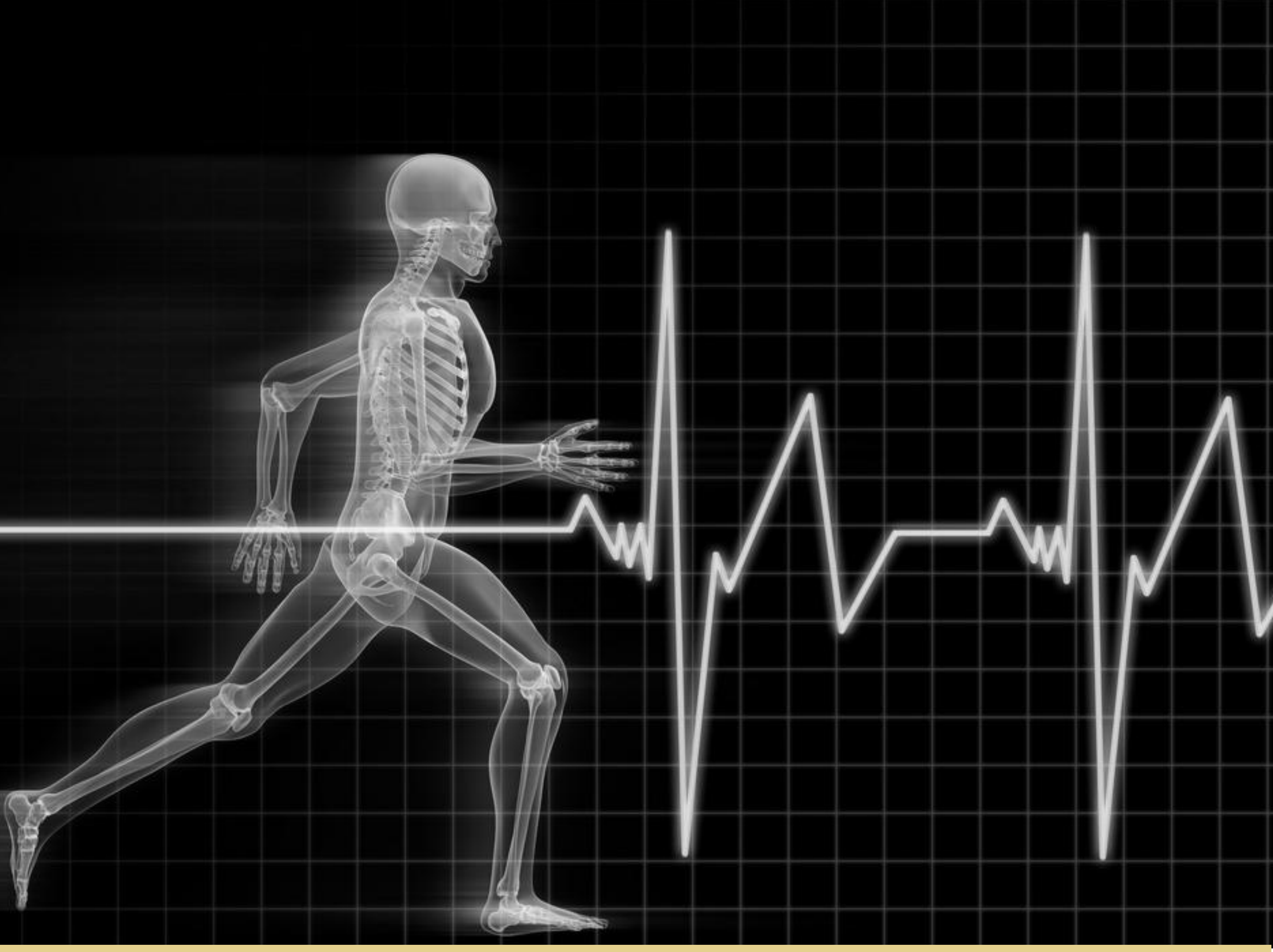

João Alberto Valente dos Santos

BODY SIZE, COMPOSITION, CARDIAC MORPHOLOGY AND FUNCTIONAL CAPACITIES
Scaling and modelling developmental changes during the pubertal years

Thesis for the degree of Doctor of Sport Sciences in the branch of Sports Training, supervised by Prof. Dr. Manuel J. Coelho-e-Silva, Prof. Dr. Marije T. Elferink-Gemser and Prof. Dr. Robert M. Malina and submitted to the Faculty of Sport Sciences and Physical Education of the University of Coimbra.

June 2014

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UNIVERSIDADE DE COIMBRA

UNIVERSITY OF COIMBRA

Faculty of Sport Sciences and Physical Education

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June 2014

Valente-dos-Santos, J. (2014). *Body size, composition, cardiac morphology and functional capacities: scaling and modelling developmental changes during the pubertal years*. Thesis for the degree of Doctor of Sport Sciences in the branch of Sports Training. University of Coimbra. Coimbra, Portugal.

To my Beloved Grandfather and to my Dear Friend

† *Florentino de Oliveira e Silva* (1937 – 2014)

† *Filipe Rafael Lopes Simões* (1987 – 2013)

Acknowledgments

There are many people who made this research possible for their generous support and guidance in my academic pursuits. Therefore, I would like to express my sincere thanks to those who have been very helpful during these past years.

First of all, I would like to sincerely thank my supervisor, Prof. Dr. Manuel J Coelho e Silva (MJCS), for his continuous support. He has been extremely committed to overseeing my progress on this PhD project providing a variety of scientific, professional and personal challenges. I also acknowledge the confidence that Prof. Dr. MJCS has had in me as a research student, the encouragement he provided, the pressure and his unique concerned manner throughout this past ten years. I will not promise you a statue but you can definitely rely on my loyalty and friendship.

I would also like to acknowledge the contributions of my co-supervisors, Prof. Dr. Marije T. Elferink-Gemser (MTEG) and Prof. Dr. Robert M. Malina (RMM). Professor MTEG provided me constant encouragement, sound advice and ideal scientific conditions throughout my periods in The Netherlands, at the Faculty of Medical Sciences of the University of Groningen. Her enthusiasm, simplicity and tending attitude are remarkable and inspiring. Prof. Dr. RMM, whose career and accomplishments are outstanding and unparalleled by many in our field, provided a valuable supervision since the submission of the PhD project. His supportive, cordial and straightforward attitude to this research project must be emphasized and was strongly appreciated. I feel genuinely honoured at having the opportunity to work with all my supervisors.

Special thanks must be extended to Prof. Dr. Lauren B. Sherar (LBS) and Prof. Dr. Adam Baxter-Jones. Professor LBS provided me several opportunities to improve my analytical and biological knowledge during my stays at the University of Saskatchewan, College of Kinesiology, Canada and at the School of Sport, Exercise and Health Sciences, Loughborough University, United Kingdom. Also, her "last hour revisions" must not be forgotten. To Professor ABJ for his enthusiasm, sense of humour, generosity and his great effort to explain things clearly and simply; he helped to make longitudinal data analyses fun for me.

I would like to highlight Prof. Dr. Vasco Vaz and Prof. Dr. Amândio Santos for the long-term friendship, academic support and voluntary help. Also, to MHSc Joaquim Castanheira for the help on conducting the echocardiographic assessments, the friendship and subtle style.

Special thanks are extended to Prof. Dr. Analiza Silva, Prof. Dr. André Seabra, Prof. Dr. António Figueiredo, Prof. Dr. António Gomes Ferreira, Prof. Dr. Armanda Pinto da Mota Matos, Prof. Dr. Cláudia Minderico, Prof. Dr. Dale Esliger, Prof. Dr. Daniel Courteix, MSc Dieter Deprez, Dr. Fátima Rosado, MSc Fernando Leite, Prof. Dr. Fernando Maia, Prof. Dr. Joaquim Paüls, Prof. Dr. Luís B Sardinha, Prof. Dr. Luís Rama, Prof. Dr. Raúl Martins, MSc Sónia Fernandes and Prof. Dr. Stuart Biddle.

I would like to express my recognition to clubs, schools (particularly, Colégio Nossa Senhora da Assunção), coaches and teachers (especially, Paula Silva) that participated in this project and to the adolescents with whom we have the opportunity to contact during the research. Without their cooperation, the study was not possible.

My gratitude also includes Hóquei Clube da Mealhada. More than the unique opportunity to work daily with Elite (Voluntary) Players, I appreciate the friendship and patience across this couple of years. Special thanks to the first team: Alexandre Santos, Daniel Homem, David Ribeiro, Gonçalo Louzada (Father and Son), Filipe Almeida, Filipe Vaz, João Pereira, Paulo Santiago, Pedro Coelho and Tiago Sousa.

To my friends and colleagues that helped me across this research: Aristides Machado Rodrigues, Bruno Silva, Gonçalo Dias, Ivo Rêgo, João Duarte, João Rafael, Nuno China, Rafael Baptista, Ricardo Rebelo Gonçalves, Rui Fernandes and Vítor Severino.

Further, I would like to show my enormous gratitude to my brother's Rui Pinho and Jorge Faria. Several of our professional and personal accomplishments were not possible without their friendship and love, their unconditional support and belief in my ambitions.

To my beloved Grandfather Florentino de Oliveira e Silva and my dear friend Filipe Lopes Simões. To me personally, I lost two members of my family. I only hope to be able to honour their memories.

To my Parents, that dedicated their whole life working more than they could for the welfare of their children. They lead by example and enabled me to develop essential personal and educational characteristics. Also, to my Sister and Brother: Sandra and Miguel. I truly regret the absence of my "presence" as an older brother but I am profoundly proud of both of you.

Lastly, and the most important, special thanks to my future wife: Maria de La Salete Quintas Remelhe. Probably, I will never be able to reattribute the overtime work, as well as the week-ends and holidays that we did not enjoy. Nevertheless, this is the fourth thesis that we accomplished together and now it is time to build another chapter in our life's; our own family. To her, I dedicate these publications.

I thank you all.

The work presented in this thesis was partially supported by the Portuguese Foundation for Science and Technology (Ministry of Education and Science)
Grant: SFRH/BD/64648/2009

Abstract

The present thesis has been divided into four parts. Part I serves to introduce basic concepts, the overall purpose of this thesis encompassed by the terms growth, maturation, physical activity, performance, geometric and elastic similarity and to provide an overview of growth and functional development in adolescent males. This section also includes specific descriptions of the studied samples as well as the materials and methods used for adolescents' assessment and data record. Part II is applied in the context of cross-sectional studies (study 1 to 5) and aimed: (*i*) to examine the contribution of biological maturation and body size to explain the inter-individual variability in left ventricular mass (LVM) and peak oxygen uptake (VO_{2peak}) in adolescent male athletes and non-athletes; (*ii*) to present allometric models to normalize LVM and VO_{2peak}; (*iii*) to derive and cross-validate a new model to predict lower limbs lean soft tissue (LST), using dual-energy X-ray absorptiometry as the reference method. Part III of the thesis (mixed-longitudinal approaches; study 6 to 10), presents longitudinal data on the development changes of: (*i*) LVM and (*ii*) cardiorespiratory fitness (CRF) among healthy adolescent boys; (*iii*) several functional and soccer-specific skills of male adolescent players. The final section, Part IV, comprises the general discussion in which the findings of the various studies are summarized, put into context and their implications discussed.

Overall, the cross-sectional studies highlighted that: (*i*) stature, fat mass (FM) and fat-free mass seems the best combination for normalizing LVM in adolescent boys and that when components of body mass are not available, an indicator of biological maturity should be included with stature; (*ii*) when stature and FM are used for indexing LVM in a sample of male adolescent athletes, biological maturity status should also be considered; (*iii*) appendicular descriptors and skeletal maturity or that lean body mass and appendicular descriptors combined with sexual maturity explain most of the inter-individual variability in of VO_{2peak} among youth roller hockey and soccer players, respectively; (*iv*) the new model derived from anthropometry and somatic maturation accurately predicts lower limbs LST in circumpubertal males.

As for the longitudinal studies: (*i*) individual differences in allometric growth of body size dimensions, adiposity and biological maturation influence growth of LVM among male adolescents; (*ii*) the longitudinal decrease of sedentary time and subcutaneous adiposity had a significant impact in maximizing CRF among male adolescents who were classified as fit; (*iii*) several functional capacities and skills of adolescent soccer athletes seems partially explained by age, maturation, components of body mass, annual volume of training and playing position.

Analytically, the present thesis outlined that multiplicative allometric modelling improves the statistical fit of regression models and provides plausible interpretations of cross-sectional and longitudinal data on cardiac morphology and functional capacity of adolescent boys. Multilevel modelling is a promising statistical technique for the derivation of developmental curves. It has the potential to provide useful information both for clinicians, coaches and conditioning trainers in the explanation of inter-individual differences at a certain age and probably more important to predict and explain changes over time, allowing a critical interpretation of variation within and between individuals.

Keywords: Youth · Young Athletes · Growth · Cardiovascular Growth · Body Composition · Biological Maturation · Aerobic Fitness · Physical Activity · Skills · Echocardiography · Dual-Energy X-Ray Absorptiometry · Accelerometry · Scaling · Allometry · Deming Regression · Longitudinal Analysis · Multilevel Modelling

Resumo

A presente tese está organizada em quatro partes. A Parte I procurou introduzir os conceitos de base e estabelecer o objetivo geral da tese considerando matérias em torno do crescimento, maturação, atividade física, desempenho, similaridade geométrica e elástica. Adicionalmente, é disponibilizada uma perspetiva geral sobre crescimento e desenvolvimento funcional em adolescentes masculinos. Esta secção inclui, ainda, uma descrição detalhada das amostras estudadas, bem como dos materiais e métodos utilizados. A Parte II desenvolve-se no contexto de estudos transversais (estudos 1 a 5) e visa: (*i*) analisar a contribuição da maturação biológica e tamanho corporal para explicar a variabilidade inter-individual na massa do ventrículo esquerdo e pico de consumo de oxigénio em atletas e não atletas adolescentes do sexo masculino; *(ii)* apresentar modelos alométricos para normalizar a massa do ventrículo esquerdo e o pico de consumo de oxigénio; *(iii)* validar um novo modelo para determinar a massa magra dos membros inferiores, usando densitometria radiológica de dupla energia como método de referência. A Parte III da tese (modelos longitudinais mistos; estudos 6 a 10) apresenta dados longitudinais sobre o desenvolvimento: *(i)* da massa do ventrículo esquerdo *(ii)* e da aptidão cardiorrespiratória em rapazes adolescentes saudáveis; *(iii)* de várias capacidades funcionais e habilidades motoras específicas do futebol em atletas adolescentes masculinos. A secção final, Parte IV, compreende uma discussão geral, na qual os resultados dos diversos estudos são sumariados, contextualizados e, com efeito, discutidas as suas implicações.

A partir da análise dos principais resultados, os estudos transversais mostram que: (*i*) a estatura, massa gorda e massa isenta de gordura se evidenciam como a melhor combinação de preditores para normalizar a massa do ventrículo esquerdo em rapazes adolescentes e que quando a informação sobre as componentes da massa corporal não se encontra disponível, deve ser considerado, em conjunto com a estatura, um indicador de maturação biológica; (*ii*) quando a estatura e massa gorda são utilizados para a normalização da massa do ventrículo esquerdo em amostras de adolescentes atletas do sexo masculino (e.g., hóquei em patins), o estatuto

maturacional deve também ser considerado; (*iii*) indicadores apendiculares e a idade esquelética ou indicadores de componentes da massa corporal e indicadores apendiculares combinados com os estádios de desenvolvimento da pilosidade púbica explicam grande parte da variabilidade inter-individual no pico de consumo de oxigénio em jovens atletas (hóquei em patins e futebol, respetivamente); *(iv)* a nova equação, derivada da antropometria e maturação somática, prediz com precisão a massa magra dos membros inferiores em rapazes pré-adolescentes.

Quanto aos estudos longitudinais: *(i)* as diferenças individuais no crescimento alométrico das dimensões de tamanho corporal, adiposidade e maturação biológica, influenciam o crescimento da massa do ventrículo esquerdo em adolescentes masculinos; *(ii)* a diminuição longitudinal do sedentarismo e adiposidade subcutânea revelam ter um impacto significativo na melhoria da aptidão cardiorrespiratória em adolescentes masculinos classificados como aptos; *(iii)* várias capacidades funcionais e habilidades motoras específicas do futebol parecem ser parcialmente explicadas pela idade, maturação biológica, componentes da massa corporal, volume anual de prática e posição ocupada em campo.

Analiticamente, conclui-se que as abordagens alométricas se revelam apropriadas e oferecem interpretações biológicas plausíveis, de dados transversais e longitudinais da morfologia cardíaca e capacidade funcional, em adolescentes masculinos. A modelação multinível é uma técnica estatística promissora para a extrapolação de curvas do desenvolvimento, detendo o potencial para dispor informações úteis, tanto no âmbito clínico como no âmbito desportivo. Com efeito, permite explicar diferenças inter-individuais e, provavelmente mais importante, antecipar e explicar alterações ao longo do tempo, possibilitando uma interpretação crítica da variação intra e inter-individual.

Palavras-chave: Adolescência · Jovens Atletas · Crescimento · Crescimento Cardiovascular · Composição Corporal · Maturação Biológica · Aptidão Aeróbia · Atividade Física · Habilidades Motoras · Ecocardiografia · Densitometria Radiológica de Dupla Energia · Acelerometria · Scaling · Alometria · Regressão de Deming · Análise Longitudinal · Modelação Multinível

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List of Abbreviations

Adj. R^2	– Adjusted coefficients of determination
AIC	– Akaike information criterion
ANTH	– Anthropometry
APHV	– Age at peak height velocity
BMC	– Bone mineral content
BMD	– Bone mineral density
BMI	– Body mass index
BMI*z*	– BMI-for-age *z*-score
CA	– Chronological age
$CC_{\text{m-t}}$	– Corrected mid-thigh circumference
CI	– Confidence intervals
$C_{\text{m-t}}$	– Mid-thigh circumference
CO_2	– Carbon dioxide
CRF	– Cardiorespiratory fitness
CT	– Computerized axial tomography
CV	– Coefficients of variation
DF	– Defenders
DST	– Dribbling speed test
DXA	– Dual energy X-ray absorptiometry
ES-*r*	– Effect size correlation
EST	– Explosive strength
FFM	– Fat-free mass
FM	– Fat mass
FW	– Forwards
G	– Genital
HR	– Heart rate
IGLS	– Iterative generalized least squares
k	– Allometric coefficients

k'	– Ontogenetic allometric coefficients
K-S	– Kolmogorov-Smirnov
LBM	– Lean body mass
LLLM	– Lean lower limbs mass
LOA	– Limits of agreement
LST	– Lean soft tissue
LV	– Left ventricular
LVIDd	– Left ventricular internal dimension at end diastole
LVM	– Left ventricular mass
MANCOVA	– Multivariate analysis of covariance
MANOVA	– Multivariate analysis of variance
MF	– Midfielders
MRI	– Multi-scan magnetic resonance imaging
MVPA	– Moderate-to-vigorous physical activity
NE	– Not entered
NM	– New model
O_2	– Oxygen
PA	– Physical activity
PH	– Pubic hair
PHV	– Peak height velocity
PMS	– Predicted mature stature
PRESS	– Predicted residuals sum of squares
PWTd	– Posterior wall thickness at end-diastole
PWV	– Peak weight velocity
r	– Pearson's product moment correlation coefficient
R	– Model correlation coefficients
R^2	– Coefficient of determination
RAE	– Relative age effect
RER	– Respiratory exchange ratio
RSA	– Repeated-sprint ability
RSS	– Repeated-sprint sequences
RSST	– Repeated shuttle sprint training

RWT	– Relative wall thickness
RUS	– Radius-Ulna-Short Bone
S	– Skinfold
SA	– Skeletal age
SEE	– Standard error of estimate
SEM	– Standard error of measurement
SHR	– Shuttle-run test
SWTd	– Septal wall thickness at end-diastole
TOYA	– Training of Young Athlete
TW3	– Second revision of the Tanner-Whitehouse method
U	– Under
VIF	– Variance inflation factor
1/VIF	– Tolerance
VO_2	– Oxygen uptake
VO_{2peak}	– Peak oxygen uptake
VO_{2max}	– Maximal oxygen uptake

Part I

Introduction and Methods

Chapter I

General introduction

1. General introduction

1.1. The adolescent years

The second decade of life is a period where children change into physically mature adults. The age at which these changes take place may be termed as "puberty" or "adolescence". Puberty is a particular period when reproductive glands begin to function and subjects become ready to reproduce (Buckler, 1987). Adolescence can be understood as a wider concept, a time when extensive changes occur in physiological, biochemical and behavioural domains (Malina et al., 2004). The termination of adolescence is quite variable so that is difficult to specify when adulthood begins. Biologically, some boys are sexually mature by 14 years of age; i.e., they are biologically adult. Yet, they are adolescents in the eyes of society.

Growth, maturation and development

Children and adolescents experience three interacting processes: They grow, mature and develop. These terms are often treated as having the same meaning. They are, however, three different tasks (Table 1.1.) in the daily lives of children and adolescents (Malina et al., 2004). Nevertheless, the three processes occur at the same time and interact. They interact to influence the child's self-concept, self-esteem, body image, and perceived competence (Malina, 2010a).

Stature and body mass are the most commonly body dimensions used to monitor children's and adolescent's growth (Baxter-Jones et al., 1995; Bayer and Bayley, 1959; Kuczmarski et al., 2002; Tanner, 1962). As children grow, they become taller and heavier, they increase in lean and fat tissues, and their organs increase in size, and so on (Malina, 2010a).

Table 1.1. Universal tasks of childhood and adolescence.

Growth	Maturation	Development
Size	Sexual	Cognitive
Proportions	Somatic	Emotional
Physique	Skeletal	Social
Composition	Neuroendocrine	Motor
Systemic	Neuromuscular	Moral
	Self-Esteem	
	Body image	
	Perceived Competence	

Adapted from Malina et al. (2004).

Different segments of the body and components of body mass, vary in the tempo and timing of growth. During the interval of maximum growth in stature (i.e., 13-15 years in boys) boys gain about 14 kg in fat-free mass (FFM) and 1.5 kg of fat mass (FM) (Malina et al., 2004). Therefore, variation within and between individuals in body size, composition, proportions and biological maturation can be considerable. A mismatch, for example, between the demands of a specific sport and those of normal growth and maturation may be a source of strain among young athletes. How an adolescent is coping with his sexual maturation or adolescent growth spurt may influence sport-related behaviours and performance (Elferink-Gemser et al., 2011).

Youth sport and variation among adolescents

Many children and adolescents participate in sports and for a majority it is the major opportunity of physical activity. Reasonably regular participation in sport is characteristic of youth in many European countries (Seabra et al., 2007; Telama and Yang, 2000). In Portugal, participants who engaged in organized physical activity reported more participation in team activities, whereas adolescents in non-organized physical activities reported more participation in individual activities (Santos et al., 2004; Seabra et al., 2007).

Participation in organized sports tends to decline with increasing age from late childhood through adolescence. This reflects changing interests as youth enter adolescence and the selective or exclusive nature of sport (Malina, 2009, 2010b).

From a biological perspective, adolescence includes the onset of sexual maturation, the growth spurt, and eventual attainment of sexual and skeletal maturity. Individual differences in the timing (when) and tempo (progress) of sexual maturation and the growth spurt, and in the time (age) of attaining sexual and skeletal maturity are considerable (Malina et al., 2004).

1.2. Pediatric exercise sciences and biological maturation

Inter-individual variation in biological maturation is a common theme in research pediatric exercise sciences, in general, and also in research with young athletes. This is evident in the relative success of boys advanced in maturation in several sports, e.g., soccer, ice hockey, American football, among others, and of girls later in maturation in artistic gymnastics and figure skating, especially as level of competition increases (Malina, 1994, 1998, 2011). There is also a need to control for variation in biological maturation in studies of the growth and performance characteristics of youth athletes.

Biological maturation can be viewed from two perspectives, status and timing. The former refers to the state of maturation at the time (chronological age, CA) of observation, while the latter refers to the CA at which specific maturational landmarks are attained. The two most commonly used indicators of maturity status are skeletal age (SA) and stage of development of secondary sex characteristics. Two commonly used indicators of maturity timing are age at menarche and age at peak height velocity (APHV); both, however, require longitudinal data to obtain estimates for individuals (Malina et al., 2004).

Sexual maturity

Sexual maturation is a continuous process that extends from sexual differentiation in the period of the embryo through puberty to full sexual maturity and fertility (Malina et al., 2004). The assessment of sexual maturity in growth studies is based in secondary sex characteristics; penis, genital (G) and pubic hair (PH) development in boys. These characteristics are most often evaluated at clinical examination on a five stage scale using the criteria described by Tanner (1962); 1=no development, 2=initial appearance or development, … 5=mature state). Note, however, self-assessments using the criteria of Tanner are increasingly used as are several other modified scales of pubertal development. Given the sensitivity of evaluating pubertal status, the issue of the reproducibility of assessments and potential error has not received sufficient attention.

Overt manifestation of genital (G2) development marks the transition into puberty in boys. Timing and sequence are variable among individuals; pubic hair (PH2) may precede G2 in some youth. Stages 3 and 4 reflect maturity progress on the path towards stage 5 (the mature state), and are more difficult to differentiate than stages 2 and 5 (Coelho-e-Silva et al., 2013). Testicular volume is an additional indicator of sexual maturation in boys but it requires direct palpation.

In addition to stages per se, sampling strategies and limitations (Malina et al., 2004; Sherar et al., 2004), pubertal status of youth is variably reported among studies, which limits comparability. Some studies simply note pubertal status (pre-pubertal, pubertal, post-pubertal) of subjects based on G and/or PH. At times, this is done without specifying the characteristic (s) that was (were) assessed (Slaughter et al., 1988). Note that pre-pubertal children of the same CA are not uniform in maturity status; they can vary by as much as four or five years in SA (Malina et al., 2004. Stages of G and PH are occasionally combined into a single score, e.g., 1.8 or 2.6, or reported as means and standard deviations (Malina et al., 2013; Quitério et al., 2009). As noted, stages are discrete categories. There are no intermediate stages equivalent to 1.8 or 2.6.

Youth are also often grouped by stage of PH or G independent of CA. Variation in CA among youth at the same stage of puberty is problematic. Assume two boys in PH3, one 11 and the other 14 years; the older youngster at the same stage of puberty has had three more years of growth. Older youth within a stage are, on average, taller and heavier. It is also difficult to assume variation in maturity timing (i.e., early or late) based on CA within a specific stage. For example, a study addressing the relationship between overweight and sexual maturation among youth

10-15 years used quartiles of CA adjusted for pubertal stage to differentiate youth by maturity timing (Ribeiro et al., 2006). Within each sex and statistically adjusting for stage of puberty, youth in the first quartile (youngest) of CA were considered "early maturers" while those in the fourth quartile (oldest) of CA were considered "late maturers". As noted, stages indicate only status at the time of observation and provide no information on how long the youngster has been in a particular stage.

The tempo of maturation - the rate of progress through puberty, is a related concept. Observations from two longitudinal studies indicate that progress from G and PH stages 2 (onset) to 5 (maturity) can take less than 2 years in some boys, and 5 years and perhaps more in others (Largo and Prader, 1983; Marshall and Tanner, 1970). Moreover, there is little relationship between the age at which a secondary sex characteristic begins and the length of time required to pass through the stage.

In summary, stages of puberty provide an indication of maturity status at the time of observation. Youth of the same CA can vary considerably in pubertal status, just as youth of the same pubertal status can vary considerably in CA. Both realities need to be recognized in studies of pubertal development of young athletes. Information on the age of transition from one stage to the next, or on the duration of a stage is limited.

Somatic maturity

Body size by itself is not an indicator of maturity. Still, if longitudinal data that span adolescence are available, specifically for stature, the inflection in growth curve that marks the adolescent growth spurt can be used to derive indicators of somatic maturity such as age at onset of growth spurt and age at maximum rate of growth using the spurt (Baxter-Jones et al. 2011; Malina et al., 2004; Sherar et al., 2005).

Recently, a method of assessing biological maturity has been developed that requires CA of an adolescent and a measurement of stature, sitting stature, and body mass (Mirwald et al., 2002). The timing of leg length velocity and sitting stature velocity is used to predict years from APHV (the adolescent growth spurt in stature), which is an indicator of somatic maturity. The standard error of estimate of the equation was 0.592 (95% confidence intervals: 1.18 years) (Mirwald et al., 2002).

Applicability of the method appears, however, to be useful during the interval of growth spurt, approximately 12–15 years (Malina and Kozieł, 2014).

Similarly, if adult stature is available, or can be estimated, the percentage of adult size attained at different ages during growth can also be used as a maturity indicator. Bayley (1962) and Roche et al. (1983) proposed percentage of adult stature as a valid indicator of morphological or somatic maturity because it reaches the same endpoint in all adults (100%), and increases monotonically with age (Beunen et al., 2010). Several non-invasive techniques to predict adult stature have been developed (Beunen et al., 1997a; Khamis and Roche, 1994; Roche et al., 1983; Sherar et al., 2005; Wainer et al., 1978). The most popular predictive equation (Khamis and Roche, 1994), estimate adult stature from current age, stature, body mass and mid-parent stature (adjusted mean height of the parents). The inclusion of midparent stature has been shown to reduce error in the prediction (Bielicki and Welon, 1976; Susane, 1975). The median error bound (median absolute deviation) between actual and predicted mature stature at 18 years of age using the Khamis–Roche protocol is 2.2 cm in males (Khamis & Roche, 1994). Percentage of predict mature (adult) stature attained at a given age is positively related to skeletal and sexual maturation in boys (Bielicki et al., 1984). Percentage of attained predict mature (adult) stature is also related to skeletal age in youth football (American) and soccer players (Malina et al., 2012; Malina et al., 2007).

Skeletal maturity

Skeletal age is an indicator of biological maturity status at the time of observation. SA is used most often to evaluate the level of maturity of the bones of the hand-wrist relative to the reference sample upon which the method of assessment was developed (see below). Assume a youth soccer player has a CA of 15.4 years and an SA of 16.5 years. Although he is chronologically 15.4 years of age, his level of skeletal maturity is equivalent to a boy with a CA of 16.4 years in the reference sample. He is advanced in skeletal maturation relative to the reference. Conversely, a youth player with a CA of 15.4 years may have an SA of 14.2 years. His SA is equivalent to a boy with a CA of 14.2 years in the reference sample; he is late or delayed in skeletal maturation for his CA.

Three methods for the estimation of SA are commonly used. All require a standard radiograph of the hand-wrist skeleton: distal radius and ulna, carpals, metacarpals and phalanges. Two methods are based on samples of American youth (Greulich-Pyle and Fels methods). The third (Tanner-Whitehouse) was initially based on British children, although the most recent version is based on a combined sample of British, Belgian, Spanish, Argentine, American and Japanese youth. The three methods are similar in the principle, but criteria and procedures vary so that SAs assigned with each method are not equivalent.

Details of the methods are beyond the scope of this introduction and are summarized elsewhere (Malina, 2011; Malina et al., 2004). In brief, the Greulich-Pyle and Fels methods use the carpals, radius, ulna and short bones in assessing SA. The most recent version of the Tanner-Whitehouse method (TW3) has two separate protocols, one limited to seven carpal bones and the other based on the radius, ulna and metacarpals and phalanges of the first, third and fifth digits. The former provides a Carpal SA while the latter provides a Radius-Ulna-Short Bone (RUS) SA.

