliability (ICC = 0.57). The differences observed in the 20 m and 40 m run may be explained by the degree of precision needed when assessing these tests. As slight differences in assessment may lead significant differences, it is usual to use more advanced materials (e.g. photoelectric cells) other than chronometers for assessing speed. However, the objective of the FITescola® is to be low cost, and with minimal required equipment, the use of chronometers was chosen.

This investigation has some limitations. First, the sample size is not sufficient to ensure a representative distribution of the young athletes from all types of sports and competition levels. In addition, of the young athletes included in our investigation, there was non-homogeneity in the distribution of sports backgrounds and competition levels between the sexes. Furthermore, maturity was not assessed. Maturity is an important variable associated with physical fitness performance (Bergeron et al., 2015; Björkman et al., 2018). It would be interesting that future studies in the athlete population stratify the analysis accordingly to the maturity status, to see the effect of this variable in the validity and reliability of fitness tests. Other limitation of this investigation is regarding the number of elite and super-elite athletes in the sample and the transferability of these findings to these levels of competition.

TABLE 2 Reliability of physical fitness test in youth athletes by sex

	Trial 1	Trial 2	*p*-value	Mean (T2–T1)	ICC	95% CI
All						
PACER (laps)	59.07 ± 25.6	59.6 ± 27.0	.694	0.49	0.85	0.80, 0.90
Push-up(reps)	23.3 ± 10.6	24.0 ± 10.5	.119	0.74	0.86	0.81, 0.90
Sit and reach (cm)	26.4 ± 9.9	26.8 ± 9.8	.056	0.36	0.98	0.97, 0.98
Horizontal jump (cm)	186.3 ± 38.9	186.5 ± 38.3	.847	0.18	0.96	0.95, 0.97
Vertical jump (cm)	38.6 ± 10.4	38.9 ± 10.0	.719	0.33	0.72	0.63, 0.70
Shuttle run (4 × 10 m)	11.8 ± 1.2	11.8 ± 1.2	.826	−0.01	0.83	0.77, 0.88
Speed test (20 m)	3.6 ± 0.5	3.7 ± 0.5	<.001	0.09	0.80	0.74, 0.86
Speed test (40 m)	6.7 ± 0.9	6.8 ± 0.9	.132	0.04	0.93	0.90, 0.95
Boys						
PACER (laps)	66.5 ± 25.8	67.3 ± 27.3	.643	0.84	0.79	0.70, 0.86
Push-up(reps)	23.8 ± 9.8	24.8 ± 10.2	.100	0.99	0.83	0.76, 0.89
Sit and reach (cm)[a]	23.5 ± 8.7	24.0 ± 8.7	.099	0.45	0.96	0.93, 0.97
Horizontal jump (cm)	1,997 ± 33.4	200.1 ± 34.5	.755	0.42	0.93	0.90, 0.95
Vertical jump (cm)	40.7 ± 10.5	40.9 ± 10.4	.821	0.18	0.75	0.65, 0.83
Shuttle run (4 × 10 m) (s)	11.4 ± 1.1	11.4 ± 1.0	.952	−0.01	0.78	0.69, 0.85
Speed test (20 m) (s)	3.4 ± 0.5	3.6 ± 0.5	<.001	0.12	0.82	0.69, 0.89
Speed test (40 m) (s)	6.5 ± 0.9	6.5 ± 0.9	.908	0.01	0.92	0.87, 0.94
Girls						
PACER (laps)	44.3 ± 17.7	44.0 ± 18.4	.799	−0.22	0.95	0.91, 0.97
Push-up (reps)	22.2 ± 12.0	22.4 ± 10.9	.757	0.24	0.90	0.82, 0.94
Sit and reach (cm)	32.2 ± 9.6	32.4 ± 9.6	.221	0.17	0.99	0.99, 0.99
Horizontal jump (cm)	159.5 ± 35.3	162.0 ± 38.7	.742	−0.29	0.99	0.97, 0.99
Vertical jump (cm)	34.3 ± 8.9	34.9 ± 8.02	.387	0.65	0.88	0.80, 0.93
Shuttle run (4 × 10 m) (s)	12.6 ± 1.0	12.6 ± 1.0	.764	−0.03	0.76	0.60, 0.86
Speed test (20 m) (s)	3.8 ± 0.3	3.8 ± 0.4	.477	0.04	0.57	0.34, 0.74
Speed test (40 m) (s)	7.3 ± 0.6	7.4 ± 0.7	.008	0.13	0.87	0.76, 0.93

Abbreviations: ICC, Intraclass correlation coefficient; PACER, Progressive Aerobic Cardiovascular Endurance Run; T1, Trial one; T2, Trial two.
[a]The average of left and right side was used to analyse; *p*-value for the *t* test in continuous variables to compare differences between T1 and T2 for all groups.

Notwithstanding, in the investigated ages very few athletes are elite and super-elite.

Despite limitations, this is the first investigation to demonstrate the reliability of a group of physical fitness tests in young athletes. Given the advantages of these physical fitness tests over laboratory tests, including their safety, minimal required equipment and being able to be performed in an ecologic context (school or training facility), these fitness tests may be useful tools to assess physical performance and overall health in athletic youth.

Additionally, for the governments, sports clubs and parents have been expressing interest in sports promotion and intervention programs in adolescents (Bergeron et al., 2015). There is a need for adequate age and sex appropriate measures to test and improve sports participation and health-related physical fitness during this specific period of growth.

This investigation assessed the test-retest reliability of the FITescola® battery among young athletes. The FITescola® battery demonstrated to be a valid alternative to laboratory tests assessing physical fitness in young athletes. Findings showed that the battery tests had good or excellent reliability and concordance of the ICC between the two trials (ICC ≥ 0.75) with exception for the 20 m speed run in girls that presented moderate reliability (ICC = 0.57).

PRACTICAL APPLICATIONS

The FITescola® is a digital platform aiming to monitor fitness and promote health and sports performance in youth aged 10 to 18 years-old attending Portuguese public schools. This tool combines physical fitness test of upper and lower strength, aerobic cardiorespiratory fitness, ability and speed. Results of this investigation indicate that the FITescola® battery reliability is high and can be used for young athletes from several sports background. Thus, this battery may be a useful tool for authorities, clubs, federations and professionals to use when aiming to promote sports participation, find young

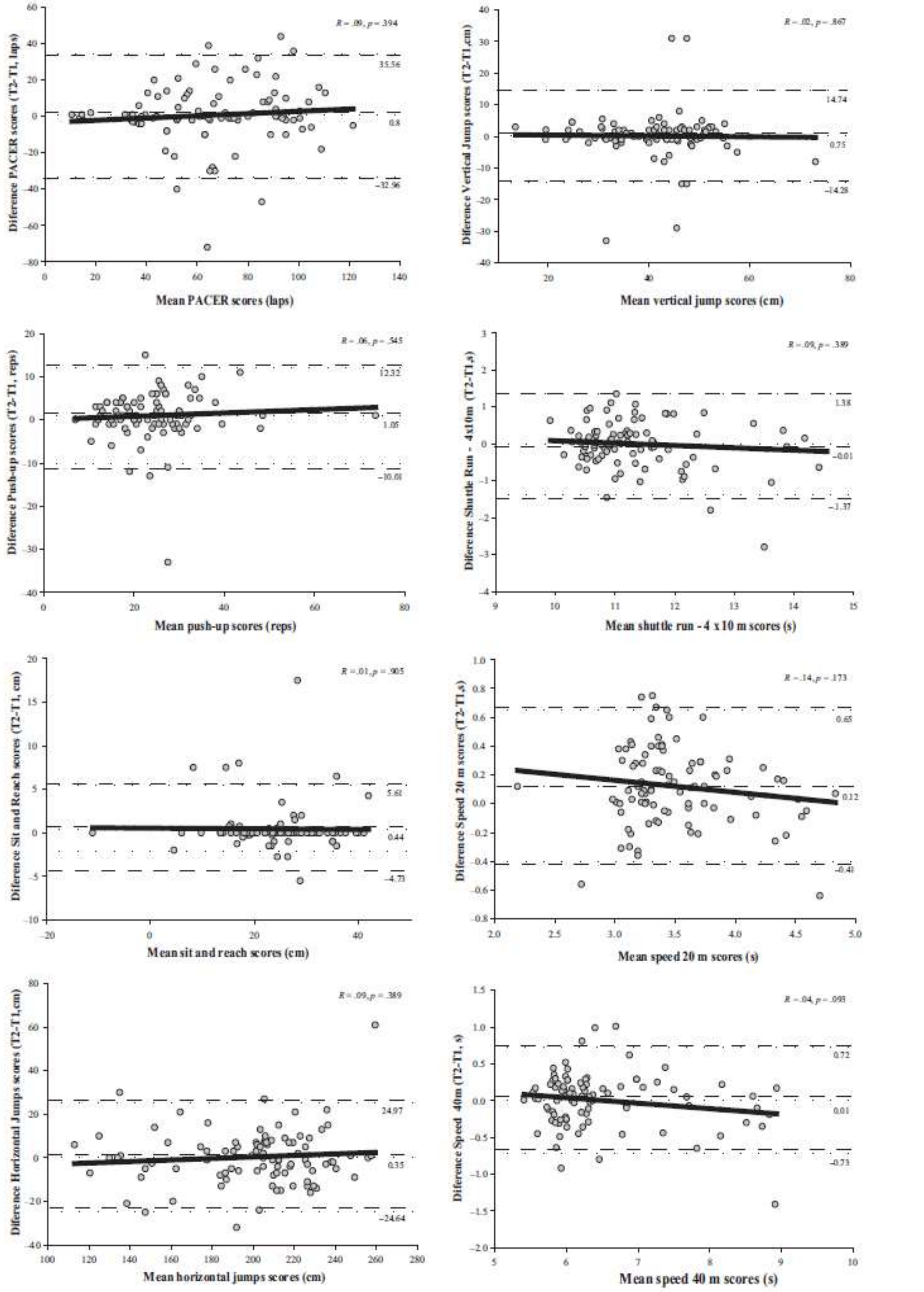

FIGURE 1 Bland–Altman plot of all the FITescola® physical fitness tests for boys. *Central dotted line represents the mean differences between T2–T1; the upper and lower dotted lines represent the upper and lower 95% limits of agreement (mean differences ± 1.96 *SD* of the differences)

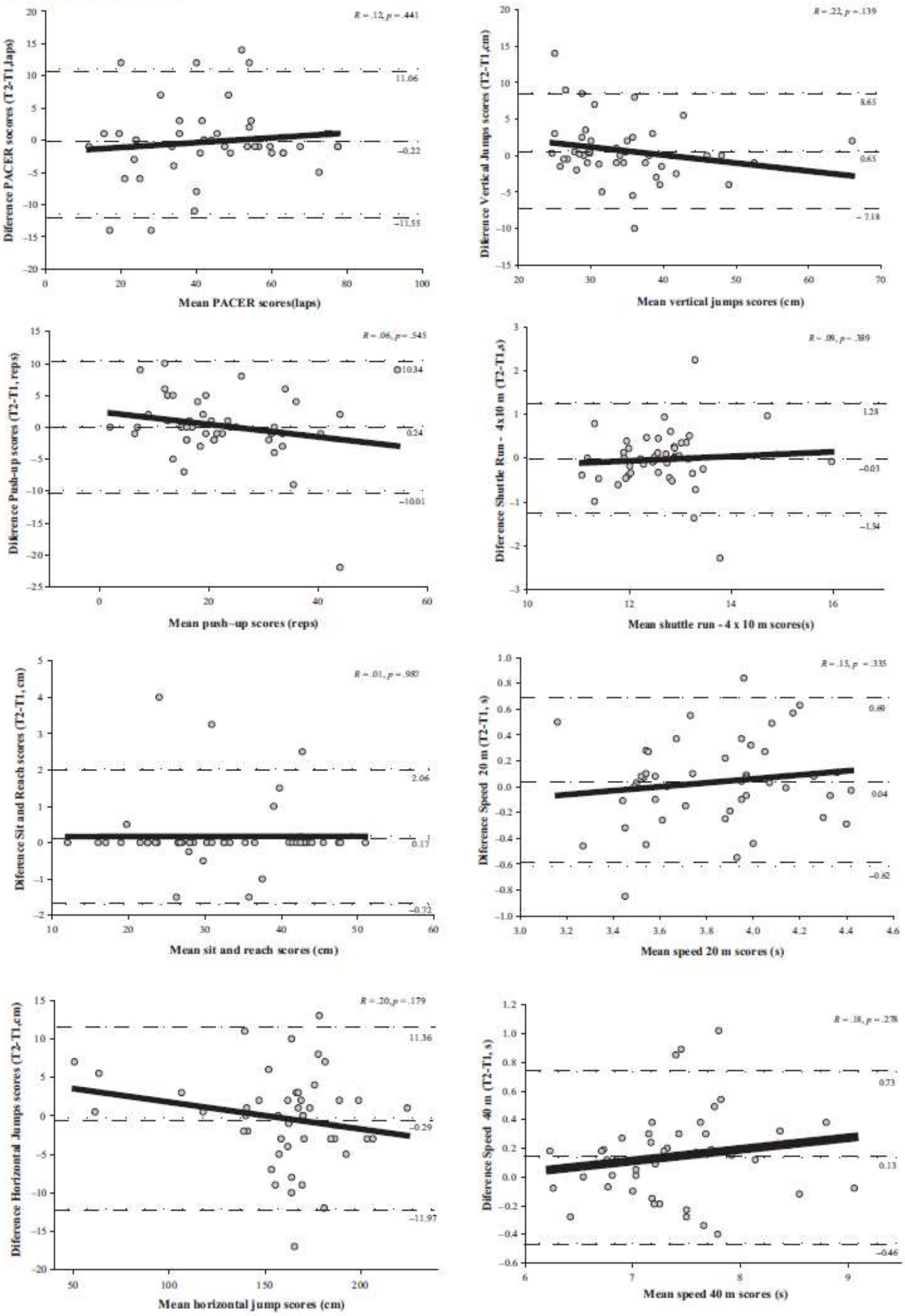

FIGURE 2 Bland–Altman plot of all FITescola® physical fitness tests for girls. *Central dotted line represents the mean differences between T2–T1; the upper and lower dotted lines represent the upper and lower 95% limits of agreement (mean differences ± 1.96 *SD* of the differences)

sports talents or when designing talent identification programs with greater confidence in its reliability.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

AUTHORS' CONTRIBUTIONS

Research concept and study design: DHN, LBS. Literature review: DHN, AM, MP. Data collection: DHN, CM, MP. Data analysis and interpretation: LBS, AM, CM. Writing of the manuscript: DHN. Reviewing/editing a draft of the manuscript: DHN, LBS, CM, AM, MP.

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CHAPTER 5

Predicting sports potential in youth through physical fitness tests

[2] Duarte Henriques-Neto, Megan Hetherington-Rauth, João Pedro Magalhães, Pedro B. Júdice, Luís B. Sardinha. (submited) Predicting sports potential in youth through physical fitness tests.

PREDICTING SPORTS POTENTIAL IN YOUTH THROUGH PHYSICAL FITNESS TESTS

Duarte Henriques-Neto, Megan Hetherington-Rauth, João Pedro Magalhães, Pedro B. Júdice, Luís B. Sardinha

ABSTRACT

Background: Promoting youth sports participation is an excellent strategy to improve health and high-level sports competition around the world.

Objective: The aim of this investigation was to analyse the potential of commonly used physical-fitness-tests (PF) to discriminate adolescents with athletic potential.