If an individual has attained skeletal maturity, a SA is not assigned; the individual is simply noted as skeletally mature since in cross-sectional samples (as in athletes or medico-legal cases) it is not known when maturity was attained. With the Greulich-Pyle method, age at maturity is somewhat variably defined. With the Fels method, an SA of 18.0 years indicates maturity. With the TW3 RUS method, an SA of 16.5 years in boys is associated with skeletal maturity. Carpal maturity is attained earlier than RUS maturity in TW3.

A factor underlying the discrepancy between the Fels and TW3 methods relates to the final stage of maturation of the radius and ulna. With the Tanner-Whitehouse protocol, the criteria for the final stage of maturation of the distal radial and ulnar epiphyses are that "…fusion of the epiphysis and metaphysis has begun." The time lag between the onset and completion of epiphyseal union is not considered. In contrast, the Fels method has specific criteria from beginning through complete union. Note that the radius is the last bone of the hand-wrist complex to reach maturity (i.e., complete union). With the TW3 protocol, many youth are classified as skeletally mature even though the process of epiphyseal union is not fully completed.

Although the Greulich-Pyle method is widely used, its application is variable. The protocol calls for the assessment of the SA of each individual bone relative to standard plates in the atlas; the median of the SAs is then the SA of the individual. In practice, however, the protocol is applied by comparing the hand-wrist radiograph of an individual as a whole to the standard plates in the atlas; the SA of the plate to which is most closely matches is then assigned as the SA of the individual. This practice is problematic as variation in level of maturity among individual bones is overlooked; there is also a need for interpolation between standard plates.

The preceding reflects methodological issues. A more problematic issue is variation in SA within a CA group. Normal variation in SA is generally accepted as plus/minus three standard deviations, except as maturity is approached (Roche et al., 1988). Standard deviations for SA within single year CA groups 10-17 years range from approximately 0.7 to 1.4 years with the Greulich-Pyle and Fels methods (Malina, 2011). Standard deviations approximate 1 year from childhood through adolescence for Tanner-Whitehouse RUS SAs, but age-specific values were not reported (Tanner et al., 2001). Allowing for differences among methods, it is likely that the range of variation in SA within a CA group can exceed 4 or perhaps 5 years. Adolescent male athletes in a variety of sports (with the exception of artistic gymnastics) tend to be, on average, advanced in skeletal maturity; they are also advanced in sexual maturity and timing of the growth spurt (Malina et al., 2004).

1.3. Functional development during childhood and adolescence

Besides dimensional modifications, adolescence is also marked by important physiological transformations. An appreciation of functional development is essential for understanding the responses of children to exercise. Left ventricular mass (LVM) is an important morphological characteristic of the heart from a functional perspective (Malina et al., 2004). This influence is particularly evident during adolescence and young adulthood (de Simone et al., 1998). LVM is similar in boys and girls until age 9 to 12 years, but it then grows faster in boys even when expressed per body mass (de Simone et al., 1995).

Long-term training in adult athletes is associated with adaptive changes in cardiac structure (D'Andrea et al., 2013; Limongelli et al., 2006). Adaptations in response to the hemodynamic loading conditions induced by chronic exercise include increased cavity size, wall thickness, and mass (Giada et al., 1998; Sharma et al., 2002). Generally, increments in LVM during adolescents are strongly correlated with increases in body mass which, among other factors, are associated with growth related changes in skeletal muscle mass (Malina et al., 2004). Similar to aerobic fitness (Rowland, 2005), LVM and cardiac output is strongly determined by the demands of metabolically active tissues (Daniels et al., 1995). Even though stature is strongly correlated with lean body mass, the relationship is not perfect.

A cross-sectional study of healthy children 6-17 years of age showed that a 10 kg increase in fat mass corresponded to 5 g increase in LVM after adjusting for lean body mass and systolic blood pressure (Daniels et al., 1995). Longitudinal echocardiographic data for youth are limited (Dai et al., 2009; Gardin et al., 2002; Urbina et al., 1995). A 5-year follow-up of biracial cohorts of the Bogalusa Heart (Urbina et al., 1995) and CARDIA Study (Gardin et al., 2002) noted that the onset of obesity predicted longitudinal changes in LVM in healthy children and young adults. Data from Project HeartBeat showed a mean change in LVM of 11–14 g for a 10 kg change in fat mass, when fat-free mass and other covariates (CA, sex or body size descriptors) were statistically controlled (Dai et al., 2009).

In children and adolescents, peak aerobic fitness (VO_{2peak}) increases as a function of body size, regardless of engagement in regular physical training (Armstrong and Welsman, 1994; Malina et al., 2004). Increments in absolute VO_{2peak} in adolescents are strongly correlated with increases in body mass which, among other factors, are connected to growth related changes in lungs, heart and skeletal muscle mass (Rowland, 2005).

Longitudinal data on boys from the Leuven Longitudinal Twin Study, suggested a simultaneous regulation of the timing of maximum growth in body dimensions and aerobic fitness during adolescence (Geithner et al., 2004). In general, the available data indicates that age-related increases in the maximal O_2 consumption are mostly mediated by changes in size dimensions, as the haematological components of oxygen delivery and the oxidative mechanisms of the exercise muscle are related to body dimensions and muscle mass (Armstrong and Welsman, 1994; Eisenmann et al., 2001).

Methodologically, it has been shown, for example, that maximum aerobic power of professional soccer players did not change from pre- to post-5 weeks of aerobic training; however, time to exhaustion on the maximal O_2 uptake test on the treadmill increased significantly by 12% (Edwards et al., 2003). These findings suggested that measured maximal O_2 uptake is not a sensitive indicator of developmental changes in mid and long-term maximal efforts in soccer players. Cardiorespiratory fitness (CRF), the capacity of the cardiovascular and respiratory systems to carry out prolonged stenuous exercise (Ortega et al., 2008), is normally assessed by performance in a 20-m multi-stage continuous shuttle-run (e.g., Albarwani et al., 2009; Machado-Rodrigues et al., 2012). This motor task also combines coordination, lower limb strength and motivation and was reported to be strongly correlated with directly measured maximal O_2 uptake ($r = 0.80$), suggesting that it could be used as a surrogate measure of aerobic capacity in children and adolescents (Ahmaidi et al., 1992).

Human performance related fitness often involves activities of short-term duration (e.g., 10 s to 1 min; Van Praagh, 1996); these activities have been termed “anaerobic” (Bar-Or, 1987), assuming that the biochemical pathways for the energy yield is mainly derived from anaerobic process (Van Praagh, 1998). Nevertheless, the aerobic capacity also exerts an important contribution to the execution of tasks that are very intense and of brief duration (Bar-Or, 1987) and this aerobic contribution is greater among children and adolescents than among adults (Hebestreit et al., 1993). Inbar and Bar-Or (1986) suggest that the improvement in short-term performance in children and adolescents is not only dependent on quantitative factors (i.e., increments in body and muscular mass) but also in qualitative aspects of the muscle or the activation of motor units.

The distinction between age and training effects on functional capacities is complex. Based on a small sample (7 youth soccer players), the effect of a 10-week specific training with one session per week of 2-3 sets of 5-6 x 15-20-m shuttle sprints interspersed with 14-23 seconds recovery produced a beneficial impact on maximal sprinting speed and repeated shuttle sprint performance (Buchheit et al., 2010a). Longitudinal observations for youth soccer players are limited to running speed, aerobic endurance and lower limb explosive strength, which, on average, tended to demonstrate maximal gains close to APHV (Philippaerts et al., 2006). However, the knowledge on developmental changes in functional characteristics are still limited in the literature that typically does not consider the interaction between growth, biological maturation and skills.

1.4. Maturity-associated variation in growth and performance

Stature and body mass of early- and mid-adolescent male athletes in a variety of sports tend to fluctuate, on average, above and below reference medians for the general population, but in late adolescence and young adulthood players (e.g., soccer, roller hockey) tend to have, on average, more body mass-for-stature (Coelho-e-Silva et al., 2012; Malina et al., 2000). The trend may reflect late adolescent growth in muscle mass. The inter-individual variation in body size is related, in part, to individual differences in biological maturation during adolescence (Figueiredo et al., 2009).

The literature suggests that the elite adolescent male athletes in team sports, including soccer, tend to be advanced in skeletal maturation especially after 14 years age (Malina, 1968). Moreover, the relative age effect evident in many sports represents age and possibly maturational advantages compared to peers (Baxter-Jones, 1995).

Boys advanced in biological maturation compared to age peers tend to perform better in tests that place a premium on strength, power and speed during adolescence (Beunen and Malina, 1988; Lefevre et al., 1990; Malina et al., 2004). Differences are especially marked between 13 and 15 years that correspond to maximal rate of growth in stature and body mass (Philippaerts et al., 2006). Maturity-related differences in strength, speed and power during the interval of the growth spurt in stature are attenuated in later adolescence and young adulthood (Lefevre et al., 1990), thus highlighting the transient nature of maturity-associated variation in performance during adolescence.

It has been recently suggested that pubertal maturation has no independent influence on VO_{2peak} (Cunha et al., 2011), but previous studies based on multilevel modelling (Armstrong et al., 1999; Baxter-Jones et al., 1993) noted an independent influence of pubertal maturation on VO_{2peak}.

Coelho-e-Silva et al. (2008) examined basketball players aged 12-13 years of age and after testing differences between participants in stages PH2, PH3 and PH4 basketball in functional capacities (strength, aerobic fitness) and sport-specific skills. The analysis was repeated using CA as covariate. In parallel, another study with young soccer players aged 11-14 years (Figueiredo et al., 2009) examined inter-individual variability in physical performance associated with skeletal maturity given by the discrepancy between SA and CA (delayed, on-time, advanced). Evidences of maturity related variation of performance in the soccer and basketball studies are not comparable, since stage of puberty used in youth basketball did not inform about the tempo and, not surprisingly, demonstrated a different influence on functional capacities compared to the study of young soccer players who did not differ in speed, agility, power and four soccer skills, but did differ in aerobic performance assessed by a 20-m intermittent shuttle-run protocol, with ‘early’ maturing athletes performing better than ‘on time’ and ‘late’ maturers.

Longitudinal observations in youth literature are scarce. From the Ghent Youth Soccer Project (Philippaerts et al., 2006), it was possible to determine peak velocities and age at maximum peak velocities for stature, body mass, and functional capacities of the EUROFIT in 33 soccer players aged 10.4 to 13.7 years at the start of the study. Nevertheless, the question of the specific contribution of biological maturation to performance, independent of body size and CA, or in interaction with body size remains to be fully investigated. On the other hand, the effect of maturation and body size on sport-specific skills is even less documented and the trend is not as consistent as it is for studies devoted to functional capacities (Figueiredo et al., 2009, Malina et al., 2007).

1.5. Adjusting and interpreting physiological functions for variation in body size

Ratio standards, isometry and elastic similarity

Physiological functions are routinely expressed relative to stature or body mass. For example, LVM has been presented as a per-stature (g/m; Lauer et al., 1991) or per-surface area ratio standard ($g{\cdot}m^{-2}$; Devereux et al., 1986). Peak oxygen uptake, on the other hand, is commonly reported in millilitres per minute per unit body mass ($L{\cdot}kg^{-1}{\cdot}min^{-1}$). Fat-free mass, body surface are and stature are also used as denominators. However, issues related to the validity of estimates FFM and body surface area need to be considered if these variables are used as denominators.

Simple ratio standards have been strongly criticised (Katch and Katch, 1974; Tanner, 1949). Expressing functions relative to anthropometric dimensions is meant to control for inter-individual variability in body size and is based on the assumption of geometric similarity. The ratios rely on the assumption that variables expressed as the ratio are linearly related, but these standards are theoretically fallacious and in practice misleading (Tanner, 1949); as smaller individuals receive an artefactual arithmetic advantage. Although ratio standards are commonly used to control for the effects of body size on performance, other methods provide plausible alternatives.

Relationships among length, surface area and volume have implications for metabolism and thermoregulation. All linear anthropometric dimensions of the body, such as stature, segment lengths, and breadths have the dimension d. All areas including body surface area and muscle cross-sectional areas have the dimension d^2. Total body volume given by air displacement plethysmography and other volumes (lung, heart, lower limb) have the dimension d^3. According to the second Newtonian law, time has the dimension d^1. For example, VO_{2peak} measured as volume per unit time ($L{\cdot}min^{-1}$) should be proportional to d^3-d^1. In order to dissociate VO_{2peak} from body size given by stature (m), values should be expressed in m^2. In isometric bodies stature corresponds to mass raised to 2/3 power function (assumption of geometric similarity) and an alternative denominator for VO_{2peak} would be $mL{\cdot}kg^{-0.67}$. However, a scaling coefficient of mass raised to the power 0.75 has also been suggested. Observations suggest that metabolic rate in many species of homeotherms conforms to mass raised to the power of 0.75 and not to the expected surface law of mass raised to the power of 0.67. A rationale for this apparent departure from theoretical predictions was offered by a model of elastic similarity (McMahon, 1973). This model using a 0.75 coefficient was proposed in zoology, but has been questioned as a statistical artefact because it was based on a single allometric model applied to data for a number of different species. When the data from specific species was interpreted separately, it had an intra-specific coefficient of 0.67.

Assumptions for ratio standards and allometric modelling

Performance and physiological data often have a normal distribution. If the data have longer tail to the right side of the distribution, the data are described as positively skewed. In contrast, if the longer tail occurs on the left side of the distribution, the data are described as negatively skewed. Certain transformations can be used to overcome the problems of skewed data to correct the asymmetry and provide a normal distribution. For example, a log transformation will frequently correct positively skewed data such as body mass (kg) or maximal oxygen uptake ($L \cdot min^{-1}$) into a 'log-normal' distribution.

Validity of ratio standards ('Tanner's exceptional circumstance') can be analysed based on the ratio of coefficients of variation (CV). The ratio standard is valid when the CV (%) of the anthropometric characteristic (*x*) dived by the CV (%) of the performance or physiological variable (*y*) is equal to Pearson's product moment correlation coefficient ($r\ x,y$) for the two variables (Tanner, 1949).

Allometric models present an alternative to ratio standards in assessing the effects of size-related increments on performance. Allometry (i.e., relationship between variables that are affected by proportional changes due to variation in body size and growth rate; Gunther, 1975), successfully accommodates nonlinear relationships between body size descriptors and LVM and apparently overcome the heteroscedastic (non-constant variance) errors observed with such size descriptors, thus improving normalization (Batterham and George, 1998).

An example of the allometric model is presented in Equation 1.1, where *a* is the intercept of the regression line on the *y*-axis and *k* is the slope of the line used to model the relationship between performance outputs and variables associated with body size, and ε is a multiplicative error term. Values of *a* and *k* are derived from linear regressions of the logarithmic regression transformations of Equation 1.1 that are given in Equation 1.2.

$$y = a \cdot x^k \cdot \varepsilon \qquad \textbf{(1.1)}$$

$$\ln (y) = \ln (a) + b \cdot \ln (k) + \ln (\varepsilon) \qquad \textbf{(1.2)}$$

Usually, single size descriptors alone do not partition out correctly the influence of body size on performance outputs. Consequently, proportional allometric models (Nevill et al., 1998) can be explored (Equation 1.3). Multiple stepwise regressions can be conducted using the linearized equation with log-transformations (Equation 1.4).

$$y = \text{size descriptor}_1{}^{k1} \cdot \text{size descriptor}_2{}^{k2} \cdot \varepsilon \qquad \textbf{(1.3)}$$

$$\ln (y) = k_1 \cdot \ln (\text{size descriptor}_1) + k_2 \cdot \ln (\text{size descriptor}_2) + \ln (\varepsilon) \qquad \textbf{(1.4)}$$

Other models that incorporate CA or SA can also be explored (Equation 1.5). Equation 1.5 can be linearized with a log-transformation as in Equation 1.6.

$$y = \text{size descriptor}^k \cdot \exp (a + b \cdot \text{CA or SA}) \cdot \varepsilon \qquad \textbf{(1.5)}$$

$$\ln (y) = k \cdot \ln (\text{size descriptor}) + a + b \cdot (\text{CA or SA}) + \ln \varepsilon \qquad \textbf{(1.6)}$$

Static and ontogenetic allometry

The concept has been used to study ontogenetic and phylogenetic changes in morphological, physiological or biochemical characteristics (Beunen et al., 1997b).

Ontogenetic allometry refers to differential growth in the individual (Balasekaran et al., 2005; Beunen et al., 1997) and is most often applied to longitudinal data (Gould, 1966). Static allometric coefficients (phylogenetic) reflects the dimensional relation between performance or physiological data and size descriptors (Beunen et al., 1997), in conspecific individuals at the same developmental (ontogenetic) stage (Pelabon et al., 2013). In summary, ontogenetic allometry describes differential growth in the individual growth process but cannot quantify group developmental changes. When different individuals are measured at the same developmental stage within a population it is called a static allometry. These allometric procedures can be used to account for intra- and inter-individual variability, respectively. However, allometric multilevel modelling offers many advantages evaluating longitudinal changes as it allows simultaneous estimation of the within and among individuals variation.

Multilevel and allometric multilevel modelling

With appropriate statistical techniques, a longitudinal design can identify changes in performance or physiological functions and, for example, partition the relative contributions of training from those associated with growth and maturation. Multilevel modelling (Goldstein, 1995) is appropriate for the analysis of longitudinal observations, i.e., repeated measurements. The technique is an extension of multiple regression analysis and is appropriate for analysing hierarchically structured data.

Multilevel modelling effectively captures the feature that the variance of the observations increases with time, and permits individual slopes and intercepts. It thus provides the opportunity to determine the effects of each predictor variable on the slope and intercept and its significance can be determined by relating the observed effects to the respective change in standard errors (Baxter-Jones et al., 1993). Thus group effects larger than within-individual variation can be identified.

Longitudinal studies often deal with missing data and experience dropout occurrences. In the multilevel model technique, the number of observations and temporal spacing between measurements can vary among subjects. All available data can thus be incorporated into the analysis. It is assumed that the probability of being missing is independent of any of the random variables in the model. As long as a full information estimation procedure is used, such as maximum likelihood in MLwiN for Normal data, the actual missing mechanism can be ignored (Rasbash et al., 1999).

The following additive polynomial random-effects multi-level regression model is usually adopted to describe the developmental changes (Rasbash et al., 1999):

$$y_{ij} = \alpha + \beta_j x_{ij} + k_1 \zeta_{ij} + \cdots k_n \zeta_{ij} + \mu_j + \varepsilon_{ij} \quad \textbf{(1.7)}$$

where y is the performance parameter on measurement occasion i in the jth individual; α is a constant; $\beta_j x_{ij}$ is the slope of the performance parameter with age for the jth individual; and k_1 to k_n are the coefficients of various explanatory variables at assessment occasion i in the jth individual. Both μ_j and ε_{ij} are random quantities, whose means are equal to zero; they form the random parameters in the model. They are assumed to be uncorrelated and follow a normal distribution; μ_j is the level 2 and ε_{ij} the level 1 residual for the ith assessment of performance in the jth individual. The model was built in a stepwise procedure, i.e., predictor variables (k fixed effects) were added one at a time, and likelihood ratio statistics were used to judge the effects of including further variables (Baxter-Jones and Mirwald, 2004).

It is suggested that multiplicative allometric rather than additive models would provide a superior fit and more plausible interpretation of such longitudinal data (Nevill and Holder, 1995; Nevill et al., 1998). They argue that because variables such as aerobic fitness are known to be proportional to but nonlinear with body mass, an additive polynomial model is unlikely to satisfactorily explain developmental changes over time. The authors propose an alternative multiplicative (proportional) model with allometric body size components to describe these developmental changes that should successfully accommodate the nonlinear but proportional changes with body mass and naturally help overcome the heteroscedastic (multiplicative) errors observed with such variables:

$$y_{ij} = \text{body mass}^{k1} \cdot \text{stature}^{k2} \cdot \exp(a_{ij} + b_j \cdot \text{CA}) \cdot \varepsilon_{ij} \quad \textbf{(1.8)}$$

where, as with the additive model structure, all the parameters were fixed with the exception of the constant (intercept term, a_{ij}) and age or maturity offset (b_j), which were allowed to vary randomly from subject to subject (level 2), and the multiplicative error ratio ε_{ij}, which was used to describe the error variance between observations (level 1).

The model can be linearized with a logarithmic transformation (Nevill et al., 1998). The transformed log-linear multilevel becomes:

$$\ln(y_{ij}) = k_1 \cdot \ln(\text{body mass}) + k_2 \cdot \ln(\text{stature}) + a_{ij} + b_j \cdot (\text{CA}) + \ln \varepsilon_{ij} \quad \textbf{(1.9)}$$

As with the additive polynomial models described above, categorical factors can be incorporated into subsequent analyses by introducing them as fixed indicator variables.

1.6. Rationale, objective and outline of the thesis

Simple ratio standards often remain size dependent thus confounding interpretations. Linear regression scaling is limited by its assumption of an additive error term and taking into account that most performance parameters are characterized by heteroscedastic (multiplicative) error terms, allometric (log-linear) scaling techniques facilitate the construction of appropriately size-adjusted ratio. Also, the relationships between body size and organ dimensions are often nonlinear and the error term does not present constant variance trough the range of observations (de Simone et al., 1992). The decision of the most appropriate variable(s) against which to normalize LVM (Foster et al., 2013; Lang et al., 2005) is still an open question especially during years of maximal changes in different size descriptors that do not occur simultaneously; further evaluation of appropriate indexing variables for LVM in children and adolescents is needed. Moreover, research dealing with the left ventricular structure of adolescent athletes is rather limited (Basavarajaiah et al., 2006; Zdravkovic et al., 2010) and does not systematically consider variation associated with inter-individual differences in growth and maturation.

Our knowledge of the relationships among, for example, aerobic fitness, growth, maturation and training is also limited. Moreover, aerobic fitness depends on the oxidative mechanisms of exercising muscle (Armstrong and Welsman, 2001) and leg muscle mass has been indicated as a major contributor to the explained inter-individual variability in aerobic fitness (Nevill et al., 2004). Considering the extent to which the legs contribute to performance (Winter et al., 1991), it is surprising that scaling analyses which include an estimate of lean lower limbs mass are lacking.

The use of longitudinal designs is systematically recommended, although such research is limited. With appropriate statistical techniques, a longitudinal design can identify changes in specific parameters and partition the relative contributions, for example, of training from those associated with growth and maturation. As previously noted, multilevel modelling (Goldstein, 1995) is appropriate for the analysis of longitudinal observations, i.e., repeated measurements, but it is suggested that multiplicative allometric rather than additive models would provide a superior fit and more plausible interpretation of such longitudinal data (Nevill and Holder, 1995; Nevill et al., 1998).

The sparse amount of data and the broad analytical approaches in pediatric exercise sciences research, the inter-individual variation in biological maturation and its influence on size, organ dimensions and performance of adolescent male athletes and non-athletes, raise many research questions that may provide clinicians, physical education teachers, trainers, coaches, parents and other interested parties, with more accurate and precise methods and information's.

Therefore, the overall purpose of this thesis is to gain more insight into the relationship between (the development of) LVM and several functional capacities with body size, biological maturation, physical activity and/or sedentary time in adolescent male athletes and non-athletes, using allometric and multilevel modelling approaches.

The thesis is presented in manuscripts format and is organized in two sections; the first section comprises the cross-sectional studies (study 1 to 5) and the second section the mixed-longitudinal studies (study 6 to 10). The manuscripts presented in this thesis (Chapter 3 to 12) have a common structure with minor modifications according to the style of the journal where the manuscripts were published or submitted for publication. Each of the ten manuscripts addresses a specific component of the overall purpose of the study.

Chapter 2 provides a comprehensive description of the studied samples as well as the materials and methods used for adolescents' assessment and data record.

In Chapter 3, the adequacy of proportional allometric model structures for scaling LVM is presented; and, in addition, the independent and combined effects of biological maturation and different body descriptors to explain variability in LVM among adolescent boys are explored.

Chapter 4 features a study that focuses on the independent and combined effects of CA and SA with various body descriptors on LVM of male adolescent roller hockey players, using proportional allometric modelling.

Chapter 5 examines the extent to which CA, SA, stature and thigh volume interdependently and interactively accounts for the inter-individual variation in peak VO_2 in male adolescent roller hockey players.

Chapter 6 present a study that investigate the independent and interactive contributions of pubertal maturity status, stature, body mass, lean body mass and appendicular descriptors to the inter-individual variation in VO_{2peak} in youth soccer players. Additionally, static allometric models to normalize VO_{2peak} were developed, using alternative descriptors and, in the case of lean body mass, concurrent estimates derived from anthropometry and dual energy X-ray absorptiometry (DXA).

In the studies described in Chapters 5 and 6, aerobic fitness was shown to be strongly determined by appendicular volume and composition. Accordingly, in Chapter 7, a non-invasive estimate of lower limbs lean soft tissue was derived in circumpubertal males and the new model was cross-validated using DXA measures as the reference method.

In Chapter 8, longitudinal data are presented on adolescent boys that have been followed across time. In the preceding context, this study aimed to investigate the independent and interactive contributions of stature, body mass, adiposity and biological maturity to inter- and intra-individual development of LVM and to present allometric models for normalizing LVM.

Chapter 9 document the characteristics of adolescent healthy boys aged 11-15 years and examines the independent and combined effects of age, biological maturity status, adiposity and objectively measured PA and sedentary time on the development of CRF.

Given the relatively limited data for youth athletes, especially covering early, middle and late pubertal development of functional capacities, in Chapter 10 the longitudinal changes of an activity of short-term duration (i.e., repeated-sprint ability) in young soccer players was considered from 11 to 17 years of age using multilevel modelling designs; initially aligned for CA and afterwards by SA.

Chapter 11 evaluates the longitudinal changes in several functional and soccer-specific skills of male youth soccer players that can be described as a developmental pool for potential talent identification. The purpose was to document the characteristics of young soccer players aged 11-17 years and to identify the longitudinal predictors of functional capacities and skills. Finally, to examine developmental curves by maturity status and playing position.

Chapter 12 explores the utility of multiplicative allometric model structures for the derivation of developmental curves for agility and dribbling performance of youth soccer players between 10 and 18 years of age.

Chapter 13 comprises the general discussion in which the findings of the various studies are summarized and put into context and their implications discussed.

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Chapter II

Methods

2. Methods

2.1. Study design and sampling

All studies included in the present thesis were conducted within projects partially funded by the Portuguese Foundation for Science and Technology (SFRH/BD/64648/2009; PTDC/DES/121772/2010; PTDC/DTP-DES/1178/2012). These projects consisted of cross-sectional and mixed-longitudinal approaches conducted in accordance with ethical procedures of the Declaration of Helsinki for human studies by the World Medical Association (2008) and in accordance with ethical standards for sports medicine (Harriss and Atkinson, 2011).

Data collection was conducted using equipment from three different research units, in Portugal:

(1) The Research Unit for Sport and Physical Activity, Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra (study 1 to 3 and 6 to 10);

(2) The Research Centre in Physical Activity, Health and Leisure, Faculty of Sport, University of Porto, Porto (study 4);

(3) The Exercise and Health Laboratory, Faculty of Human Kinetics, University of Lisbon, Lisbon (study 5).

All participants and parents/legal guardians were informed about the objectives experimental protocol and procedures of the studies and provided appropriate informed assent and written informed parental consent, respectively.

In Table 2.1 are summarized the basic characteristics of each study regarding design, sampling and studied variables.

Table 2.1. Basic characteristics of each study.

Study	Design	Sample characteristics; sex	Age	Studied variables
1	Cross–sectional	110; males	11–15 years	Anthropometry; heart dimensions; biological maturation
2	Cross–sectional	73 athletes; males	14.5–16.5 years	Anthropometry; heart dimensions; biological maturation
3	Cross–sectional	73 athletes; males	14.5–16.5 years	Anthropometry; biological maturation; peak aerobic fitness
4	Cross–sectional	81 athletes; males	8–18 years	Anthropometry; body composition; biological maturation; peak aerobic fitness
5	Cross–sectional	75; males	10–13 years	Anthropometry; body composition; biological maturation;
6	Mixed–longitudinal	87; males	12–15 years	Anthropometry; heart dimensions; biological maturation
7	Mixed–longitudinal	110; males	11–15 years	Anthropometry; biological maturation; intensity levels of physical activity; sedentary time; cardiorespiratory fitness
8	Mixed–longitudinal	135 athletes; males	11–17 years	Anthropometry; biological maturation; functional capacities
9	Mixed–longitudinal	135 athletes; males	11–17 years	Anthropometry; biological maturation; functional capacities; skills
10	Mixed–longitudinal	83 athletes; males	11–17 years	Anthropometry; biological maturation; functional capacities; skills

2.2. Anthropometry

Anthropometric assessment was performed by a single experienced observer following standard procedures (Lohman et al., 1988). Stature and sitting stature were measured to the nearest 0.1 cm with a Harpenden stadiometer (model 98.603, Holtain Ltd, Crosswell, UK and Harpenden sitting height table, model 98.607, Holtain Ltd, Crosswell, UK, respectively) and body mass was measured to the nearest 0.1 kg using an electronic scale [SECA balance, model 770, Hanover, MD, USA (study 1 to 3 and 6 to 10); Tanita, BC-418, MA, USA (study 4 and 5)]. The body mass index (BMI) was calculated and expressed as a BMI-for-age *z*-score (BMIz) for a reference population (Kuczmarski et al., 2002). BMIz has been suggested as a reasonable index of adiposity for youth (Freedman and Sherry, 2009).

Leg length was estimated as stature minus sitting stature. Circumference at the gluteal furrow (highest possible horizontal circumference), mid-thigh (largest mid-thigh circumference) and distal thigh (minimum circumference above the knee) were measured in the orthogonal plane, on the right site of the body. Mid-thigh circumference ($C_{\text{m-t}}$) was corrected for subcutaneous adipose tissue thickness, according to Lee et al. (2000). The corrected mid-thigh circumference ($CC_{\text{m-t}}$) was calculated as $CC_{\text{m-t}} = C_{\text{m-t}} - \pi S$, where S stands for the skinfold measurement, which is assumed to be twice the subcutaneous adipose tissue thickness. Lengths between each circumference level were also measured to estimate total thigh volume (study 3) of the dominant leg (Jones and Pearson, 1969). The method was already used in pediatric sport science (Martin et al., 2004).

Skinfolds (triceps, subscapular, suprailiac, anterior and posterior mid-thigh and mid-calf) were measured to the nearest mm using a Lange Caliper (Beta Technology, Ann Arbor, MI, USA). The logarithm of sum of the four skinfolds was used as an estimate of overall subcutaneous adiposity (study 6).

Technical errors of measurement for a subsample of participants (n = 22) measured one week apart were as follows: stature, 0.3 cm; sitting stature, 0.3 cm; body mass, 0.5 kg; skinfolds, 0.5 to 0.7 mm. The magnitude of errors was well within the range of several health surveys in the United States and a variety of field surveys (Malina, 1995).

2.3. Body composition

Estimated fat and fat-free mass

Percentage body fat was estimated from triceps and subscapular skinfold thicknesses using the protocol of Slaughter and colleagues (1988). Fat-free mass (FFM) was derived in kg (study 1, 2, 8-10).

Estimated lean body mass

Lean body mass estimated from anthropometry (study 4) was obtained from logarithmic transformed values of stature and body mass, BMIz and chronological age using the protocol of Foster et al. (2012), as presented in Equation 2.1:

$$\ln \text{lean body mass} = -2.9585 + 0.8208 \cdot \ln \text{stature} + 0.5607 \cdot \ln \text{body mass} + 0.0000184 \cdot \text{body mass}^2 - 0.0159 \cdot \text{BMIz}^2 + 0.0135 \cdot \text{chronological age} \quad \textbf{(2.1)}$$

Dual energy X-ray absorptiometry

Research Centre in Physical Activity, Health and Leisure

A Hologic QDR 4500A DXA scanner (Hologic Inc., Waltham, MA, USA) with v.9.10 software was used to perform total body scans (study 4). DXA is an accepted measure of body composition (Ellis, 2000; Leahy et al., 2012). The procedure allows measurement of segmental composition: arms, legs and trunk. Daily calibration of the scanner was performed using a phantom spine containing composites of bone, fat and lean tissue. Participants were positioned on the scanner bed according to manufacturer recommendations. The lower limbs on each image were sectioned as follows: all tissue distal to a line drawn through and perpendicular to the axis of the femoral neck and angled with the pelvic brim to the phalange tips. Lean lower limbs mass was obtained by adding the left and right legs. Mean variation between measured and reconstructed absolute whole-body mass with DXA software was 0.7%. Based on test-retest in 15 individuals, the coefficients of variation for percent body fat and lean body mass were 3.1% and 1.1%, respectively.

The Exercise and Health Laboratory

A Hologic Explorer-W, fan-bean densitometer, software QDR for Windows version 12.4 (Hologic, Waltham, MA, USA) was used to perform total body scans (study 5). The procedure allows measurement of segmental composition: arm, leg and trunk. Daily calibration of the scanner was performed using a phantom spine containing composites of bone, fat and lean tissue. Participants were positioned on the scanner bed according to manufacturer recommendations. The same lab technician positioned the participants, performed the scans and executed the analyses according to the operator’s manual. The lower limbs on each image were sectioned as follows: all tissue distal to a line drawn through and perpendicular to the axis of the femoral neck and angled with the pelvic brim to the phalange tips. Total lower limb composition was estimated by adding the mass of the left and right legs. Mean variation between measured and reconstructed absolute whole-body mass with DXA software was 0.9%. Based on test-retest in 10 individuals, the coefficients of variation for percent body fat and LST were 1.6% and 0.8%, respectively.

2.4. Heart dimension

A comprehensive resting echocardiography study was performed using a Vivid 3 ultrasound machine with a 1.5-3.6 MHz transducer (GE Vingmed Ultrasound, Horten, Norway). M-mode echocardiograms were derived from 2-dimensional images under direct visualization and were recorded at 100 mm · s $^{-1}$ (study 1, 2 and 6). Measurements of LV internal dimension at end diastole (LVIDd), septal wall thickness at end-diastole (SWTd) and posterior wall thickness at end-diastole (PWTd) were obtained in accordance with recommendations of the American Society of Echocardiography (Lang et al., 2005). The LVM was calculated with the formula proposed by Devereux and colleagues (Devereux et al., 1986). Relative wall thickness (RWT) was the ratio of the average ventricular septal and posterior free wall thickness to the radius of the ventricular cavity. To assess intra-observer variability, 20 randomly chosen subjects were measured again after a one week interval by the same observer. Intra-observer variability was determined as the difference and percentage difference (with a denominator a mean of the two measurements) between the two measurements along with their 95% limits of agreement (LOA) (Bland and Altman, 2012). Intra-observer variability for LVIDd, SWTd and PWTd was 0.17 mm (95% LOA – 1.95 to 2.28 mm), 0.02 mm (95% LOA – 0.30 to 0.34 mm), and 0.06 mm (95% LOA – 0.45 to 0.56 mm), respectively. For the same three parameters, percentage intra-observer variability was 0.3% (95% LOA – 4.1 to 4.8%), 0.3% (95% LOA – 4.2 to 4.8%), and 0.8% (95% LOA – 6.5 to 8.1%), respectively.

2.5. Biological maturation

Sexual maturity

Pubic hair (PH) development (study 4) was assessed by an experienced physician using the criteria described by Tanner (1962).

Somatic maturity

Maturity offset [time before or after age at peak height velocity [(APHV; study 6)] was predicted from a sex-specific equation based on Canadian and Belgian boys

(Mirwald et al., 2002); maturity offset minus chronological age (CA) provide an estimate of APHV (study 1). The standard error of estimate (SEE) of the equation was 0.592 (95% confidence intervals: 1.18 years) (Mirwald et al., 2002). Maturity offset can be used to classify adolescents as pre- or post-APHV, while individuals can also be grouped by years before or after APHV rather than CA. Applicability of the method appears to be useful during the interval of growth spurt, approximately 12–15 years (Malina and Kozieł, 2014).

Chronological age, stature, and body mass of each boy and midparent stature were used to predict mature (adult) stature (study 5) using the Khamis–Roche protocol (Khamis and Roche, 1994). The median error bound (median absolute deviation) between actual and predicted mature stature (PMS) at 18 years of age is 2.2 cm in males (Khamis and Roche, 1994). Percentage of PMS attained at a given age is positively related to skeletal and sexual maturation in boys (Bielicki et al., 1984). Percentage of attained PMS is also related to skeletal age in youth football (American) and soccer players of the same age range as the study sample (Malina et al., 2012; Malina et al., 2007). Finally, percentage of attained PMS expressed as a *z*-score relative to age-specific (half-year intervals) means and standard deviations from the Berkeley Guidance Study (Bayley and Pinneau, 1952), was also used as indicator of maturity status (study 7).

Skeletal maturity

A posterior-anterior radiograph of the left hand-wrist was used to assess skeletal age (SA; study 2, 3, 8-10). All films were rated using the Fels method for assessing SA (Roche et al., 1988). This method assigns grades to specific maturity indicators for the radius, ulna, carpals, metacarpals plus phalanges of the first, third and fifth rays, and utilizes ratios of linear measurements of the widths of the epiphysis and metaphysis of the long bone. The protocol also notes the presence (ossification) or absence of the pisiform and addutor sesamoid bones. Ratings are entered into a program (Felshw 1.0 Software, Lifespan Health Research Center, Departments of Community Health and Pediatrics, Boonshoft School of Medicine, Wright State University, Dayton, Ohio). The treatment weights the contributions of the specific indicators, depending on CA and sex in calculating a SA and standard error of estimate (a confidence interval for the assessment). The standard error is a unique feature of this protocol and tended to slightly increase with age (11–12 years: 0.27–0.32; 13–14 years: 0.27–0.49; 15–17 years: 0.28–0.72), because at later ages the assessment is based on fewer indicators (Malina et al., 2010). Using an independent sample (*n* = 20), the mean difference in SA assessments made one week apart was 0.02±0.13 years. Additionally, intra-observer technical error of measurement was 0.03±0.04 years. The difference between SA and CA (SA minus CA) was used to classify players into maturity categories: late (delayed), SA younger than CA by > 1.0 yr; average (on time), SA ± 1.0 yr CA; early (advanced), SA older than CA by > 1.0 yr; a SA was not assigned if the individual had attained skeletal maturity. The band of 1.0 year is consistent with age-specific standard deviations for SAs in adolescent boys and allows for errors associated with assessments (Malina et al., 2010).

2.6. Physical activity and sedentary time

Physical activity and time spent sedentary were measured for seven consecutive days (study 7) using ActiGraph GT1M accelerometer (ActiGraph^{TM}, LLC, Fort Walton Beach, FL, USA). Participants wore the accelerometer over the right hip and were instructed to remove the sensor while showering and performing aquatic activities. KineSoft program (version 3.3.20; Loughborough, UK) was used to reduce the 15-second epoch data. Non-wear was defined as 60 minutes of consecutive zeros, allowing for 2 minutes of non-zero interruptions. The criterion for a valid day was 600 wear minutes per day. All participants had at least three valid days (two weekdays and one weekend day) of monitoring. Minutes spent in each intensity were determined using age-specific regression equations (Trost et al., 2002). The threshold of sedentary activity was established at 100 counts·min^{-1} (Trost et al., 2011).

2.7. Peak aerobic fitness

Research Unit for Sport and Physical Activity

Peak VO_2 was determined using an incremental running test (study 3) on a motorized treadmill (Quasar, HP Cosmos, Germany). Participants started with 2 minutes at 8 km/h, with subsequent increments of 2 km/h every minute until 16 km/h. Exercise intensity was subsequently increased through increasing the treadmill grade by 2º every minute until exhaustion (Gore, 2000). Attainment of peak VO_2 was confirmed if the athlete met any two of the following criteria: 1) respiratory exchange ratio (RER) $\geq$ 1.05; 2) heart rate (HR) within 10% of the age predicted maximum; 3) plateau in oxygen consumption despite increased exercise intensity or volitional exhaustion. Expired oxygen (O_2) and carbon dioxide (CO_2) flow and concentrations were measured every 10 seconds using a mixing chamber system (MetaMax System, Cortex Biophysics, Leipzig, Germany). Calibration and ambient air measurements were conducted before each testing session according to the manufacturer's guidelines. Before each test, flow and volume were calibrated using a 3-L capacity syringe (Hans Rudolph, Kansas City, USA). Gas analysers were calibrated using gases of known concentrations. HR was measured throughout exercise with a commercially available HR-monitor (Polar Electro, Finland).

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VO_{2peak} was determined using an incremental running test (study 4) on a motorized treadmill (Quasar-Med, Nussdorf, Germany) to voluntary exhaustion. Participants started at 2.2 $m \cdot s^{-1}$ with subsequent increments of 0.2 $m \cdot s^{-1}$ every minute until exhaustion. Expired oxygen (O_2) and carbon dioxide (CO_2) flow and concentrations were measured using an open circuit breath-by-breath automated gas analyser (Cortex, Metalyser, 3*B*, Leipzig, Germany). Calibration and ambient air measurements were conducted before each testing session according to the manufacturer guidelines. Before each test, flow and volume were calibrated using a 3-L capacity syringe (Hans Rudolph, Kansas City, USA). Gas analysers were calibrated using gases of known concentrations. Heart rate was measured every 5 s using a heart rate monitor (Vantage NV, Polar Electro, Kempele, Finland) connected to the gas-analyser system. Attainment of VO_{2peak} was confirmed if the athlete met at least two of the following criteria: respiratory exchange ratio ≥ 1.05; blood lactate concentrations > 8 mmol·L^{-1}; heart rate within 10% of the age predicted maximum; plateau in oxygen consumption despite increased exercise intensity, or volitional exhaustion.

2.8. Functional capacities

Cardiorespiratory fitness

Cardiorespiratory fitness (CRF; study 7-9) was measured using the 20-m multi-stage continuous shuttle endurance test (Léger et al., 1988), a standard field test included in the European fitness test battery (Council of Europe, 1988) and in the Portuguese physical education curriculum. In brief, 5–10 subjects performed a series of runs across a 20m track, changing direction at the end of each run to coincide with an audio signal that was getting progressively faster. Subjects started running at a speed of 8.5 km · h^{-1}, and speed increased at various stages (0.5 km · h^{-1} every minute). Each stage was made up of several shuttle runs, and players were instructed to keep pace with the signals as long as possible. The results were recorded as laps taken to complete the 20-m shuttle-run test. CRF was expressed as the number of completed laps achieved in the shuttle-run test.

Repeated-sprint ability

The repeated-sprint ability (RSA; study 8 and 9) was assessed using the 7-sprint protocol, also called the Bangsbo Sprint Test (Bangsbo, 1994). The time for each sprint was recorded with a digital chronometer connected to photoelectric cells (Globus Ergo Timer Timing System, Codogné, Italy) to a resolution of 0.01 s. The photocells were positioned 0.8 m above the floor, which typically corresponded to the hip level. The first pair was positioned along the starting line and the second pair along the finish line. The trials were initiated after the exact end of the recovery interval with the subject positioning the lead foot 0.3 m behind the starting line. Performance was expressed as the sum of the seven sprints.

Agility: shuttle-run test

The shuttle-run test (SHR; study 9 and 10) was assessed according to the EUROFIT test battery (Council of Europe, 1988). Players wore indoor shoes and were tested individually. The player started the test with the feet on the starting line. On the command “Go”, subjects ran 10 consecutive shuttle sprints of 5 m (total 50 m). Both feet had to fully cross the line at each end of the shuttle course. The time to complete the 10 shuttles was recorded with a digital chronometer connected to photoelectric cells placed on the start/finish line (Globus Ergo Timer Timing System, Codogné, Italy). Times were recorded to a resolution of 0.01 s. Two trials were performed and the faster of the two was retained for analysis.

Lower limb explosive strength

Lower limb strength (study 8 and 9) was assessed with the vertical counter movement jump using the ergo-jump (Globus, Glo1.etest, Italy) protocol (Bosco et al., 1983). Three trials were performed and the best score was retained for analysis (estimated jump height). The position of the jumper on the platform was the same for the take-off and landing. Participants were instructed to keep the hands on hips from the starting position through counter movement phase, jump and end of the flight trajectory. Prior to jumping, subjects were in a standing position and counter-moved until the knee was flexed approximately to 90°.

Test-retest protocols

Test-retest protocols of the functional capacities (one week apart) were performed with 32 participants. The 95% limits of agreement (LOA) where adopted to examine the test-retest agreement (Bland and Altman, 1986; Nevill and Atkinson, 1997). A visual inspection of Bland-Altman plots (Bland and Altman, 1986) showed that measurement differences (error) against the respective means where homoscedastic (Nevill and Atkinson, 1997). This was confirmed by the correlation coefficients between the absolute differences and the corresponding means (Nevill and Atkinson, 1997). The test-retest LOA were –12 to 9 ($r = 0.18$, $p = 0.32$) for number of laps (i.e. CRF); –5.04 to 6.01 s ($r = 0.10$, $p = 0.60$) for RSA; –1.46 to 2.25 s ($r = 0.05$, p = 0.78) for SHR; –6.42 to 3.56 cm ($r = –0.08$, p = 0.66) for lower limb strength.

2.9. Soccer-specific skills

Four tests of soccer-specific skills were administrated: ball control with the body, without using the arms or hands, dribbling speed, shooting accuracy (Federação Portuguesa de Futebol, 1986) and wall pass (Kirkendall et al., 1987).

Ball control

Ball control (Federação Portuguesa de Futebol, 1986) was tested (study 9) within a 9 x 9 m square. The player had to keep the ball in the air without using the arms or hands. The score was the number of hits of the ball before it fell to the floor.

Dribbling speed

For the dribbling speed test (Federação Portuguesa de Futebol, 1986), a cone was placed on each corner of the 9 x 9 m square (study 9 and 10). A fifth cone was placed midway (4.5 m) on the line of the square where the test began. Beginning at one corner, the participant had to move the ball with the feet (dribble) around the three cones (corner directly opposite the starting cone, the cone placed midway and the cone diagonally opposite the starting cone) in slalom fashion, and then dribble the ball into the fifth cone (i.e., not with a pass). The objective was to complete the drill in the fastest time possible by controlling the ball only with the feet. The time for each trial was recorded with the photoelectric cells. The first pair of the photocells was positioned along the starting line and the second pair along the finish line.

Shooting accuracy

Shooting accuracy (Federação Portuguesa de Futebol, 1986) was measured (study 9) in five attempts at kicking the ball at a 2 x 3 m goal located at the end line of a 9 x 9 m square. The target was divided by ropes into six sections. One rope was placed horizontally between the posts at a height of 1.5 m. Two ropes were dropped from the crossbar, 0.5m from each post. Five points were allocated for the upper right and left sections, and two for the upper middle section. Three points were allocated for the lower right and left sections, and one for the lower middle section. Kicks were attempted with the player standing outside of the square at the line opposite the goal. The maximum score was 25 points. The test was recorded and scored subsequently.

Wall pass

The wall pass test (Kirkendall et al., 1987), involved a 1.22m high (starting from the floor) and 2.44m wide target drawn on a flat wall (study 9). At a distance of 1.83m from the marked wall, an area of 1.83m in length and 4.23m in width was marked on the floor. The player had to remain in this area. The test consisted of making as many passes to the wall in 20 s. Players could use all body parts except the hands to make a pass to the wall. The best of two trials of each test was retained for analysis.

Test-retest protocols

Replicates were obtained from 32 participants within 1 week and the test-retest agreement for the soccer-specific skills were determined (Bland and Altman, 1986; Nevill and Atkinson, 1997). The test-retest LOA were –10.28 to 5.78 **(**r = 0.29, p = 0.11) for number of hits (i.e. ball control), –0.64 to 2.16 s (r = 0.15, p = 0.40) for dribbling speed, –4.79 to 2.42 points (r = –0.21, p = 0.26) for shooting accuracy and –4.19 to 3.06 (r = –0.17, p = 0.36) for number of passes.

2.10. Statistical analyses

Different statistical analisis were performed according to the aims of each specific study (Table 2.2.). Statistical analyses were performed using IBM SPSS version 19.0 software (SPSS, Inc., IBM Company; NY, USA), GraphPad Prism version 5.03 software (GraphPad Software, Inc.; La Jolla, CA, USA), MedCalc version 12.2.1 software (MedCalc; Mariakerke, Belgium) and MLwiN version 2.26 software (Centre for Multilevel Modelling; University of Bristol, United Kingdom). Alpha level was set at 0.05.

Table 2.2. Statistical analysis used in each study.

Analyses	Study									
	1	2	3	4	5	6	7	8	9	10
Normal Q-Q plots					●					
De-trended normal Q-Q plots					●					
Kolmogorov-Smirnov test			●		●					
Coefficient of variation	●									
z-scores									●	
Pearson´s correlation	●	●	●	●	●	●	●	●	●	
Paired samples *t*-tests				●			●	●	●	
Bland-Altman plot		●		●	●				●	●
MANOVA										●
MANCOVA										●
Effect size correlations										●
Tolerance		●		●	●	●	●	●	●	●
Variance inflation factor		●		●	●	●	●	●	●	●
Allometric (log-linear) scaling	●	●	●							
Static allometry				●		●				
Ontogenetic allometry						●				
Proportional allometric modelling	●	●	●		●					
Multiple linear regression	●	●	●							
Deming regression				●	●					
Homoscedasticity of residuals		●	●	●	●					
Predicted residuals sum of squares					●					
Multiplicative allometric multilevel modelling						●				●
Additive polynomial multilevel modelling							●	●	●	

MANOVA, multivariate analysis of variance; MANCOVA, Multivariate analysis of covariance.

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Part II

Cross-sectional Studies

Chapter III

Study 1

Scaling left ventricular mass in adolescent boys aged 11-15 years

3. Scaling left ventricular mass in adolescent boys aged 11-15 years

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Journal: Valente-dos-Santos J, et al. 2014. Scaling left ventricular mass in adolescent boys aged 11-15 years. Ann Hum Biol.
doi: 10.3109/03014460.2013.866694

3.1. Abstract

Background: Normalizing left ventricular mass (LVM) for inter-individual variation in body size is a central issue in human biology. During the adolescent growth spurt, variability in body size descriptors needs to be interpreted in combination with biological maturation.

Aim: To examine the contribution of biological maturation, stature, sitting stature, body mass, fat-free mass (FFM) and fat mass (FM) to inter-individual variability in LVM in boys, using proportional allometric modelling.

Subjects and methods: The cross-sectional sample included 110 boys 11-15 years (12.9 ± 1.0 years). Stature, sitting stature, body mass, cardiac chamber dimensions and LVM were measured. Age at peak height velocity (APHV) was predicted and used as an indicator of biological maturation. Percentage fat was estimated from triceps and subscapular skinfolds; FM and FFM were derived.

Results: Coefficients for body size descriptors were k = 2.33 for stature, k = 2.18 for sitting stature, k = 0.68 for body mass, k = 0.17 for FM and k = 0.80 for FFM (adjusted R^2 = 19% to 62%). The combination of body descriptors and APHV increased the explained variance in LVM (adjusted R^2 = 56% to 69%).

Conclusion: Stature, FM and FFM are the best combination for normalizing LVM in adolescent boys; when body composition is not available, an indicator of biological maturity should be included with stature.

Keywords: Youth · Growth · Maturation · Echocardiography · Allometry

3.2. Introduction

Interpretation of left ventricular mass (LVM) should be performed in relation to body size descriptors (de Simone et al., 1992; Foster et al., 2013). Historically LVM has been presented as a per-stature (g/m; Lauer et al., 1991) or per-surface area ratio standard (g/m^2; Devereux et al., 1986). Accurate quantification and analysis is essential to distinguish normal variants from clinical disease (Foster et al., 2013). This is particularly important in children and adolescents (Daniels et al., 1995), among whom body proportions and composition change with growth and with individual differences in biological maturation (Malina et al., 2004).

Simple ratio standards have been used to facilitate the analysis of LVM from individuals of different body size. However, these standards are theoretically fallacious and in practice misleading (Tanner, 1949); as smaller individuals receive an artefactual arithmetic advantage. Tanner (1949) proposed an alternative by adding to the group mean the individual's residual errors, taken from the fitted least-squares regression line. However, relationships between body size and organ dimensions are often nonlinear and the error term does not present constant variance trough the range of observations (de Simone et al., 1992; Valente-dos-Santos et al., 2013). Allometric modelling is a valid alternative as size descriptors are assumed to be proportional and errors multiplicative (Nevill and Holder, 1994; Nevill et al., 1992).

The decision of the most appropriate variable(s) against which to normalize LVM (Foster et al., 2013; Lang et al., 2005) is still an open question especially during years of maximal changes in different size descriptors that do not occur simultaneously. Besides stature and body surface area, body mass (de Simone et al., 1992), fat-free mass (FFM) (Foster et al., 2013) and fat mass (FM) (Dai et al., 2009) are often used for scaling LVM in children and adolescents with little considerations of appropriateness. Therefore, further evaluation of appropriate indexing variables for LVM in children and adolescents is needed. This study was designed to examine the adequacy of proportional allometric model structures for scaling LVM; and, in addition, to explore the independent and combined effects of biological maturation and different body descriptors to explain variability in LVM among adolescent boys.

It was hypothesized that proportional allometric modelling offers an analytical framework that provides more plausible estimates of LVM than simple ratio standards. It was also hypothesized that biological maturation would be a positive and independent predictor of LVM.

3.3. Methods

Participants

Boys in grades 6-8 from schools in the Portuguese Midlands were invited to participate in the study. The sample included 110 Caucasian boys. No boy had any symptom of underlying structural or cardiovascular abnormalities or a family history of premature death from heart disease; none had blood pressure > 125 mm Hg systolic or > 85 mm Hg diastolic. The experimental protocol followed the Declaration of Helsinki of the World Medical Association for research with humans and was approved by the Scientific Committee of the University of Coimbra. All participants were fully informed about the aims, experimental protocol and procedures; parents provided written informed consent.

Anthropometry

Stature (Harpenden stadiometer, model 98.603, Holtain Ltd, Crosswell, UK), sitting stature (Harpenden sitting height table, model 98.607, Holtain Ltd, Crosswell, UK), body mass (SECA balance, model 770, Hanover, MD, USA) and two skinfolds (triceps and subscapular; Lange Caliper, Beta Technology, Ann Arbor, MI, USA) were measured by a single, experienced observer following standard procedures (Lohman et al., 1988). FM and FFM were estimated from skinfold thicknesses using the protocol of Slaughter et al. (1988).

Biological maturation

Age at peak height velocity (APHV) was predicted using sex-specific anthropometric equations (Mirwald et al., 2002). The equations were developed using data from a longitudinal study of children's growth and verified in two other similar longitudinal studies.

Imaging protocol

A comprehensive resting echocardiographic evaluation was performed for each boy using a Vivid 3 ultrasound machine with a 1.5-3.6 MHz transducer (GE Vingmed Ultrasound, Horten, Norway). M-mode echocardiograms were derived from 2-dimensional images with direct visualization and were recorded at 100 $mm \cdot s^{-1}$. Measurements of LV internal dimension at end diastole, septal wall thickness at end-diastole and posterior wall thickness at end-diastole were obtained following recommendations of the American Society of Echocardiography (Lang et al., 2005). LVM was calculated (Devereux et al., 1986).

Statistical analysis

Data were expressed as means, standard deviations and ranges. Validity of ratio standards ('Tanner's exceptional circumstance') was reported based on the ratio of coefficients of variation (CV). The ratio standard is valid when the CV (%) of the maturational or anthropometric characteristic (x) dived by the CV (%) of LVM (y) is equal to Pearson's product moment correlation coefficient ($r\ x,y$) for the two variables (Tanner, 1949). An initial allometric model based on linear regressions of logarithmic regression transformations (Nevill et al., 1992), was adopted to examine relationships between body descriptors and LVM:

$$ln\,(\text{LVM}) = ln\,a + k \cdot ln\,(\text{body descriptor}) + ln\,\varepsilon \qquad \textbf{(3.1)}$$

where a was the scaling constant, and k the scaling coefficient of the body size or composition descriptor. Subsequently, stepwise multiple linear regression on ln *y* based on proportional allometric models was used (Nevill and Holder, 1994); with the inclusion of APHV as exponential term in addition to the selected body descriptor:

$$ln\ (\text{LVM}) = k \cdot ln\ (\text{body descriptor}) + a + b \cdot (\text{APHV}) + ln\ \varepsilon \qquad \textbf{(3.2)}$$

Finally, different combinations of body size and composition descriptors (e.g., stature, FM and FFM) and the exponential term (APHV) were simultaneously considered:

$$ln\ (\text{LVM}) = k_1 \cdot ln\ (\text{body descriptor}_1) + k_2 \cdot ln\ (\text{body descriptor}_2) + k_3 \cdot ln\ (\text{body descriptor}_3) + a + b \cdot (\text{APHV}) + ln\ \varepsilon \qquad \textbf{(3.3)}$$

A 2-tailed P value < 0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS version 19.0 software (SPSS Inc., IBM Company, NY, USA).

3.4. Results

Descriptive statistics for the total sample and the validity of ratio standards are summarized in Table 3.1. Simple ratio standards were not valid as the ratio between CV's (*x/y*) did not correspond to Pearson's product moment correlation coefficient (*r x,y*). Coefficients for stature, sitting stature, body mass, FFM and FM from the independent allometric models of LVM are summarized in Table 3.2 (Models 1-5 derived from equation 3.1). Overall, the independent variables explained 19% to 62% of variance in the LVM. The allometric models combining stature, sitting stature or FFM with APHV (Models 6-8 derived from equation 3.2, Table 3.2) increased the explained variance in LVM to about 56% – 64%. The model with stature, FM and FFM explained the largest proportion of variance in LVM (69%; Model 10 derived from equation 3.3, Table 3.2).

Table 3.1. Descriptive statistics for the total sample (n = 110), 95% confidence intervals (95% CI) for mean values, relationship of left ventricular mass with age and body size descriptors and validity of ratio standards.

	Range (min – max)	Mean ± SD	95% CI for mean	CV (%)	CV*x*/CV*y*	*r x,y*	Ratio standard (Qualitative inference)
Chronological age (years)	11.15 to 15.12	12.87 ± 0.97	12.69 to 13.05	7.54	0.38	+ 0.46	Not valid
Age at peak height velocity (years)	12.02 to 15.28	13.85 ± 0.53	13.75 to 13.95	3.83	0.19	– 0.60	Not valid
Stature (cm)	133.0 to 185.1	156.4 ± 10.7	154.4 to 158.4	6.84	0.34	+ 0.78	Not valid
Sitting stature (cm)	64.5 to 94.3	80.5 ± 5.5	79.5 to 81.5	6.83	0.34	+ 0.74	Not valid
Body mass (kg)	28.6 to 91.1	49.7 ± 12.0	47.5 to 51.9	24.15	1.21	+ 0.76	Not valid
Percentage of fat mass (%)	7.9 to 40.1	22.9 ± 9.4	21.1 to 24.7	41.05	2.06	+ 0.18	Not valid
Fat mass (kg)	3.3 to 38.5	12.0 ± 7.5	10.6 to 13.4	62.50	3.14	+ 0.42	Not valid
Fat-free mass (kg)	22.8 to 57.4	37.7 ± 7.6	36.3 to 39.1	20.16	1.01	+ 0.78	Not valid
Left ventricular mass (g)	62.3 – 168.0	104.9 ± 20.9	101.0 to 108.8	19.92			

CV, coefficient of variation (%); CV *x*, CV for age or body size descriptors; CV *y*, CV for left ventricular mass; *r*, Pearson's product moment correlation coefficient.

Table 3.2. Scaling coefficients (k), 95% confidence intervals (95% CI), standard error of estimate (SEE), model correlation coefficients (R), and adjusted coefficients of determination (adj. R^2) for LVM in boys 11-15 years of age.

Equation	Model *	Descriptors †	k (95% CI)	SEE	Model summary		
					R	adj. R^2	P
Equation 3.1	1	Stature	2.33 (1.98 to 2.68)	0.18	0.79	0.61	<0.001
	2	Sitting stature	2.18 (1.80 to 2.56)	0.19	0.74	0.54	<0.001
	3	Body mass	0.68 (0.58 to 0.78)	0.05	0.79	0.61	<0.001
	4	Fat mass	0.17 (0.10 to 0.23)	0.03	0.45	0.19	<0.001
	5	Fat-free mass	0.80 (0.68 to 0.92)	0.06	0.79	0.62	<0.001
Equation 3.2	6	Stature	2.02 (1.59 – 2.45)	0.22	0.80	0.63	<0.001
		APHV	– 0.07 (– 0.12 to – 0.01)	0.03			
	7	Sitting stature	1.82 (1.34 to 2.31)	0.24	0.75	0.56	<0.001
		APHV	– 0.07 (– 0.14 to – 0.01)	0.03			
	8	Fat-free mass	0.69 (0.55 to 0.83)	0.07	0.81	0.64	<0.001
		APHV	– 0.07 (– 0.13 to – 0.02)	0.03			
Equation 3.3	9	Stature	1.30 (0.77 to 1.82)	0.26	0.83	0.68	<0.001
		Body mass	0.38 (0.23 to 0.53)	0.08			
	10	Stature	0.92 (0.08 to 1.76)	0.43	0.83	0.69	<0.001
		Fat mass	0.09 (0.04 to 0.13)	0.02			
		Fat-free mass	0.44 (0.16 to 0.73)	0.15			
	11	Sitting stature	1.01 (0.51 to 1.50)	0.25	0.82	0.66	<0.001
		Body mass	0.46 (0.31 to 0.60)	0.07			
	12	Fat mass	0.09 (0.05 to 0.13)	0.02	0.82	0.67	<0.001
		Fat-free mass	0.73 (0.62 to 0.85)	0.06			

* Non-significant models are not presented.
† Non-significant predictors are not presented.
APHV, age at peak height velocity.

3.5. Discussion

The present study investigated the influence of biological maturation and several indicators of body size and estimated body composition on LVM in boys 11-15 years of age using proportional allometric modelling. 'Tanner's exceptional circumstance' (Tanner, 1949) was not satisfied, and thus precludes the use of simple ratio scaling for normalization of LVM. Statistically significant effects of maturation (APHV), body size and composition depend largely on the particular combination of predictors selected for the models.