Methods: Participants consisted of 1831 youth (boys: 514 non-athletes and 401 athletes; girls: 722 non-athletes and 194 athletes) aged 10-18 years (y). Cardiorespiratory-fitness (CRF), muscular-fitness (MF), agility and speed were tested using PF tests. Application of ROC curves were used to assess the discriminatory potential of each PF for distinguishing athletes from non-athletes, with an area under the curve (AUC) higher than 65% (0.65).

Results: In the oldest groups (≥16y), the speed test at 20m in boys (AUC= 0.70) and horizontal jump test in girls (AUC=0.75) were the best discriminators, while the push-up-test (AUC 10-11y boys=0.68, AUC 14-15y boys=0.68, AUC 10-11y girls=0.73, AUC 12-13y girls=0.87) and the PACER (AUC boys: 12-13y=0.68 and AUC girls 14-15y=0.73) appeared to be better discriminators than other PF tests, for the younger age-groups.

Conclusion: The speed-test at 20m and the horizontal-jump were the best PF to identify older adolescents with athletic potential, while the PACER and push-up tests were the most discriminatory for younger adolescents.

Keywords: PACER, adolescents, athletes, sports, talent.

INTRODUCTION

The number of young people participating in sports has been raising significantly in the last decades (Bayt & Bell, 2016). There is a consensus that regular participation in organised sports plays an essential role in building teamwork and interpersonal skills, promoting healthy lifestyles, and improving physical activity (PA) and physical fitness (PF) levels among children and adolescents (J. Baker et al., 2017; J. L. Baker, Hebert, Møller, Andersen, & Wedderkopp, 2015; Brenner, 2016; Patton et al., 2016; Schmidt et al., 2017). Notwithstanding, one of the main goals of organized sports participation in youth is to provide a basis for developing latent sports talents (Phillips, Davids, Renshaw, & Portus, 2010).

To achieve this goal, several sporting entities around the world have developed programs to identify talented young athletes based on PF performance (Gulbin, Croser, Morley, & Weissensteiner, 2013; Tamara Kramer, Huijgen, Elferink-Gemser, & Visscher, 2016). It is a common practice to analyze the physical profile of young athletes, compare this with adult athletes when aiming to predict potential success (B. Jones et al., 2018; Li et al., 2018). However, this type of methodology is heavily rooted in assumptions (Tribolet, Bennett, Watsford, & Fransen, 2018), as several characteristics that distinguish top athletes in adults may not be observed during adolescence (J. Baker et al., 2017; Johnston et al., 2017). Additionally, adolescence is a dynamic period characterised by the growth and development of different organic systems (e.g. bone tissue, muscle tissue), which rarely progresses at the same time or in a linear fashion (Patton et al., 2016). Thus, assessing physical fitness in youth and comparing it with their peers may be a better alternative.

PF is an essential marker of health and sports performance in youth (N. Armstrong & McManus, 2011; B. Ortega, R. Ruiz, J. Castillo, & M. Sjostrom, 2008). Adequate PF monitoring can be an excellent strategy to promote health and to identify young athletic talents for all types of sports (Bergeron et al., 2015). Thus, the first aim of the present investigation was to analyse the diagnostic accuracy of PF tests to discriminate between children and adolescent athletes and non-athletes, independent of sports type and competitive level. The second aim was to define the age group and sex-specific cutoff values for identifying athletes by each PF test.

METHODS

Participants and investigation design

This cross-sectional investigation comprised a sample of 1831 children and adolescents (915 boys, 916 girls), aged between 10- and 18-years-old (yrs). From the 1831, 595 were registered athletes (401 boys, 193 girls) from 25 different sports (athletics, badminton, basketball, boxing, canoeing, cycling, equestrian, fencing, football/soccer, gymnastics, handball, hockey, judo, rowing, rugby, sailing, swimming, table tennis, taekwondo, tennis, trampoline, volleyball, water polo, weightlifting, and wrestling). Participants were considered athletes if they achieved the following criteria: (1) were registered in the national association of their sport, and the association was registered in the National Institute of Sport and Youth; (2) participated in competitions regularly; (3) had two or more training sessions per week; (4) had completed at least one sporting season before assessment. All participants were recruited from several public schools or official federative club teams within the Porto and Lisbon district areas. PF tests were assessed using the protocols from the

FITescola® program, which comprehend PF tests and anthropometric evaluations, such as, cardiorespiratory fitness (CRF), upper and lower muscular fitness (MF), flexibility, agility, speed and body mass index (BMI). The FITescola® program is a free online platform used in all public schools by physical education teachers, which provides a set of tools to assess and monitor the health-related PF of Portuguese children and adolescents aged 10-18 yrs (http://fitescola.dge.mec.pt/home.aspx). Data were collected during training sessions in the schools or sports clubs' facilities by trained physical education teachers and sports coaches. All participants were informed about the aim of the project, and written consent was obtained from their legal guardians before participation in the investigation. The investigation followed the Declaration of Helsinki for Human Investigations (WMA, 2008) and was approved by the Ethics Committee of Faculty of Human Kinetics, University of Lisbon (CEFMH-Approved; N.º19/2018).

Measures

Body composition

All participants were weighed to nearest 0.01 kg on an electronic scale (model 799 SECA, Hamburg, Germany) while wearing minimal clothes. Height was measured to the nearest 0.1 cm with a stadiometer (model 220 SECA, Hamburg, Germany) (Lohman TG, 1998). BMI was calculated as body mass (kg)/height^2 (m).

Competitive level and training frequency

Competitive level and weekly training frequency were self-reported. Competitive level was categorised based on past individual performance (Super-elite: Olympics or world championship; Elite: senior international competition; Junior-elite: junior national or international competition; Non-elite: juniors or seniors under national competition) (Rees et al., 2016) and weekly training duration and frequency was counted as the usual number of training sessions per week at the time of the investigation.

Physical fitness

The FITescola® program was used to assess PF. The specific tests from the FITescola® program used to evaluate each component of PF have been described in detail elsewhere (Lisbon, 2015) and validated in previous investigations for adolescents (Gracia-Marco, Vicente-Rodríguez, et al., 2011; Vanhelst et al., 2016).

Statistical analysis

Data analysis was performed using IBM SPSS Statistics version 24.0 (SPSS Inc., an IBM Company, Chicago, Illinois, USA). Descriptive statistics (including mean, standard deviation and percentages) were calculated for all variables for the overall sample and for each sex separately. Differences between sexes were assessed using Students' T-test for continuous variables and Chi-square test for categorical variables. ROC curves were created to assess the predictive capacity of each PF test to distinguish athletes from non-athletes. The ROC curves, which created an area under the curve (AUC) higher than 65% (0.65)(Pearce, Sinclair, Leicht, & Woods, 2018) for each PF test, were used to signal a greater probability of discrimination. Coefficient interval (CI) was set at 95% and performed by using the nonparametric approach (Hoo, Candlish, & Teare, 2017). The cut-offs determined for the PF tests correspond to the best tradeoff between sensitivity and specificity for identifying athletes from non-athletes in each age-group. These values were calculated using the receiver operating characteristics (ROC) with Youden's index (Ruopp, Perkins, Whitcomb, & Schisterman, 2008). Statistical significance was set at 5%.

RESULTS

Descriptive data of the participants' characteristics and their distribution, according to sex are presented in Table 5.1. For both sexes, age, weight, height, and BMI were not significantly different among athletes and non-athletes.

Table 5.1 – Anthropometric characteristics, level of sports competition and type of sports of youth participants

	Mean ± SD or n						
		Boys (n=915)			Girls (n=916)		
	All (n=1831)	Non-Athletes (n=514)	Athletes (n=401)	p-value	Non-Athletes (n=722)	Athletes (n=194)	p-value
Age (years)	14.1±2.3	14.1±2.3	14.0±2.2	NS	14.3±2.3	13.9±2.2	0.023
Weight (kg)	54.5±13.5	56.3±15.3	56.5±14.2	NS	52.9±12.1	51.7±11.2	NS
Height (cm)	161±11.4	164.5±12.6	164.7±13.0	NS	158.4±8.6	157.5±9.0	NS
BMI (kg/m^2)	20.7±3.6	20.5±3.8	20.5±3.1	NS	20.9±3.8	20.6±3.2	NS
Week Frequency Training (times)	2.0±1.9	1.2±1.7	3.8±1.2	<0.001	1.1±1.6	3.8±1.6	<0.001
Week Duration Training (minutes)	191.3±250.6	98.7±152.3	372.7±188.9	<0.001	86.9±153.5	451.8±415.0	<0.001
Level Sports Competition (n)*							<0.001
Non-Elite	418	-	312		-	107	
Junior-Elite	178	-	86		-	82	
Elite	3	-	2		-	1	
Super-Elite	5	-	1		-	4	

p-value for the T-test in continuous variables to compare differences between non-athletes and athletes.
*Chi-square test to compare differences in proportions level sports competition between boys and girls.
SD, standard deviation; NS, non-significant; BMI, body mass index.

The AUC estimates and the cut-offs for each PF test in the four age-groups of boys and girls are presented in Tables 5.2 and 5.3. The push-up test (for boys 10-11 and 14-15 yrs; for girls 10-11 and 12-13 yrs) and the PACER (for boys 12-13 yrs and for girls 14-15 yrs) appeared to be better discriminators than the other PF tests, while for the oldest age group (≥16 yrs), the PF tests were the speed test at 20m for boys and horizontal jump test for girls. All AUC values in both sexes were over 0.5, suggesting informative diagnostic discriminatory power, except for the speed test at 40m (AUC= 0.50) and vertical jump test (AUC=0.50) in boys 10-11 yrs.

The cut-offs based on the ROC curves showed that for certain PF tests, the values used to identify athletes from non-athletes were either increased or decreased with ageing. For example, the cut-offs for the shuttle-run test (4x10m), and the speed at 20m and 40m all decreased with age, except for the shuttle-run test in girls ≥ 16 yrs, where the cut-off value increased. Conversely, the cut-offs for PACER increase with age for the boys, but not in girls. In both sexes, the cut-offs for horizontal jump increased with age, with the exception of the oldest age group for girls, where the horizontal jump distance decreased 4cm when compared with 14-15 yrs girls. The cut-offs for vertical jump increased with age in girls, while in boys, the values decreased in 12-13 and 14-15 yrs and increased in ≥ 16 yrs, when compared with the youngest age group.

Table 5.2 – Physical fitness tests used to differentiate athlete and non-athlete boys by age group

	Boys (total n=915)							
	10-11 years (non-athletes n=76; athletes n =61)							
Physical Fitness Tests	Non-Athletes	Athletes	p-value	AUC	95% CI	Cut-off	Sensitivity	Specificity
PACER (laps)	29.4±14.3	38.0±20.0	0.004	0.621	0.523-0.718	37.0	0.475	0.197
Push-up (reps)	12.4±10.5	17.0±11.6	0.016	0.678	0.587-0.770	12.0	0.721	0.342
Horizontal jump (cm)	140.2±21.0	147.8±20.0	0.032	0.618	0.523-0.713	147.5	0.590	0.342
Vertical jump (cm)	27.9±6.3	28.4±7.0	NS	0.502	0.403-0.600	34.3	0.230	0.145
Sit and reach (cm)	19.6±6.8	22.4±5.9	0.013	0.618	0.524-0.712	18.3	0.820	0.592
Shuttle run – 4x10m (s)	13.1±1.0	12.7±0.9	0.007	0.622	0.528-0.716	12.5	0.508	0.289
Speed at 20m (s)	4.2±0.4	4.2±0.3	NS	0.523	0.427-0.620	4.5	0.869	0.724
Speed at 40m (s)	7.5±0.7	7.4±0.6	NS	0.496	0.399-0.594	7.1	0.443	0.368
	12-13 years (non-athletes n=143 ; athletes n =103)							
PACER (laps)	41.3±15.9	52.9±19.3	<0.001	0.681	0.612-0.751	52.0	0.553	0.231
Push-up (reps)	13.0±9.6	15.2±8.6	NS	0.594	0.523-0.666	12.0	0.670	0.469
Horizontal jump (cm)	160.9±31.4	161.7±34.1	NS	0.527	0.454-0.601	171.8	0.398	0.308
Vertical jump (cm)	33.6±8.0	33.8±7.5	NS	0.520	0.447-0.593	27.5	0.835	0.748
Sit and reach (cm)	18.6±7.2	20.0±6.8	NS	0.559	0.486-0.638	20.8	0.583	0.392
Shuttle run – 4x10m (s)	12.6±1.0	12.2±1.1	0.013	0.608	0.537-0.680	12.0	0.466	0.280
Speed at 20m (s)	3.9±0.4	3.8±0.4	NS	0.543	0.469-0.616	3.8	0.495	0.385
Speed at 40m (s)	6.9±0.7	6.8±0.7	NS	0.529	0.452-0.601	7.5	0.903	0.825

p-value for the T-test in continuous variables between non-athletes and athletes. AUC, area under curve; CI, confidence interval
NS, non-significant.

(Continued)

Table 5.2 – Physical fitness tests used to differentiate athlete and non-athlete boys by age group (continued)

	Boys Groups (n=915)							
	14-15 years (non-athletes n=127 ; athletes n =127)							
Physical Fitness Tests	Non-Athletes	Athletes	p-value	AUC	95% CI	Cut-off	Sensitivity	Specificity
PACER (laps)	55.5±16.9	71.0±19.3	<0.001	0.723	0.661-0.785	70.0	0.520	0.137
Push-up (reps)	16.0±7.5	22.4±1.0	<0.001	0.688	0.624-0.753	18.0	0.654	0.402
Horizontal jump (cm)	185.1±41.1	197.7±34.0	0.009	0.625	0.556-0.694	195.5	0.638	0.378
Vertical jump (cm)	41.3±8.6	42.8±8.0	NS	0.547	0.476-0.618	31.8	0.953	0.811
Sit and reach (cm)	21.1±8.0	23.1±7.9	NS	0.581	0.510-0.651	30.8	0.142	0.126
Shuttle run – 4x10m (s)	11.9±1.0	11.3±0.7	<0.001	0.662	0.596-0.729	11.9	0.858	0.567
Speed at 20m (s)	3.6±0.4	3.5±0.3	<0.001	0.654	0.586-0.721	3.6	0.787	0.480
Speed at 40m (s)	6.3±0.6	6.1±0.5	0.011	0.587	0.516-0.657	6.1	0.685	0.488
	≥ 16 years (non-athletes n=110 ; athletes n =168)							
PACER (laps)	61.6±18.1	75.4±20.5	<0.001	0.691	0.627-0.755	74.0	0.555	0.220
Push-up (reps)	20.6±9.2	23.3±7.0	0.010	0.600	0.533-0.666	18.0	0.873	0.685
Horizontal jump (cm)	183.0±62.0	186.5±73.0	NS	0.595	0.527-0.664	200.5	0.654	0.440
Vertical jump (cm)	45.5±9.4	47.9±8.1	0.030	0.578	0.510-0.646	42.3	0.655	0.500
Sit and reach (cm)	24.6±8.3	25.9±8.0	NS	0.539	0.470-0.608	23.9	0.625	0.554
Shuttle run – 4x10m (s)	11.4±0.8	10.9±0.7	<0.001	0.672	0.607-0.738	10.8	0.564	0.256
Speed at 20m (s)	3.6±0.4	3.4±0.3	<0.001	0.702	0.640-0.765	3.4	0.564	0.238
Speed at 40m (s)	6.1±0.5	5.9±0.4	0.001	0.615	0.548-0.683	5.9	0.609	0.405

p-value for the T-test in continuous variables between non-athletes and athletes. AUC, area under curve; CI, confidence interval
NS, non-significant.