Although LVM/stature$^{2.7}$ is the most widely accepted indexing method in older children and adolescents (de Simone et al., 1992), the physiological basis for normalization LVM on stature alone is not particularly strong. Results of the present study (Table 3.2) and others (Foster et al., 2013) indicated that LVM/stature$^{2.7}$ did not adequately normalize LVM for stature in boys 11-15 years. Moreover, the proportional allometric analyses indicated that after adjusting for the effects of stature, it was also necessary to consider APHV (Model 6, Table 3.2), body mass (Model 9, Table 3.2) or FM and FFM (Model 10, Table 3.2) as simultaneous covariates. These results are contrasting with a report showing no influence of maturation on LVM (Daniels et al., 1995), and consistent with other studies that demonstrated the relevance of body size descriptors as correlates of LMV: body mass (de Simone et al., 1992), FFM (Foster et al., 2013) and FM (Dai et al., 2009). Further, LVM, like cardiac output, is determined primarily by the demands of metabolically active tissues (Dewey et al., 2008). Even though stature is strongly correlated with FFM, the correlation is not perfect and may be a biased surrogate.

After adjusting for the effects of FFM in the proportional allometric analyses, it was also necessary to consider APHV (Model 8, Table 3.2) or FM (Model 12, Table 3.2) as simultaneous covariates. Note, however, that errors associated with estimates of body composition based on skinfolds (Slaughter et al., 1988) are a major limitation. As such, additional variables were considered in the regression models used to normalize LVM. Sitting stature and APHV (Model 7, Table 3.2) as combined predictors were important determinants of LVM among the explanatory variables evaluated. This was consistent with observations on late adolescent athletes (Valente-dos-Santos et al., 2013) among whom sitting stature and skeletal maturation had a major influence on LVM. Nevertheless, the interpretation of LVM in adolescent boys may also be expressed relative body mass (Model 11, Table 3.2) if indicators of body composition or biological maturation are not available.

The method for estimating biological maturity used in the present study was non-invasive, but its applicability among boys appear to be limited to the interval of growth spurt (approximately 12–15 years; Malina and Koziel, 2014). Finally, this study only included Caucasian boys and the allometric models put forward in this paper need to be validated in different samples (e.g., females, other ethnic groups).

3.6. Conclusions

Results suggested that non-linear allometric modelling procedures were statistically appropriate to account for the inter-individual variability in body dimensions. Nevertheless, the extensive use of allometric models based on a single descriptor for indexing LVM can be questioned. The current study clearly identified biological maturation as an independent correlate of LVM. Results also confirmed stature, FM and FFM as the best combination of body descriptors for normalizing LVM. Whenever body composition is not available, LVM should be expressed relative to stature and APHV.

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Chapter IV

Study 2

Ventricular mass in relation to body size, composition and skeletal age in adolescent athletes

4. Ventricular mass in relation to body size, composition and skeletal age in adolescent athletes

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Journal: Valente-dos-Santos J, et al. 2013. Ventricular mass in relation to body size, composition, and skeletal age in adolescent athletes. Clin J Sport Med 23:293–299. doi: 10.1097/JSM.0b013e318280ac63

4.1. Abstract

Objective: To examine the contribution of chronological age (CA), skeletal age (SA), stature, sitting stature, fat-free mass (FFM) and fat mass (FM) to inter-individual variability in left ventricular mass (LVM) in a male adolescent roller hockey players using allometric models.

Design: Cross sectional.

Setting: Training and competitive sport during adolescence.

Participants: Seventy-three Portuguese male roller hockey players aged 14.5-16.5 years.

Independent Variables: Stature, sitting stature, body mass, estimated FM and FFM, and skeletal age (SA) assessed by the Fels method.

Main Outcome Measures: Allometric modelling of left ventricular mass (LVM) assessed in accordance with recommendations of the American Society of Echocardiography.

Results: Hockey players (CA: 15.4 ± 0.6 y; SA: 16.4 ± 1.5 y) showed an eccentric remodelling of left ventricular (LV) structure within the reference range (i.e., 0.24 – 0.42), a dilated LV chamber, but no LVM increase. Coefficients for body size descriptors were 2.69 for stature (R^2=27%; P<0.001), 2.49 for sitting stature (R^2=37%; P<0.001), 0.76 for FFM (R^2=31%; P<0.001) and 0.22 for FM (R^2=26%; P<0.001). The combination of size descriptors with CA and SA increased the explained variance in LVM slightly (26% to 45%).

Conclusions: When stature and FM are used for indexing LVM in a sample of adolescent athletes, biological maturity status should also be considered.

Keywords: Youth Sports · Growth · Bone Age · Left Ventricular Mass · Allometry

4.2. Introduction

Long-term training in adult athletes is associated with adaptive changes in cardiac structure (D'Andrea et al., 2013; Limongelli et al., 2006). Adaptations in response to the hemodynamic loading conditions induced by chronic exercise include increased cavity size, wall thickness, and mass (Giada et al., 1998; Sharma et al., 2002). Among adults, a variety of methods have been used to normalize left ventricular mass (LVM) to body size, including dividing LVM by a body size variable such as stature (Lauer et al., 1991) or body surface area (Devereux et al., 1984). However, body proportions change dramatically with growth and maturation (Malina et al., 2004), and the relationship between the LVM and stature differs at different stages of development (Dewey et al., 2008). Further, adjusting or scaling cardiac measurements to body surface area is influenced by body composition (Lang et al., 2005; Rowland and Roti, 2010), which in turn is related to growth and maturation.

Adolescent athletes within a sport tend to be relatively homogeneous in training experience, but variation in body size, composition, proportions and biological maturation can be considerable. Ratio standards are routinely used to interpret physiological and morphological measurements of athletes contrasting in body size and composition descriptors. Allometric models are effective for partitioning the effects of body size and have been recommended as providing a “size-free” expression of physiological parameters (de Simone et al., 1992; Malina et al., 2004). Dividing LVM by stature to a power of 2.7 (de Simone et al., 1992) is the most widely accepted indexing method in older children and adolescents. Recent studies (Dai et al., 2009; Daniels et al., 1995; Rowland and Roti, 2010) have shown that fat-free mass (FFM), a surrogate for metabolically active tissue, is most closely and positively related to LVM. Nevertheless, fat mass (FM) is also an independent and positive predictor of LVM in children and adolescents (Dai et al., 2009; Chinali et al., 2006). The latter reflects the relationship between absolute fat mass and body size. At present, limited data are available on the ventricular characteristics of adolescent athletes. Research dealing with the left ventricular (LV) structure of adolescent athletes is rather limited (Basavarajaiah et al., 2006; Zdravkovic et al.,

2010) and does not systematically consider variation associated with inter-individual differences in growth and maturation.

Skeletal age (SA) is perhaps the best maturity indicator as it can be used from childhood through adolescence. It reflects the maturation of the skeletal system, specifically the ossification of endochondral bones of the hand-wrist (Malina, 2011). Athletes who are advanced in SA relative to CA (higher SA/CA ratio) tend to be taller, heavier, stronger, more powerful and faster than athletes late or delayed in skeletal maturation (Malina, 2011; Malina et al., 2004).

The purpose of this study was to assess the influence of various body-size and body-composition indicators on LVM and, to explore the independent and combined effects of CA and SA with the body indicators on LVM, using proportional allometric modelling. It was hypothesized that skeletal maturation would exert a positive influence on LVM independently of body size and composition. It was also hypothesized that sitting stature, an indicator of upper body length, would be more strongly associated with LVM than overall body size.

4.3. Methods

Participants

During the peak of the 2008 junior roller hockey competitive season, 73 Caucasian male athletes 14.5 to 16.5 years of age from 15 clubs in Portugal were enrolled in the study. The study was approved by the Scientific Committee of the University of Coimbra and subsequently by the Portuguese Foundation for Science and Technology [SFRH/BD/64648] taking into account the Declaration of Helsinki. The Portuguese Skating Federation, clubs, parents and athletes provided written consent.

No athlete had any symptoms of underlying cardiovascular disease or a family history of premature death from heart disease; none had blood pressure > 125 mm Hg systolic or > 85 mm Hg diastolic. All athletes were engaged in formal training and competition at the national level for at least five years. They participated in regular training sessions (4 – 6 sessions · week $^{-1}$; ~ 360 – 510 min · week $^{-1}$) and typically played one or two games per week over a 10 month period (mid-September to June). Moreover, this was the group from which the Portuguese selections for the 2007 and 2008 national teams for the U-17 European League competitions were selected.

Age and skeletal maturity

Chronological age (in decimals) was calculated as the difference between date of birth and the date on which a posterior-anterior radiograph of the left hand-wrist was taken. SA was assessed with the Fels method for estimating skeletal maturity (Roche et al., 1988). Details of the method and applications to youth athletes are presented elsewhere (Malina et al., 2010). Assessments were made by a single individual experienced with the Fels method. The mean difference in SA between independent assessments made at least 1 week after the initial assessments in a random sample of 20 subjects was 0.02±0.13 years and the intra-observer technical error of measurement for the replicate measures was 0.03±0.04 years.

Physical examination

Stature, sitting stature, body mass, and two skinfolds (triceps and subscapular) were measured by a single, experienced individual following standard procedures (Lohman et al., 1998). Stature was measured to the nearest 0.1 m with a Harpenden stadiometer (model 98.603, Holtain Ltd, Crosswell, UK) and body mass was measured to the nearest 0.1 kg with a SECA balance (model 770, Hanover, MD, USA). Skinfolds were measured to the nearest mm using a Lange Caliper (Beta Technology, Ann Arbor, MI, USA). Technical errors of measurement for stature (0.3 cm), sitting stature (0.3 cm), body mass (0.5 kg), and skinfolds (0.5-0.7 mm) were well within the range of several health surveys in the United States and a variety of field surveys (Malina et al., 2004). Percentage body fat was estimated from triceps and subscapular skinfold thicknesses using the protocol of Slaughter and colleagues (1988). Fat mass and fat-free mass (kg) were derived.

Imaging protocol

A comprehensive resting echocardiography study was performed using a Vivid 3 ultrasound machine with a 1.5-3.6 MHz transducer (GE Vingmed Ultrasound, Horten, Norway). M-mode echocardiograms were derived from 2-dimensional images under direct visualization and were recorded at 100 mm · s $^{-1}$. Measurements of LV internal dimension at end diastole (LVIDd), septal wall thickness at end-diastole (SWTd) and posterior wall thickness at end-diastole (PWTd) were obtained in accordance with recommendations of the American Society of Echocardiography (Lang et al., 2005). The LVM was calculated with the formula proposed by Devereux and colleagues (Devereux et al., 1986). Relative wall thickness (RWT) was the ratio of the average ventricular septal and posterior free wall thickness to the radius of the ventricular cavity. To assess intra-observer variability, 20 randomly chosen subjects were measured again after a one week interval by the same observer. Intra-observer variability was determined as the difference and percentage difference (with a denominator a mean of the two measurements) between the two measurements along with their 95% limits of agreement (LOA) (Bland and Altman, 2012). Intra-observer variability for LVIDd, SWTd and PWTd was 0.17 mm (95% LOA – 1.95 to 2.28 mm), 0.02 mm (95% LOA – 0.30 to 0.34 mm), and 0.06 mm (95% LOA – 0.45 to 0.56 mm), respectively. For the same three parameters, percentage intra-observer variability was 0.3% (95% LOA – 4.1 to 4.8%), 0.3% (95% LOA – 4.2 to 4.8%), and 0.8% (95% LOA – 6.5 to 8.1%), respectively.

Statistical analysis

Data were expressed as means, standard deviations and ranges. The Kolmogorov-Smirnov test was used to confirm the Gaussian distribution of variables. A tolerance > 0.10 and a variance inflation factor < 10 was set to avoid collinearity between the explanatory variables (Slinker and Glantz, 1985). The incidence of a high bivariate correlation between body mass and FFM (r = 0.85) suggested unacceptable collinearity; body mass was thus excluded from the regression. Before the allometric analysis, correlation coefficients (Pearson) were calculated to examine the linearity of relationships between body size (stature and sitting stature), body mass components (FFM and FM) and age with LVM. An initial allometric model was adopted to examine the relationship between body descriptors and LVM:

$$y = a \cdot x^{k} \cdot \varepsilon \qquad \textbf{(4.1)}$$

Values of *a* and *k* were derived from linear regressions of the logarithmic regression transformations in the form of:

$$\log y = \log a + k \cdot \log x + \log \varepsilon \qquad \textbf{(4.2)}$$

where *y* was the dependent variable of LVM (log LVM, i.e., natural logarithms), *a* was the scaling constant and *k* was the scaling coefficient of the body size or body composition descriptor (i.e., log stature, log sitting stature, log FFM or log FM). Subsequently, stepwise multiple linear regression on log(*y*) based on proportional allometric models was used to fit the unknown parameters (Nevill and Holder, 1994). The models incorporated CA or SA as exponential terms in addition to body descriptors:

$$\log (\text{LVM}) = k \cdot \log (\text{body descriptor}) + a + b \cdot (\text{CA or SA}) + \log \varepsilon \qquad \textbf{(4.3)}$$

Finally, different combinations of body size and body composition descriptors (e.g., stature and FFM, sitting stature and FM) and both exponential terms (i.e., CA and SA) were simultaneously considered:

$$\log (\text{LVM}) = k_1 \cdot \log (\text{body descriptor}_1) + k_2 \cdot \log (\text{body descriptor}_2) + a + b_1 \cdot (\text{CA}) + b_2 \cdot (\text{SA}) + \log \varepsilon \qquad \textbf{(4.4)}$$

Validation of the allometric models was determined by examining the association between the residuals of each model. The respective scaling denominators were then calculated using Pearson's product-moment correlations to check the assumptions of scaled LVM independent of CA or SA, stature, sitting stature, FFM or FM as well as homoscedasticity of residuals in the log-linear regressions (Nevill et al., 1992). The coefficient of determination (R^2) provides an indication of the explained variance achieved by the independent variables in each proportional allometric model. The magnitude of correlations was interpreted as follows: trivial ($r < 0.1$), small ($0.1 < r < 0.3$), moderate ($0.3 < r < 0.5$), large ($0.5 < r < 0.7$), very large ($0.7 < r < 0.9$), and nearly perfect ($r > 0.9$) (Hopkins et al., 2009). A 2-tailed P value < 0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS version 19.0 software (SPSS Inc., IBM Company, NY, USA).

4.4. Results

Descriptive statistics

Chronological age, SA, body size and composition and echocardiographic characteristics of the roller hockey players are summarized in Table 4.1. The range of SA was 4.8 years (13.1 – 17.9 years) while the range of CA was 2 years (14.5 – 16.5 years). Athletes had mean statures between the 25th and 50th centiles and mean body masses between the 50th and 75th US age specific percentiles (Kuczmarski et al., 2002). LVIDd ranged from 44.2 to 64.8 mm and exceeded the clinically accepted upper limits (i.e., 39 – 53 mm; Lang et al., 2005) in a substantial proportion of athletes (33; 45.2%), including 6 (8.2%) with markedly dilated LV cavity > 62 mm. Both SWTd and PWTd did not exceed normal limits in all athletes (i.e., > 10 mm; Lang et al., 2005). Absolute LVM was also within normal limits in all athletes (i.e., 88 – 224 g; Lang et al., 2005). RWT showed an eccentric remodelling (i.e., dilated LV chamber, but the LV mass was not increased) within the reference range (i.e., 0.24 – 0.42; Lang et al., 2005).

Table 4.1. Descriptive statistics for the total sample of adolescent roller hockey players (*n* = 73).

	Mean ± SD	Range	Kolmogorov -Smirnov	*P*
Chronological age (years)	15.4 ± 0.6	14.5 – 16.5	0.96	NS
Skeletal age (years)	16.4 ± 1.5	13.1 – 17.9	1.20	NS
Years of training (years)	8.6 ± 1.2	5 – 11	1.24	NS
Stature (m)	1.70 ± 0.69	1.44 – 1.82	0.72	NS
Sitting stature (m)	0.89 ± 0.46	0.75 – 0.97	0.98	NS
Body mass (kg)	63.7 ± 10.7	38.4 – 90.3	0.63	NS
Fat-free mass (kg)	52.1 ± 7.9	34.2 – 68.7	0.50	NS
Fat mass (kg)	11.8 ± 6.2	3.2 – 20.8	1.07	NS
Heart rate (b.p.m.)	61 ± 9	43 – 85	1.26	NS
Left ventricular internal dimension at end-diastole (mm)	54.7 ± 4.6	44.2 – 64.8	0.40	NS
Septal wall thickness at end-diastole (mm)	8.1 ± 0.8	6.0 – 10.4	1.12	NS
Posterior wall thickness at end-diastole (mm)	7.6 ± 0.7	5.5 – 9.3	1.09	NS
Left ventricular mass (g)	156.3 ± 31.6	83.1 – 216.7	0.60	NS
Relative wall thickness	0.28 ± 0.06	0.21 – 0.38	0.89	NS

Correlation analysis

Relationships between log LVM and CA, SA, log stature, log sitting stature, log FFM and log FM are illustrated in panels a through f of Figure 4.1. Linear relationships were observed between FFM and LVM. Non-linear relationships were apparent between stature, sitting stature, FM, CA and SA with LVM. Correlations between the preceding variables and LVM ranged from 0.36 and 0.61 ($P < 0.01$).

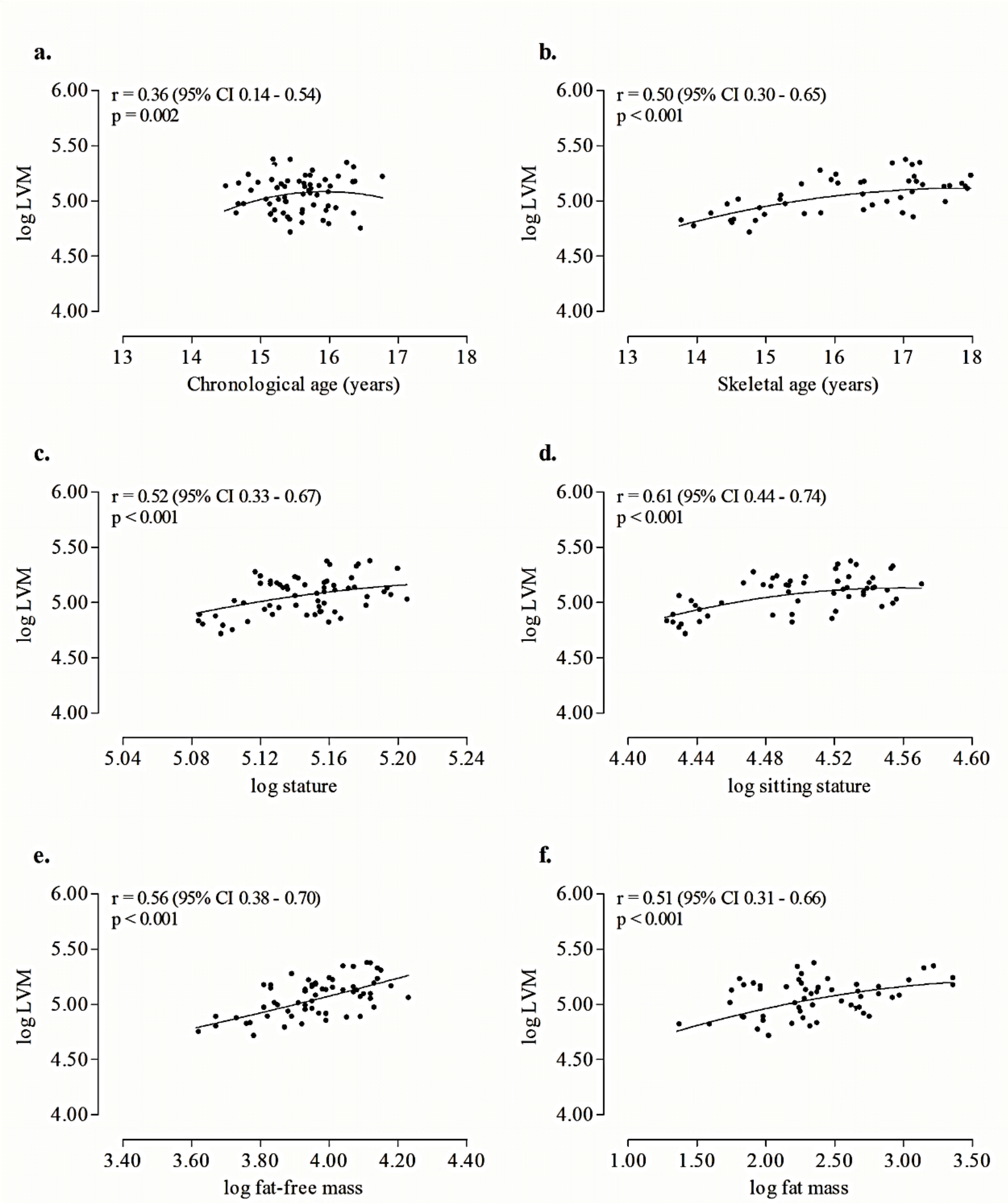

Figure 4.1. Relationship of the log transformed left ventricular mass (LVM) with chronological (panel a) and skeletal age (panel b) and with the log transformed stature (panel c), sitting stature (panel d), fat-free mass (panel e) and fat mass (panel f).

Determinants of ventricular mass

The coefficients for stature, sitting stature, FFM and FM resulting from the independent allometric models of LVM are summarized in Table 4.2, while the allometric models combining body descriptors with CA or SA are summarized in Table 4.3. Overall, the independent variables explained 26% to 41% of variance in the LVM. Chronological age and SA were not significant predictors once stature and sitting stature were respectively accounted for. Skeletal age was a significant predictor of LVM when included in the model with stature, FFM or FM ($P < 0.05$). In contrast to SA, CA was a significant predictor of LVM when included with sitting stature, FFM or FM ($P < 0.05$). As expected, all descriptors were positively correlated with LVM.

Significant allometric models combining more than one body size and composition descriptor in addition to CA and SA are summarized in Table 4.4. Compared with the previous models limited to two variables (Table 4.3), CA and SA were no longer significant predictors of LVM, but the explained variance of LVM increased to about 40% – 45%. The model with sitting stature and FM explained the largest proportion of variance in LVM (45%, Table 4.4). The estimated effects of sitting stature and FM on LVM were higher than those estimated from the previous models.

Table 4.2. Allometric modelling* of the left ventricular mass (LVM) using different body size and composition descriptors.

Constant	Body descriptor	Coefficient (95% CI)	*R*	R^2	*P*
-8.78	Stature	2.69 (1.65 – 3.73)	0.52	0.27	< 0.001
-6.16	Sitting stature	2.49 (1.72 – 3.26)	0.61	0.37	< 0.001
2.03	Fat-free mass	0.76 (0.49 – 1.03)	0.56	0.31	< 0.001
4.51	Fat mass	0.22 (0.13 – 0.31)	0.51	0.26	< 0.001

* $\log(\text{LVM}) = \log a + k \cdot \log(\text{body descriptor}) + \log \varepsilon$.

Table 4.3. Multiplicative allometric modelling* of the left ventricular mass (LVM) combining body descriptors with an age variable.

Predictors						Model summary			
Constant	Body descriptor	Age variable	β Unstandardized	SEE	*Partial correlation*	*R*	R^2	Adjusted R^2	*P*
-4.98	Stature		1.81	0.61	0.35	0.58	0.33	0.31	< 0.001
		Skeletal age	0.04	0.01	0.30				
-6.07	Sitting stature		2.26	0.40	0.55	0.66	0.42	0.41	< 0.001
		Chronological age	0.08	0.03	0.24				
1.08	Fat-free mass		0.69	0.13	0.50	0.61	0.37	0.35	< 0.001
		Chronological age	0.08	0.03	0.24				
2.26	Fat-free mass		0.55	0.17	0.40	0.59	0.35	0.33	< 0.001
		Skeletal age	0.04	0.01	0.25				
3.33	Fat mass		0.19	0.05	0.44	0.56	0.31	0.29	< 0.001
		Chronological age	0.08	0.03	0.24				
3.79	Fat mass		0.16	0.04	0.37	0.61	0.37	0.35	< 0.001
		Skeletal age	0.05	0.02	0.36				

* $\log(\text{LVM}) = k \cdot \log(\text{body descriptor}) + a + b \cdot (\text{age variable}) + \log \varepsilon$.
† Non-significant models are not presented.
SEE, standard error of estimate.

Table 4.4. Multiplicative allometric models* of the left ventricular mass (LVM) combining more than one body descriptor with age variables.

		Predictors			Model summary ‡			
Constant	Variables †	β Unstandardized	SEE	*Partial correlation*	*R*	R^2	Adjusted R^2	*P*
-6.33	Stature	2.14	0.49	0.41	0.64	0.41	0.40	<0.001
	Fat-free mass	0.17	0.04	0.39				
-4.32	Sitting stature	2.01	0.38	0.49	0.68	0.47	0.45	<0.001
	Fat mass	0.14	0.04	0.33				
2.14	Fat mass	0.63	0.12	0.46	0.68	0.46	0.44	<0.001
	Fat-free mass	0.17	0.04	0.39				

* $\log(\text{LVM}) = k_1 \cdot \log(\text{body descriptor}_1) + k_2 \cdot \log(\text{body descriptor}_2) + a + b_1 \cdot (\text{chronological age}) + b_2 \cdot (\text{skeletal age}) + \log \varepsilon$.
† Non-significant predictors are not included.
‡ Non-significant models are not presented.

4.5. Discussion

The present study investigated the influence of CA, SA and several indicators of body-size and composition on LVM in well trained adolescent athletes using proportional allometric modelling procedures. Results of cross-sectional analyses of data for adolescent roller hockey players were consistent with previous studies of children and adolescents in showing that FFM and FM as simultaneous covariates were robust determinants of LVM (Chinali et al., 2006; Dai et al., 2009; Daniels et al., 1995; de Simone et al., 1992; Rowland and Roti, 2010), and also that both CA and SA exerted independent effects on LVM. In contrast to a report showing no influence of pubertal maturation on LVM (Daniels et al., 1995), this study indicated a major influence of skeletal maturation (SA) on LVM of adolescent roller hockey players. The variable results may reflect sampling and age variation. The study of pubertal maturation included a combined sample of American Black and White boys and girls 6 to 17 years of age, whereas the present study was limited to late adolescent athletes of European (White) ancestry. Maturity indicators in the two studies also differed, categorical stages of secondary sex characteristics compared to continuous SAs. The studies also differed in analytical strategies and predictor variables.

The estimated effect of body size, body composition, SA and/or CA on LVM depended largely on the particular combination of predictors selected for the allometric models. Sitting stature, an indicator of upper body length, was more strongly associated with LVM than overall stature, body composition or SA. Additionally, a modest increase in LV cavity (LVIDd: 54.7 ± 4.6 mm) and lower values of wall thickening (SWTd: 8.1 ± 0.8 mm; PWTd: 7.6 ± 0.7 mm) in the roller hockey players compared to Italian (LVIDd: 51.9 ± 2.6 mm; SWTd: 9.2 ± 1.0 mm; PWTd: 9.0 ± 0.8 mm) and African (LVIDd: 51.0 ± 3.6 mm; SWTd: 9.7 ± 1.3 mm; PWTd: 9.6 ± 1.4 mm) soccer players of the same CA grouping (Di Paolo et al., 2012). The eccentric remodelling in roller hockey players likely represented a physiological adaptation to the hemodynamic loading conditions associated with chronic exercise (Giada et al., 1998; Pelliccia et al., 2010; Prakken et al., 2011; Sharma et al., 2002).

The results for the allometric models were consistent in verifying the most widely accepted coefficient for normalizing LVM (i.e., 2.7, Table 4.2) in older children and adolescents (de Simone et al., 1992). However, the proportional allometric analyses indicated that after adjusting for the effects of stature, it was also necessary to consider SA (Table 4.3) or FM (Table 4.4) as a simultaneous covariate. The physiological basis for normalization LVM on stature alone is not particularly strong. LVM, like cardiac output, is determined primarily by the demands of metabolically active tissues or FFM (Daniels et al., 1995). Although stature is strongly correlated with FFM, the correlation is not perfect and the inclusion of additional easily measured variables should be considered in the regression model used to normalize LV for body size and composition. Sitting stature, as a single predictor (Table 4.2), was the most striking determinant of LVM among the explanatory variables evaluated (i.e., stature, FFM and FM).

The variables incorporated in the proportional allometric models (Tables 4.3 and 4.4) explained between 26% and 45% of variance in LVM, which emphasized the importance of the inter-relationships among size, composition, CA and SA with LVM even in the latter period of adolescence. A recent report (Dai et al., 2009) based on longitudinal data from Project HeartBeat! (678 children aged 8–14 years at baseline), showed that the mean change in LVM for a 10 kg change in FFM was about 21–24 g when FM and other covariates (CA, sex or body size indicators) were held constant. Findings on an independent effect of body fatness on LVM in child and adolescent samples have been inconsistent. It has been reported (Daniels et al., 1995) that a 10-kg increase in FM would result in a 5 g increase in LVM in healthy children and adolescents 6–17 years of age after adjusting for effects of FFM and systolic blood pressure. Estimated changes in LVM with every 10 kg change in FM were about 11 to 14 g in Project Heart Beat! (Dai et al., 2009). The size of both the fat-free and fat components of body mass apparently exerts an impact on LVM. FFM is the stronger determinant of LVM, while FM, although weaker, is an additional positive determinant of LVM. However, inter-individual differences in biological maturation were not considered in the preceding studies.

Changes in LVM are also associated with the type and duration of training programs (Basavarajaiah et al., 2006; Giada et al., 1998; Zdravkovic et al., 2010;

Pellicia et al., 2010). The mechanisms underlying LV remodelling with training and the differential effects of pressure and volume overload are currently being explored, but are beyond the scope of this discussion. Such information will improve the understanding of mechanisms controlling the relationship between the stimulus (overload) and pattern of LV remodelling in youth athletes.

The present study differentiated the overall impact of stature and sitting stature on LVM from the effects of FFM and FM, as well as effects associated with CA and SA. A larger impact of upper body length (in the form of sitting stature) was noted in models with CA (Table 4.3) and FM (Table 4.4) for the prediction of LVM. The effect of the upper body length was significant and positive. In the models containing FFM, CA or SA (Table 4.3), SA had a similar effect as CA, whereas in the models containing FM, CA or SA, CA was of minor biological importance compared to SA. It was previously suggested that greater FFM alone explained the larger LVM and dimensions in trained adult male endurance athletes (Whalley et al., 2004). FFM (r = 0.69) and $FFM^{0.3}$ (r = 0.62) were also the strongest predictors of LVM in combined groups of male and female adult cyclists (Rowland and Roti, 2010). Similar associations were observed in the adolescent roller hockey players when LVM was adjusted simultaneously to sitting $stature^{2.01}$ and $FM^{0.14}$ (r = 0.68) or $FFM^{0.63}$ and $FM^{0.17}$ (r = 0.68; Table 4.4). The different allometric scaling coefficients for FFM and the different combinations of covariates in the present study strengthen the rationale that conclusions derived from studies of adult athletes cannot be directly extrapolated to adolescent athletes who are most likely physically and physiologically less mature and were generally exposed to shorter periods of training (Malina et al., 2004; Sharma et al., 2002).

Several methodological limitations of the present study should be noted. First, body composition was estimated with prediction equations specific for adolescent males of a similar CA, pubertal stage and ethnicity (European or White ancestry; Slaughter et al., 1988) rather than a more specific body composition assessment protocol such as dual-energy X-ray absorptiometry. Second, the study was limited to a cross-sectional sample of adolescent roller hockey players 14.5–16.5 years of age. Generalizations to other athletes or other ages, and to the clinical context need to be made with care. Third, the potential impact of training was not considered in the allometric analyses. It is well established that the volume and intensity of training influence cardiac remodelling (D'Andrea et al., 2013; Giada et al., 1998; Pelliccia et al., 2010; Zdravkovic et al., 2010). It is thus likely that the observations on the select sample of elite roller hockey players may reflect training-induced variability in LVM and other cardiac characteristics.

4.6. Conclusions

This study provides perhaps the first analysis of the relationship between LVM and skeletal maturation in a sample of elite, adolescent male athletes. Upper body length measured as sitting stature was the most robust individual determinant of LVM in the roller hockey players. Upper body length and FM may be the best combination of body descriptors for normalizing LVM. Skeletal age was also an independent, positive predictor of LVM, so that when stature or FM is used as a predictor of LVM, SA should probably be included. This study may be relevant to the accuracy of clinical assessments of LVM in the context of preparticipation physical examinations of adolescent athletes, where the utility of echocardiography is still under discussion (Corrado et al., 2006; Maron et al., 2007). The approach used in the present study also offers a different statistical and biological approach to understanding LVM in youth athletes and adolescents in general.

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Chapter V

Study 3

Allometric scaling of peak oxygen uptake in male roller hockey players under 17 years old

5. Allometric scaling of peak oxygen uptake in male roller hockey players under 17 years old

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Journal: Valente-dos-Santos J, et al. 2013. Allometric scaling of peak oxygen uptake in male roller hockey players under 17 years old. Appl Physiol Nutr Metab 38:390–395. doi: dx.doi.org/10.1139/apnm-2012-0178

5.1. Abstract

Peak oxygen uptake (peak VO_2) is routinely expressed in liters per minute and also by unit of body mass (ml/kg/min) despite theoretical and statistical limitations of using ratios. Allometric modelling is an effective approach for partitioning body-size effects in a performance variable. The current study examined the relationships among chronological age (CA), skeletal age (SA), total body and appendicular size descriptors with peak VO_2 in male adolescent roller hockey players. Seventy three Portuguese, highly trained male athletes (CA: 15.4±0.6 years; SA: 16.4±1.5 years; stature: 169.9±6.9 cm; body mass: 63.7±10.7 kg; thigh volume: 4.8±1.0 L) performed an incremental maximal test on a motorized treadmill. Coefficients for body size descriptors were 2.15 for stature (R^2=0.30; P<0.01) and 0.55 for thigh volume (R^2=0.46; P<0.01). The combination of stature or thigh volume with CA or SA and with CA^2 or SA^2 increased the explained variance in peak VO_2 (R^2 ranged 0.30-0.55). The allometric model combining more than one body size descriptor (i.e., stature and thigh volume) in addition to SA and CA^2 was not significant. Results suggested that thigh volume and skeletal age are the main contributors to explain inter-individual variability in aerobic fitness.

Keywords: Young Athletes · Biological Maturation · Growth · Peak Oxygen Uptake · Allometry

5.2. Introduction

Roller (rink) hockey is played on a rectangular rink (40 m x 20 m) surrounded by a barrier one meter high. Games have two periods of 25-minutes duration and are played by two teams of five players (two defenders, two attackers, one goalkeeper). Players must wear four-wheeled squad skates (in contrast to inline hockey skates) and use a two-sided stick to play the ball. As in other team sports, the performance structure of roller hockey is complex (Mendo and Argilaga, 2002). Match analysis of Seniors (23.4 ± 2.1 years old) indicated high intensity non-continuous actions with an estimated total skating distance of 16 km (Kingman and Dyson, 1997), suggesting that aerobic fitness is an important component of hockey performance. In contrast to ice hockey (Burr et al., 2008; Cox et al., 1995), the literature on energetic requirements of the roller hockey is still limited. Under simulated game conditions among 14 Spanish players (20–32 years), lactate concentration and heart rate were, on average, 4.20 ± 0.95 mmol/L and 163.5 ± 10.4 beats/min, respectively (Bonafonte et al., 1994). These values were similar to those reported for other team sport such as handball (Póvoas et al., 2012) and basketball (Abdelkrim et al., 2007). In other team sports (e.g., ice hockey), a wide range of inter-individual variability in physical fitness is observed both among young (Burr et al., 2008) and adult (Cox et al., 1995) players.

Aerobic fitness depends upon the integration of the pulmonary, cardiovascular, and hematological components of oxygen delivery and the oxidative mechanisms of the exercising muscle (Armstrong and Welsman, 2001). An important aspect of training-induced changes in peak VO_2 is caused by an increase in the oxidative profile of skeletal muscle (Holloszy et al., 1977). Peak VO_2 is routinely expressed as a ratio of total body mass (e.g., mL/kg/min) despite theoretical and statistical limitations (Nevill et al., 1992). Allometric models are an effective approach for partitioning the effects of body size and have been recommended as providing a 'size-free' expression of physiological parameters (Armstrong and Welsman, 2001; Nevill et al., 1992). Additionally, leg muscle mass was noted as a major contributor to explain inter-individual variability in peak VO_2 (Nevill et al., 2004). Thus the next step is to examine the contribution of more than one covariate (i.e., body mass, leg muscle mass), simultaneously.

Most of the previous research dealing with aerobic fitness in children and adolescents include participants who are not regularly involved in intensive training programs (Beunen et al., 2002; Geithner et al., 2004; Nevill et al., 1998). Armstrong and Welsman (1994) summarized cross-sectional and longitudinal peak VO_2 data on over 10,000 children and adolescents. 'Untrained' boys and girls showed a progressive, linear increase in peak VO_2 in relation to chronological age (8-16 years), with higher levels in boys after the 10 years of age. Adolescent athletes within a sport tend to be relatively homogeneous in training history, functional capacity and sport-specific skills, but variation in size and biological maturation may be considerable (Malina et al., 2004).

Skeletal age (SA) assessment is considered the most accurate maturity indicator and is the only method that spans the whole growth period (Malina et al., 2004). The assessment of skeletal maturity is based on the observation that a person more advanced in maturity will have greater bone development and a smaller amount of cartilage than a less mature person. Skeletal ages ranging from 9 to 16 years have been demonstrated in a group of 13- and 14-year olds, thus illustrating the wide variation in skeletal age evident in children of a similar chronological age (CA) (Kemper and Verschuur, 1981). Athletes who are advanced in SA relative to CA (higher SA/CA ratio) tend to be taller, heavier, stronger, more powerful and faster than athletes late or delayed in skeletal maturation (Malina, 2011; Malina et al., 2004).

The relationship between SA, as a measure of biological maturation, and the peak VO_2 in young athletes engaged in organized sport is still lacking in the literature. The purpose of this study was to determine the extent to which CA, SA, stature and thigh volume interdependently and interactively accounts for the inter-individual variation in peak VO_2 in male adolescent roller hockey players. It was hypothesized that SA is positively correlated with peak VO_2 independently of body size. It was also hypothesized that thigh volume is a significant size descriptor to be considered in allometric modelling of peak VO_2.

5.3. Methods

Participants

The sample comprised 73 Caucasian male competitive roller hockey players aged 14.5 to 16.5 years from 15 clubs in Portugal. All players participated in regular training sessions (4–6 sessions per week; ~ 360–510 min per week) and typically played one or two games per week over a 10-month period (mid-September to June). All participants were engaged in formal training and competition for at least five years. Moreover, the Portuguese national team selected to participate in the 2007 and 2008 editions of the U-17 European League were selected from this sample of young roller hockey players. The Portuguese team finished second in 2007 and first in 2008 in the European league. The study received ethical approval from the Scientific Committee of the University of Coimbra and afterwards from the Portuguese Foundation for Science and Technology [SFRH/BD/64648]. The Portuguese Skating Federation, clubs and parents provided written consent.

Age and skeletal maturity

Chronological age (in decimals) was calculated as the difference between date of birth and the date on which a posterior-anterior radiograph of the left hand-wrist was taken. Skeletal age was assessed with the Fels method (Roche et al., 1988). This method assigns grades to specific maturity indicators of the radius, ulna, carpals, metacarpals plus phalanges of the first, third and fifth digits, and utilizes ratios of linear measurements of the widths of the epiphysis and metaphysis of the long bone. The protocol also notes the presence (ossification) or absence of the pisiform and addutor sesamoid bones. Ratings are entered into a program (Felshw 1.0 Software, Lifespan Health Research Center, Departments of Community Health and Pediatrics, Boonshoft School of Medicine, Wright State University, Dayton, Ohio). The standard error is a unique feature of this protocol and tended to slightly increase with age (11–12 years: 0.27–0.32; 13–14 years: 0.27–0.49; 15–17 years: 0.28–0.72), because at later ages the assessment is based on fewer indicators (Malina et al., 2010). Using an independent sample (n=20), the mean difference in SA assessments made one week apart was 0.02±0.13 years. Additionally, intra-observer technical error of measurement was 0.03±0.04 years.

Anthropometry

Anthropometric assessment was performed by a single experienced observer following standard procedures (Lohman et al., 1988). Players wore shorts and T-shirt and shoes were removed. Stature was measured to the nearest 0.1 cm using a Harpenden stadiometer (model 98.603, Holtain Ltd, Crosswell, UK) and body mass was measured to the nearest 0.1 kg using a SECA balance scale (model 770, Hanover, MD, USA). Circumferences at the gluteal furrow (highest possible horizontal circumference), mid-thigh (largest possible mid-thigh circumference) and lower thigh (minimum circumference above the knee) were measured. Lengths between each circumference level were also measured to estimate total thigh volume of the dominant leg (Jones and Pearson, 1969). The method was already used in pediatric sport science (Martin et al., 2004).

Peak aerobic fitness

Peak VO_2 was determined using an incremental running test on a motorized treadmill (Quasar, HP Cosmos, Germany). Participants started with 2 minutes at 8 km/h, with subsequent increments of 2 km/h every minute until 16 km/h. Exercise intensity was subsequently increased through increasing the treadmill grade by 2º every minute until exhaustion (Gore, 2000). Attainment of peak VO_2 was confirmed if the athlete met any two of the following criteria: 1) respiratory exchange ratio (RER) ≥ 1.05; 2) heart rate (HR) within 10% of the age predicted maximum; 3) plateau in oxygen consumption despite increased exercise intensity or volitional exhaustion. Expired oxygen (O_2) and carbon dioxide (CO_2) flow and concentrations were measured every 10 seconds using a mixing chamber system (MetaMax System, Cortex Biophysics, Leipzig, Germany). Calibration and ambient air measurements were conducted before each testing session according to the manufacturer's guidelines. Before each test, flow and volume were calibrated using a 3-L capacity syringe (Hans Rudolph, Kansas City, USA). Gas analysers were calibrated using gases of known concentrations. HR was measured throughout exercise with a commercially available HR-monitor (Polar Electro, Finland).

Statistical analysis

Descriptive statistics were calculated for the total sample (mean, standard deviation and range) and Kolmogorov-Smirnov test was used to examine degree of normality. Correlation coefficients (Pearson) were calculated to examine the linearity of relationships among body descriptors (natural logarithms, i.e., log transformed stature and log transformed thigh volume), CA and SA and the natural logarithms of absolute peak VO_2 (L/min). An initial allometric model was adopted to examine the relationship between body descriptors and peak VO_2:

$$y = a \cdot x^{k} \cdot \varepsilon \quad \textbf{(5.1)}$$

Values of *a* and *k* were derived from linear regressions of the logarithmic regression transformations in the form of:

$$\log y = \log a + k \cdot \log x + \log \varepsilon \quad \textbf{(5.2)}$$

where *y* was the dependent variable of peak VO_2 (log transformed peak VO_2, i.e., natural logarithms), *a* was the scaling constant and *k* the scaling coefficient of the body descriptors (i.e., log transformed stature or log transformed thigh volume). Afterwards, a stepwise multiple linear regression on log(*y*), based on proportional allometric models, was used to fit the unknown parameters (Nevill and Holder, 1994). The models incorporated CA or SA and CA^2 or SA^2 as exponential terms in addition to stature and thigh volume. Age was incorporated into the model as a quadratic polynomial to allow the nonlinear relationship with peak VO_2:

$$\log(\text{peak } VO_2) = k_1 \cdot \log(\text{body descriptor}_1) + k_2 \cdot \log(\text{body descriptor}_2) + a + b_1 \cdot (\text{CA or SA}) + b_2 \cdot (\text{CA}^2 \text{ or SA}^2) + \log \varepsilon \quad \textbf{(5.3)}$$

The association between the residuals of each proportional allometric model and the respective scaling denominators were calculated using Pearson correlations. This was conducted to check the assumptions of scaled power variables independency of the participants' body size and age descriptors, as well as homoscedasticity of residuals in the log-linear regressions. If the allometric model is successful in partitioning out the influence of CA, SA and body size, then the correlation between the residuals and each independent variable used in the model, separately, should approach zero, which indicates there is little or no residual size correlation. Correlation coefficients that do not approach zero, regardless of whether they are statistically significant, indicate that the proportional allometric model is not successful in scaling peak VO_2 independent of age and body size (Nevill et al., 1992). The coefficient of determination (R^2) was reported to provide an indication of the explained variance. The magnitude of correlations was interpreted as follows: trivial ($r < 0.1$), small ($0.1 < r < 0.3$), moderate ($0.3 < r < 0.5$), large ($0.5 < r < 0.7$), very large ($0.7 < r < 0.9$), and nearly perfect ($r > 0.9$) (Hopkins, 2002). Statistical analyses were performed using IBM SPSS version 19.0 software (SPSS Inc., IBM Company, NY, USA).

5.4. Results

Descriptive statistics for the total sample are summarized in Table 5.1. Relationships between log transformed peak VO_2 with CA, SA, log transformed stature and log transformed thigh volume are illustrated in Figure 5.1 (panels a-d). A linear relationship was observed between stature and peak VO_2. Non-linear relationships were apparent between thigh volume, CA and SA with peak VO_2. Correlations between the descriptors and peak VO_2 ranged from 0.34 to 0.68 ($P < 0.01$).

Table 5.1. Descriptive statistics for the total sample (n = 73) and results of Kolmogorov-Smirnov test for checking the normality of the distribution.

	Mean	Standard deviation	Range	Kolmogorov-Smirnov	
				Value	*P*
Chronological age (years)	15.4	0.6	14.5 to 16.5	0.96	0.32
Skeletal age (years)	16.4	1.5	13.1 to 17.9	1.20	0.11
Years of training (years)	8.6	1.2	5 to 11	1.24	0.11
Stature (cm)	169.9	6.9	143.6 to 182.2	0.72	0.67
Body mass (kg)	63.7	10.7	38.4 to 90.3	0.63	0.82
Thigh volume (L)	4.8	1.0	2.9 to 7.8	0.89	0.40
Peak oxygen uptake (L/min)	3.89	0.62	2.48 to 5.46	0.73	0.67

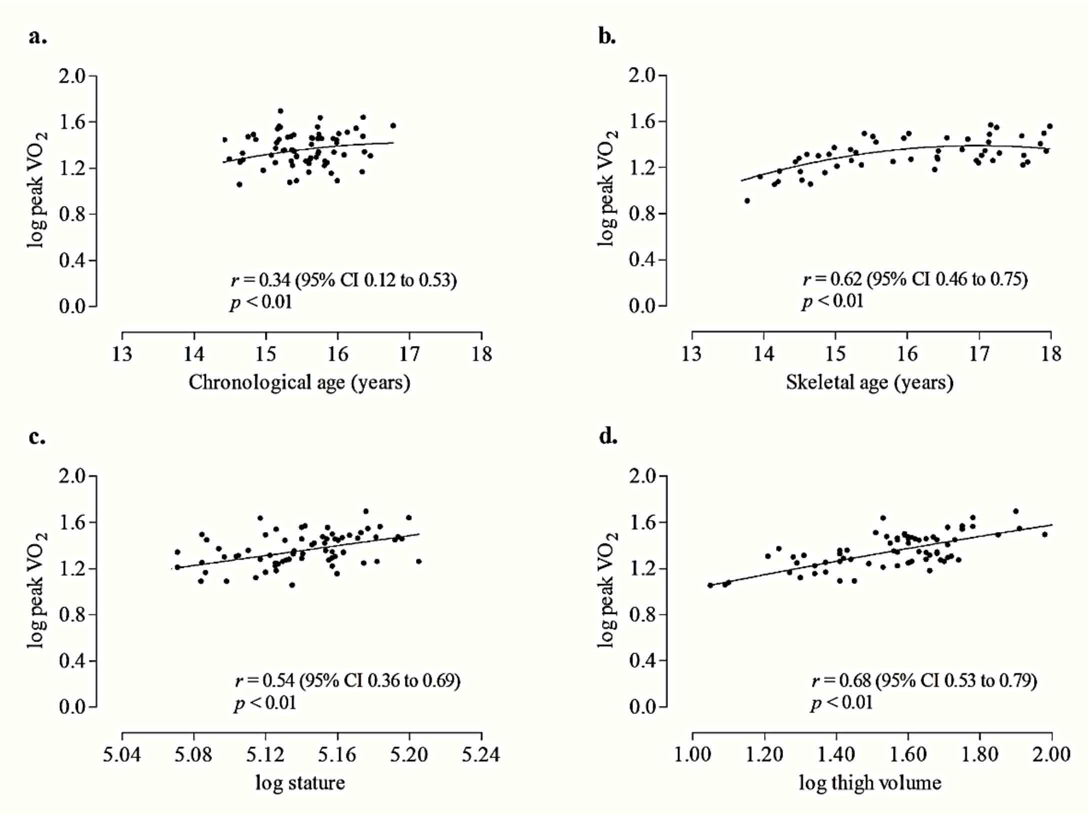

Figure 5.1. Relationship (r) of the log transformed peak VO_2 with chronological (panel a) and skeletal age (panel b) and with the log transformed stature (panel c) and thigh volume (panel d). The 95% confidence intervals (95% CI) of the correlation coefficients are also presented.

The coefficients for stature and thigh volume from the independent allometric models of peak *V*O_2 are summarized in Table 5.2 (Models 1 and 2 derived from Equations 5.2). The residuals of the simple allometric models presented no residual correlation to their respective body size variables. Visual inspection of absolute residuals showed no heteroscedasticity, indicating that stature and thigh volume can be used to derive peak *V*O_2 "size free scores". However, substantial residual size correlations was present when residuals were correlated with other size variable (e.g., residuals of peak VO_2 modelled for thigh volume against stature) to other size variables ($0.15 < r < 0.33$), indicating that stature or thigh volume alone did not correctly partition out the influence of body size in peak *V*O_2.

The allometric models combining body descriptors with SA, CA and their respective squared terms explained 44% to 55% of variance in the peak *V*O_2 (Models 3-5 derived from Equation 5.3). Chronological age and SA^2 did not enter as significant predictors. Stature was a significant predictor when combined with SA and thigh volume entered two times, and when combined with CA^2 and SA. Finally, allometric models simultaneously combining stature and thigh volume were not significant.

Table 5.2. Allometric modelling [Equation 5.2] and proportional multiplicative allometric modelling [Equation 5.3] of the peak VO_2 for body size variables and for body size variables and age, respectively.

Equations	Model	Descriptor	Coefficients (95% CI)	r	Model Summary R^2	Model Summary (95% CI)
[Equation 5.2]: $\log y = \log a + k \cdot \log x + \log \varepsilon$	1	Stature	2.15 (1.37 to 2.94)	0.54	0.30	(0.13 to 0.47)
	2	Thigh volume	0.55 (0.41 to 0.69)	0.68	0.46	(0.30 to 0.62)
[Equation 5.3]: $\log(\text{peak } VO_2) = k_1 \cdot \log(\text{body descriptor}_1) + k_2 \cdot \log(\text{body descriptor}_2) + a + b_1 \cdot (\text{CA or SA}) + b_2 \cdot (\text{CA}^2 \text{ or SA}^2) + \log \varepsilon$	3	Stature	1.11 (0.25 to 1.97)	0.28	0.44	(0.28 to 0.60)
		SA	0.05 (0.03 to 0.08)	0.46		
	4	Thigh volume	0.51 (0.38 to 0.65)	0.64	0.50	(0.35 to 0.66)
		CA^2	0.002 (0.000 to 0.003)	0.20		
	5	Thigh volume	0.66 (0.38 to 0.94)	0.49	0.55	(0.40 to 0.70)
		SA	0.02 (0.01 to 0.06)	0.36		

5.5. Discussion

The results of the allometric models of this study identified SA and body dimensions as significant predictors of peak VO_2, whereas CA was systematically removed (P > 0.05), with one exception of CA^2. The independent variables incorporated in the proportional allometric models explained between 30% and 55% of variance in peak VO_2. These results emphasize the importance of the inter-relations between size variables and the variability caused by biological age (SA).

Players of this study presented mean statures between the 25th and 50th centiles and mean body masses between the 50th and 75th using US age specific percentiles (Kuczmarski et al., 2002). The elevated mass-for-stature probably reflected the advanced biological age of the players and perhaps the influence of systematic training on lean mass development (Malina et al., 2004). Absolute values for peak VO_2 (Table 5.1), in the present sample, were higher than those previously reported for similar age range in the general population (Beunen et al., 2002; Geithner et al., 2004), However, the peak VO_2 values were comparable to male athletes of similar age in several team sports but lower than athletes in endurance-based individual sports - triathlon, long-distance running, cross country skiing, swimming (Bunc, 2004). Although roller hockey is not an endurance sport per se, it has been suggested that high values of cardiopulmonary functions may be important for players to maintain a high level of activity during the entire game (Kingman and Dyson, 1997) and for effective recovery from high-intensity, short burst movements (Bonafonte et al., 1994).

It is expected that absolute peak VO_2 increases as a function of body size during childhood and adolescence, regardless of whether youth are engaged in organized sports. Longitudinal data based on Belgian boys suggested that the timing of maximum growth in body dimensions is related to the timing of peak aerobic fitness during adolescence (Geithner et al., 2004). Studies using multilevel modelling have demonstrated size-independent effects of sexual and somatic maturation on peak VO_2 (Armstrong and Welsman, 2001; Beunen et al., 2002). Results from the Training of Young Athlete (TOYA) study indicated that peak VO_2, adjusted for age and body dimensions, increases with pubertal status in male athletes (Baxter-Jones et al., 1993; Nevill et al., 1998). In general, the available data indicates that age-related increases in peak VO_2 are mostly mediated by changes in size dimensions; however, individuality of timing and tempo of maturation and year-to-year changes in body mass and peak VO_2 may be masked by maturity effects (Eisenmann et al., 2001).

Since important inter-individual variability in body dimensions is observed during pubertal years (Malina et al., 2004), the non-linear allometric modelling procedures used in this study were statistically appropriate to account for differences in body dimensions. The null correlation between the residuals of the power function models and their respective body dimension variables indicate that power functions (Nevill et al., 1992) can be used to derive peak VO_2 “size free scores” for each of the adopted size variables. Also, the proportional allometric models, derived from stepwise regressions, were adjusted to fit peak VO_2 data, as absolute residuals from each of the models were uncorrelated with log transformed independent variables used in each of the models. These results are consistent with previously published allometric models for partitioning body-size effects on VO_2 (Chamari et al., 2005; Cunha et al., 2011; Nevill et al., 1992).

The size coefficients derived from the allometric models confirmed the observations in the literature that the relationship between body size dimensions and peak VO_2 is not proportional. This supports the well-acknowledged limitations of ratio standards, such as the penalization of larger individuals (Nevill et al., 1992; Welsman et al., 1996). In the present study, thigh volume coefficient was 0.55. However, the contribution of thigh volume is substantially improved when this size descriptor is combined with SA. The multiplicative allometric model emerged from stature and SA also explained more variance (44%) than the model defined by stature (30%). Together, these suggest that SA significantly explains inter-individual variability in peak VO_2, and also that multiplicative models provide a better understanding than allometric models based on a single size descriptor. The contribution of lower limb volume may be an effect of interactions among growth, maturation and systematic training (Nevill et al., 2004).

The stepwise multiple regression analysis (Table 5.2) suggests that the quadratic term of CA only has a small (4%), but significant, effect on peak aerobic fitness via thigh volume. Likewise, the allometric coefficients for stature and thigh volume derived from stepwise regressions compared to the initial allometric model (Equation 5.2) suggests that the linear influence of CA on peak VO_2 was negligible. This finding, to some extent, likely reflects the relatively narrow CA range of the sample.

The inclusion of the biological age (i.e. SA) indicator term in the final model (Table 5.2: Models 3 and 5) indicates that biological age likely exerts the most important effect on peak VO_2. In contrast, a recent report using allometric modelling showed no influence of pubertal maturation on peak VO_2 (Cunha et al., 2011). However, the study by Cunha and colleagues (2011) used less accurate, categorical stages of secondary sex characteristics whereas the present study used continuous SAs, a more objective and accurate measure of biological maturity.

We recognize that although of SA is considered the best maturational index, it is costly, requires specialized equipment and interpretation which may hinder its use in some field situations. Nevertheless, when SA is not available, peak aerobic fitness can be expressed relative to thigh volume and the quadratic term of CA. A limitation of this study is also the measure of limb volumes by anthropometry. A previous report comparing the anthropometric protocol to magnetic resonance imaging in a small sample of athletes, showed an over-estimation in limb volume by anthropometry (Tothill and Stewart, 2002). Lastly, the allometric models put forward in this paper need to be validated in different samples (e.g., in females) and sports contexts and a wider matrix of predictors should be explored.

5.6. Conclusion

The present study clearly identified an independent effect of body size (using both stature and thigh volume) and skeletal age on peak VO_2 of male U-17 roller hockey players. Proportional, multiplicative allometric models are recommended for scaling VO_2 data. The interpretation of peak aerobic fitness of adolescent hockey athletes can be expressed relative to thigh volume and the quadratic term of chronological age, whenever skeletal age is not available. Peak aerobic fitness established a curvilinear increase in relation to CA in late adolescence suggesting a plateau. The information derived from this study offers a new biological approach to normalize or adjust peak VO_2 data in youth athletes and adolescents in general.

5.7. References

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Chapter VI

Study 4

Allometric modelling of peak oxygen uptake in male soccer players 8-18 years of age

6. Allometric modelling of peak oxygen uptake in male soccer players 8-18 years of age

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Journal: Valente-dos-Santos J, et al. 2014. Allometric modelling of peak oxygen uptake in male soccer players 8-18 years of age. Ann Hum Biol.
(Accepted)

6.1. Abstract

Background: Peak oxygen uptake (VO_{2peak}) is routinely scaled as mL O_2 per kilogram body mass despite theoretical and statistical limitations of using ratios.

Aim: To examine the contribution of maturity status and body size descriptors to age-associated inter-individual variability in VO_{2peak} and to present static allometric models to normalize VO_{2peak} in male youth soccer players.

Subjects and methods: Total body and estimates of total and regional lean mass were measured with dual energy X-ray absorptiometry in a cross-sectional sample of Portuguese male soccer players. The sample was divided into three age groups for analysis: 8–12 years, 13–15 years, and 16–18 years. VO_{2peak} was estimated using an incremental maximal exercise test on a motorized treadmill. Static allometric models were used to normalize VO_{2peak}.

Results: The independent variables with the best statistical fit explained 72% in the younger group (lean body mass: k=1.07), 52% in mid-adolescent players (lean body mass: k=0.93) and 31% in the older group (body mass: k=0.51), of variance in VO_{2peak}. The inclusion of the exponential term pubertal status marginally increased the explained variance in VO_{2peak} (adjusted R^2 = 36% to 75%) and provided statistical adjustments to the size descriptors coefficients.

Conclusion: The allometric coefficients and exponents evidenced the varying interrelationship among size descriptors and maturity status with aerobic fitness from early to late-adolescence. Lean body mass, lean lower limbs mass and body mass combined with pubertal status explain most of the inter-individual variability in of VO_{2peak} among youth soccer players.

Keywords: Youth Athletes · Growth · Body Composition · Aerobic Fitness · Static Allometry

6.2. Introduction

In children and adolescents, VO_{2peak} increases as a function of body size, regardless of engagement in regular physical training (Armstrong and Welsman, 1994; Malina et al., 2004). Increments in absolute VO_{2peak} in adolescents are strongly correlated with increases in body mass which, among other factors, are connected to growth related changes in lungs, heart and skeletal muscle mass (Rowland, 2005). Similar to left ventricular mass and cardiac output (Daniels et al., 1995), VO_{2peak} is strongly determined by the demands of metabolically active tissues (Rowland, 2005). Even though stature is strongly correlated with lean body mass, the relationship is not perfect and may be a biased surrogate.

In boys, cross-sectional and longitudinal data for VO_{2peak} show an increase in VO_{2peak} with chronological age from 8–16 years, with an accelerated increase after 10 years of age (Malina et al., 2004). Longitudinal data shows that adolescent maximal growth in VO_{2peak} overlaps with the timing of peak height velocity (Geithner et al., 2004; Mirwald and Bailey, 1986). Although it should be noted that the variation in estimated parameters of VO_{2peak} may be related to sampling, testing protocols, equipment, analytical procedures and, in the case of longitudinal studies, intervals between observations and age at baseline (Malina et al., 2004). In addition, interpretation of VO_{2peak} is confounded by variation in body size (Welsman et al., 1996).

The adoption of ratios to obtain an output of peak oxygen uptake per unit of body mass or fat-free mass (i.e., $mL \cdot kg^{-1} \cdot min^{-1}$) are often used in the literature, despite the several theoretical and statistical limitations (Nevill et al., 1992) which have been long recognized (Tanner, 1949; Malina, 2012). Comparisons of VO_{2peak} using a ratio approach tend to penalize heavier athletes (Nevill et al., 2003) and proper scaling approaches are promoted although not always adopted. Allometric modelling successfully accommodates the nonlinear relationships between body size descriptors and VO_{2peak} and helps to minimize the impact of heteroscedastic (non-constant variance) errors, thus improving normalization (Nevill et al., 1992; Valente-dos-Santos et al., 2013). In brief, allometry refers to the relationship between variables that are affected by proportional changes due to variation in body size and growth rate (Gunther, 1975). The concept has been used to study ontogenetic and phylogenetic changes in morphological, physiological or biochemical characteristics (Beunen et al., 1997). Ontogenetic allometry refers to differential growth in the individual (Balasekaran et al., 2005; Beunen et al., 1997) and is most often applied to longitudinal data (Gould, 1966). Static allometric coefficients (phylogenetic) reflects the dimensional relation between VO_{2peak} and size descriptors (Beunen et al., 1997), in conspecific individuals at the same developmental (ontogenetic) stage (Pelabon et al., 2013).