Table 5.3 – Physical fitness tests used to differentiate athlete and non-athlete girls by age group

	Girls (total n=916)							
	10-11 years (non-athletes n=100; athletes n =31)							
Physical Fitness Tests	Non-Athletes	Athletes	p-value	AUC	95% CI	Cut-off	Sensitivity	Specificity
PACER (laps)	22.6±9.0	33.0±18.5	0.005	0.638	0.512-0.763	43.0	0.355	0.040
Push-up (reps)	9.9±7.7	17.5±10.1	<0.001	0.733	0.632-0.834	15.0	0.581	0.200
Horizontal jump (cm)	133.3±19.3	145.7±20.1	0.002	0.674	0.565-0.783	152.0	0.419	0.130
Vertical jump (cm)	26.6±6.1	30.0±3.9	0.004	0.719	0.624-0.813	30.0	0.667	0.250
Sit and reach (cm)	24.1±6.0	28.3±6.5	0.001	0.688	0.572-0.803	28.0	0.548	0.250
Shuttle run – 4x10m (s)	13.4±0.9	12.8±0.7	0.001	0.707	0.608-0.806	13.3	0.839	0.440
Speed at 20m (s)	4.3±0.3	4.2±0.3	0.009	0.657	0.552-0.762	4.3	0.774	0.470
Speed at 40m (s)	7.8±0.7	7.4±0.4	0.006	0.688	0.585-0.791	7.7	0.806	0.460
	12-13 years (non-athletes n=174 ; athletes n =48)							
PACER (laps)	31.8±11.0	43.0±14.6	<0.001	0.725	0.643-0.806	40.0	0.583	0.213
Push-up (reps)	8.9±5.5	20.3±10.9	<0.001	0.865	0.809-0.921	14.0	0.771	0.213
Horizontal jump (cm)	142.4±29.7	164.7±17.5	<0.001	0.757	0.685-0.828	156.0	0.708	0.333
Vertical jump (cm)	28.5±7.3	32.1±6.0	0.002	0.633	0.545-0.720	32.8	0.500	0.282
Sit and reach (cm)	24.9±7.4	29.1±7.3	0.001	0.638	0.543-0.733	29.9	0.479	0.236
Shuttle run – 4x10m (s)	13.2±1.0	12.3±0.7	<0.001	0.776	0.707-0.845	12.7	0.771	0.322
Speed at 20m (s)	4.1±0.4	4.0±0.4	NS	0.551	0.463-0.640	4.2	0.771	0.615
Speed at 40m (s)	7.2±0.6	7.1±0.5	NS	0.576	0.486-0.665	7.0	0.542	0.379

p-value for the T-test in continuous variables between non-athletes and athletes. AUC, area under curve; CI, confidence interval
NS, non-significant.

(Continued)

Table 5.3 – Physical fitness tests used to differentiate athlete and non-athlete girls by age group (continued)

	Girls Groups (n=916)							
	14-15 years (non-athletes n=195 ; athletes n =63)							
Physical Fitness Tests	Non-Athletes	Athletes	p-value	AUC	95% CI	Cut-off	Sensitivity	Specificity
PACER (laps)	33.4±11.5	44.4±13.5	<0.001	0.731	0.661-0.802	39.0	0.683	0.297
Push-up (reps)	9.7±7.1	15.4±9.3	<0.001	0.689	0.615-0.764	10.0	0.746	0.472
Horizontal jump (cm)	146.5±34.7	163.4±40.8	0.001	0.679	0.601-0.756	161.5	0.651	0.328
Vertical jump (cm)	30.8±7.1	35.0±5.4	<0.001	0.697	0.626-0.769	33.0	0.635	0.338
Sit and reach (cm)	29.3±14.0	31.4±7.7	NS	0.571	0.489-0.652	36.2	0.286	0.138
Shuttle run – 4x10m (s)	13.0±1.1	12.4±0.9	<0.001	0.678	0.602-0.755	12.2	0.524	0.215
Speed at 20m (s)	4.1±0.3	3.8±0.3	<0.001	0.725	0.656-0.795	4.0	0.730	0.410
Speed at 40m (s)	7.2±0.6	7.0±0.5	0.001	0.651	0.576-0.726	7.1	0.651	0.405
	≥ 16 years (non-athletes n=253 ; athletes n =52)							
PACER (laps)	36.4±11.7	42.0±16.4	0.023	0.602	0.516-0.689	34.5	0.673	0.510
Push-up (reps)	11.5±6.4	16.0±8.1	<0.001	0.662	0.578-0.746	15	0.654	0.316
Horizontal jump (cm)	133.3±49.9	165.1±43.0	<0.001	0.753	0.678-0.828	157.5	0.750	0.300
Vertical jump (cm)	30.7±6.1	37.7±9.8	<0.001	0.727	0.641-0.812	36.8	0.596	0.150
Sit and reach (cm)	29.8±8.1	31.2±9.9	NS	0.553	0.459-0.646	39.3	0.231	0.079
Shuttle run – 4x10m (s)	12.9±1.0	12.2±1.0	<0.001	0.701	0.621-0.781	12.9	0.904	0.597
Speed at 20m (s)	4.1±0.3	3.8±0.4	<0.001	0.699	0.623-0.776	3.9	0.519	0.229
Speed at 40m (s)	7.3±0.5	7.0±0.6	0.003	0.640	0.553-0.726	6.7	0.404	0.150

p-value for the T-test in continuous variables between non-athletes and athletes. AUC, area under curve; CI, confidence interval
NS, non-significant.

Our results indicate that athletes had better PF than non-athletes, however, this was not verified for all tests, especially in boys. The PACER, the push-up test, and the shuttle run test (4x10m) were significantly higher in athletes for both sexes and across all age groups.

DISCUSSION

To our knowledge, this is the first investigation to use a ROC curve analysis to identify the discriminatory power and the cut-off values of several PF tests for distinguishing young athletes from non-athletes of different ages and by sex. It is a misconception that young athletic talent can be identified by comparing their athletic profile to adult athletes (N. Armstrong & McManus, 2011; Reilly, Bangsbo, & Franks, 2000). Some investigations have shown that approaches comparing or associating adolescent characteristics with adult characteristics have a higher probability of misclassification when it comes to identifying future athletes (J. Baker et al., 2017; Pearson, Naughton, & Torode, 2006). Thus, using a sample of athlete and non-athlete children and adolescents to generate ROC curves in order to discriminate athletic status may be a more suitable approach. In this investigation, the ROC curves generated an AUC higher than 65% (Pearce et al., 2018) in at least one PF test per sex and age group, suggesting that PF tests can be used as a surrogate to differentiate athletes from non-athletes. Our findings suggest that athlete identification was more accurate in girls than in boys, and it is interesting to verify the different ability of specific PF tests to discriminate athletes among groups. From all tests, the PACER and the push-up test were the ones that showed higher ability to discriminate athletes from non-athletes.

High levels of aerobic capacity are commonly associated with excellent indicators of sports performance and recovery (Mohr, Krustrup, & Bangsbo, 2003), which explains why PACER presented itself as a good discriminatory test for identifying athletes. The push-up test usually expresses the capacity to produce upper muscular strength and power in several sporting events, thus working as a tool for identification purposes (Wang et al., 2017). Nevertheless, it is essential to note that there were differences in the discriminatory ability of each PF test across age groups and sexes. For boys in age groups 10-11 and 14-15 yrs, the push-up test had the best discriminatory ability, whereas, for boys in the age groups 12-13 and ≥16 yrs, the PACER was better able to discriminate the athletic condition. The 20m speed test and shuttle run (4x10m) test were also strongest on identifying athletes in boys ≥ 16 yrs. Compared to boys, a higher number of PF tests presented the ability to discriminate between athletes and non-athletes girls. The push-up test had the best predictive ability in the 10-11 yrs girls. For the 12-13 yrs age group, four tests, including the PACER, push-up, horizontal jump, and shuttle run (4x10m) presented the ability to discriminate the athletic condition. For the older age group of girls, the push-up, horizontal jump, vertical jump, shuttle-run (4x10m), and 20m speed test were able to discriminate between athletes and non-athletes. Of all PF tests, the speed at 20 and 40m in the 12-13 yrs age group, the shuttle-run (4x10m) in the 14-15 yrs, and the sit and reach in the 14-15 and ≥ 16 yrs groups were the tests with the AUC below 65%, meaning that they do not reached the minimal criteria to identify athletes.

The observed differences between boys and girls in the discriminatory capacity of the PF tests, i.e., the fact that for girls more PF tests were able to discriminate the athletic condition, may be explained by two main reasons. First, boys have higher levels of leisure-time physical activity than girls (Telford, Telford, Olive, Cochrane, & Davey, 2016), which could possibly blur PF differences between athletes and non-athletes in boys. This factor stands out on a higher number of PF tests that were significantly different between athletes and non-athletes in girls compared to boys. Secondly, in this investigation a higher percentage of boys were non-elite athletes when compared to girls. Considering that the intensity and frequency of the training sessions are lower in this athletic category, this could lead to a lower specificity of the PF tests to identify the athlete population in boys (Pearce et al., 2018; Ramos, Volossovitch, Ferreira, Fragoso, & Massuça, 2019).

Along with the discriminatory power analysis, specific cut-off values for each PF test are proposed for identifying athletes. These cut-off values can have practical utility for talent identification programs but also for promoting youth sports participation, because adolescents that do not reach one specific test-related cut-off may still attain the cut-off of other tests that are also relevant and in this way integrate another sport that matches his abilities. This includes, for instance, government bodies, sports federations, parents, physical education teachers and coaches (Bergeron et al., 2015; Bonney, Berry, Ball, & Larkin, 2019). Although these cut-off values have high practical utility, some aspects must be taken into consideration when using them. As these values were generated from a ROC curve analysis, it is expected that similarly to the discriminatory power, they have better applicability in girls than in boys. According to previous investigations,, even in boys some tests, such as the PACER, push-up, and speed at 20m depending on the age group, have demonstrated to be valid discriminatory tools (Gil et al., 2014; J. Pion, Lenoir, Vandorpe, & Segers, 2015; Till, Cobley, Morley, et al., 2015). To the best of our knowledge, this is the first investigation proposing cut-off values for PF tests to be used for identifying young athletes considering specific age groups and based on a heterogeneous sample including different sports.

Despite the practical utility of the findings from this investigation, it is essential to recognise some limitations. First, this is a cross-sectional investigation making it impossible to observe all biologic changes across adolescence. The second limitation consists of the non-homogeneous distribution of competition levels between sexes and age groups, which impacts the cut-off values. Moreover, another limitation is the lack of information about other characteristics, which may be related with a youth athlete profile (i.e. maturity, psychologic profile, social environment), that could further aid in the discrimination of athletes from non-athletes. It is relevant to highlight that this investigation included a large sample of youth, which is a strength. As per the results in this investigation, it is possible to establish that PF plays an important role in identifying athletes in youth, supporting some previous organization models in this field (Bonney et al., 2019). Further investigations should assess more variables with a multidimensional approach (e.g. psychological, sociological, morphological, and motor) and include a more homogeneous group of sports type and competition levels across the sexes and age groups (Johnston et al., 2017).

CONCLUSION

The present investigation examined the ability of commonly used PF tests that are currently used to identify healthy from non-healthy youth, to discriminate young athletes, while proposing cut-off values for each of these tests. The ROC curves generated an AUC higher than 65% in at least one PF test per sex and age group, demonstrating that it is possible to differentiate athletes from non-athletes using some of these tests. Furthermore, the speed test at 20m and the horizontal jump were the best tests to identify older adolescents with athletic potential, while the PACER and push-up tests were the most discriminatory for the younger adolescents. The ability of specific PF tests to discriminate athletes among distinct age groups and sex must be taken into account. Thus, some PF tests can be a viable resource for government agencies, sports federations, physical education teachers, and coaches not only to monitor health status in youth but also to identify potential athletes and in this way increase the number of young athletes included in the high-performance sport system.

PRACTICAL APPLICATIONS

There are three essential considerations of this investigation. First, the PF tests from FITescola® battery can be used by physical education teachers, coaches, and government bodies to better identify young athletes from schools or sports entities. For example, adolescents with potential athletic ability can be identified by physical education teachers during physical education classes as they already perform these tests to monitor health status. Secondly, for the coaches and athletes, the thresholds generated in the present investigation for each PF test, by sex and age, can be applied to track their sport progression. Finally, FITescola® PF tests are simple, low cost, and can be easily applied in several settings.

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CHAPTER 6

Physical Fitness and Bone Health in Young Athletes and Non-athletes

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Physical Fitness and Bone Health in Young Athletes and Nonathletes

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Background: Physical activity (PA) and physical fitness (PF) are crucial for bone health. However, children participating in competitive sports with high PA are at a greater risk of fracture from trauma or overuse. Given the importance of bone development during adolescence, associations between commonly used physical fitness tests with distal third radius (R-SoS) and midshaft tibia (T-SoS) speed of sound by quantitative ultrasound were assessed in adolescent athletes and nonathletes.

Hypothesis: The relationship between physical fitness tests and R-SoS and T-SoS will differ depending on sex and athletic status.

Study Design: Cross-sectional study.

Level of Evidence: Level 3.

Methods: Physical fitness of 285 boys (156 athletes) and 311 girls (74 athletes) aged 10 to 18 years was assessed through strength, speed, agility, and cardiorespiratory tests. Linear regression was used to assess the associations of physical fitness tests with R-SoS and T-SoS.

Results: For boys, favorable associations were observed between physical fitness tests with R-SoS in athletes and T-SoS in both athletes and nonathletes ($P < 0.05$). For nonathlete girls, favorable associations were found for handgrip (R-SoS and T-SoS, both $P < 0.05$), whereas the progressive aerobic cardiovascular endurance run, vertical jump, speed at 20 m and 40 m were only favorably associated with T-SoS. For athlete girls, the association between handgrip ($P = 0.03$), vertical jump, and 4 x 10 m shuttle run ($P < 0.05$) with T-SoS was significantly related to a bone outcome.

Conclusion: The handgrip test and vertical jump were associated with T-SoS in boys and girls independent of sport status. These results suggest that physical fitness is associated with bone health in adolescents, particularly boys, and that the relationship between physical fitness and bone may differ depending on sex and athletic status.

Clinical Relevance: Physical fitness tests are simple, easy-to-use tools for monitoring bone health and should be used by sport and health professsionals to promote healthy sport participation and prevent bone injuries.

Keywords: adolescent; PACER; muscular fitness; bone health; quantitative ultrasound

Although osteoporosis symptoms usually do not manifest until later in life, the disease is thought to have roots at the beginning of childhood.[16] Osteoporosis and bone fractures are considered a significant public health matter worldwide,[3,24] with 1 in 2 women and 1 in 5 men older than 50 years of age expected to suffer an osteoporotic fracture in their lifetime. Primetime for osteoporosis prevention is in childhood, particularly during adolescence, as this is a time characterized by a fast-growing skeleton[7] where the majority of the adult bone mineral is being laid down.[20] Hence, maximizing bone

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mineral mass during this time will have a significant impact on bone health later in adulthood and older age.[30]

A healthy lifestyle with regular physical activity (PA), including sports participation, and healthy physical fitness levels is crucial for bone health.[25] Upper limb strength,[8] lower limb strength,[26] agility,[13] and speed[13] have all been positively associated with several bone health outcomes in youth.[19] Thus, promoting youth sports participation has been a strategy to increase physical fitness levels, decrease patterns of inactivity,[10] and increase peak bone mass in youth.[28] However, it is essential to note that despite these positive benefits, there are transient regional deficits in the bones of children. Increased porosity and thinning of cortical bone, especially during the rapid longitudinal growth period of adolescence,[11] may put children participating in high levels of PA, such as competitive sports, at a greater risk of fracture from a single episode of trauma or repetitive overuse.[27] Indeed, an increased incidence in stress fractures has been reported in children due to sport participation.[21] Given the importance of the adolescent period for bone development,[12] it is crucial to understand how various levels of PA influences bone health.