Cross-sectional studies in young people (Armstrong and Welsman, 1994; Welsman et al., 1996) produced coefficients that are thought to reflect relationships between VO_{2peak} and size descriptors which approximate the theoretical range of geometric similarity (e.g., body mass to the power 0.67; Schmidt-Nielsen, 1975) and elastic similarity (e.g., body mass to the power 0.75; Kleiber and Rogers, 1961). However, other allometric coefficients are reported for VO_{2peak}: 1.01 (Cooper et al., 1984), 0.90 (Cunha et al., 2011); which may be a consequence of varying experimental conditions (Rowland, 1998). Meantime, the differential growth rates of size descriptors in relation to the developmental changes in this physiological parameter, suggest that the same scaling coefficient may not be generalized for all ages (Thomis et al., 2000).

Aerobic fitness depends on the oxidative mechanisms of exercising muscle (Armstrong and Welsman, 2001) and leg muscle mass has been indicated as a major contributor to the explained inter-individual variability in aerobic fitness (Nevill et al., 2004). Considering the extent to which the legs contribute to performance (Winter et al., 1991), it is surprising that scaling analyses which include an estimate of lean lower limbs mass in young athletes are lacking. The aim of the current study is to investigate the independent and interactive contributions of pubertal maturity status, stature, body mass, lean body mass and appendicular descriptors to the inter-individual variation in VO_{2peak}. A secondary aim is to present static allometric models to normalize VO_{2peak} in youth soccer players, using alternative descriptors and, in the case of lean body mass, concurrent estimates derived from anthropometry and dual energy X-ray absorptiometry (DXA).

6.3. Methods

Participants

The sample included 81 healthy male soccer players aged 8-18 (14.98 ± 2.71) years of European (Caucasian) ancestry. The players were members of eight soccer clubs from the metropolitan area of Porto (Portugal). All subjects were engaged in formal training (3 – 5 sessions · week $^{-1}$; ~ 225 – 450 min · week $^{-1}$) and had competed for at least three years (usually 1 – 2 competitive games per week over a 10-month season). At the time of the measurements, no players suffered from chronic diseases, or had been taking medication known to affect bone metabolism (e.g., corticosteroids) within 6 months of the testing date. The experimental protocol fitted the recommendations from the Declaration of Helsinki produced by the World Medical Association for research with humans. In addition, the procedures were approved by the Institutional Review Board of the Faculty of Sport, University of Porto. Participants were fully informed about the aims, experimental protocol and procedures, and their parents provided written informed consent.

Age and maturity status

Chronological age was determined as the difference between date of birth and date of testing. The sample was divided into three age groups: 8.50–12.49 years, 12.50–15.49 years, and 15.50–18.49 years to approximate early-, mid- and late-adolescence. Pubic hair (PH) development was assessed by an experienced physician using the criteria described by Tanner (1962).

Anthropometry

Anthropometry was performed by a single experienced observer following standard procedures (Lohman et al., 1988). Stature was measured to the nearest 0.1 cm with a Harpenden stadiometer (model 98.603, Holtain Ltd, Crosswell, UK) and body mass was measured to the nearest 0.1 kg using an electronic scale (Tanita, BC-418, MA, USA). The body mass index (BMI) was calculated and expressed as a BMI-for-age *z*-score (BMIz) for a reference population (Kuczmarski et al., 2002). BMIz has been suggested as a reasonable index of adiposity for youth (Freedman and Sherry, 2009). Lean body mass estimated from anthropometry was obtained from logarithmic transformed values of stature and body mass, BMIz and chronological age using the protocol of Foster et al. (2012), as presented in Equation 6.1:

$$\ln \text{lean body mass} = -2.9585 + 0.8208 \cdot \ln \text{stature} + 0.5607 \cdot \ln \text{body mass} + 0.0000184 \cdot \text{body mass}^2 - 0.0159 \cdot \text{BMIz}^2 + 0.0135 \cdot \text{chronological age} \quad \textbf{(6.1)}$$

Dual energy X-ray absorptiometry

A Hologic QDR 4500A DXA scanner (Hologic Inc., Waltham, MA, USA) with v.9.10 software was used to perform total body scans. DXA is an accepted measure of body composition (Ellis, 2000; Leahy et al., 2012). The procedure allows measurement of segmental composition: arms, legs and trunk. Daily calibration of the scanner was performed using a phantom spine containing composites of bone, fat and lean tissue. Participants were positioned on the scanner bed according to manufacturer recommendations. The lower limbs on each image were sectioned as follows: all tissue distal to a line drawn through and perpendicular to the axis of the femoral neck and angled with the pelvic brim to the phalange tips. Lean lower limbs mass was obtained by adding the left and right legs. Mean variation between measured and reconstructed absolute whole-body mass with DXA software was 0.7%. Based on test-retest in 15 individuals, the coefficients of variation for percent body fat and lean body mass were 3.1% and 1.1%, respectively.

Peak aerobic fitness

VO_{2peak} was determined using an incremental running test on a motorized treadmill (Quasar-Med, Nussdorf, Germany) to voluntary exhaustion. Participants started at 2.2 $m \cdot s^{-1}$ with subsequent increments of 0.2 $m \cdot s^{-1}$ every minute until exhaustion.

Expired oxygen (O_2) and carbon dioxide (CO_2) flow and concentrations were measured using an open circuit breath-by-breath automated gas analyser (Cortex, Metalyser, 3*B*, Leipzig, Germany). Calibration and ambient air measurements were conducted before each testing session according to the manufacturer guidelines. Before each test, flow and volume were calibrated using a 3-L capacity syringe (Hans Rudolph, Kansas City, USA). Gas analysers were calibrated using gases of known concentrations. Heart rate was measured every 5 s using a heart rate monitor (Vantage NV, Polar Electro, Kempele, Finland) connected to the gas-analyser system. Attainment of VO_{2peak} was confirmed if the athlete met at least two of the following criteria: respiratory exchange ratio ≥ 1.05; blood lactate concentrations > 8 $mmol \cdot L^{-1}$; heart rate within 10% of the age predicted maximum; plateau in oxygen consumption despite increased exercise intensity, or volitional exhaustion.

Statistical analyses

Descriptive statistics were calculated for all variables by age groups. Paired samples *t*-tests were used to compare lean body mass derived by DXA and anthropometry. Agreement between the reference method and the anthropometric estimate was assessed using Deming regression (i.e., least products regression method which accounts for the error of both *x* and *y* variables) and standard error of estimation ($S_{y \cdot x}$) (Ricker, 1973). Data were then visually inspected by plotting the errors against the DXA and anthropometric estimated means using the Bland–Altman plot (Bland and Altman, 2012). Heteroscedasticity was examined by calculating the correlation coefficients between the absolute differences and the corresponding mean, to check if errors were associated with the magnitude of the values. Finally, if measurement differences were normally distributed, reliability was assessed using the standard error of measurement and 95% limits of agreement (Bland and Altman, 2012).

Static (inter-individual) allometry coefficients were calculated for the natural logarithms of absolute VO_{2peak} ($L \cdot min^{-1}$) and natural logarithms of body descriptors (ln transformed stature, ln transformed body mass, ln transformed lean body mass, ln transformed and ln transformed lean lower limbs mass) in each age group. The static coefficients reflect the dimensional relationship between VO_{2peak} and body descriptors in a given age group, and are not identical with ontogenetic allometry coefficients which reflect proportional changes over time (Beunen et al., 1997). An initial allometric model was adopted, as follows:

$$y = a \cdot x^{k} \cdot \varepsilon \qquad \textbf{(6.2)}$$

Values of *a* and *k* were derived from linear regressions of the logarithmic regression transformations in the form of:

$$\ln y = \ln a + k \cdot \ln x + \ln \varepsilon \qquad \textbf{(6.3)}$$

where *y* was the dependent variable of VO_{2peak} (ln transformed VO_{2peak}, i.e., natural logarithms), *a* was the scaling constant and *k* the scaling coefficient of the body descriptors. Residuals VO_{2peak} (predicted – observed VO_{2peak}) were converted to absolute values and correlated with the predictor variables (i.e., ln transformed body descriptors) to examine the data for heteroscedasticity. Pearson correlations were also calculated between each allometric function and the respective scaling denominators, as a diagnostic test. Constant error variance (homoscedasticity), in the first case, and scaled power variables independency from size descriptors, in the second case, can be assumed if the correlations approach zero (Batterham et al., 1997; Nevill et al., 1992). Subsequently, a stepwise multiple linear regression based on proportional allometric models, was used to fit the unknown parameters (Nevill and Holder, 1994). The models incorporated stage of PH (pubertal development: dummy coded) as an exponential term, in addition to body descriptors:

$$\ln (VO_{2peak}) = k_1 \cdot \ln (\text{body descriptor}_1) + k_2 \cdot \ln (\text{body descriptor}_2) + a + b \cdot \text{PH stage (dummy coded)} + \ln \varepsilon \qquad \textbf{(6.4)}$$

A tolerance > 0.10 and a variance inflation factor < 10 was set to avoid collinearity between the explanatory variables (Slinker and Glantz, 1985). The incidence of high bivariate correlations between body composition indicators (r = 0.97 to r = 0.99) suggested unacceptable collinearity; separate regression models were thus used. The coefficient of determination (R^2) was reported to provide an indication of the explained variance. Magnitude of correlations was interpreted as follows: trivial ($r < 0.1$), small ($0.1 < r < 0.3$), moderate ($0.3 < r < 0.5$), large ($0.5 < r < 0.7$), very large ($0.7 < r < 0.9$), and nearly perfect ($r > 0.9$) (Hopkins et al., 2009). Statistical analyses were performed using IBM SPSS version 19.0 software (SPSS, Inc., IBM Company; NY, USA), GraphPad Prism version 5.03 software (GraphPad Software, Inc.; La Jolla, CA, USA) and MedCalc version 12.2.1 software (MedCalc; Mariakerke, Belgium). Alpha level was set at 0.05.

6.4. RESULTS

Chronological age, training experience, maturity status, body size and composition, and aerobic fitness of the soccer players by age group are described in Table 6.1. As expected, mean values of VO_{2peak} increased with age group. The majority of players aged 13–15 years (74%) were classified as late pubertal (PH4). In the older group (16–18 years) players tended to be classified as mature (PH5). In contrast, only 6 (30%) and 9 (45%) players 8–12 years were, respectively, classified as pre-pubertal (PH1) and early pubertal (PH2). Mean statures and mean body masses fall between the 50th and 75th age specific percentiles (Kuczmarski et al., 2002) which is consistent with other samples of adolescents Portuguese soccer players (Malina et al., 2013). Despite a trend for elevated mass-for-stature in all age groups, mean BMIz was within the normal range for boys of the same age (– 1.0 < BMIz < + 1.0). Estimates of lean body mass were lower when assessed by the anthropometric protocol than those derived from DXA ($P<0.05$).

Table 6.1. Descriptive statistics of soccer players by age group.

	8 – 12 years (n = 20)			13 – 15 years (n = 31)			16 – 18 years (n = 30)			All (n = 81)		
	Mean	SD	f	Mean	SD	f	Mean	SD	f	Mean	SD	f
Chronological age (years)	10.50	1.29		14.57	0.76		17.39	0.83		14.61	2.83	
Years of training (years)	1.7	1.2		4.7	2.3		7.1	2.8		4.8	3.1	
Stage of pubic hair												
PH 1			6 (30%)			—			—			6 (7%)
PH 2			9 (45%)			—			—			9 (11%)
PH 3			5 (25%)			8 (26%)			—			13 (16%)
PH 4			—			23 (74%)			10 (33%)			33 (41%)
PH 5			—			—			20 (67%)			20 (25%)
Stature (cm)	144.7	11.1		171.8	5.2		176.8	7.2		166.4	14.7	
Stature-for-age (*z*-score)	0.46	0.89		0.61	0.68		0.02	0.97		0.34	0.88	
Stature-for-age (percentile)	64	28		69	20		51	30		60	27	
Body mass (kg)	40.5	9.5		61.8	6.9		69.7	9.0		59.5	14.2	
Body mass-for-age (*z*-score)	0.71	0.82		0.66	0.48		0.29	0.68		0.54	0.67	
Body mass-for-age (percentile)	73	24		72	15		60	23		68	21	
BMI (kg/m^2)	19.1	2.4		20.9	1.7		22.7	2.2		21.1	2.5	
BMI-for-age (*z*-score)	0.71	0.75		0.41	0.50		0.30	0.65		0.44	0.64	
BMI-for-age (percentile)	72	23		64	17		60	22		65	21	
Lean body mass$_{ANTH}$ (kg)	29.1	6.4		46.9	4.8		54.1	6.7		45.2	11.4	
Body mass$_{DXA}$ (kg)	41.3	9.7		62.3	7.0		69.7	8.5		59.9	13.8	
Percentage of fat mass$_{DXA}$ (%)	21.7	4.7		16.0	2.5		15.7	2.8		17.3	4.1	
Fat mass$_{DXA}$ (kg)	8.8	2.0		10.0	2.2		11.0	2.8		10.1	2.5	
Lean body mass$_{DXA}$ (kg)	31.2 *	8.6		50.0 *	5.4		56.0 *	6.4		47.6 *	11.8	
Lean lower limbs mass$_{DXA}$ (kg)	11.0	3.2		18.1	2.3		19.9	2.6		17.0	4.4	
Peak oxygen uptake (L·min^{-1})	2.00	0.57		3.33	0.47		3.83	0.40		3.19	0.86	

Where applicable, results are expressed as frequencies (percentages) or mean ± SD.

DXA, dual x-ray absorptiometry; ANTH, anthropometry.

* Significant difference DXA *vs.* ANTH (paired samples *t*-test, $P<0.05$).

Deming regression analyses between estimates of lean body mass (Figure 6.1, panel a) showed a very large correlation (*r* = 0.98). The intercept (0.02, 95% CI -0.14 to 0.18) and slope (1.01, 95% CI 0.97 to 1.05) did not differ ($P > 0.05$) from the identity line (i.e., *y*-intercept = 0 when *x* = 0 and slope = 1). Therefore, the possibility of systematic or proportional bias was rejected (Ludbrook, 2002). Additionally, the Bland-Altman plot (Figure 6.1, panel b) for concurrent measurements of lean body mass showed that the error against the mean assumed a homoscedastic distribution (*r* = 0.03, $P > 0.05$) (Batterham et al., 1997; Nevill et al., 1992).

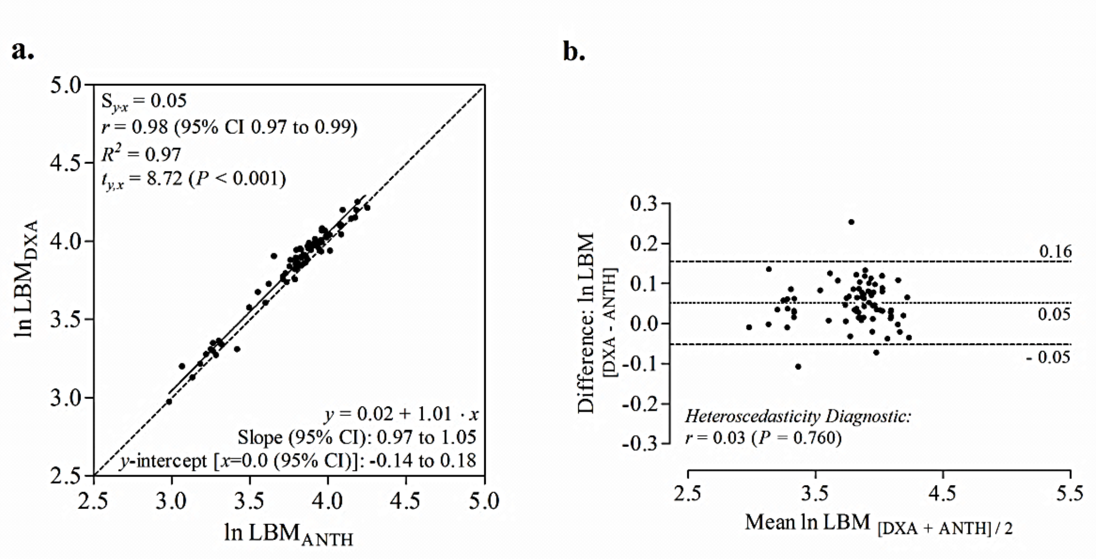

Figure 6.1. Deming regression between lean body mass (LBM) measured by dual-energy X-ray absorptiometry (DXA) and the estimated LBM using the protocol of Foster et al. (2012; panel **a**). The standard errors of estimation ($S_{y.x}$), correlations (*r;* 95% CI), coefficient of determination (R^2), paired samples *t*-test ($t_{y,x}$) and the reference line from the equation are also presented (95% CI). The right panel (panel **b**; Bland-Altman Plot) illustrate the relation between residuals (mean differences between measured and predicted LBM) and the corresponding mean (heteroscedasticity diagnostic). The dashed lines represent 95% limits of agreement (± 1.96 SD).

Static (inter-individual) allometric coefficients (*k*) were determined for the different age groups and are summarized in Table 6.2. The coefficients (*k*) suggested a non-linear association between VO_{2peak} and size descriptors. Coefficients consistently decreased with age and are as follows (i.e., for 8-12, 13-15 and 16-18 years, respectively): 2.71, 2.03 and 0.86 for stature; 0.98, 0.86, 0.51 for body mass; 1.07, 0.93, 0.42 for lean body mass derived by anthropometry; and 0.77, 0.70, 0.39 for lean lower limbs mass. Overall, the independent variables explained 60-72% in the younger group, 19-52% in players aged 13-15 years and 11-31% in the older group, of variance in VO_{2peak}.

Table 6.2. Allometric models* of the log transformed VO_{2peak} using log transformed total body and regional size descriptors.

y	x_i	8 – 12 years				13 – 15 years				16 – 18 years			
		$r_{y,x}$	k (95% CI)	R^2	SEE	$r_{y,x}$	k (95% CI)	R^2	SEE	$r_{y,x}$	k (95% CI)	R^2	SEE
ln VO_{2peak}	ln stature	0.78	2.71 (1.62 to 3.80)	0.60	0.17	0.44	2.03 (0.45 to 3.61)	0.19	0.13	0.33	0.86 (-0.09 to 1.80)	0.11	0.10
	ln body mass	0.84	0.98 (0.66 to 1.29)	0.70	0.15	0.66	0.86 (0.49 to 1.24)	0.43	0.11	0.58	0.51 (0.23 to 0.79)	0.31	0.09
	ln LBM_{ANTH}	0.85	1.07 (0.74 to 1.40)	0.72	0.14	0.65	0.93 (0.51 to 1.35)	0.52	0.11	0.48	0.42 (0.12 to 0.72)	0.23	0.09
	ln $LLLM_{DXA}$	0.78	0.77 (0.46 to 1.07)	0.60	0.17	0.61	0.70 (0.36 to 1.05)	0.38	0.11	0.46	0.39 (0.10 to 0.69)	0.21	0.09

r, Pearson's product moment correlation coefficient; LBM, lean body mass; LLLM, lean lower limbs mass; ANTH, anthropometry; DXA, dual x-ray absorptiometry

* $\ln (VO_{2peak}) = \ln a + k \cdot \ln (\text{body descriptor}) + \ln \varepsilon$.

The absolute residuals derived from the allometric models (Table 6.2) were plotted against the best predictor variables (Figure 6.2, body mass: panel a–c; lean body mass: panel d–f; lean lower limbs mass: panel g–i). The absolute residuals did not suggest heteroscedasticity (r = 0.001 to 0.36; P = 0.990 to 0.111) indicating that body mass, lean body mass (by anthropometry) and lean lower limbs mass by DXA can be used to obtain VO_{2peak} "size-free scores" in young soccer players.

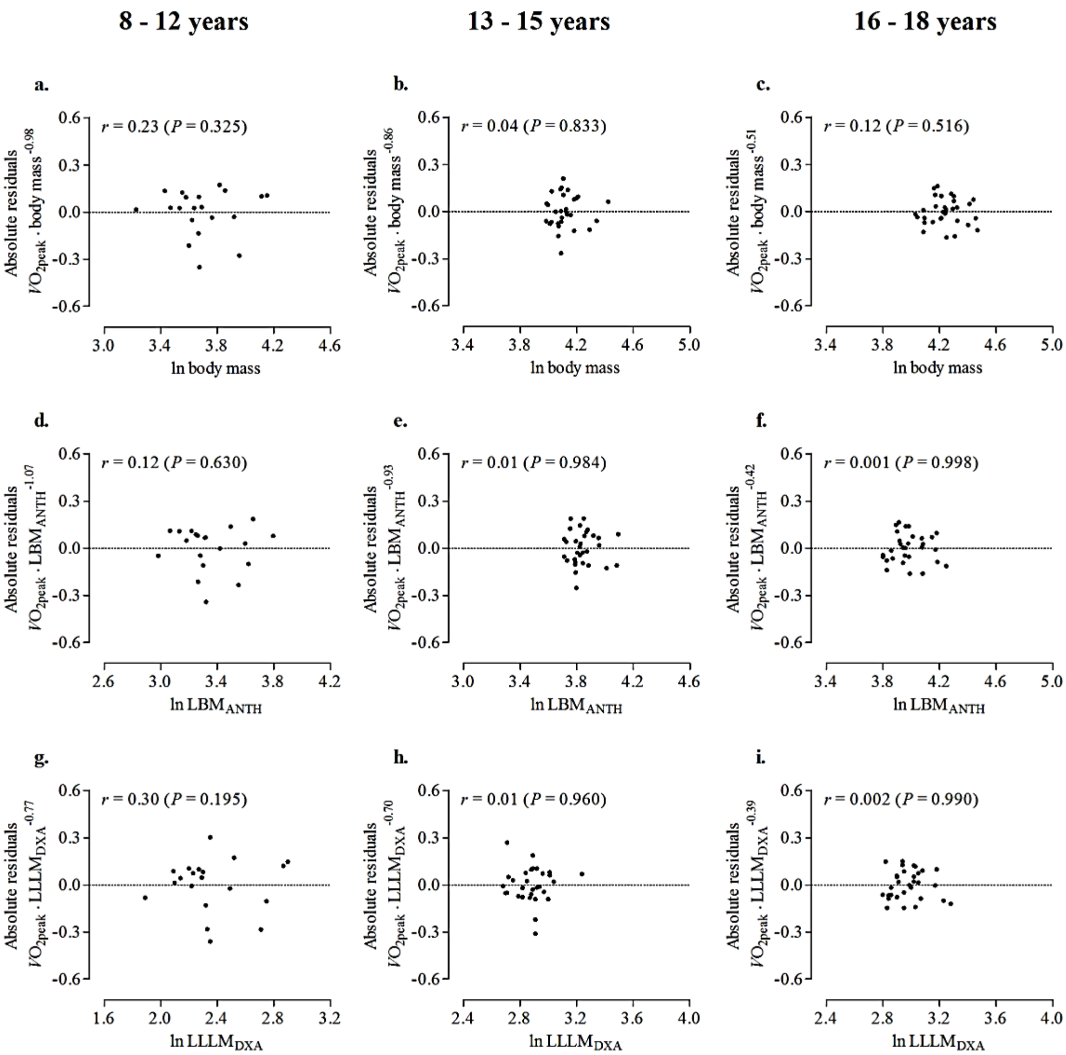

Figure 6.2. Relationship (r) of the absolute residuals from the simple static allometric models (VO_{2peak}·body descriptor^k; Equation 6.3) with ln transformed body descriptors: ln transformed body mass (panel a – c), ln transformed lean body mass derived by anthropometry (LBM_{ANTH}; panel d – f) and ln transformed lean lower limbs mass measured by dual-energy X-ray absorptiometry ($LLLM_{DXA}$; panel g – i).

Significant static allometric models combining more than one size descriptor in addition to PH stage (dummy coded) are summarized in Table 6.3. Compared with previous models that considered a single variable (Table 6.2), the explained variance of VO_{2peak} is larger (36–75%) in the models presented in Table 6.3. Distinct multiplicative static allometricc models emerged at each age group: lean body mass by anthropometry (k = 1.04) and PH development (PH3 *vs*. PH1; b = 0.07) in players aged 8–12 years (75% of explained variance); advanced pubertal maturity (PH4 *vs*. PH3; b = 0.06) and lean lower limbs mass (k = 0.66) explained 41% of variance in VO_{2peak} in players aged 13–15 years; finally, among older soccer aged 16–18 years, body mass (k = 0.59) and pubertal maturity status (PH5 *vs*. PH4; b = 0.04) explained 36% of variance in VO_{2peak}.

Table 6.3. Significant multiplicative allometric models for scaling VO_{2peak}.

Age Group	Model	Predictors	k (95% CI)	R	Adj. R^2	SEE
8 – 12 years						
	Model 1 †	ln LBM_{ANTH}	1.04 (0.71 to 1.37)	0.87	0.75	0.14
		PH3 *vs*. PH1	0.07 (-0.07 to 0.21)			
13 – 15 years						
	Model 2 ‡	ln $LLLM_{DXA}$	0.66 (0.32 to 1.01)	0.65	0.41	0.11
		PH4 *vs*. PH3	0.06 (-0.04 to 0.15)			
16 – 18 years						
	Model 3 §	ln body mass	0.59 (0.27 to 0.90)	0.60	0.36	0.09
		PH5 *vs*. PH4	0.04 (-0.04 to 0.12)			

LBM, lean body mass; LLLM, lean lower limbs mass; ANTH, Anthropometry; DXA, dual x-ray absorptiometry; PH, pubic hair development.
† $\ln (VO_{2peak}) = k_1 \cdot \ln(\text{stature}) + k_2 \cdot \ln(LBM_{ANTH}) + a + b \cdot$ PH stage (dummy coded) $+ \ln \varepsilon$;
‡ $\ln (VO_{2peak}) = k_1 \cdot \ln(\text{stature}) + k_2 \cdot \ln(LLLM_{DXA}) + a + b \cdot$ PH stage (dummy coded) $+ \ln \varepsilon$;
§ $\ln (VO_{2peak}) = k_1 \cdot \ln(\text{stature}) + k_2 \cdot \ln(\text{body mass}) + a + b \cdot$ PH stage (dummy coded) $+ \ln \varepsilon$.

Figure 6.2 corresponds to the estimated curve for VO_{2peak} obtained from the antilog function of the allometric models that produced the best statistical fit for each age group. Predicted VO_{2peak} increased substantially from 8–12 to 13–15 years (on average: 1.13 L·min^{-1}, 52.3%), and then increments were more modest (from 13–15 to 16–18 years, on average: 0.51 L·min^{-1}, 15.5%).

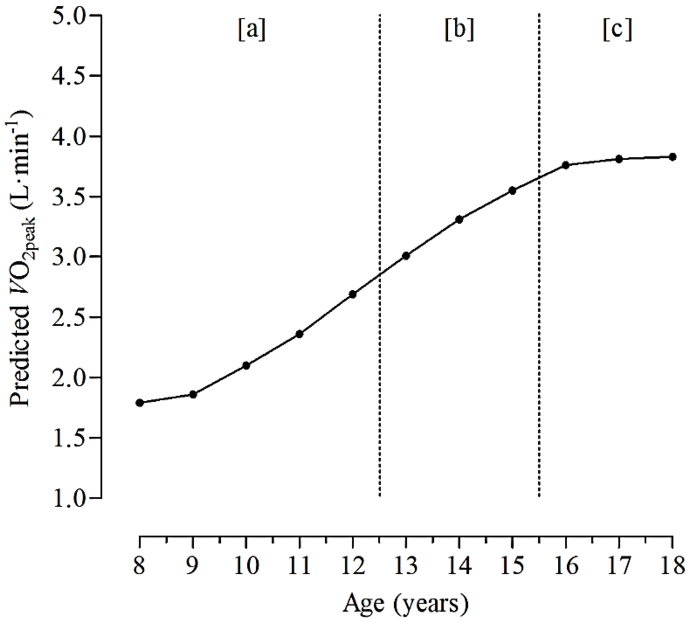

Figure 6.3. Predicted VO_{2peak} (L·min^{-1}) of soccer players by chronological age. Data are predicted from the static allometric models in Table 6.2 and 6.3: [a] ln (VO_{2peak}) = 1.04 · ln (LBM_{ANTH}) – 2.80 + 0.07 · PH stage (PH3 *vs*. PH1); [b] ln (VO_{2peak}) = – 2.39 + 0.93 · ln (LBM_{ANTH}); and, [c] ln (VO_{2peak}) = 0.59 · ln (body mass) – 1.12 + 0.04 · PH stage (PH5 *vs*. PH4).

6.5. Discussion

The present study examined the age-associated variation in VO_{2peak} using static allometric modelling in male soccer players aged 8-18 years. Results were consistent with previous studies in young people that evidenced determinants of aerobic fitness: e.g., stature (Nevill, 1994; Valente-dos-Santos et al., 2013), total body mass, appendicular mass and composition (Chamari et al., 2005; Cunha et al., 2011; Nevill et al., 2004) and pubertal status (Armstrong et al., 1999; Baxter-Jones et al., 1993). The non-linear allometric modelling procedures used in this study substantially accounted for inter-individual differences across ages (i.e., 8–12, 13–15 and 16–18 years). Therefore, the power functions (Nevill et al., 1992), can be used to derive VO_{2peak} 'size free scores' for each of the adopted size and composition variables in youth soccer players. This was also confirmed by homoscedasticity of the data (i.e., the null correlation between the absolute residuals of the power function models and their respective body dimension variables – see Figure 6.2).

Growth, pubertal status and body size of young soccer players

Physical performances that require movement of body mass through space will benefit individuals who have greater stature-to-mass ratios (Nevill et al., 2009). Soccer players of the current study had mean statures and mean body masses between the 50^{th} and 75^{th} percentiles (Kuczmarski et al., 2002). Despite the trend for elevated mass-for-stature [73^{th} *vs*. 64^{th} (8–12 years), 72^{th} *vs*. 69^{th} (13–15 years) and 60^{th} *vs*. 51^{th} percentiles (16–18 years), respectively], mean BMIz was within the normal range for boys of the same age (– 1.0 < BMIz < + 1.0). BMI-for-age (8–18 years) ranged from the 12^{th} to 97^{th} percentiles, but exceeded the ≥ 95^{th} percentile in only two players in the youngest group (22±5% of fat mass). This was consistent with observations for other samples of youth soccer players (Malina et al., 2013).

Inter-individual variability in body size and composition is substantial during puberty (Malina et al., 2004). The literature also suggests that individuals with greater fat mass-for-stature also have greater lean body mass-for-stature (Chinali et al., 2006). The elevated mass-for-stature likely reflects the advanced maturity status of the players (consistent with other data for youth soccer players, Malina, 2011; Malina et al., 2013) and perhaps the influence of systematic training on lean body mass (Malina et al., 2004). About 66% of the athletes were in PH4 and PH5 (33 and 20, respectively). In addition, 16% of players were in PH3 (n = 13) and 18% in PH2 and PH1 (respectively 9 and 6). This distribution by pubertal status confirms the trend for the adolescent soccer players for being advanced in pubertal which likely reflects preferential selection of early maturers (Malina et al., 2013). Stage of pubic air does not provide information on when a player entered that stage or how long he has been in that stage (Sherar et al., 2004). Nevertheless, the trend for pubertal development was consistent with skeletal age of Portuguese players of the same age acting in clubs and regional selections (Coelho-e-Silva et al., 2010) and also with skeletal age data of adolescent soccer players in general (Malina, 2011; Malina et al., 2010).

Whole body and regional size descriptors and static allometric models

Absolute values for VO_{2peak} in the current study were higher than values reported for the general population of the same age (Beunen et al., 2002; Geithner et al., 2004). Mean values of this sample of soccer players were comparable to adolescent male athletes of other team sports (Valente-dos-Santos et al., 2013), but substantially lower than in professional soccer players (Tonnessen et al., 2013) and endurance-athletes in individual sports (Bunc, 2004). Although soccer is not an endurance sport per se, it has been suggested that high values of cardiorespiratory functions are important for players to maintain a high level of activity during an entire match (Castagna et al., 2009; Helgerud et al., 2001; Meckel et al., 2009; Stolen et al., 2005). The estimated curve for VO_{2peak} in the present sample (Figure 6.3) was consistent with corresponding trends in cross-sectional and longitudinal studies (Armstrong and Welsman, 1994; Baxter-Jones et al., 1993; Geithner et al., 2004; Malina et al., 2004; Valente-dos-Santos et al., 2013) and also with the longitudinal development of aerobic performance assessed with the 20-m multi-stage continuous shuttle endurance test in 10 to 18 years male soccer players (Valente-dos-Santos et al., 2012). In this last study, it was possible to produce a multi-level model that predicted aerobic performance of adolescent male soccer players taking into account chronological or skeletal age, body size and training.

The independent variables in the simple static allometric regression models explained, on average, 66%, 38% and 22% of variance in VO_{2peak} in players aged 8-12 years, 13-15 years and 16-18 years, respectively. This decrement in explained variance may be influenced by sport selection that systematically exclude players who are shorter, delayed in terms of maturation and spend less minutes in training and playing sessions. Late adolescence is also a period of decrements in annual changes on stature and body mass. However, stature and body mass are not the only descriptors that should be used in scaling approach of physiological parameters (Nevill et al., 2005).

Theoretical allometric coefficients of $k = 0.66$ or $k \geq 0.75$ for body mass have been proposed as appropriate for the expression of oxygen consumption (e.g., Armstrong and Welsman, 1994; Batterham and Jackson, 2003; Cunha et al., 2011; Kleiber and Rogers, 1961; Nevill et al., 2005; Rowland, 2005; Schmidt-Nielsen, 1975). Studies with children and adolescents have noted coefficients ranging from $k = 0.37$ to $k = 1.17$ (Beunen et al., 2002; Cooper et al., 1984; Cunha et al., 2011; Eisenmann et al., 2001; Rowland, 2005; Sjodin and Svedenhag, 1992; Welsman et al., 1996). Given the substantial variability in individual growth patterns in scaled VO_{2peak}, derivation of a single scaling factor is problematic.

For log transformed VO_{2peak} and body mass, coefficients decreased from $k = 0.98$ at 8–12 years, to $k = 0.86$ at 13–15 years and to $k = 0.51$ at 16–18 years. The coefficients indicate that VO_{2peak} was proportionally higher at 8–15 years and proportionally lower at 16–18 years than theoretically expected from body mass. The trend was consistent with three important concepts. First, the same scaling coefficient may not be appropriate for all ages (Thomis et al., 2000). Second, scaling to either the traditional ratio standard ($mL \cdot kg^{-1} \cdot min^{-1}$) or to one of the theoretical coefficient ($mL \cdot kg^{-0.67} \cdot min^{-1}$ and $mL \cdot kg^{-0.75} \cdot min^{-1}$) may not be valid. And third, allometric coefficients should be developed for each particular sample (Balasekaran et al., 2005; Rowland, 1998).

VO_{2peak} may be confounded by variability in total body composition (Dencker et al., 2011). Thus scaling VO_{2peak} on lean body mass has been suggested as an appropriate method for children and adolescents independent of ethnic ancestry, age, sex or body fat (McMurray et al., 2011). Lean body mass assessed by anthropometry provided the best statistical fit from 8-to 15-years-old (R^2 = 72–52%) than other predictors (i.e., stature, R^2 = 19-60%; body mass, R^2 = 70-43%; lean lower limbs mass, R^2 = 60-38%). The best model was obtained with an anthropometric estimate of lean body mass (Foster et al., 2012), that was noted as a non-biased assessment using DXA protocol as a reference (Bland and Altman, 2012; Holiday et al., 1995; Ludbrook, 2002; Ricker, 1973). By inference, lean body mass derived by anthropometry may be a reasonable and acceptable descriptor of the maximal metabolic capacity of muscles. The contribution of the muscle mass of the exercising lower limbs (Armstrong and Welsman, 2001; Nevill et al., 2004; Winter et al., 1991) and more specifically, the metabolically active tissue (McMurray et al., 2011), was an additional relevant fact supported by the present study. Of relevance to soccer, enlargement of leg muscle mass in professional soccer may increase at a greater rate than that predicted by geometric similarity (1/3 power law) (Nevill et al., 2004). This trend was confirmed in the present results at early (8-12 years) and mid-adolescence (13-15 years) with relationships between VO_{2peak} and appendicular lean mass that overcome the theoretical range (lean lower limbs mass: k = 0.77 and 0.70, respectively), but not, unequivocally, at 16-18 years (k = 0.39; 95% CI 0.10 to 0.69).

Proportional allometric modelling across adolescence

The proportional static allometric models (Beunen et al., 1997; Nevill and Holder, 1994) provided larger explained variance than those models obtained using simple allometric modelling. At 16-18 years the proportional static allometric model emphasizes the interactions between growth, pubertal maturation and systematic training (Nevill et al., 2004; Malina et al., 2004). It has been recently suggested that pubertal maturation has no independent influence on VO_{2peak} (Cunha et al., 2011), but previous studies based on multilevel modelling (Armstrong et al., 1999; Baxter-Jones et al., 1993) noted an independent influence of pubertal maturation on VO_{2peak}. The current study was consistent with previous research on adolescent roller hockey players which used skeletal age as the maturity indicator in an allometric analysis (Valente-dos-Santos et al., 2013). Longitudinal studies adopting skeletal age as a more sensitive indicator than pubertal status are needed. The proportional static allometric models adopted in the present study, provided statistical adjustments to the size descriptors coefficients [8-12 years: k = 1.04 (lean body mass by anthropometry); 13-15 years: k = 0.66 (lean lower limbs mass); 16-18 years: k = 0.59 (body mass)] with the inclusion of the exponential term PH stage [8-12 years: b = 0.07 (PH3 *vs*. PH1); 13-15 years: b = 0.06 (PH4 *vs*. PH3); 16-18 years: b = 0.04 (PH5 *vs*. PH4)].

Allometric scaling of VO_{2peak} showed lean body mass and body mass as preferential size descriptors compared with appendicular lean mass. Body mass, among other factors, is influenced by growth and maturity related changes in size of the lungs, heart and skeletal muscle (Rowland, 2005), but lean body mass represents more closely the active metabolically cell mass than body mass (Sheng and Huggins, 1979). Training is associated with adaptive changes in cardiac structure (Giada et al., 1998). Moreover, heart size is positively correlated with aerobic fitness, cardiac output and stroke volume in childhood, adolescence and adulthood (Osborne et al., 1992). Longitudinal studies spanning childhood and adolescence are needed to further understand curve trajectories of VO_{2peak} with a focus on intra-individual or ontogenetic allometric coefficients (k') derived multilevel multiplicative models that may be more appropriate for the analysis of VO_{2peak} during adolescence.

Although the current investigation has a number of strengths (e.g., objective measures, analytical procedures), it is not without limitations. Specifically, DXA has limitations related to costs and time and, consequently, non-invasive assessments can be used to determine lean mass in youth soccer players. Future studies should consider the development and cross-validation of anthropometric equations for estimating regional size descriptors such as lean lower limbs mass using DXA assessments as a reference in trained athletes of different ages. This approach has the potential to provide reliable, inexpensive and useful information. The current study used a non-invasive equation to determine lean body mass that produced a promising size descriptor for allometric scaling.

6.6. Conclusions

This study examined the relationship between VO_{2peak} and body size descriptors, estimates of total and regional lean mass and pubertal status in a sample of soccer players aged 8-18 years. The results confirmed the limitations of expressing VO_{2peak} per unit of body mass and suggested scaling coefficients and allometric models to interpret aerobic fitness in relation to different size descriptors. Additionally, results confirmed the important relationship between body size descriptors and pubertal maturity status for soccer players at different ages. Proportional, multiplicative allometric models are recommended for scaling VO_2 data as they allow a more critical interpretation of inter-individual variability. The approach offers an analytical framework for understanding VO_{2peak} in soccer players from late childhood through adolescence.

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Chapter VII

Study 5

Determination of lower limbs lean soft tissue in circumpubertal boys using anthropometry and biological maturation

7. Determination of lower limbs lean soft tissue in circumpubertal boys using anthropometry and biological maturation

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Journal: Valente-dos-Santos J, et al. 2014. Determination of lower limbs lean soft tissue in circumpubertal boys using anthropometry and biological maturation. PLoS One. (Accepted)

7.1. Abstract

Lean soft tissue (LST), a surrogate of skeletal muscle mass, is essentially found in appendicular body regions. Simple and accurate methods to estimate lower limbs LST are often claimed for partitioning out the influence of body size on performance outputs. The aim of current study was to derive and cross-validate a new model to predict lower limbs LST in healthy school boys aged 10-13 years, using dual-energy X-ray absorptiometry (DXA) as the reference method. Total body and segmental (lower limbs) composition were assessed with a Hologic Explorer-W QDR DXA scanner in a cross-sectional sample of 75 Portuguese boys (144.8±6.4 cm; 40.2±9.0 kg). Skinfolds were measured at the anterior (21±8 mm) and posterior mid-thigh (18±8 mm), and mid-calf (14±6 mm). Circumferences were measured at the proximal (46.8±6.2 cm), mid (41.3±5.4 cm) and distal thigh (34.3±4.4 cm). Leg length (69.6±4.2 cm) was estimated as stature minus sitting stature. Current stature expressed as a percentage of attained predicted mature stature (PMS, 81.5±2.3%) was used as an indicator of biological maturation. Backward proportional allometric models were used to identify the model with the best statistical fit: ln (lower limbs LST) = 0.838 · ln (body mass) + 0.476 · ln (leg length) – 0.135 · ln (mid-thigh circumference) – 0.053 · ln (anterior mid-thigh skinfold) – 0.098 · ln (mid-calf skinfold) – 2.680 + 0.010 · (percentage of attained PMS) (R = 0.95). The developed equation was cross-validated using the predicted residuals sum of squares statistics (PRESS) method (R^2_{PRESS} = 0.90). Deming repression analysis between predicted and measured lower limbs LST showed an standard error of estimation of 0.52 kg (95% limits of agreement: 0.77 to -1.27 kg). The new model accurately predicts lower limbs LST in circumpubertal males.

Keywords: Skeletal Muscle Mass · Growth · Biological Maturation · Dual-Energy X-Ray Absorptiometry · Deming Regression

7.2. Introduction

Muscle mass in the lower extremities is well correlated with a variety of performance measures in children and adolescents (Davies et al., 1972; Docherty et al., 1991; Tolfrey et al., 2006; Valente-dos-Santos et al., 2013; Van Praagh et al., 1990; Welsman et al., 1997). In heterogeneous samples of boys and men, it has been demonstrated that estimates of lower leg muscle (Tolfrey et al., 2006) or thigh muscle volume (Welsman et al., 1997) are more valid allometric scaling denominators than either body mass or fat-free mass for properly partitioning out the influence of body size on peak oxygen uptake. Early dissection studies indicate that the muscles in the lower extremities increase their relative contribution from about 40% at birth to 55% of the total weight of the musculature at maturity (Scammon, 1923). Other studies of regional variation in skeletal muscle tissue are based on radiography and are limited largely to the arm and calf (Malina, 1996). During adolescence, males gain about 30% in calf muscle mass width (Malina, 1996) but more recent imaging techniques have not yet been used systematically to address this issue in children and adolescents.

Multi-scan magnetic resonance imaging (MRI) and computerized axial tomography (CT) are considered the “criterion” methods for evaluating skeletal muscle mass (Lee and Gallagher, 2008). MRI is not widely available for use in research and clinical practice and CT method comprises exposure to radiation which limits its application in children and adolescents (Lee et al., 2000; Shih et al., 2000). Other approaches have been developed to evaluate skeletal muscle mass, including laboratory methods (Wang et al., 2003), bioelectrical impedance (Salinari et al., 2002) and air displacement plethysmography analysis (Dempster and Aitkens, 1995). Densitometric techniques rely on specific assumptions concerning fat-free mass density (1.1 g/mL), assumed to be stable for adults but may substantially vary in children of different maturity status, gender, and ethnicity (Lohman, 1986; Wells et al., 1999). From birth to 22 years of age, the density of the fat-free mass increases from 1.063 to 1.102 g/mL in boys (Fidanza et al., 1953). Dual-energy X-ray absorptiometry (DXA) is a non-invasive protocol and implies a minimal radiation dose (1 mSv or 1/100th of the equivalent radiation exposure of a chest x-ray) (Goran, 1998). It allows measurements of both regional and total lean soft tissue (LST) (Ellis, 2000; Kulkarni et al., 2013; Leahy et al., 2012; Quiterio et al., 2009); a surrogate of skeletal muscle mass (Kim et al., 2006). Moreover, DXA-determined appendicular LST have been previously validated against criterion methods, including four-compartment body composition models and CT (Visser et al., 1999; Wang et al., 1996).

The availability of DXA in field settings is limited. Therefore, estimates of LST in youth based on anthropometry are required. A popular anthropometric method (Jones and Pearson, 1969) characterizes the lower limb as the sum of six truncated cones based on lengths, circumferences and skinfold thickness. The method was originally developed with a sample of 32 male and 15 female young adults and has been applied in children and adolescents (Dore et al., 2001; Martin et al., 2004; Valente-dos-Santos et al., 2013). Recently, an attempt to cross-validate the regression equation of Jones and Pearson (1969) in 83 girls and 85 boys (11.0±0.7 years of age) was not successful (Coelho-e-Silva et al., 2013). Thigh volume by DXA (4.43±1.23 L) was significantly higher than the anthropometric estimate (4.39±1.22 L). Addition of body mass and the sum of anterior and posterior thigh skinfolds to the model resulted in a new predictive equation (R^2 = 0.95). Anthropometric models for appendicular LST (upper and lower limbs combined) in young athletes of both sexes have also been developed and cross-validated (Quitério et al., 2009). The available research has not systematically considered the potential contribution of maturation to explain inter-individual variability in total and lean soft tissues. Correlations between skeletal age and soft tissue components were noted to increase from childhood (r = 0.13 – 0.16) to adolescence (r = 0.30 – 0.51) in boys (Malina et al., 2004). The changes in correlations across chronological age (CA) groups suggest that differences between children of contrasting skeletal maturity status become more pronounced with age. Allowing the limited data on youth, the aim of the present study was to develop a non-invasive estimate of lower limbs LST in circumpubertal males and to cross-validate the new model using DXA measures as the reference method.

7.3. Methods

Ethics statement

The study was approved by the Portuguese Foundation for Science and Technology [PTDC/DTP-DES/1178/2012] and by the scientific board of the Faculty of Human Kinetics at the Technical University of Lisbon. The study was conducted in accordance with the Declaration of Helsinki for human studies by the World Medical Association (2008). All participants and parents/legal guardians were informed about the objectives of the study and provided appropriate informed assent and written informed parental consent, respectively.

Participants

Participants were recruited voluntarily from the school population in the Lisbon metropolitan area (Portugal). The sample comprised 75 healthy boys of European ancestry, 10-13 years of age.

Age and anthropometry

Chronological age was calculated as the difference between date of birth and date of measurement. Anthropometry was performed by a single experienced observer following standard procedures (Lohman et al., 1988). Stature and sitting stature was measured to the nearest 0.1 cm with a Harpenden stadiometer (model 98.603, Holtain Ltd, Crosswell, UK and Harpenden sitting height table, model 98.607, Holtain Ltd, Crosswell, UK, respectively) and body mass was measured to the nearest 0.1 kg using an electronic scale (Tanita, BC-418, MA, USA). Leg length was estimated as stature minus sitting stature. Circumference at the gluteal furrow (highest possible horizontal circumference), mid-thigh (largest mid-thigh circumference) and distal thigh (minimum circumference above the knee) were measured in the orthogonal plane, on the right site of the body. Mid-thigh circumference ($C_{m\text{-}t}$) was corrected for subcutaneous adipose tissue thickness, according to Lee et al. (2000). The corrected mid-thigh circumference ($CC_{m\text{-}t}$) was calculated as $CC_{m\text{-}t} = C_{m\text{-}t} - \pi S$, where S stands for the skinfold measurement, which is assumed to be twice the subcutaneous adipose tissue thickness. Lengths between each circumference level were also measured to estimate total thigh length. Anterior and posterior mid-thigh and mid-calf skinfolds were measured to the nearest mm using a Lange Caliper (Beta Technology, Ann Arbor, MI, USA). Technical errors of measurement were previously reported for the anthropometric dimensions (Coelho-e-Silva et al., 2013) and all were well within the range of several health surveys in the United States and a variety of field surveys (Malina, 1995).

Somatic maturation

Chronological age, stature, and body mass of each boy and midparent stature were used to predict mature (adult) stature using the Khamis–Roche protocol (Khamis and Roche, 1994). The median error bound (median absolute deviation) between actual and predicted mature stature (PMS) at 18 years of age is 2.2 cm in males (Khamis and Roche, 1994). Percentage of PMS attained at a given age is positively related to skeletal and sexual maturation in boys (Bielicki et al., 1984). Percentage of attained PMS is also related to skeletal age in youth football (American) and soccer players of the same age range as the study sample (Malina et al., 2012; Malina et al., 2007). Finally, percentage of attained PMS expressed as a *z*-score relative to age-specific (half-year intervals) means and standard deviations from the Berkeley Guidance Study (Bayley and Pinneau, 1952) was the indicator of maturity status.

Dual energy X-ray absorptiometry

A Hologic Explorer-W, fan-bean densitometer, software QDR for Windows version 12.4 (Hologic, Waltham, MA, USA) was used to perform total body scans. The procedure allows measurement of segmental composition: arm, leg and trunk. Daily calibration of the scanner was performed using a phantom spine containing composites of bone, fat and lean tissue. Participants were positioned on the scanner bed according to manufacturer recommendations. The same lab technician positioned the participants, performed the scans and executed the analyses according to the operator's manual. The lower limbs on each image were sectioned as follows: all tissue distal to a line drawn through and perpendicular to the axis of the femoral neck and angled with the pelvic brim to the phalange tips. Total lower limb composition was estimated by adding the mass of the left and right legs. Mean variation between measured and reconstructed absolute whole-body mass with DXA software was 0.9%. Based on test-retest in 10 individuals, the coefficients of variation for percent body fat and LST were 1.6% and 0.8%, respectively.

Statistical analyses

Statistical analyses were performed using IBM SPSS version 19.0 software (SPSS, Inc., IBM Company; NY, USA), GraphPad Prism version 5.03 software (GraphPad Software, Inc.; La Jolla, CA, USA) and MedCalc version 12.2.1 software (MedCalc; Mariakerke, Belgium). Alpha level was set at 0.05.

Visual inspection of data was made using stem-and-leaf diagram and box-and-whisker plots to examine central tendency, variability and distributional form, as well as pointing to potential outliers. Afterwards, Gaussian distribution of variables was confirmed with normal Q-Q plots, de-trended normal Q-Q plots and Kolmogorov-Smirnov (*K-S*) test with Lilliefors significance correction. Descriptive statistics were calculated for the total sample (mean, standard deviation and range).

A backward multiple linear regression on ln *y*, based on proportional allometric models (Nevill and Holder, 1994; Valente-dos-Santos et al., 2013), was used to fit the unknown parameters that best predict lower limb LST determined with DXA. Based on analytical [i.e., Pearson's product moment correlation coefficients ($r_{y,x}$) between lower limbs LST_{DXA} (*y*) with age, maturation/maturity status and body descriptors (*x*)] and biological assumptions, the new model (NM) considered CA, CA^2, percentage of attained PMS or z-score for age (% attained PMS) as exponential terms in addition to body mass, leg length, $C_{m\text{-}t}$, stature × $CC_{m\text{-}t}^2$, anterior and posterior mid-thigh and mid-calf skinfolds:

$$\ln(\text{lower limbs LST}) = k_1 \cdot \ln(\text{body mass}) + \ldots k_7 \cdot \ln(\text{mid-calf skinfold}) + a + b_1 \cdot (\text{CA}) + b_2 \cdot (\text{CA}^2) + b_3 \cdot (\%\ \text{attainded PMS}) + \ln \varepsilon \qquad \textbf{(7.1)}$$

A tolerance > 0.10 and a variance inflation factor < 10 was set to avoid collinearity between the explanatory variables (Slinker and Glantz, 1985).

Internal cross-validation of the model was performed using the predicted residuals sum of squares (PRESS) statistics method (Holiday et al., 1995). PRESS statistics were obtained by fitting the proposed equations with the deletion of an observation for obtaining the predicted value of the deleted observation. Residuals for each predicted value were then calculated. The PRESS statistic is a function of the sum of squares (SS) of all residuals. The PRESS statistic is always higher than the error (i.e., SS) from MANOVA results. Alternative measures of model adequacy (R^2_{PRESS}) and standard error of estimation ($\text{SEE}_{\text{PRESS}}$) were also calculated (Holiday et al., 1995).

Agreement between the reference method (lower limbs LST_{DXA}) and the estimation (lower limbs LST_{NM}) was assessed using Deming regression (i.e., least products regression method; accounts the error of both x and y variables) and standard error of estimation ($S_{y \cdot x}$) (Ricker, 1973). Data were then visually inspected by plotting residuals (prediction errors) against predicted values. This plot is similar to the plot suggested by Bland and Altman (2012), except that predicted values of lower limbs LST (using the new developed equation) are plotted on the x-axis rather than the average of predicted and measures of lower limbs LST (Quiterio et al., 2009). If errors were associated with the magnitude of the values, heteroscedasticity was examined by calculating the correlation coefficients between the absolute differences and the predicted values of lower limbs LST. Constant error variance (homoscedasticity) can be assumed if the correlations approach zero (Batterham et al., 1997; Nevill et al., 1992). Correlation coefficients were interpreted as follows: trivial ($r < 0.1$), small ($0.1 < r < 0.3$) moderate ($0.3 < r < 0.5$), large ($0.5 < r < 0.7$), very large ($0.7 < r < 0.9$), nearly perfect ($r > 0.9$) and perfect ($r = 1$) (Hopkins et al., 2009). Finally, if measurement differences were normally distributed, reliability was assessed on the original scale using the standard error of measurement and 95% limits of agreement (Bland and Altman, 2012).

7.4. Results

Chronological age, somatic maturation and composition for the total sample are described in Table 7.1 and 7.2. All variables fitted a normal distribution (*K-S* = 0.37 to 1.18; $p > 0.05$). Mean percentage of attained PMS (81.5 ± 2.3 %) for the present sample was equivalent of that of the sample on which the physical maturity-prediction protocol was based (81.6±1.2 % at 11.0 years) (Roche et al., 1983). The means and standard deviations for percent of total body fat_{DXA}, LST_{DXA}, lower limbs fat $percentage_{DXA}$ and lower limbs LST_{DXA} were 26.4 (± 8.1 %), 29.0 (± 4.2 kg), 31.7 (± 8.3 %) and 10.0 (± 1.7 kg), respectively.

Table 7.1. Descriptive statistics (*n* = 75) for chronological age and somatic maturation, and results of the Kolmogorov–Smirnov test for checking the normality of the distribution.

	Mean ± SD	Range (min – max)	Kolmogorov–Smirnov	
			Value	*P*
Chronological age (years)	11.12 ± 0.69	10.27 to 13.08	1.18	0.12
Percentage of attained PMS (%)	81.5 ± 2.3	77.3 to 90.3	0.70	0.72
z-score for age (% attained PMS)	0.04 ± 0.92	-2.07 to 2.40	0.74	0.65

Abbreviations: PMS, predicted mature stature.

Table 7.2. Descriptive statistics (n = 75) for body composition and results of the Kolmogorov–Smirnov test for checking the normality of the distribution.

	Mean ± SD	Range (min – max)	Kolmogorov–Smirnov	
			Value	p
DXA total body				
BMD ($g\cdot cm^{-2}$)	0.84 ± 0.06	0.69 to 0.96	0.37	0.99
BMC (kg)	1.22 ± 0.17	0.86 to 1.66	0.55	0.93
Fat (%)	26.4 ± 8.1	14.0 to 44.6	0.77	0.60
Fat (kg)	11.2 ± 5.8	3.6 to 27.6	1.03	0.24
Lean soft tissue (kg)	29.0 ± 4.2	20.7 to 39.8	0.73	0.66
DXA lower limbs				
BMD ($g\cdot cm^{-2}$)	0.84 ± 0.07	0.68 to 1.04	0.51	0.96
BMC (kg)	0.44 ± 0.08	0.30 to 0.68	0.87	0.43
Fat (%)	31.7 ± 8.3	16.0 to 50.2	0.56	0.92
Fat (kg)	4.9 ± 2.3	1.3 to 10.9	1.16	0.13
Lean soft tissue (kg)	10.0 ± 1.7	6.9 to 13.9	0.74	0.64

Abbreviations: DXA, dual x-ray absorptiometry; BMD, bone mineral density; BMC, Bone mineral content.

Relationships between ln lower limbs LST_{DXA} and the chronovariables [i.e., CA, percent of attained PMS and z-score for age (% attained PMS)] are illustrated in panels a through c of Figure 7.1. Linear relationships were observed between CA and lower limbs LST_{DXA}. Non-linear relationships were apparent between percent of attained PMS and z-score for age (% attained PMS) with lower limbs LST_{DXA}. Correlations between the preceding variables and LVM ranged from 0.30 and 0.71 (p < 0.01).

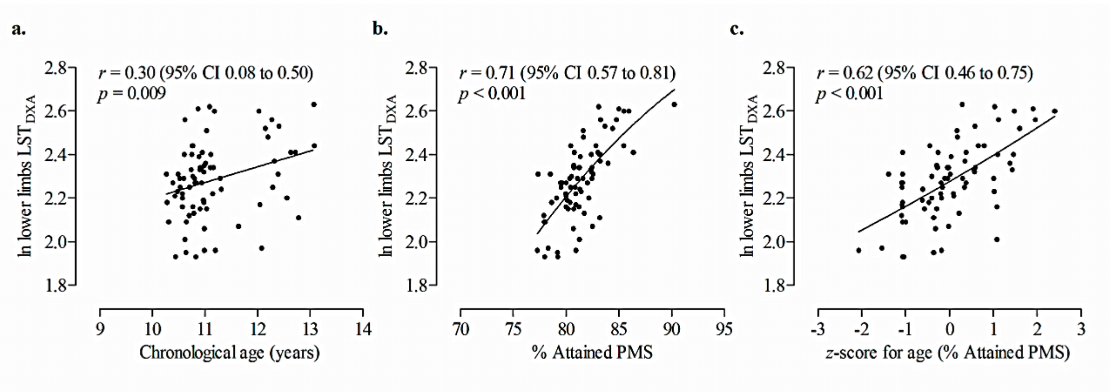

Figure 7.1. Relationship of the ln transformed lower limbs lean soft tissue (LST_{DXA}) with chronological age (panel a), percent of attained predicted mature stature (% Attained PMS, panel b), and z-score for age of the percent of attained PMS (panel c).

Anthropometric characteristics for the total sample, results of the Kolmogorov–Smirnov test, and bivariate correlations of anthropometry with the chronovariables are shown in Table 7.3. Boys of the current study had mean statures and mean body masses correspondent to the 56^{th} and 60^{th} age specific percentiles, respectively (Kuczmarski et al., 2002). Correlations between the chronovariables and anthropometry ranged from trivial (r = 0.01) to very large (r = 0.73), suggesting the possibility of collinearity occurrence between some exponential terms and body size descriptors.

Table 7.3. Descriptive statistics (n = 75) for anthropometry, results of the Kolmogorov–Smirnov test for checking the normality of the distribution, and bivariate correlations of anthropometry with chronological age and maturation.

	Mean ± SD	Range (min – max)	Kolmogorov–Smirnov		Correlation					
					Chronological age		% Attained PMS		*z*-score for age (% Attained PMS)	
			Value	*p*	*r*	95% CI	*r*	95% CI	*r*	95% CI
Stature (cm)	144.8 ± 6.4	127.9 to 163.5	0.52	0.95	0.36	0.15 to 0.55	0.73	0.60 to 0.82	0.58	0.41 to 0.71
Body mass (kg)	40.2 ± 9.0	24.4 to 66.5	1.17	0.13	0.15	-0.01 to 0.37	0.64	0.48 to 0.76	0.71	0.57 to 0.81
Lengths (cm)										
Leg	69.6 ± 4.2	60.2 to 78.5	0.43	0.99	0.34	0.13 to 0.53	0.63	0.47 to 0.75	0.49	0.30 to 0.65
Thigh	27.8 ± 3.9	17.3 to 36.2	0.58	0.89	0.12	-0.11 to 0.34	0.15	-0.08 to 0.36	0.06	-0.17 to 0.29
Circumferences (cm)										
Proximal thigh	46.8 ± 6.2	36.5 to 66.7	0.97	0.30	0.05	-0.18 to 0.27	0.54	0.35 to 0.68	0.68	0.54 to 0.79
Mid-thigh	41.3 ± 5.4	26.0 to 54.0	0.85	0.47	0.16	-0.08 to 0.37	0.52	0.33 to 0.67	0.54	0.35 to 0.68
Distal thigh	34.3 ± 4.4	27.5 to 47.0	1.19	0.12	0.01	-0.22 to 0.23	0.47	0.27 to 0.63	0.64	0.49 to 0.76
Skinfold thickness (mm)										
Anterior mid-thigh	21 ± 8	7 to 42	0.85	0.47	-0.19	-0.40 to 0.04	0.21	-0.02 to 0.41	0.51	0.32 to 0.66
Posterior mid-thigh	18 ± 8	6 to 37	1.09	0.19	-0.07	-0.30 to 0.16	0.31	0.09 to 0.50	0.53	0.34 to 0.68
Mid-calf	14 ± 6	4 to 28	1.11	0.17	-0.04	-0.27 to 0.19	0.41	0.20 to 0.58	0.62	0.46 to 0.74

Abbreviations: PMS, predicted mature stature; *r*, Pearson's product moment correlation coefficient; 95% CI, 95% confidence interval.

The allometric regression model for the prediction of lower limbs LST is given in Table 7.4. The NM included ln body mass, ln leg length, ln mid-thigh circumference, ln anterior mid-thigh skinfold, ln mid-calf skinfold and percent of attained PMS. Its association with lower limbs LST_{DXA} was nearly perfect (R = 0.95). The prediction equation was:

$$\ln(\text{lower limbs } LST_{DXA}) = 0.838 \cdot \ln(\text{body mass}) + 0.476 \cdot \ln(\text{leg length}) - 0.135 \cdot \ln(\text{mid-thigh circumference}) - 0.053 \cdot \ln(\text{anterior mid-thigh skinfold}) - 0.098 \cdot \ln(\text{mid-calf skinfold}) - 2.680 + 0.010 \cdot (\%\text{ attainded PMS}) \quad \textbf{(7.2)}$$

Equation 7.2 (new model) was validated using PRESS statistics method (Table 7.4). The excellent accuracy of the model is illustrated by the high R^2 and low SEE observed after using the PRESS statistics (R^2 = 0.90 and SEE = 0.27).