Some physical fitness batteries were developed with the goal of monitoring health, given the associations between physical fitness levels and several health outcomes, such as adiposity and bone health.[14,26] These field-based physical fitness batteries are easy to administer, require minimal equipment, and have demonstrated good validity and reliability in a school setting.[22] However, how well these physical fitness batteries are related to bone health in children is not clear. Therefore, the aim of this investigation was to assess the associations between different physical fitness tests with bone strength of the radius and tibia using quantitative ultrasonography (QUS) in adolescents with different levels of PA (ie, athletes and nonathletes).

METHODS

Participants

The total sample consisted of 596 participants, aged 10 to 18 years, with no history of stress fractures (ascertained by questionnaire). Of these, 285 were boys (156 athletes) and 311 were girls (74 athletes). The participants were recruited from several schools or official federative club teams within the greater Lisbon and Porto area. All participants who were considered athletes needed to be engaged in organized sports (eg, athletics, basketball, handball, football, swimming), with regular participation in the official competition organized by each national federation recognized by the Portuguese Olympic Committee and the Portuguese Institute of Sports and Youth in the past 12 months. Participants gave their assent, and informed consent was obtained from their legal guardians.

This cross-sectional investigation was conducted according to the Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects[31] and approved by the ethics committee of the Faculty of Human Kinetics, University of Lisbon (CEFMH approved; No. 19/2018).

Body Composition

All participants were weighed to the nearest 0.01 kg on an electronic scale (model 799; Seca) while wearing minimal clothing and no shoes. Height was measured twice to the nearest 0.1 cm with a stadiometer (model 220; Seca). Final height was obtained by using the mean value of the 2 measurements. Body mass index (BMI) was calculated as body mass (kg)/height^2 (m^2). All assessments were conducted by the same trained researcher.

Maturity

Height and decimal age were used to calculate the age of peak height velocity (PHV) using sex-specific equations established by Moore et al.[17]

Type of Physical Exercise

The different types of physical exercise were categorized as follows: nonosteogenic (eg, swimming, water polo, equestrian) and osteogenic (ie, football, handball, volleyball). Those who did not participate regularly in any sport were classified as nonexercisers. This categorization has been used previously in bone health studies in adolescents.[29]

Physical Fitness

Handgrip strength (HG) was assessed using an analog adjustable dynamometer (Lafayette Hand Dynamometer; Lafayette Instrument Company, Lafayette, Indiana). Participants were given a demonstration and verbal instructions before the individual test. The test was performed with the participant in a standing position with the shoulders neutrally rotated in abduction and arms parallel without being in contact with the body. The participants were asked to squeeze the handle for a maximum of 3 to 5 seconds. Verbal encouragement was given during the test. Two trials were performed for each limb, and the maximum score was recorded in kilograms.

As far as the other physical fitness tests were concerned, we used the FITescola fitness battery, which is currently being used in the Portuguese school setting. Progressive aerobic cardiovascular endurance run (PACER) test, horizontal jump (HJ) and vertical jump (VJ) tests, 4 x 10-m shuttle run test, and 20-m and 40-m speed tests were used to examine cardiorespiratory fitness, upper body and lower body muscular fitness, agility, and speed, respectively. A full description of each test can be found in the study by Gracia-Marco et al.[13]

Bone Speed of Sound

Bone speed of sound (SoS) was assessed by QUS at the radial distal third (R-SoS) and tibial midshaft (T-SoS) on the nondominant limb using a sonometer (Omnisense 8000s; Sunlight Omnisense, BeamMed). The same researcher conducted all measurements, which have been previously described,[4] after a daily calibration and according to specific manufacturer's instructions. Before performing SoS analyses, the participant's age, height, weight, and sex were entered into the program. Therefore, SoS analyses were adjusted to these variables.

Calcium Intake

A semiquantitative questionnaire used on Portuguese adolescents in a similar investigation[15] was used to assess the intake of a full set of typical Portuguese foods in the previous month.

Statistical Analysis

Statistical analyses were performed using SPSS (Version 24.0; IBM Corp). Descriptive statistics, including means ± SD, were calculated for all outcome measurements. Comparison between athlete status and sex were made by using analysis of variance for all variables. Also, given the existence of an interaction effect for sex ($P < 0.01$) and athlete group ($P < 0.01$), all analyses were performed separately for these groups. Linear regression analysis was used to examine the associations between the physical fitness tests and R-SoS and T-SoS according to sports status and sex.

Two models were tested. Model 1 was unadjusted, and model 2 was adjusted for type of physical exercise, number of minutes exercising per week, age of PHV, and calcium intake. A variance inflation factor for each independent variable was calculated to evaluate multicollinearity, and the <5.0 cutoff was considered an indicator of nonmulticollinearity. Significance was set at $P < 0.05$.

RESULTS

Table 1 presents the sample characteristics for the total sample, by sex and by athlete status. In boys and girls, the values for the time of physical exercising per week, PACER, HJ, VJ, 4 x 10-m shuttle run test, and speed at 20 m test were higher in athletes than for nonathletes ($P < 0.05$). Significant ($P < 0.05$) differences between nonathlete and athlete girls were observed for maturity offset and handgrip values. Inversely, athlete girls showed lower values of R-SoS and T-SoS than nonathlete girls ($P = 0.04$).

As far as the type of physical exercise (nonosteogenic and osteogenic) was concerned, and considering only the main groups who engaged in federative sport, 89.2% of girls engaged in osteogenic physical exercise and 10.8% in nonosteogenic physical exercise, whereas 94.9% of the boys engaged in osteogenic physical exercise and only 5.1% in nonosteogenic physical exercise. Among nonathlete boys, 48.1% were nonexercisers. From the remaining, 36.4% engaged in osteogenic physical exercise while 19.5% were in nonosteogenic physical exercise. On the other hand, 47.7% of girls did not practice any physical exercise, while 13.5% engaged in nonosteogenic physical exercise and 38.8% practiced osteogenic physical exercise.

The unadjusted and adjusted (type of physical exercise, number of minutes exercising per week, PHV age, and calcium intake) results of linear regression analyses assessing the associations of physical fitness tests with R-SoS and T-SoS are presented in Tables 2 and 3 for boys and girls, respectively. After adjusting for type of physical exercise, the number of minutes exercising, PHV age, and calcium intake, we observed favorable associations between physical fitness tests and T-SoS in both athlete and nonathlete boys, except the 4 x 10-m shuttle run test in male nonathletes ($P = 0.05$). For the radial bone, only the HG and the speed at 40 m tests were associated with higher R-SoS in the athlete boys ($P < 0.05$), whereas no associations were observed in nonathletes.

For girls, when considering the adjusted model, we observed favorable associations for the HG test with R-SoS, but only in nonathletes ($P < 0.05$). However, the HG test and VJ test were positively associated with T-SoS in both the girl athletes and nonathletes. Regarding the other tests, higher number of laps in the PACER test ($P = 0.02$) and a shorter time in speed at 20 m ($P < 0.01$) and speed at 40 m ($P < 0.01$) were associated with higher T-SoS in girl nonathletes, while positive association with the shorter time in 4 x 10-m shuttle run ($P < 0.01$) was observed in girl athletes.

Figure 1 summarizes the results of the associations of R-SoS and T-SoS with physical fitness tests for boys and girls and between athletes and nonathletes. Overall, we observed stronger associations of the physical fitness tests with R-SoS and T-SoS in boys, especially those who engaged in federative sports, compared with girls. Of the 2 bone locations, stronger relationships were observed between the T-SoS and physical fitness tests versus R-SoS in both male and female athletes and nonathletes.

DISCUSSION

Most of the physical fitness tests in boys were significantly associated with T-SoS in both athletes and nonathletes. On the other hand, fewer associations were found for girls between the physical fitness tests and T-SoS, especially in the athletic population, where HG, VJ, and 4 x 10-m shuttle run were the only significant tests. As for R-SoS, most associations with the physical fitness tests (HG and 4 x 10-m shuttle run) were observed in boys who were involved in federative sports, while only the nonathlete girls presented 1 association (HG).

Several studies have suggested that higher test results for HG,[13] HJ,[8] and VJ,[5] as well as faster times for the speed tests[13] and 4 x 10-m shuttle run,[13] were positively associated with bone health in adolescents. Our results are in line with these findings, even when using a different method to assess bone parameters (ie, QUS vs dual-energy X-ray absorptiometry [DXA]), especially in the associations between physical fitness tests and bone parameters in boys. The speed of sound by QUS is highly correlated with several bone characteristics assessed by DXA[23] and reflects a range of bone status factors, such as elasticity, structure, microstructure, and cortical thickness, which are essential aspects of overall bone strength.[6]

Despite the overall beneficial associations between the physical fitness tests and SoS, these associations differed depending on sex and athlete status. In girls, the HJ was not significantly related to bone SoS. In boys, however, most of the physical fitness tests were significantly correlated with T-SoS, with more associations being observed in athletes. Additionally, the associations observed between the physical fitness tests and

Table 1. Descriptive characteristics of youth participants

		Boys (n = 285)		Girls (n = 311)	
	All (N = 596), Mean ± SD	Nonathletes (n = 129), Mean ± SD	Athletes (n = 156), Mean ± SD	Nonathletes (n = 237), Mean ± SD	Athletes (n = 74), Mean ± SD
Age, y[a]	14.8 ± 2.3	14.09 ± 2.4	14.7 ± 2.3	15.1 ± 2.3	13.9 ± 2.4
Weight, kg[b,c]	53.5 ± 12.5	55.6 ± 14.3	56.4 ± 13.4	51.7 ± 10.5	49.3 ± 11.4
Height, cm[b,c]	160.8 ± 11.5	163.5 ± 12.9	165.0 ± 12.9	158.0 ± 8.6	156.1 ± 9.6
BMI, kg/m^2	20.4 ± 3.2	20.5 ± 3.6	20.4 ± 2.9	20.5 ± 3.1	20.1 ± 3.4
Maturity offset, y[a,b]	1.6 ± 2.0	0.8 ± 1.9	1.9 ± 0.2	2.4 ± 1.9	1.5 ± 1.9
PHV age, y[a,b,c]	13.2 ± 0.9	14.0 ± 0.6	13.8 ± 0.5	12.7 ± 0.6	12.4 ± 0.6
Total calcium intake, mg/d	918.9 ± 437.7	882.3 ± 442.4	966.8 ± 409.7	912.0 ± 445.1	903.4 ± 462.8
Volume of exercise, min/wk[a,d]	199.8 ± 139.2	112.44 ± 152.6	354.2 ± 160.8	101.3 ± 138.1	342.4 ± 174.2
Type of exercise, %					
Nonexercisers	29.4	48.1	NA	47.7	NA
Nonosteogenic exercise	11.4	15.5	5.1	13.5	10.8
Osteogenic exercise	59.2	36.4	94.9	38.8	89.2
Physical fitness					
Handgrip, kg[b,c]	31.3 ± 8.6	34.3 ± 9.6	35.2 ± 10.6	28.0±5.0	27.8 ± 5.4
PACER, laps[a,b,c,d]	45.3 ± 20.5	48.5 ± 18.7	62.4 ± 22.7	33.8 ± 11.4	40.4 ± 13.8
Horizontal jump, cm[a,b,c,d]	166.6 ± 33.6	179.2 ± 34.9	188.48 ± 31.9	146.0 ± 21.6	164.4 ± 26.8
Vertical jump, cm[a,b,c,d]	35.0 ± 9.6	37.7 ± 10.1	40.7 ± 10.6	30.1 ± 6.4	33.6 ± 5.9
Shuttle run, 4 × 10 m, s[a,b,c,d]	12.3 ± 1.1	12.1 ± 1.0	11.5 ± 1.1	12.9 ± 0.9	12.4 ± 0.9
Speed at 20 m, s[a,b,c,d]	3.9 ± 0.4	3.9 ± 0.5	3.6 ± 0.4	4.2 ± 0.3	4.0 ± 0.3
Speed at 40 m, s[a,b,c]	6.9 ± 0.8	6.7 ± 0.9	6.3 ± 0.7	7.3 ± 0.6	7.0 ± 0.5
Bone health					
Radial speed of sound, m/s[b]	3866.7 ± 198.0	3839.6 ± 139.4	3814.0 ± 139.9	3914.0 ± 256.6	3873.8 ± 128.0
Tibial speed of sound, m/s[a,b,c]	3768.2 ± 133.0	3753.6 ± 121.1	3717.7 ± 120.7	3810.4 ± 138.8	3764.8 ± 117.2

BMI, body mass index; PACER, progressive aerobic cardiovascular endurance run; PHV, peak height velocity; NA, not applicable.
[a] $P \leq 0.05$, significant difference between female nonathletes and female athletes.
[b] $P \leq 0.05$, significant difference between male nonathletes and female nonathletes.
[c] $P \leq 0.05$, significant difference between male athletes and female athletes.
[d] $P \leq 0.05$, significant difference between male nonathletes and male athletes.

Table 2. Associations for physical fitness with ultrasound variables in boy nonathletes and athletes[a]

Physical Fitness Tests	Third Distal Radius Ultrasound (R-SoS)											
	Nonathletes						Athletes					
	Model 1 *B*	95% CI	R^2	Model 2 *B*	95%CI	R^2	Model 1 *B*	95%CI	R^2	Model 2 *B*	95% CI	R^2
Handgrip, kg	4.5**	2.07, 6.93	0.31	1.32	−1.66, 4.30	0.44	4.1**	2.13, 6.15	0.31	2.78*	0.41, 5.15	0.40
PACER, laps	1.70*	0.43, 2.97	0.23	0.05	−1.43, 1.53	0.43	1.18	0.22, 2.14	0.19	0.37	−0.67, 1.14	0.36
Horizontal jump, cm	0.87*	0.19, 1.55	0.22	0.08	−0.69, 0.85	0.43	1.37**	0.71, 2.03	0.31	0.72	−0.15, 1.59	0.38
Vertical jump, cm	3.86	1.54, 6.17	0.28	1.02	−1.68, 3.72	0.43	3.21*	1.18, 5.23	0.24	1.44	−0.88, 3.75	0.37
Shuttle run, 4 × 10 m, s	−35.78*	−59.20, −12.35	0.26	−6.47	−33.54, 20.60	0.43	−36.07**	−55.34, −16.80	0.29	−18.30	−44.67, 4.06	0.38
Speed at 20 m, s	−69.43*	−120.85, −18.01	0.23	−4.60	−63.44, 54.25	0.43	112.93**	−161.48, −64.39	0.35	−72.11*	−133.65, −10.61	0.40
Speed at 40 m, s	−50.36**	−76.40, −24.32	0.32	−18.68	−50.99, 13.65	0.44	−60.25**	−89.95, −30.56	0.31	−34.29	−71.42, 2.84	0.38
	Midshaft Tibia Ultrasound (T-SoS)											
	Nonathletes						**Athletes**					
Physical Fitness Tests	**Model 1 *B***	**95% CI**	R^2	**Model 2 *B***	**95% CI**	R^2	**Model 1 *B***	**95% CI**	R^2	**Model 2 *B***	**95% CI**	R^2
Handgrip, kg	7.01**	5.16, 8.86	0.55	4.33**	2.09, 6.58	0.63	6.22**	4.69, 7.75	0.54	3.90**	2.22, 5.57	0.66
PACER, laps	3.45**	2.49, 4.41	0.53	2.15**	1.04, 3.26	0.62	2.11**	1.33, 2.89	0.40	0.88*	0.12, 1.65	0.61
Horizontal jump, cm	1.79**	1.27, 2.31	0.52	1.13**	0.55, 1.70	0.63	2.07**	1.56, 2.57	0.55	1.06*	0.43, 1.69	0.63
Vertical jump, cm	6.60**	4.86, 8.35	0.55	4.18**	2.17, 6.20	0.63	5.76**	4.20, 7.31	0.51	3.29**	1.65, 4.93	0.64
Shuttle run, 4 × 10 m, s	−5.75**	−70.75, −32.74	0.43	−20.93	−42.12, 0.26	0.58	−55.78**	−70.68, −40.89	0.51	−29.80**	−45.84, −13.77	0.64
Speed at 20 m, s	133.37*	−172.85, −93.90	0.51	−77.46*	−122.11, −32.81	0.61	−170.66**	−206.09, −135.22	0.61	−111.47**	−154.31, −68.63	0.67
Speed at 40 m, s	−79.95**	−99.28, −60.62	0.59	−54.49**	−78.36, −30.54	0.64	−100.14**	−121.83, −78.45	0.59	−64.96**	−90.77, −39.16	0.67

PACER, progressive aerobic cardiovascular endurance run.

[a]Model 1 is unadjusted, and model 2 is adjusted for type of exercise, time of practice per week, peak height velocity, age, and total calcium intake.

*$P < 0.05$, **$P < 0.01$.

Table 3. Associations for physical fitness with ultrasound variables in girl nonathletes and athletes[a]

	Third Distal Radius Ultrasound (R-SoS)											
	Nonathletes						Athletes					
Physical Fitness Tests	Model 1 *B*	95% CI	R^2	Model 2 *B*	95% CI	R^2	Model 1 *B*	95% CI	R^2	Model 2 *B*	95% CI	R^2
Handgrip, kg	7.6*	1.03, 14.20	0.15	6.83*	0.14, 13.53	0.23	3.89	−1.57, 9.35	0.17	3.32	−1.10, 7.74	0.65
PACER, laps	2.93*	0.06, 5.80	0.13	1.75	−1.30, 4.79	0.20	0.28	−1.90, 2.46	0.03	−0.32	−2.24, 1.60	0.63
Horizontal jump, cm	0.34	−1.18, 1.87	0.03	0.04	−1.49, 1.57	0.18	0.34	−0.78, 1.46	0.07	0.14	−0.78, 1.05	0.63
Vertical jump, cm	0.64	−4.52, 5.80	0.02	0.30	−4.85, 5.45	0.19	−0.17	−5.24, 4.91	0.01	−0.91	−5.03, 3.20	0.63
Shuttle run, 4 × 10 m, s	0.23	−35.24, 35.69	0.01	2.65	−32.75, 38.04	0.19	−2.25	−37.12, 32.61	0.02	−3.18	−32.13, 25.77	0.63
Speed at 20 m, s	−21.49	−123.26, 80.28	0.03	−8.22	−110.88, 94.43	0.19	−63.81	−169.12, 41.50	0.14	−2.11	−90.83, 86.61	0.63
Speed at 40 m, s	−41.69	−100.62, 17.25	0.09	−30.75	−90.26, 28.76	0.20	−20.94	−82.90, 41.03	0.08	10.86	−40.32, 62.04	0.63
	Midshaft Tibia Ultrasound (T-SoS)											
	Nonathletes						**Athletes**					
Physical Fitness Tests	**Model 1 *B***	**95% CI**	**R^2**	**Model 2 *B***	**95% CI**	**R^2**	**Model 1 *B***	**95% CI**	**R^2**	**Model 2 *B***	**95% CI**	**R^2**
Handgrip, kg	7.42**	3.95, 10.89	0.27	5.74*	2.36, 9.11	0.42	5.39*	0.48, 10.30	0.25	4.90*	0.39, 9.41	0.52
PACER, laps	2.92**	1.40, 4.44	0.24	1.83*	0.28, 3.37	0.39	1.03	−0.95, 3.02	0.12	0.91	−1.07, 2.89	0.48
Horizontal jump, cm	1.02*	0.21, 1.84	0.16	0.77	−0.01, 1.55	0.39	0.77	−0.24, 1.78	0.18	0.74	−0.19, 1.68	0.50
Vertical jump, cm	3.28*	0.68, 5.88	0.16	3.28*	0.68, 5.88	0.40	5.19*	0.71, 9.67	0.26	4.56*	0.41, 8.69	0.53
Shuttle run, 4 × 10 m, s	17.44	−36.48, 1.61	0.12	−17.43	−35.40, 0.53	0.39	−46.22*	−76.26, −16.17	0.34	−47.02*	−74.88, −19.16	0.58
Speed at 20 m, s	−96.51**	−150.15, −42.88	0.23	77.72*	−129.27, −26.17	0.41	−104.79*	−199.07, −10.52	0.25	−65.66	−156.49, 25.17	0.50
Speed at 40 m, s	−65.77**	−96.64, −34.91	0.26	−51.08*	−80.87, −21.29	0.42	−64.69*	−119.56, −9.81	0.27	−42.03	−94.32, 10.26	0.50

PACER, progressive aerobic cardiovascular endurance run.
[a]Model 1 is unadjusted, and model 2 is adjusted for type of exercise, time of practice per week, peak height velocity, age, and total calcium intake.
*$P < 0.05$, **$P < 0.01$.

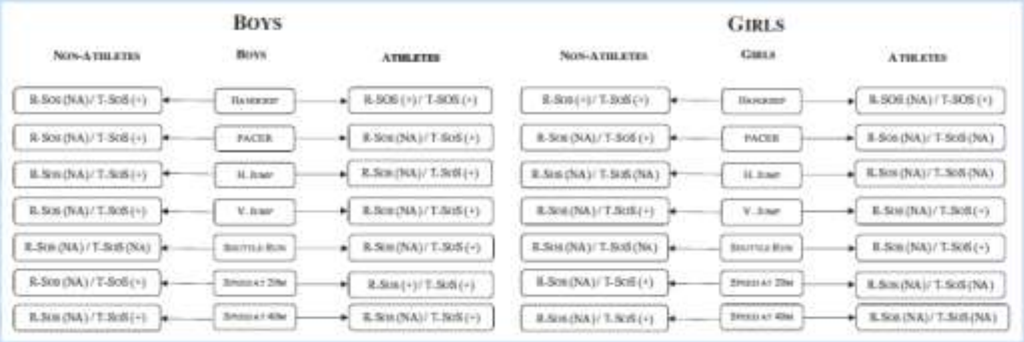

Figure 1. Overall associations of R-SoS (radial distal third speed of sound) and T-SoS (tibial midshaft speed of sound) with physical fitness tests. H. Jump, horizontal jump; NA, no significant association; V. Jump, vertical jump; +, positive and significant association.

SoS were overall stronger in the boys than the girls. This suggests that physical fitness may be a better marker for bone health in boys when compared with girls, and that, among girls, physical fitness is more related to bone health in the nonathletic population than in the athletic population. These differences between sexes and between girl athletes and nonathletes may be due to differences in the timing of growth and development during adolescence.[18] Particularly in girls, engaging in sports that emphasize thinness or weight control behaviors increases the risk for low energy availability and delayed onset of pubertal maturation.[2]

Given that 89.2% of our female athletic population was involved in higher volumes of training per week, and in an esthetic sport (ie, gymnastics) where leanness is highly emphasized, these athletes may have delays in their maturation, resulting in the suppression of key hormones (ie, estrogen) involved in enhancing the sensitization of bone to the loading stimuli brought about by PA.[1]

Nevertheless, after adjusting for the age of PHV, an indicator of biological maturation, the number of significant associations between physical fitness tests in female athletes were less than those observed in nonathletes. It is also possible that in the competitive youth athlete, the overloading training process and having insufficient time to recovery may contribute to the deregulation of bone tissue modeling and remodeling, leading to increased risk of injury and lower bone mineral density in athletes.[2] However, we did not observe lower associations between physical fitness and bone health in the male athletes.

Beyond differences in sex and athlete/nonathlete girls, site-specific differences in the relationships of the physical fitness tests with bone were also observed, such that higher associations were found between T-SoS than with R-SoS. These results might be due to the specific characteristics of the physical fitness tests, in which most of them (ie, PACER, jumps, agility, and speed) were a reflection of lower limb performance, except for the HG test, which is highly correlated with overall body strength.

Since the osteogenic effect of exercise is site specific, one can expect that higher values for these physical fitness tests measuring lower limb performance would be associated more with the strength of bones in the lower limbs (ie, T-SoS) rather than those of the upper limbs (ie, R-SoS). In addition, differences in the rates of growth of various bone locations, especially during the rapid longitudinal growth period of adolescence, with certain areas of bone having higher porosity than others,[11] may be influencing the relationships of physical fitness with T-SoS and R-SoS.

The present investigation has limitations that should be acknowledged. First, this is a cross-sectional study, making it impossible to induce causality. Second, other factors known to influence bone, such as smoking habits, the use of oral contraceptives or other hormone therapy, and hormonal levels such as estradiol, testosterone, growth hormone, and other metabolic factors were not assessed. In addition, we did not have a measure indicative of muscle mass, which is known to mediate the associations between PA and bone strength in children.[32] Despite these limitations, this investigation assessed a relatively large sample of children/adolescents, who had different levels of PA. Finally, this investigation paves the way for a better understanding of the relationship between several components of physical fitness with R-SoS and T-SoS in 2 types of youth populations (nonathletes and athletes), allowing the tests to be used as indicators of bone-related health outcomes.

CONCLUSION

This investigation demonstrates that physical fitness is differentially associated with R-SoS and T-SoS among boys and girls and athletes and nonathletes. Regardless of athletic status, all associations between physical fitness and bone health in boys were higher than those in girls. Furthermore, the HG and VJ tests were the best indicators of bone health in adolescents, independent of sport status. Upper and lower body strength may be important indicators of bone health and should be considered for monitoring and promoting the bone health status in adolescent nonathletes and athletes of both sexes.

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CHAPTER 7

Mediating role of physical fitness on the associations between physical activity and bone health in youth

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Mediating role of physical fitness and fat mass on the associations between physical activity and bone health in youth

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ABSTRACT

We aimed to assess if the relationship between VPA and bone health is simultaneously mediated by PF and fat mass in adolescents. Bone health was assessed by quantitative ultrasound (QUS) in 412 participants (221 girls) aged 10–18 years. VPA was assessed by accelerometry and PF was measured using specific protocols from FITescola®. Fat mass (%) was assessed using two skinfolds (triceps and calf). Parallel mediation analysis was performed by Hayes' PROCESS (V.3.3-model 4) for SPSS. We observed that in boys, handgrip mediated the associations of VPA with speed of sound on the third distal radius (R-SoS). While, speed at 20 m and handgrip mediated the relationship of VPA with speed of sound on the tibial midshaft (T-SoS). Body fat (%) only acted as a mediator when handgrip integrates the mediation model. For girls, the only mediating variable for the relationship between VPA and R-SoS or T-SoS was the PACER test. Handgrip, speed and fat mass (%) in boys, and cardiorespiratory fitness in girls mediates the relationships between VPA and bone health assessed by QUS. Promoting muscular fitness and cardiorespiratory fitness and decrease of fat mass through VPA in adolescents may be an important strategy to improve bone health.

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Muscular fitness; speed; ultrasonography; adolescence

Introduction

Osteoporosis remains a major public health concern due to its relation with fragility fractures (Cauley, 2017). Promoting healthy behaviours during adolescence reduces the risk of fractures and optimises peak bone mass, which may lead to a decreased risk of osteoporosis in adulthood (Bielemann et al., 2013).

Adolescence is an essential period for skeletal development (Santos et al., 2017), where various environmental and behavioural factors can either augment or interfere with gains in bone density and structure (Rizzoli et al., 2010). In particular, physical activity (PA) of moderate to vigorous intensity has been associated with better bone mineral density (BMD) and bone mineral content (BMC) (Binkley & Specker, 2004; Muñoz-Hernandez et al., 2018). Based on the mechanostat theory, it is recognized that physical activities, typically those of vigorous-intensity, which promote impact (e.g., jumps or direction changes) (Vlachopoulos et al., 2018) and are weight-bearing (Hong & Kim, 2018) with adequate loads, are paramount for developing healthy bones (Goodman et al., 2015).

Physically active adolescents usually have higher levels of physical fitness (PF) (Judice et al., 2017). Moreover, a positive relationship between PF attributes and bone outcomes has been described in adolescents (Baptista et al., 2016; Alghadir et al., 2018). These associations seem to be linked to cardiorespiratory fitness (CRF) (Vlachopoulos et al., 2017) and muscular fitness (MF) (Baptista et al., 2016), in which both are strong predictors of bone health during this period of growth and development. On the other hand, fat mass is also an important factor when considering bone health in adolescents, however this relationship is still unclear (Sioen et al., 2016). Fat mass can have both positive and negative effects on BMC and BMD (Sioen et al., 2016; Torres-Costoso et al., 2015). The relationship between fat mass and bone health outcomes has been widely discussed. Variables such as sex, weight status, lean mass, PF level and the measure of bone health used are factors that influence partially or totally the relationship between fat mass and bone health and that can explain the contradicting findings (Gracia-Marco et al., 2011; Sioen et al., 2016).

The relationship of vigorous physical activity (VPA) with fitness and fat mass and VPA with bone outcomes has been extensively studied (Judice et al., 2017; Muñoz-Hernandez et al., 2018). However, to the best of our knowledge, no previous investigation has jointly analysed the associations between VPA, fitness, fat mass and bone outcomes, namely, the speed of sound in the third distal radius (R-SoS) and in the midshaft tibia (T-SoS) in adolescents, nor has any investigation analysed the possible mediating effect of fitness and fatness in the relationship between VPA and R-SoS and T-SoS. Furthermore, knowing if PF and fat mass are mediators of VPA and bone health will help in determining which PF attributes should be promoted in youth in order for them to achieve the maximal bone health potential from VPA. Therefore, the present investigation aimed to assess if the relationship between VPA and R-SoS and T-SoS are parallel mediated by PF and fat mass in

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adolescents. Due to the logical relationship of VPA with PF and fat mass it is hypothesised that the associations between VPA with R-SoS and T-SoS are partially mediated by PF and fat mass.

Material and methods

Participants and study design

This cross-sectional investigation included a total sample of 412 adolescents aged 10 to 18 years old (n = 221 girls) with valid data on PF tests (handgrip [HG], PACER, vertical jump, 4x0m shuttle run, and speed at 20 m) and PA measured by accelerometry. Participants were recruited from public schools in the Lisbon district area. The investigation was conducted respecting the Declaration of Helsinki for Human Subjects (WMA, 2008) and was approved by the Ethics Committee of Faculty of Human Kinetics, University of Lisbon (CEFMH-Approved; N.º 19/2018). All participants were informed about the aim of the project, and written consent was obtained from their legal guardians before participation in the investigation.

Anthropometrics measurements

All participants were weighted to the nearest 0.01 kg on an electronic scale (*Seca*, Hamburg, Germany) while wearing minimal clothing and without shoes. Height was measured to the nearest 0.1 cm with a stadiometer (*Seca*, Hamburg, Germany) (Lohman & Martorell, 1998). BMI was calculated as body mass (kg)/height (Bielemann et al., 2013) (m).

Body composition

The triceps and calf skinfolds measurements were conducted by the same anthropometrist to reduce the risk of bias. Two measurements were performed for each location point, and if the two measures differed by more than 2 cm, a third measurement was performed. The average value for the 2 or 3 measurements was recorded. All skinfold measurements were according to the procedures described by Lohman et al (Lohman & Martorell, 1998). Values corresponding to body fatness in adolescents were estimated by specific mathematic equations (Slaughter et al., 1988).

Maturity

Height and age, were used to calculate the maturity offset in years using sex-specific equations established by Moore et al. (Moore et al., 2015). Age of peak of height velocity (PHV) was estimated by subtracting maturity offset from the chronologic age.

Calcium and vitamin D intake

A semi-quantitative questionnaire specific for Portuguese adolescents (Lopes et al., 2017) was used to assess the intake of a full set of typical Portuguese foods in the previous month (e.g., 1- How many supplements of vitamins, D, and calcium do you usually take per day?; 2- How many milk cups (250 ml) do you usually take per day?; 3 How many yoghurts do you usually take per day?).

Accelerometer analysis

Total wear time and VPA were assessed by accelerometry (ActiGraph, GT3x model, Fort Walton Beach, FL). All participants were asked to wear the accelerometer on the right side, near the iliac crest for 7 consecutive days (5 weekdays and 2 weekend day), and were instructed to remove the devices for water activities and during sleep. The accelerometers were activated on the morning of the first day. The devices were activated on raw mode with a 100 Hz frequency using the normal filter as default and posteriorly downloaded into 15-s epochs (Actilife v.6.9.1). Periods of at least 60 consecutive minutes of zero counts were considered as non-wear time. A valid day was considered when there were at least 10 hours (600 minutes) of wear time.

Participants had to present at least three valid days (including one weekend day) to be considered for analysis in this investigation. Activity levels were expressed in terms of counts per minute, and intensity thresholds were defined according to Evenson et al. (Evenson et al., 2008). Accelerometer counts ≥ 100 counts.min^{-1} were identified as active time, and participants were in VPA if the accelerometer counted ≥4012 counts. min^{-1} (Evenson et al., 2008).

Type of physical exercise

The different types of physical exercise were categorized as follows: non-osteogenic (i.e. swimming, water polo, equestrian) and osteogenic (i.e. football, handball, volleyball). Those who did not participate regularly in any sport were classified as non-exercisers. This categorization has been used previously in bone health studies in adolescents (Hong & Kim, 2018; Vlachopoulos et al., 2018).

Physical fitness

All participants performed a specific warm-up for at least 10 minutes before PF testing, which was coordinated by a member of the research group. PF was assessed using specific tests from the *FITescola*® fitness battery, which is currently being used in the Portuguese school setting. Progressive aerobic cardiovascular endurance run PACER test, vertical jump test, and the speed at 20 m tests were used to examine CRF, lower body MF, and speed, respectively. A full description of each test can be found elsewhere (Gracia-Marco et al., 2011; Ruiz et al., 2011). Additionally, the upper body MF strength test (HG) was assessed using an analogue adjustable dynamometer (Lafayette Hand Dynamometer – Instrument Company, Lafayette, Indiana, United States of America). Participants were given a previous demonstration and verbal instructions before the individual test. The test was performed with the participant in a standing position with shoulder neutrally rotated in abduction and arms parallel without being in contact with the body. The participants were asked to squeeze the handle for a maximum of 3–5 seconds. Verbal encouragement was given during the test. Two trials were performed for each limb and the maximum score was recorded in kilograms (kg).

Bone health–speed of sound

Bone speed of sound (SoS) was assessed by quantitative ultrasound (QUS) at the radial distal third (R-SoS) and tibial midshaft (T-SoS) of the non-dominant limb by sonometer *Omnisense* 8000s (*Sunlight Omnisense*, BeamMed, Tel Aviv, Israel). The same researcher conducted all measurements, after a daily calibration, and according to specific manufacturer's instructions, further details can be found elsewhere (Baptista et al., 2011). Before performing SoS, the participant's age, height, weight and sex were entered in the program. Therefore, SoS analyses were adjusted for these variables. All SoS analyses were conducted on a different day from PF assessments but on the same week.

Statistical analysis

Statistical analyses were completed using SPSS (24.0, Chicago, IL, USA). Descriptive analysis included mean and standard deviation for all variables. In the preliminary analysis, an interaction effect between sexes was observed, thus subsequent analysis were stratified by sex. Independent sample t-tests were used to compare continuous variables and chi-square test for categorical variables between sexes. To analyse whether each PF test and fat mass (%) mediated the relationship between VPA time with R-SoS and T-SoS, a parallel mediation model was used, where VPA time was set as the independent variable (X), each PF test (M1) and fat mass (M2) were set as the mediating variables, and each bone variable was set as the dependent variable (Y). Age of PHV, BMI, daily intake of calcium and vitamin D and type of PA were added as covariates. Also, in a parallel mediation model the mediators (M1 and M2) serve as covariates of each other when assessing their effect on bone outcomes (i.e. *path b* in Figure 1). The parallel mediation model used in the analysis is presented in Figure 1.

Before assessing the mediation effect, *path a* (i.e. the effect of VPA time on each PF test) and *path b* (i.e. the effect of each PF and fat mass (%) on bone outcomes) were tested using unstandardized regression coefficients. Posteriorly, we tested the total (*path c*) and direct (*path c′* – with PF adjustment) effect

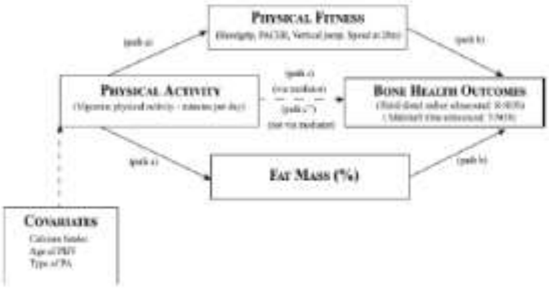

Figure 1. Overall mediation model.

between X (VPA time) and Y (R-SoS and T-SoS) to understand if there was an association of VPA time with bone health outcomes by QUS. Mediation analysis was performed only when both *path a* and *path b* were significant. Mediation analysis was performed using the PROCESS SPSS macro (v3.3 – model 4) developed by Preacher and Hayes (Preacher & Hayes, 2004). The PROCESS macro analyses the total and specific indirect effects using bootstrap procedures (resampling of 5000 bootstrap samples was used), which do not require assumptions of normality of the sampling (Hayes, 2017).

Results

Descriptive statistics on the characteristics of the participants are presented in Table 1. Significant differences between sexes were observed for weight, height, fat mass (%), maturity offset, age of PHV, VPA, PF tests, and bone outcomes ($p < 0.05$). Boys had higher values in weight, height, age of PHV, VPA, and PF tests, while girls had higher values in fat mass (%), maturity offset, R-SoS, and T-SoS ($p < 0.001$).

Parallel mediation analysis

Figure 2 depicts the results of the parallel mediations analysis of PF and fat mass in the association between VPA and R-SoS (panel a) or T-SoS (panel b) for boys. VPA was not associated with R-SoS, when adjusted for the covariates (*path c*). The only PF test that mediated the relationship between VPA and R-SoS, was the HG (*path a* = 0.12, $p = 0.008$; *path b* = 2.62, $p = 0.031$). Fat mass did not act as a mediator between the relationship between VPA and R-SoS. On the other hand, VPA was associated with T-SoS, when adjusted forthe covariates (*path c*). However, when considering the PF tests and fat mass (%), this association loss significance (*path c′*), except for the PACER test model. The speed at 20 m (*path a* = −0.02, $p = 0.046$; *path b* = −86.11, $p < 0.001$) was a mediator between VPA and T-SoS, while the HG (*path a* = 0.12, $p = 0.008$; *path b* = 4.33, $p < 0.001$) and fat mass (%) (*path a* = −0.12, $p = 0.005$; *path b* = −2.53, $p = 0.004$) jointly mediated the association between VPA and T-SoS.

Figure 3 summarizes the results for the parallel mediation analysis of PF and fat mass (%) in the association between VPA and R-SoS (panel a) or T-SoS (panel b) for girls. Similar to boys, VPA was not associated with R-SoS, when adjusted for the covariates (*path c*). The PACER test was the only mediator of the relationship between VPA and R-SoS (*path a* = 0.20, $p = 0.026$; *path b* = 1.27, $p = 0.023$). Fat mass did not act as a mediator between the relationship of VPA and R-SoS. Regarding the T-SoS, PACER was also the only mediator for its association with VPA (*path a* = 0.20, $p = 0.026$; *path b* = 1.39, $p = 0.020$). VPA was not associated with T-SoS, when adjusted for the covariates (*path c*).

Overall, we observed that in boys, HG mediated the associations of VPA with R-SoS, while speed at 20 m and HG mediated the relationship of VPA with T-SoS. Body fat (%) only acted as a mediator when HG integrates the mediation model. For girls, the only mediating variable for the relationship between VPA and R-SoS or T-SoS was the PACER test.

Table 1. Sample characteristics by sex and age group.

	Mean (SD)			
Anthropometry	All (n = 412)	Boys (n = 191)	Girls (n = 221)	P value
Age (yrs)	14.59 ± 2.36	14.46 ± 2.38	14.69 ± 2.35	NS
Weight (kg)	52.64 ± 12.48	54.56 ± 13.98	51.24 ± 10.86	0.016
Height (m)	159.89 ± 11.69	162.49 ± 13.74	157.63 ± 9.02	<0.001
BMI (kg/m²)	20.33 ± 3.22	20.24 ± 3.30	20.41 ± 3.15	NS
Fat mass (%)	20.28 ± 8.20	16.52 ± 8.15	23.54 ± 6.73	<0.001
Maturity offset (yrs)	1.41 ± 2.09	0.59 ± 1.98	2.13 ± 1.92	<0.001
Age of PHV (yrs)	13.17 ± 0.88	13.88 ± 0.56	12.56 ± 0.62	<0.001
Calcium intake (mg/day)	894.54 ± 456.24	896.60 ± 455.62	892.76 ± 457.80	NS
Type of Physical Activity				
Non-exercisers (%)	30.1	23.6	35.7	NS
Non-osteogenic (%)	12.6	12.6	12.7	NS
Osteogenic (%)	57.3	63.9	51.6	NS
Physical Activity				
VPA (min/day)	16.14 ± 13.10	20.86 ± 14.65	12.06 ± 9.96	<0.001
Total wear time (min/day)	853.54 ± 64.44	857.83 ± 67.86	849.84 ± 61.52	NS
Physical Fitness				
Handgrip (kg)	30.58 ± 8.03	33.33 ± 9.71	28.19 ± 5.18	<0.001
PACER 20 m (laps)	44.43 ± 19.88	53.81 ± 22.32	36.32 ± 12.87	<0.001
Vertical jump (cm)	34.32 ± 9.46	38.15 ± 10.62	31.00 ± 6.79	<0.001
Speed at 20 m (s)	3.96 ± 0.42	3.79 ± 0.45	4.11 ± 0.31	<0.001
Bone Health – Ultrasound				
Third distal radius ultrasound (m/s)	3874.66 ± 145.59	3819.50 ± 146.20	3922.33 ± 127.31	<0.001
Midshaft tibia ultrasound (m/s)	3767.65 ± 126.86	3727.62 ± 121.32	3802.24 ± 121.50	<0.001

Differences between sex groups were assessed by the T-test
SD, standard deviation; NS, non-significant; BMI, body mass index; PHV, peak height velocity; VPA, vigorous physical activity; PACER, Progressive Aerobic Cardiovascular Endurance Run.

Discussion

This investigation aimed to analyse whether the relationship between VPA and R-SoS and T-SoS was parallel mediated by PF and fat mass (%) in adolescents. Our results suggest that speed at 20 m, HG and fat mass (%) in boys and PACER in girls mediated the relationship between VPA and bone health assessed by QUS.

VPA, PF, fatness, and bone health in boys

Practicing regular PA, especially at higher intensities, and having healthy PF levels are among the primary modifiable factors capable of influencing body composition and bone tissue health (Joensuu et al., 2018). In our investigation, we observed a positive and significant association between time spent in VPA with T-SoS in boys, but not with R-SoS, that was mediated by speed at 20 m and jointly mediated by HG and fat mass (%). These observations are in line with previous studies, which have found higher VPA levels to be positively associated with several indicators of bone health in adolescents (Boreham & McKay, 2011). Indeed, the impact on bone generated by PA is known to promote bone modelling and remodelling processes to take place, with the overloading provided by higher PA levels promoting increases in bone strength and the underloading occurring in situations of decreased PA resulting in bone loss (Tyrovola & Odont, 2015). The high prevalence of participation in PA that involved lower limbs, may explain the lack of association of VPA with R-SoS.

Higher intensity PA is also known to improve PF levels (Judice et al., 2017), with higher values in different components of PF being associated with higher BMD and BMC (Baptista et al., 2016; Cray et al., 2015; Vlachopoulos et al., 2018). In this investigation, PACER, an PF test indicative of CRF, was positively associated to T-SoS in boys, however, it was not a mediator of the relationship between VPA and bone health. Similar to our findings, several studies have demonstrated some controversial results regarding the associations between CRF and bone health outcomes (Fonseca et al., 2008; Gracia-Marco et al., 2011). It may be hypothesized that boys who practice more aerobic PA with higher volumes of training and repetitive loads, such as in distance running, have lower levels of body mass, including muscle mass (Faustino-da-Silva et al., 2018; Tenforde et al., 2018), and as a result have less bone health benefits than more strength based sports.

Several studies have demonstrated that MF is associated with BMD and BMC (Baptista et al., 2016; Ubago-Guisado et al., 2017; Vlachopoulos et al., 2018). MF is a component of PF, focused on assessing the capacity of skeletal muscle to produce body movement, while it promotes blood flow to organs, among other biologic functions (Goodman et al., 2015; Ortega et al., 2007). In this investigation, speed at 20 m was identified as a mediator to the relationship between VPA and T-SoS, while the HG was identified as a mediator to the relationship between both bone outcomes by QUS. These tests (HG and speed at 20 m) are linked with motor performance and may give useful information regarding the strength and power capacity of the muscle (Vlachopoulos et al., 2018). For instance, grip strength can be a surrogate measure of total body muscular strength and the speed test can be a good indicator of the capacity of muscle fibres to produce power in a short period of time (Ortega et al., 2007).

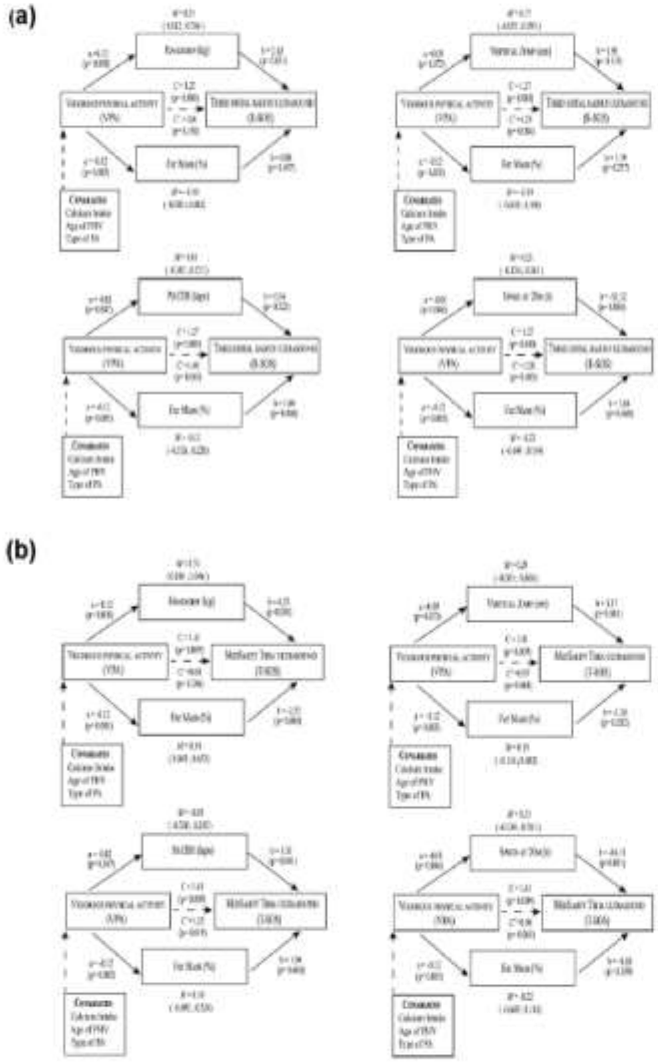

Figure 2. Parallel mediation model. The model includes one independent variable (VPA), two mediating variables (physical fitness tests and fat mass [%]), one dependent variable (third distal radius ultrasound), and covariates (daily calcium intake, age of PHV, and Type of PA) for the boys.

Additionally, all these PF tests have been previously identified as potential predictors of bone health in adolescence (Baptista et al., 2016; Saint-Maurice et al., 2018; Vicente-Rodriguez et al., 2008). The muscle-bone unit is known to play an important role in the development of bone strength in children and adolescents, with gains in muscle mass and strength preceding increases in bone mass and alterations in bone geometry (Fricke & Schoenau, 2007). Active muscle contractions contribute to the mechanical loading of bone, subjecting the bone to tensile, comprehensive and bending forces (Goodman et al., 2015). Therefore, muscles with higher levels of strength or power have a capacity to generate greater forces on bone and, thus, play a direct role in promoting bone strength (Goodman et al., 2015). Our results are supported by previous studies which demonstrate significant and positive associations for MF with BMD. This means that, in boys, VPA may be associated with R-SoS and T-SoS, in part because of its associations with HG and the speed at 20 m.

VPA, PF, fatness, and bone health in girls

Unlike in boys, VPA was not associated with bone health in girls in both the upper and lower limbs. Lower levels of VPA when compared to boys may explain the lack of association of VPA with SoS of the radius and tibia in girls. Notwithstanding, VPA is consistently associated with bone health in the literature (Joensuu et al., 2018; Tan et al., 2014), and thus should be promoted in both girls and boys.

In girls the PACER test, when used as a surrogate measure of CRF, was a mediator in the associations between VPA with R-SoS and T-SoS. Furthermore, PACER was the only mediator for the association between VPA and bone health in girls. As stated before, controversial results regarding the associations between CRF and bone health outcomes have been reported (Fonseca et al., 2008; Gracia-Marco et al., 2011). In our investigation, the PACER seems to be a more relevant indicator of bone health in girls than it is among boys.

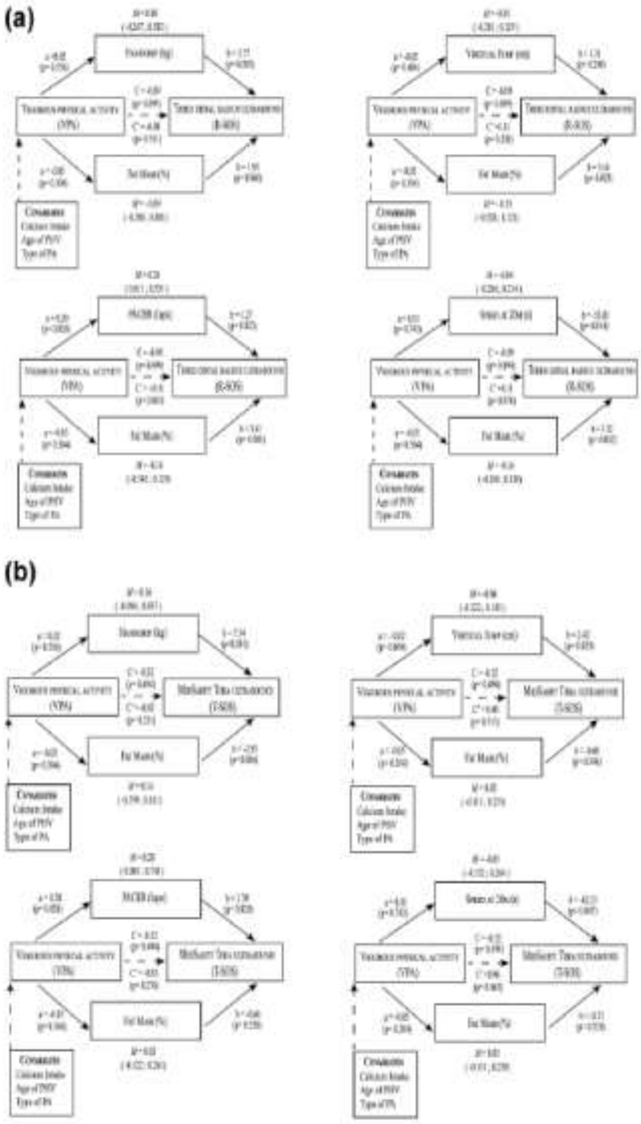

Figure 3. Parallel mediation model. The model includes one independent variable (VPA), two mediating variables (physical fitness tests and fat mass [%]), one dependent variable (third distal radius ultrasound or midshaft tibia ultrasound), and covariates (daily calcium intake, age of PHV, and Type of PA) for the girls.

Unlike in boys, there were no mediating effects of MF on the relationship between VPA and bone health found in girls. This finding is likely due to boys having a significantly higher number of minutes in VPA compared to girls in our sample of adolescents. Given that VPA is positively associated with MF, the higher VPA observed in boys allows them to develop a higher degree of MF to a level capable of mediating part of the associations of VPA with R-SoS and T-SoS. In addition, the muscle-bone interactions are influenced by hormonal factors during puberty. In particular, testosterone is largely responsible for the increased muscle mass and corresponding greater muscle force imparted on the skeleton in boys when compared to girls (Fricke & Schoenau, 2007). In girls, oestrogen lowers the level of strain on the bone needed for a bone remodelling effect to occur (Fricke & Schoenau, 2007). Hence a lower amount of muscle mass and force are needed to produce increases in bone mass in girls compared to boys. MF is likely not as important of a factor as hormonal levels for contributing to bone strength in girls, which may additionally explain why in girls MF was not a significant mediator of the VPA-bone relationship.

Strengths and limitations

The present investigation has some limitations. First, the cross-sectional design of the investigation does not make it possible to induce causality. This is an important limitation as it is not possible to determine the direction of the relationships in which variables influence each other, i.e., it is possible that those with less fat mass or higher levels of PF are more likely to engage in VPA. Furthermore, the cross-sectional design dos not allow to differentiate between a mediator and a confounder, thus it is not possible to statistically assess whether fat mass and PF are confounders or mediators in the relationship between PA and bone health. Notwithstanding, from a conceptual standpoint the indicated directions are the most expectable. Second, other factors known to influence bone, such as muscle mass, sleep, smoking habits, the use of oral contraceptive pills or other hormone therapy, and hormonal levels such as oestradiol, testosterone, growth hormone and other metabolic factors were not assessed. Also, we did not have an objective measure of body composition (muscle mass), which has previously been shown to mediate the associations between PA and bone strength in children (Zymbal et al., 2018). Finally, the fact that we do not have 24-h accelerometer data, due to participants removing the devices during water-based activities and sleep, may potentially cause some PA to be missed during these non-wear time periods.

Despite limitations, this investigation has several strengths, one being our assessment of a relatively large sample of children/adolescence. Additionally, we used QUS to assess bone, which is a non-invasive, fast, low-cost method without radiation that has been widely used to assess BMD status in adolescents (Maggioli & Stagi, 2017). The main outcome of QUS, SoS, is highly correlated with BMD by DXA, reflecting a range of bone status characteristics, such an elasticity, structure, microstructure, and cortical thickness, all of which are essential characteristics predictive of overall bone strength (Wang et al., 2014).

Conclusion

To the best of our knowledge, this is the first investigation addressing the indirect effect (through PF and fat mass) of VPA on R-SoS and T-SoS during adolescence. This investigation suggests that during adolescence VPA is associated with increased bone health, which is mediated by PF, particularly MF (i.e. HG and speed at 20 m) and fat mass in boys, and the PACER in girls. Hence physical activity that enhances muscular fitness should be especially emphasised during adolescent development for boys, while for girls an enphasis in CRF should be given. More studies are needed to clarify if there is any mediating role of skeletal muscle in the relationship between PA and bone outcomes by SoS in girls.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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CHAPTER 8

General discussion

8.1 - OVERVIEW

Physical inactivity or insufficient PA levels are identified as one of the five major risks factors for all-causes of deaths worldwide and for this reason, the WHO has defined the promotion of PA as a vital strategy to increase the quality of life and life expectancy (Guthold et al., 2018; WHO, 2015). Childhood and adolescence are crucial periods to instigate present and future healthy lifestyles. The most significant changes both physiologically and socially occur during these periods, consequently, making childhood and adolescence as optimal windows to promote the development of healthy lifestyles, which ultimately can improve quality of life and possibly increase life expectancy (National Research, Institute of Medicine Committee on Adolescent Health Care, Models of Care for Treatment, & Healthy, 2009). It is essential for governments' entities across the world to create and develop strategies and tools to promote PA at early ages (Fraser-Thomas, Côté, & Deakin, 2005; WHO, 2010, 2015). Several investigations have demonstrated youth sport participation to have excellent results on current health status, as well as in later life (Grima & Thalassinos, 2017; Schmidt et al., 2017; Rohan M. Telford et al., 2016).

It is essential to organise an integrated model for improving healthy sport participation, considering all cultural and social variables and the characteristics of the sports, with careful attention to the child or adolescent's individual characteristics (Fraser-Thomas et al., 2005; Rhodri S. Lloyd et al., 2015). School plays a crucial role in this strategy for promoting a healthy sports participation in youth because it is a place where the most significant people in children's and adolescent's life meet has facilities and tools (e.g. indoor/outdoor equipment and PF battery tests) to successfully operationalise this strategy (R. Bailey, 2006; A. F. Seabra et al., 2007).

Researchers, sports professionals, and sports authorities are recommended to determine the potential of talent identification and development systems, to promote health, by also evaluating the positive health of the athlete rather than solely focusing on performance outcomes (Rongen et al., 2018).

Monitoring the different components of PF in youth is safe and can be performed in ecologic contexts (e.g. school) with minimal equipment (Artero et al., 2010; J. R. Ruiz et al., 2010). Assessing the PF is fundamental to the promotion of youth's health and sports participation. PF plays an essential role in monitoring sports performance and training process as well as bone health (Baptista et al., 2016; Bergeron et al., 2015; Gracia-Marco, Vicente-Rodríguez, et al., 2011; F. B. Ortega, J. R. Ruiz, et al., 2008).

Despite advances in the PF assessment on sports performance and health in youth, all this information derives from investigations with different test protocols to assess the same PF attributes (Johan Pion et al., 2014). In the same way, bone health in youth has been assessed using several methods of assessment (Bachrach & Gordon, 2016; Maggioli & Stagi, 2017). There was a significant gap in the literature regarding the standardisation of PF tests on sports performance (i.e. the use of a single PF battery), as well as their relationship with bone health assessed by QUS in the youth considering several PA levels and sports backgrounds. Moreover, from a methodological point of view, the wide variety of PF batteries make interpretation of outcomes difficult and preclude comparisons between athletes with different sports backgrounds or solely between athletes and non-athletes.

The overall aim of this dissertation was to gain better knowledge of the role of PF tests on sports performance, and bone health on the radius and the tibia, in young athletes and non-athletes. To achieve this aim, four investigations were conducted. Investigation 1 (chapter 4) comprises an analysis of the reliability of FITescola® battery tests in young athletes of different sports backgrounds and competition levels. Investigation 2 (chapter 5) was conducted with a large sample (n=1831) of young athletes (M=401 and F=194) and non-athletes (M=514 and F=722). Based on the results of this investigation 2, we analysed the sensitivity and the sensibility for each PF test to identify potential young athletes, based on sex and chronologic ages.

After studying the reliability of the FITescola® in young athletes and knowing its validity in the general population, we analysed the relationship between different PF tests and the radial and tibial bone health by QUS in young athletes (M=121 and F=68) and non-athletes (M=117 and F=183) (Investigation 3; chapter 6). Since PF tests were associated with R-SoS and T-SoS and acknowledging the associations between PA and bone health, we examined the association between VPA and R-SoS and T-SoS in 413 young, as well as the potential mediating effect of PF on that association (Investigation 4; chapter 7).

In summary, the present dissertation focused on the promotion of healthy sport participation in youth and added to the scientific knowledge by exploring: 1) the reliability of a set of PF tests in young athletes; 2) the ability of the PF tests as potential discriminators of athletes by sex and chronologic age in a large sample of young athletes and non-athletes; 3) the associations of R-SoS and T-SoS with PF in young athletes and non-athletes; and finally 4) the mediation effect of PF on the association of VPA with R-SoS and T-SoS. Extensive and specific discussions of each investigation and main findings were present in the respective chapters. The main purpose of this section was to gather and integrate the contributions of the four investigations, by summarising the main results and reflect on the practical applications, implications, conclusions and future research directions. Limitations of these investigations are also disclosed.

8.2 - MAIN RESEARCH FINDINGS

In order to promote healthy sports participation in youth and the transition to healthy lifestyles in adulthood, a diversified and balanced program based on health, fun and safety is required for youth sports development. These principles are the best pathway to a sustainable sport system, promoting sports performance and healthy sports participation (I. Balyi, Way, R., and Higgs, C., 2013; Bergeron et al., 2015; M. H. Murphy et al., 2016). This process should begin with an integrated and inclusive program based on individual characteristics and specific sports demands to potentiate fun and healthy sports participation (Bergeron et al., 2015). Assessing PF attributes can determine the sports and health profile of youth according to specific goals (Gracia-Marco, Vicente-Rodríguez, et al., 2011; T. Kramer et al., 2017).

PF tests are excellent tools for monitoring sports performance and several health risks factors (Neil Armstrong & McManus, 2010; Garcia-Hermoso et al., 2019; Johnston et al., 2017; Till, Cobley, O'Hara, et al., 2015). When using this tool, biologic factors, such as sex and maturity should be taken into account. These two factors have a direct influence on PF, and thus all analysis were stratified or adjusted for them. Additionally, PA levels, an environmental factor which influences PF, should be promoted in order to improve health and performance-related PF, as the investigations have demonstrated that athletes (which have higher PA levels than non-athletes) have higher PF levels than non-athletes.

There is a lack of standardisation of PF test protocols to assess performance and this promotes misinterpretation of test results and makes it difficult to compare test outcomes (Johan Pion et al., 2014). Therefore, Investigation 1 was conducted to investigate the reliability of a set of health-related PF tests in young athletes from different sports backgrounds and competition levels. Good or excellent reliability (ICC>0.7) for all PF tests in both sexes was verified (only exception for the 20m speed run in girls), which means that this battery may be a useful tool for monitoring sports performance and health status in young non-athletes and athletes. Furthermore, PF assessments can be viewed as an excellent tool to discriminate sporting potential (I. Balyi, Way, R., and Higgs, C., 2013; Boullosa et al., 2019; Chiwaridzo et al., 2019; De Moor et al., 2012; Haycraft et al., 2017; Hoare, 2000). The potential of each PF test used in Portuguese public schools was analysed to discriminate adolescents with athletic potential from the general population while proposing cut-off values for each of these tests. It was demonstrated that at least one PF test per sex and age group could discriminate the athlete condition, showing that it is possible to differentiate athletes from non-athletes using some of these tests.

Sport is a naturally competitive environment, even in youth competitions (Caine et al., 2016; Mountjoy et al., 2008). During certain states of youth maturation, factors such as excessive training volume and intensity and having an unbalanced diet can negatively affect the sports performance as well as the bone health of young athletes (Bergeron et al., 2015). PF is a valuable tool to monitor the training process, sports performance, as well as bone health in youth (Baptista et al., 2016; Gracia-Marco, Vicente-Rodríguez, et al., 2011). Therefore, in Investigation 3, we examined the associations between PF tests with R-SoS and T-SoS for athletes and non-athletes. To our knowledge, no investigation has examined the associations between the PF tests and bone outcomes measured by QUS in non-athletes and athletes. The results showed that most of the physical fitness tests in boys were significantly associated with T-SoS in both athletes and non-athletes.

On the other hand, fewer associations were found for girls between the physical fitness tests and T-SoS, especially in the athletic population, where handgrip strength, VJ, and 4x10m shuttle run were the only significant tests.

The associations observed between the physical fitness tests and SoS were overall stronger in the boys than the girls. These findings suggest that other factors other than the PF tests may be influencing bone measures in girls (e.g. PA levels, body composition). More investigations should be carried out to understand biologically what is going on during the training process at these ages to ensure there is a balance between sports performance and health.

Given the identified the sex-biased relationship of PF with R-SoS and PF with T-SoS observed in investigation 3, investigation 4 was conducted with the main objective of investigating the parallel mediating effect of several PF attributes and fatness on the relationship of PA with R-SoS and T-SoS.

High-intensities of PA have been associated with bone health during growth (Gracia-Marco, Moreno, et al., 2011; Zymbal, Baptista, Letuchy, Janz, & Levy, 2019). However, there is a lack of knowledge about the effect of some PF attributes on the relationship between PA with peripheral bone health in youth.

In investigation 4, we assessed the PF levels, fat mass (%), R-SoS, T-SoS and PA levels in 412 young. The results of this investigation showed a positive and significant association between time spent in VPA with T-SoS in boys, but not with R-SoS, that was mediated by speed at 20m and jointly mediated by HG and fat mass (%).

The speed at 20 m was identified as a mediator to the relationship between VPA and T-SoS, while the HG was identified as a mediator to the relationship between both bone outcomes by QUS.

For girls, PACER was the only mediator for the association between VPA and bone health (R-SoS and T-SoS) in girls. There is a need to develop more prospective investigations about the relationship between PA (type, volume and intensity), PF, potential confounders (e.g. maturity, hormonal status, body composition at the four compartments) and several outcomes of youth bone health, particularly in girls.

8.3 - PRACTICAL IMPLICATIONS AND FUTURE DIRECTIONS

In this section, the practical findings derived from the studies were summarised to the real-world of young sport-performance and bone health in young athletes and non-athletes.

- **Study 1 (chapter 4)** provide reliability on a set PF test protocols from FITescola® online platform can be used to monitoring sports performance and health status, independently of level competition, sports background or type of population (athlete or non-athlete). These PF tests protocols permit a standardisation on the assessment of PF attributes in youth on ecologic context. Our new proposed allows FITescola ® battery to be used to assessing sports performance and health status in athletes and non-athletes.
- **Study 2 (chapter 5)** we observed that the PF tests from FITescola® battery could be used physical education teachers, coaches, and government bodies to better identify young athletes from schools or sports entities. Indeed, we observed that PF tests are simple, low cost and easily applied.
- **Study 3 (chapter 6)** we analysed the relationship between PF attributes with R-SoS and T-SoS for athletes and non-athletes in youth for both sexes. We observed that PF is an indicator for radius and tibia bone health in athletes and non-athletes in youth. We also highlighted the relevance of these results on the monitoring of bone health by physical education teachers and coaches in specific groups. However future research is still required to the agreement between methods of bone diagnosis in young ages for these specifics populations

- **Study 4 (chapter 7)** reinforced that the PF level is an important mediator on the relationship between high-intensity PA levels and bone health on the radius and tibia. Therefore, when designing programs that aim in improving bone health in youth, one must not only consider the intensity of PA, but also the type of exercise. This study showed that MF and speed play a crucial role as a mediating variable of bone health and thus, exercises that improve these attributes of PF should be considered.

8.4 - LIMITATIONS

This PhD dissertation was composed of 4 investigations. Therefore, all investigations are not without some limitations that should be acknowledged. In investigation 1, we tested the reliability of a set of PF test protocols in young athletes aged 10-18 years-old. Even though the sample had the appropriate power for the analysis and was representative in number, it was not representatively distributed according to age and sport. This was due to the lack of information regarding this matter from sports federations at the national level. In investigation 2, the practical utility of the PF tests for identifying athletes was recognized. However, the sample was a heterogeneous distribution of sport backgrounds and competition levels, which may impact the power of discrimination for each PF test and respective cut-off values. Investigation 3 and investigation 4 were limited by their cross-sectional design in which causality cannot be assumed. Secondly, the use of ultrasound to assess bone health of the radius and tibia is limited as it is unable to distinguish between the different types of bone tissues and provide information on bone geometry and strength. However, 3D-imaging techniques to measure bone at the radius and tibia, such as pQCT, are very expensive and there is limited reference databases using this technology for children during the growing years.

8.5 - STRENGTHS

Despite the limitations cited previously, this dissertation also has some strengths. The major strength was the realiability of a PF battery from FITescola® program to assess sports performance and simultaneously to monitoring the bone health status in youth. This can be used in the school context, providing a platform for promoting sports participation and health in youth. Furthermore, all investigations were performed using a relatively large sample of Portuguese children/adolescence. Another strength included our use of QUS to assess bone, which is a non-invasive, fast, low-cost method without radiation that has been widely used to assess BMD status in adolescents. The main outcome of QUS, SoS, is highly correlated with BMD by DXA, reflecting a range of bone status characteristics, such an elasticity, structure, microstructure, and cortical thickness, all of which are essential characteristics predictive of overall bone strength. Finally, to our knowledge, this was the first investigation that examined how commonly used PF tests relate to QUS bone SoS in this population. Overall, this dissertation showed that it is possible to connect the school and sports systems, creating an ecosystem where healthy sports participation is promoted and monitored within and outside the school setting (Figure 8.1).

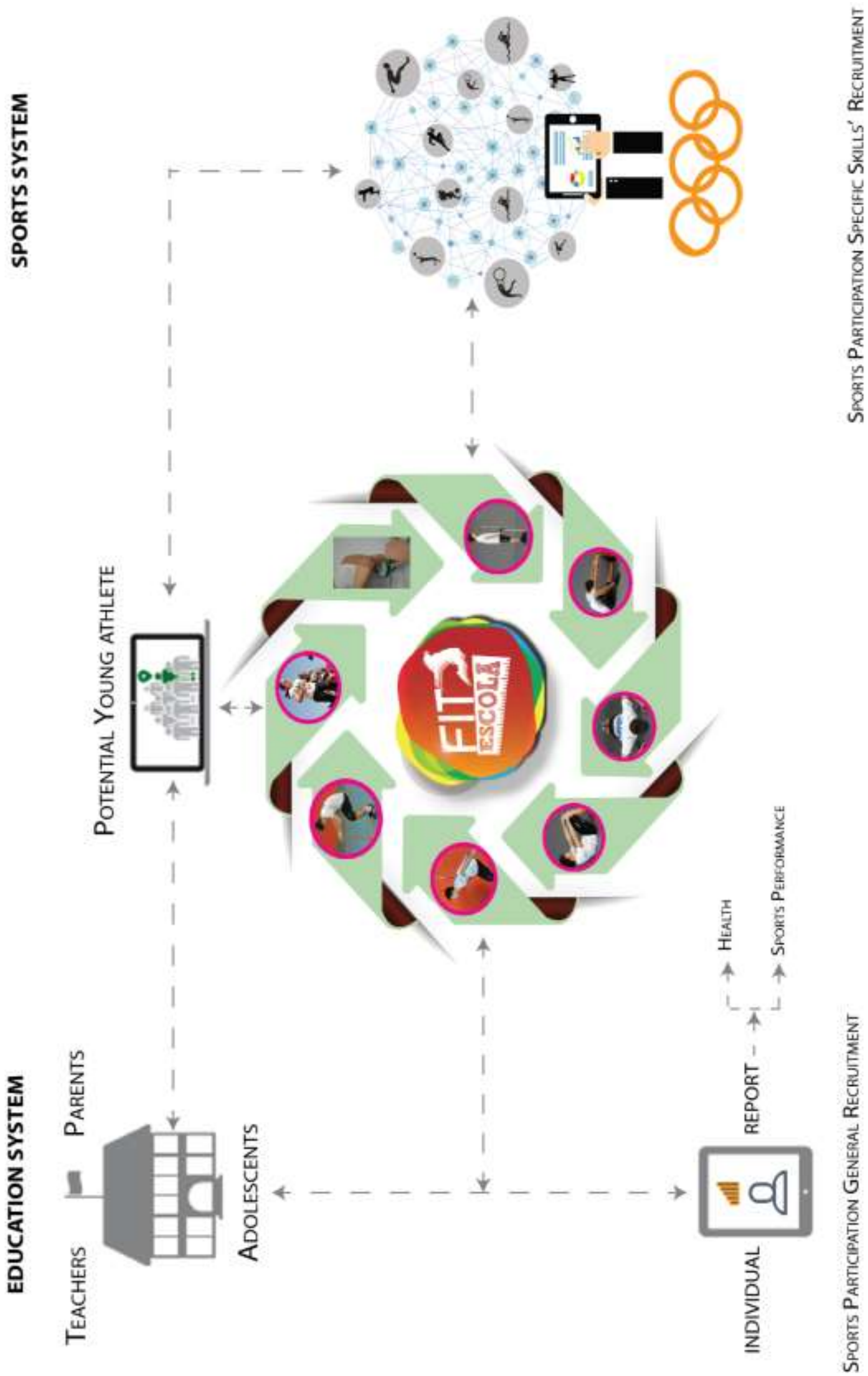

Figure 8.1 - Healthy Sports Participation

8.6 - CONCLUSIONS

This dissertation adds relevant information to the literature related to sports performance and bone health in youth, while simultaneously generating essential questions for future investigation. From the methodological perspective, this dissertation reinforced the importance of assessing and monitoring sports performance and bone health in youth. From the investigations included in this dissertation, the following conclusions can be drawn: 1) PF levels are excellent indicators of sports performance and bone health; 2) The FITescola® battery test protocols provide a reliable and standardized way of PF assessment for sports performance and health in youth. Additionally, the PF tests may be applied for use in discriminating potential athletes from the general population by sex and age; 3) PF attributes are associated with R-SoS and T-SoS in youth, however the relative contribution of specific PF attributes on R-SoS and T-SoS depend on sex and athletic status. The strength and power levels appear to be the best indicators of bone health on the radius and tibia; 4) PF, specifically in the areas of muscular strength and power, mediate the relationship of VPA with R-SoS and T-SoS, and plays an essential role in bone health of the radius and tibia in youth, however this role of PF in bone health may only be applicable to boys. Figure 8.2 summarizes the main results of the investigations presented in this dissertation. More PF and bone health investigations in youth are necessary to confirm our findings, specifically longitudinal and interventional investigations, as well as investigations assessing the relationship between PA, PF and the biologic mechanisms of bone tissue in several regions.

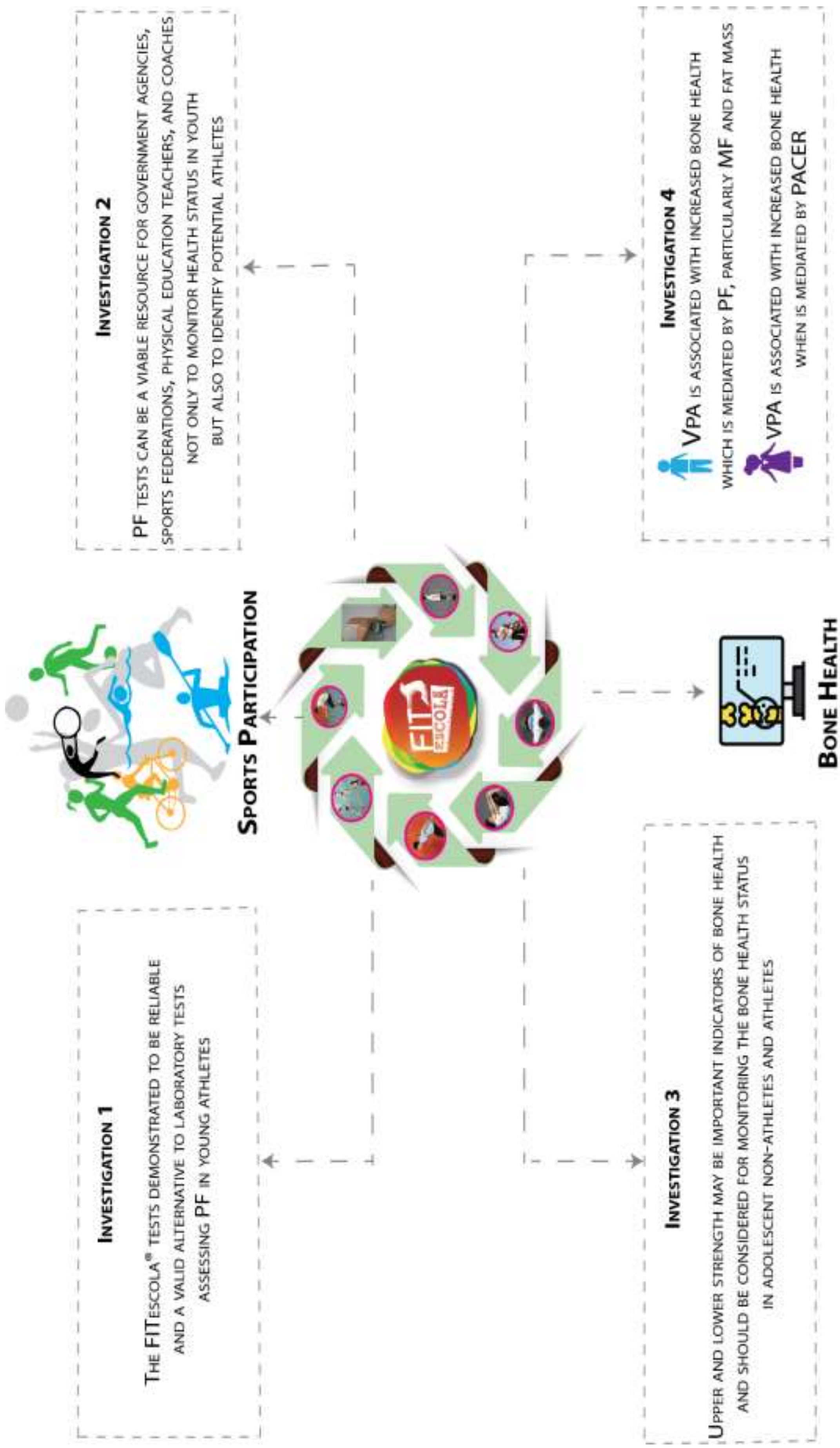

Figure 8.2 - Main results of this dissertation

8.7 - REFERENCES

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