Table 7.4. Proportional multiplicative allometric regression model and internal cross-validation for the prediction of DXA-measured lower limbs lean soft tissue.

	Predictors			Model summary			
						Cross-validation	
Y	x_i	β Unstandardized	95% CI	*R*	SEE	R^2_{PRESS}	SEE_{PRESS}
ln (lower limbs LST_{DXA})	Constant	-2.680	-4.308 to -1.052				
	ln (body mass)	0.838	0.616 to 1.061				
	ln (leg length)	0.476	0.067 to 0.884				
	ln (mid-thigh circumference)	-0.135	-0.412 to 0.143				
	ln (anterior mid-thigh skinfold)	-0.053	-0.129 to 0.024				
	ln (mid-calf skinfold)	-0.098	-0.176 to 0.020				
	% attained PMS	0.010	-0.002 to 0.021				
				0.95	0.07	0.90	0.27

Abbreviations: 95% CI, 95% confidence intervals; VIF, variance inflation factors; LST, lean soft tissue; DXA, dual x-ray absorptiometry; % attained PMS, percentage of attained predicted mature stature.

Deming repression analysis between predicted and measured lower limbs LST showed that the NM had an R^2 = 0.91 and a $S_{y \cdot x}$ of 0.52 kg (Figure 7.2, panel a). The intercept (-0.62, 95% CI -1.40 to 0.17) and slope (1.04, 95% CI 0.96 to 1.11), of the equation, did not differ significantly (p > 0.05) from the identity line (i.e., y-intercept = 0 when x = 0 and slope = 1), rejecting the possibility of systematic or proportional bias, respectively (Ludbrook, 2002). The relation between residuals and predicted lower limbs LST values is illustrated in Figure 7.2, panel b. No significant mean differences between predicted and measured values and small limits of agreement were found (bias ± 1.96 SD = -0.25 ± 1.02 kg, p > 0.05). Also, the measurement differences (error) against the predicted lower limbs LST were homoscedastic (Batterham et al., 1997; Nevill et al., 1992). This was confirmed by the correlation coefficient (r = 0.09, p = 0.398), between the absolute differences and the predicted lower limbs LST values (Nevill and Atkinson, 1997).

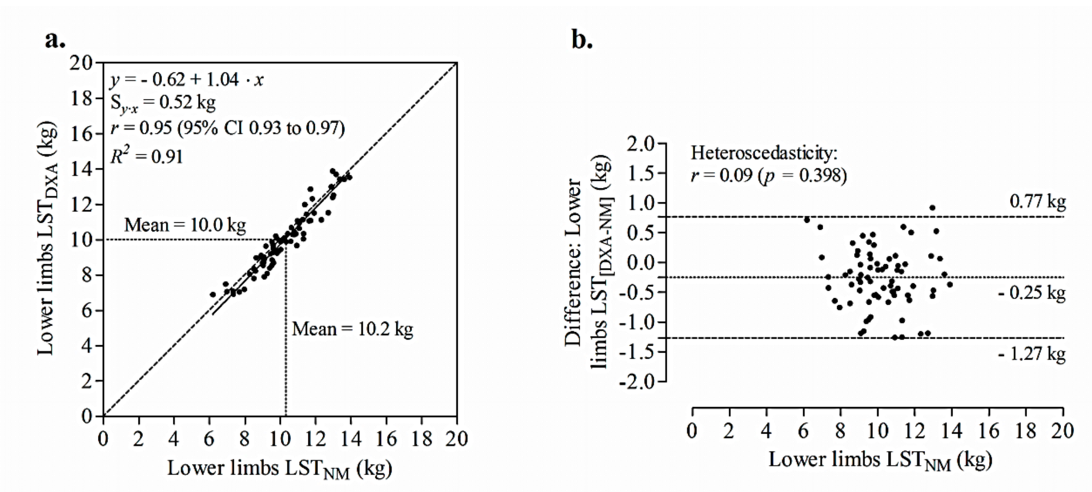

Figure 7.2. Deming regression analysis between the new anthropometric model (NM) and lower limbs lean soft tissue (LST) measured by dual-energy X-ray absorptiometry (panel a). The reference line from the equation, standard error of estimation ($S_{y \cdot x}$), correlation (r) and coefficient of determination (R^2), are also presented. The right panel (panel b) illustrate the relation between residuals (mean differences between lower limbs LST measured by DXA and predicted by the derived equation) and lower limbs LST_{NM} (heteroscedasticity diagnostic). The dashed lines represent 95% limits of agreement (± 1.96 SD).

7.5. Discussion

The data from this study detail total body and segmental (lower limbs) body composition of a population of healthy young boys. The aims of the present investigation were to develop and cross-validate an anthropometric and maturation-based model to predict lower limbs LST based on DXA as the reference method. Allometric modelling identified biological maturation as a significant predictor of lower limbs LST. Anterior mid-thigh and mid-calf skinfolds, mid-thigh circumference, leg length and body mass were also identified as significant predictors of lower limbs LST. The new model showed to be valid, non-biased and to accurately predict lower limbs LST; (*i*) a nearly perfect correlation was obtained between methods, (*ii*) the intercept and slope of the equation did no differ from the identity line, (*iii*) small limits of agreement were found and (*iv*) errors were not associated with the magnitude of the values. In addition, the equation was found to have high values of R^2_{PRESS} and low SEE_{PRESS}. To our knowledge, this is the first study that obtained and cross-validated an equation derived from anthropometry and somatic maturation to estimate lower limbs LST.

Appendicular skeletal muscle mass can be obtained from DXA (Ellis, 2000; Kulkarni et al., 2013; Leahy et al., 2012; Quiterio et al., 2009), by assuming that all non-bone and non-fat tissue is muscle mass (Heymsfield et al., 1990). This assumption is likely to be valid in the legs (Heymsfield et al., 1997; Snyder et al., 1975) and is most likely to be valid at the regions between the joints such as the mid-thigh and the calf, where the amount of tendons and cartilage is small (Visser et al., 1999). Moreover, nearly perfect correlations (r = 0.98–0.99) were found between appendicular LST assessed by DXA and skeletal muscle mass determined by MRI in multi-ethnic adolescents and children, endorsing these assumptions (Kim et al., 2006). Data specifically reporting estimates of tissue composition of the lower limbs in children and adolescents are lacking. Total body LST of the present sample was considerably less (29.0±4.2 kg) than that of young males aged 13.7±3.0 years (38.1±12.7 kg) (Machado et al., 2013), although this may be a consequence of smaller body size (stature: 144.8±6.4 kg *vs.* 158.1±17.7 cm; body mass: 40.2±9.0 kg *vs.* 50.2±17.4 kg, respectively). Despite a slightly trend for elevated mass-for-stature (56^{th} and 60^{th} age specific percentiles, respectively), the characteristics of this sample of healthy boys were consistent with other reports and reference values of comparable samples (Kuczmarski et al., 2002; Malina et al., 2004).

DXA measurements of LST, body fat and bone mineral content were made independently, producing direct sources of measurement error; this is rejected in the difference between DXA and scale mass. In the present sample the error absolute whole-body mass amounted to 0.4 kg (0.9%). Although hydration was not controlled, minimal effects are expected since, for example, it has been shown that a change of 1 kg in extracellular fluid induces an error in fat measurement of only 0.6% using DXA (Lohman and Going, 1998). Errors in the lower limbs LST prediction equation may be attributed in part to the use of skinfold measures as predictor variables. Holmes et al. (2005) stated that the use of skinfold thickness measurements to estimate human body composition is based on two assumptions: that the thickness of the subcutaneous adipose tissue reflects a constant proportion of total body fat, and that the sites selected for measurement represent the average thickness of the subcutaneous adipose. Neither assumption has been proven to be consistently generalized (Lukaski, 1987). Again, this was not believed to be a major limitation since anthropometry was performed by a single experienced observer following standard procedures (Lohman et al., 1988). Small intra-observer technical errors of measurement and coefficients of variation (CV) for skinfolds measurements were noted: 0.70–0.71 mm (%CV = 3.73–4.68) (Coelho-e-Silva et al., 2013).

There is no single definitive measure of leg length. Protocols derived from anthropometric methods like Jones and Pearson (1969), measured leg as the length from the ground to the gluteal furrow. In the present study, leg length corresponded to the difference between stature and sitting stature, measured on a specific standardized table. In DXA, the leg length is defined as the mass of tissue distal to an oblique line passing through the neck of the femur, excluding gluteal muscles. Leg circumferences were identified as suitable biological predictor variables and is interesting to note that despite the very large correlation observed between stature × $CC_{m\text{-}t}^{2}$ with lower limbs LST_{DXA} (r = 0.72, p < 0.001), the interaction term was not a significant predictor of lower limbs LST_{NM}. Both Quiterio et al.´s (2009) and Lee et al.'s (Lee and Gallagher, 2008) studies, suggested that the square of each corrected muscle girth multiplied by stature is the anthropometric parameter that most contributed to the appendicular LST and skeletal muscle mass variance. The discrepancy of results and contribution of corrected muscle circumferences and stature (components of the cylinder's dimensions of the skeletal muscle mass of the human body) may be due to the considerably larger appendicular LST of young athletes (Quitério et al., 2009) and non-obese adults (Lee et al., 2000), compared to this circumpubertal sample.

The adolescent growth spurt in stature tends to start at about 10 or 11 years of age, in boys, and reaches peak velocity at about 14 years (Malina et al., 2004). Age at peak height velocity (APHV), predicted from sex-specific anthropometric equations (Mirwald et al., 2002), was a significant predictor of total body LST in the study of Machado et al. (2013) with boys aged 8–18 years of age. However, it is relevant to note that the mentioned method for assessing somatic maturation, among boys seemed to have limited applicability and was recently recommended for the interval between 12 and 15 years of age (Malina and Koziel, 2014). Sexual maturation has been previously considered in a study designed to predict appendicular LST in adolescent athletes aged 14–17 years (Quitério et al., 2009) and skeletal muscle mass in boys aged 5–17 years (Kim et al., 2006), but the indicator failed to enter as a significant predictor.

Recognizing sex differences in the timing and tempo of sexual maturation and the growth spurt, and that components of body composition also differentially change during the period of maximum growth spurt in boys and girls (Malina et al., 2004), a more sensitive indicator than pubertal status is needed. The present study identified a significant and positive contribution of individual differences in somatic maturation (expressed as the percentage between attained current stature and PMS) in the prediction of lower limbs LST. This method is non-invasive. The prediction equation and reference values were based on white middle socio-economic class American youth in the Fels Longitudinal Study (Khamis and Roche, 1994). Validation of the prediction equations in Portuguese youth is needed. Nevertheless, Spearman rank order correlations between somatic maturity status classifications and classifications based on skeletal age were moderate to large; 0.37 in Portuguese soccer players 11-15 years (Malina et al., 2012) and 0.52 in American football players 9-14 years (Malina et al., 2007).

Finally, despite the advancements in technology that provides accurate assessments of segmental tissue mass in vivo, there is still an interest for methods that permit to acquire this information in a safe, cost-effective and non-invasive manner. The present study offers an equation that has a predictive power that compares favorably with those reported in the literature for similar purposes (Coelho-e-Silva et al., 2013; Fuller et al., 2002; Kim et al., 2006; Lee et al., 2000; Machado et al., 2013; Quitério et al., 2009; Welsman et al., 1997). No significant mean differences between measured and predicted lower limbs LST were observed. Also, the robustness of the model was not compromised by multicollinearity between independent variables. Tolerance and a variance inflation factors were well within the normal ranges (>0.10, <10, respectively) (Slinker and Glantz, 1985). The adopted method of cross-validation (Holiday et al., 1995), confirmed the effectiveness of the model to predict lower limbs LST with a high internal validity (R^2_{PRESS} = 0.90) and low proportional errors of estimation ($\text{SEE}_{\text{PRESS}}$ = 0.27). The PRESS method avoids data-splitting difficulties and provides similar unbiased estimates of future prediction equation performance. It was deemed important to maintain an appropriate participant-to-variable ratio. In multiple regressions, that ratio was recommended to be higher than 5:1 and preferably 20:1 (Vincent, 1995). The present ratio was ~ 13:1. Moreover, it is established that usually PRESS statistics generates less confident estimates of an equation's potential (Holiday et al., 1995). The least products regression (Deming regression), where random errors in both dependent and independent variables are accommodated in the regression model (Ricker, 1973), was used as an alternative to the ordinary least squares method. The NM (R^2 = 0.91 and a $S_{y \cdot x}$ of 0.52 kg) did not presented systematic or proportional bias, as the equation did not differ significantly from the identity line (Ludbrook, 2002). Lastly, the measurement differences (error) against the predicted lower limbs LST values were homoscedastic (r = 0.09, p = 0.398) (Batterham et al., 1997; Nevill et al., 1992), allowing the use of this equation with constant error variance.

7.6. Conclusions

In summary, a new model derived from anthropometry and somatic maturation was proposed for assessing lower limbs LST in research dealing with male pediatric samples. The equation was satisfactorily cross-validated at group and individual basis, ensuring its applicability to similar samples. However, it is important to note that when an equation is applied to groups the associated errors will be always smaller than those for individuals. This equation will allow accurate lower limbs-specific mass information, thus offering a valid alternative to scan participants with technologies such as DXA. Note, however, that prediction equations tend to be age, gender and population specific and additional validation studies are needed to critically validate this model in different samples (e.g., females, other ethnic groups, adults or specific populations of people with varying anthropometric characteristics).

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Part III

Longitudinal Studies

Chapter VIII

Study 6

Longitudinal development of left ventricular mass in adolescent boys

8. Longitudinal development of left ventricular mass in adolescent boys

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Journal: Valente-dos-Santos J, et al. 2014. Longitudinal development of left ventricular mass in adolescent boys. Am J Hum Biol.
(Accepted)

8.1. Abstract

Objectives: To estimate inter- and intra-individual influences of stature, body mass, adiposity and biological maturity on the development of left ventricular mass (LVM) in adolescent boys, and to present allometric models for normalizing LVM.

Methods: 87 boys (12-14 years at baseline) were assessed biannually for 2 years. Stature, body mass and triceps, subscapular, suprailiac and medial calf skinfolds were measured. Age at peak height velocity (APHV) was predicted and time relative to APHV was used as an indicator of biological maturity. LVM was obtained from M-mode echocardiograms using two-dimensional images. Inter-individual and intra-individual allometric coefficients were calculated using static, ontogenetic and multilevel models.

Results: At most age categories, inter-individual allometric coefficients for stature and body mass did not exceed k = 2.5 and k = 1.0, respectively. Intra-individual coefficients showed wide variation (k' = 0.80 to 7.84 and k' = 0.23 to 4.68, respectively). The multilevel models with the best statistical fit showed that body size descriptors, age and biological maturity had strong positive effects on LVM. When body mass was statistically controlled (growth of 10 kg of body mass predicted 27 g of LVM) additional negative effects of adiposity were noted.

Conclusions: Individual differences in allometric growth of body size dimensions, adiposity and biological maturation influence growth of LVM among male adolescents 12-15 years.

Keywords: Cardiovascular Growth · Maturation · Scaling · Echocardiography

8.2. Introduction

Left ventricular mass (LVM) is related to cardiovascular health (Gidding et al., 2013; Levy et al., 1990; Liao et al., 1997; Urbina et al., 1995). The Framingham Risk Score (FRS) is a frequently used indicator of risk for cardiovascular disease (CVD) outcomes - cerebrovascular events, peripheral artery disease and heart failure (Wilson et al., 1998). It has been suggested, more recently, that the FRS underestimates risk of CVD events in young adults followed over 20 years, and that echocardiography-derived LVM predict CVD events independent of FRS (Armstrong et al., 2014). LVM had the best performance in normal weight participants, but the 85th percentiles of LVM (men, ≥ 116 $g \cdot m^{-2}$; women, ≥ 96 $g \cdot m^{-2}$) is a more robust predictor of CVD event than currently recommended cut-points for hypertrophy (Armstrong et al., 2014). By inference, accurate quantification of LVM is essential to distinguish disease conditions from normal variation (Dewey et al., 2008; Foster et al., 2013; Khoury et al., 2009).

Although the preceding is derived from adults, it has relevance in discussions of left ventricular growth in children and adolescents (Chinali et al., 2006; Daniels et al., 1995; Foster et al., 2013; Khoury et al., 2009; Urbina et al., 1995). During adolescence body proportions and composition change with growth and with individual differences in biological maturation (Malina et al., 2004). Positive and significant interrelationships among biological maturation, body size, body composition and LVM in boys have recently been documented in a cross-sectional sample of boys 11-15 years of age (Valente-dos-Santos et al., 2014b).

Simple ratio standards have traditionally been used to facilitate the analysis of LVM relative to body size (Devereux et al., 1986; Lauer et al., 1991). However, ratio standards are theoretically fallacious and in practice misleading (Tanner, 1949); as heavier individuals tend to have an arithmetic disadvantage (Malina, 2012). Nevertheless, the most appropriate method to normalize parameters of LVM remains controversial (de Simone et al., 1992; Dewey et al., 2008; Foster et al., 2013). An alternative was suggested by adding residual errors from the fitted least-squares regression line to the group mean (Tanner, 1949). However, body size and organ dimensions are not geometrically similar during the second decade of life and the relationships between body size descriptors and heart parameters are often nonlinear (de Simone et al., 1992; Valente-dos-Santos et al., 2014b; Valente-dos-Santos et al., 2013). Allometry (i.e., relationship between variables that are affected by proportional changes due to variation in body size and growth rate; Gunther, 1975), successfully accommodates nonlinear relationships between body size descriptors and LVM and apparently overcome the heteroscedastic (non-constant variance) errors observed with such size descriptors, thus improving normalization (Batterham and George, 1998; Valente-dos-Santos et al., 2014b; Valente-dos-Santos et al., 2013).

Discussions of the most appropriate size descriptor against which LVM should be scaled during the adolescent growth spurt continue (de Simone et al., 1992; Foster et al., 2013; Lang et al., 2005). In addition to body mass, stature, body surface area and body mass index (de Simone et al., 1992; de Simone et al., 1998; Devereux et al., 1986; Foster et al., 2013; Lang et al., 2005; Lauer et al., 1991), LVM has been scaled relative to fat-free mass (Dewey et al., 2008; Foster et al., 2013; Rowland and Roti, 2010; Valente-dos-Santos et al., 2014b; Valente-dos-Santos et al., 2013) and fat mass (Dai et al., 2009; Daniels et al., 1995). Studies of interrelationships among estimates of body composition and LVM, however, have yielded inconsistent results in children and adolescents. For example, in LVM models with body mass as the size descriptor, the estimated effects of body fat were negative (Dai et al., 2009; Daniels et al., 1995), whereas in models containing stature or fat-free mass, the effects of body fat indicators were significantly positive (Dai et al. 2009; Valente-dos-Santos et al., 2014b; Valente-dos-Santos et al., 2013).

Previous research has not systematically considered biological maturation and its interaction with growth to address inter-individual variability in LVM and the limited literature lacks a longitudinal perspective during adolescence. Changes in body size, proportions and composition associated with inter-individual variation in the adolescent growth spurt are well documented (Malina et al., 2004). Age at peak height velocity (APHV) is commonly accepted as an indicator of somatic maturation. Based largely on studies of European and North American boys, APHV occurs, on average, at ~14.0±1.0 years of age (Malina et al., 2004). Body mass likewise has an adolescent spurt which occurs, on average, at ~3-6 months after APHV. Derivation of APHV for individual boys (and also age at peak weight velocity) requires longitudinal records of stature that span adolescence, although an anthropometric equation to predict time before or after APHV (labeled maturity offset) is available (Mirwald et al., 2002).

The present study considers the development of LVM in a mixed-longitudinal sample of adolescent boys using static (inter-individual), ontogenetic (intra-individual) and multilevel (hierarchical) allometric modelling (Baxter-Jones and Mirwald, 2004; Beunen et al., 2002; Pelabon et al., 2013). In brief, ontogenetic allometry refers to differential growth in the individual (Beunen et al., 1997; Balasekaran et al., 2005) and is most often applied to longitudinal data (Gould, 1966). Static allometry (phylogenetic) reflects the dimensional relationship between the dependent variable and size descriptors (Beunen et al., 1997) in individuals at the same developmental (ontogenetic) stage (Pelabon et al., 2013). Multilevel modelling (Goldstein, 1995) is appropriate for the analysis of longitudinal data, and it has been suggested that multiplicative allometric rather than additive models provide better understanding and more plausible interpretations (Nevill and Holder, 1995; Nevill et al., 1998). In the preceding context, objectives of this study are twofold: first, to investigate the independent and interactive contributions of stature, body mass, adiposity and biological maturity to inter- and intra-individual development of LVM, and second, to present allometric models for normalizing LVM in adolescent boys.

8.3. Methods

Participants and design

The study was conducted in accordance with the ethical procedures of the Declaration of Helsinki for human studies by the World Medical Association (2008) and was approved by the Scientific Committee of the University of Coimbra and by the Portuguese Foundation for Science and Technology (SFRH/BD/64648).

Participants were randomly invited to participate in this mixed-longitudinal study. The sample included 137 Caucasian boys in grades 6-8 from schools in the Portuguese Midlands (11-16 years), but the present study was limited to boys 12-14 years at baseline (*n* = 87). Tests were performed at 6-month intervals for two years (four time moments); a total of 401 observations [average 3.8 (SD = 0.5, range 2 to 4) observations per subject] were available for each variable. Informed written consent was obtained from the parents. About 56% of the sample was engaged in recreational sports; none were elite athletes (i.e., youth participating in the highest national leagues for their ages) and none participated in conditioning programs.

Physical examination

Stature (Harpenden stadiometer, model 98.603, Holtain Ltd, Crosswell, UK), sitting stature (Harpenden sitting height table, model 98.607, Holtain Ltd, Crosswell, UK), body mass (SECA balance, model 770, Hanover, MD, USA) and four skinfolds (triceps, subscapular, suprailiac and medial calf; Lange Caliper, Beta Technology, Ann Arbor, MI, USA) were measured by a single, experienced observer following standard procedures (Lohman et al., 1988). The logarithm of sum of the four skinfolds was used as an estimate of overall subcutaneous adiposity. Leg length was estimated as stature minus sitting stature. Technical errors of measurement for a subsample of participants (*n* = 22) measured one week apart were as follows: stature, 0.3 cm; sitting stature, 0.3 cm; body mass, 0.5 kg; skinfolds, 0.5 to 0.7 mm. The magnitude of errors was well within the range of several health surveys in the United States and a variety of field surveys (Malina, 1995).

Chronological age and biological maturity

Chronological age (CA) was calculated as the difference between date of birth and the date of echocardiographic assessment. Maturity offset (time before or after APHV) was predicted from a sex-specific equation based on Canadian and Belgian boys (Mirwald et al., 2002); maturity offset minus CA provide an estimate of APHV. The SEE of the equation was 0.592 (95% CI 1.18 years) (Mirwald et al., 2002).

Maturity offset can be used to classify adolescents as pre- or post-APHV, while individuals can also be grouped by years before or after APHV rather than CA. Applicability of the method appears to be useful during the interval of growth spurt, approximately 12–15 years (Malina and Kozieł, 2014). The present study was limited to boys < 12 years at baseline and > 15 years at the ending moment; CA group was defined with the whole year as the midpoint of the range, i.e., 12 = 11.50–12.49 years

Imaging protocol

Echocardiography was performed using a Vivid 3 ultrasound machine with a 1.5-3.6 MHz transducer (GE Vingmed Ultrasound, Horten, Norway) by a single trained researcher. M-mode echocardiograms were derived from 2-dimensional images. Measurements of left ventricular internal dimension at end diastole (LVIDd), septal wall thickness at end-diastole (SWTd) and posterior wall thickness at end-diastole (PWTd) were obtained following recommendations of the American Society of Echocardiography (Lang et al., 2005). LVM was calculated with the formula proposed by Devereux et al. (1986). Twenty randomly chosen subjects were measured again after a one week by the same observer. Relative intra-observer variability (Bland and Altman, 2012) for LVIDd, SWTd and PWTd was 0.3% (–4.1 to 4.8%), 0.3% (–4.2 to 4.8%), and 0.8% (–6.5 to 8.1%), respectively.

Statistical analyses

Descriptive statistics by age group are presented as means and standard deviations. Age-specific static (inter-individual) allometric coefficients (*k*) were calculated for the natural logarithms of LVM and natural logarithms of body size descriptors (ln transformed stature and ln transformed body mass). The static coefficients reflect the dimensional relationship between LVM and size descriptors in a given age group, and are not identical with ontogenetic allometry coefficients which reflect proportional changes over time (Beunen et al., 1997). Ontogenetic (intra-individual) allometric coefficients (*k*’) were calculated for each boy (Pelabon et al., 2013). The static and ontogenetic allometric analyses assumed the following model:

$$\ln(\text{LVM}) = \ln a + k \text{ or } k' \cdot \ln(\text{size descriptor}) + \ln \varepsilon \quad \textbf{(8.1)}$$

where *a* was the scaling constant and *k or k'* were the scaling coefficient of the body size descriptors. Coefficients of determination (R^2) provide an estimate of explained variance. Magnitude of correlations was interpreted as follows: trivial ($r < 0.1$), small ($0.1 < r < 0.3$), moderate ($0.3 < r < 0.5$), large ($0.5 < r < 0.7$), very large ($0.7 < r < 0.9$), and nearly perfect ($r > 0.9$) (Hopkins et al., 2009). A 2-tailed P value < 0.05 was considered significant. The analyses were performed using IBM SPSS version 19.0 software (SPSS Inc., IBM Company, NY, USA).

The multilevel regression analysis was performed using MLwiN 2.02 software. Multilevel modelling was used to identify factors associated with the development of LVM with adjustments for differences in body size, CA or biological maturity status:

$$\text{LVM} = (\text{size descriptor}_1)^{k1} \cdot (\text{size descriptor}_2)^{k2} \cdot \exp(a_{ij} + b_j \cdot \text{CA or maturity offset}) \cdot \varepsilon_{ij} \quad \textbf{(8.2)}$$

where all the parameters were fixed with the exception of the constant (intercept term, a_{ij}) and age or maturity offset (b_j), which were allowed to vary randomly from subject to subject (level 2), and the multiplicative error ratio ε_{ij}, which was used to describe the error variance between observations (level 1).

The model can be linearized with a logarithmic transformation (Nevill et al., 1998; Valente-dos-Santos et al., 2014). The transformed log-linear multilevel becomes:

$$\ln \text{LVM} = k_1 \cdot \ln(\text{size descriptor}_1) + k_2 \cdot \ln(\text{size descriptor}_2) + a_{ij} + b_j \cdot (\text{CA or maturity offset}) + \ln \varepsilon_{ij} \quad \textbf{(8.3)}$$

A tolerance > 0.10 and a variance inflation factor (VIF) < 10 were set to avoid collinearity between explanatory variables (Slinker and Glantz, 1985). Goodness of statistical fit of multilevel models was measured by the deviance between two models measured using the – 2 × log likelihood criterion. The difference in iterative generalized least squares (IGLS) deviance between two nested models has a χ^2 distribution which is compared with degrees of freedom lost to determine if one model is a significant improvement over the other. Explanatory variables were added to the models and are retained if deviance improves and/or if the variances at levels 1 and 2 are reduced.

8.4. Results

Descriptive statistics and static allometry

Descriptive characteristics of subjects by age groups are summarized in Table 8.1. Stature and body mass increase with age. Mean stature falls between the 40-60th age specific percentiles, while mean body mass falls between 65-70th age specific percentiles of US reference data (Kuczmarski et al., 2002). The trend suggests elevated mass-for-stature, although adiposity declines with age. Absolute LVM increases with age from 95.2 g to 122.6 g and is within the range of normally expected variation, 88 – 224 g (Lang et al., 2005).

Static (inter-individual) allometric coefficients (k) suggest non-linear associations between LVM and body size descriptors (Table 8.1). Static allometric coefficients for stature are highest (k = 2.47) at 12 years and lowest at 15 years (k = 1.54). Scaling coefficients for body mass as the size descriptor tend to be close to linearity at 12 years of age (k = 0.61) than at 15 years (k = 0.49). Overall, stature accounts for a decreasing proportion of the explained variance in LVM between 12 and 15 years of age, 63% to 15%. Body mass accounts for an increasing proportion of the explained variance in LVM from 12 to 14 years of age, 49% to 54%, but the contribution of body mass to variance in LVM is lowers at 15 years, 34%.

Table 8.1. Descriptive statistics (mean ± SD) by age group and age-specific static allometric coefficients of left ventricular mass (LVM) for stature and body mass based using simple models [ln (LVM) = ln a + k · ln (size descriptor) + ln ε].

Variables	Size descriptor	Models summary	Age group 12 years (n = 108)	13 years (n = 138)	14 years (n = 103)	15 years (n = 52)
Chronological age (years)			11.98 ± 0.32	12.95 ± 0.30	13.91 ± 0.29	14.88 ± 0.34
Maturity offset (years)			-1.83 ± 0.60	-0.84 ± 0.69	0.09 ± 0.69	1.00 ± 0.56
Predicted APHV (years)			13.82 ± 0.53	13.79 ± 0.61	13.82 ± 0.60	13.89 ± 0.47
Stature (cm)			149.7 ± 8.8	157.8 ± 9.9	164.1 ± 8.2	168.1 ± 6.3
Body mass (kg)			44.8 ± 9.3	50.4 ± 11.3	55.8 ± 12.7	60.7 ± 11.2
Sum of skinfolds (mm)			67.8 ± 25.0	61.4 ± 28.5	53.3 ± 28.3	49.3 ± 22.5
Left ventricular mass (g)			95.2 ± 17.4	107.0 ± 19.5	115.2 ± 18.9	122.6 ± 18.6
	Stature	*k* (95% CI)	2.47 (2.11 – 2.84)	2.05 (1.70 – 2.41)	1.84 (1.35 – 2.33)	1.54 (0.51 – 2.57)
		SEE	0.11	0.13	0.13	0.14
		R	0.79	0.70	0.65	0.39
		R^2	0.63	0.49	0.42	0.15
	Body mass	*k* (95% CI)	0.61 (0.49 – 0.73)	0.60 (0.50 – 0.70)	0.56 (0.46 – 0.66)	0.49 (0.30 – 0.69)
		SEE	0.13	0.13	0.11	0.12
		R	0.70	0.72	0.74	0.58
		R^2	0.49	0.51	0.54	0.34

Note: Chronological age is defined with the whole year as the midpoint of the range, i.e., 12 = 11.50–12.49 years

Where applicable, results are expressed as mean ± SD or scaling coefficients (k), 95% confidence intervals (95% CI), standard error of estimate (SEE), model correlation coefficients (R) and coefficients of determination (R^2).

Results of the static allometric regressions to predict LVM are shown relative to estimated time before and after predicted APHV in Figure 8.1. The static allometric coefficient (*k*) for stature is higher among boys classified as pre-APHV (*k*=2.13, R^2=0.53) compared to those classified as post-APHV (*k*=1.85; R^2=0.44). On the other hand, with body mass as the size descriptor, the scaling coefficient is lower among boys classified as pre-APHV (*k*=0.55, R^2=0.39) and higher in boys classified as post-APHV (*k*=0.68, R^2=0.53).

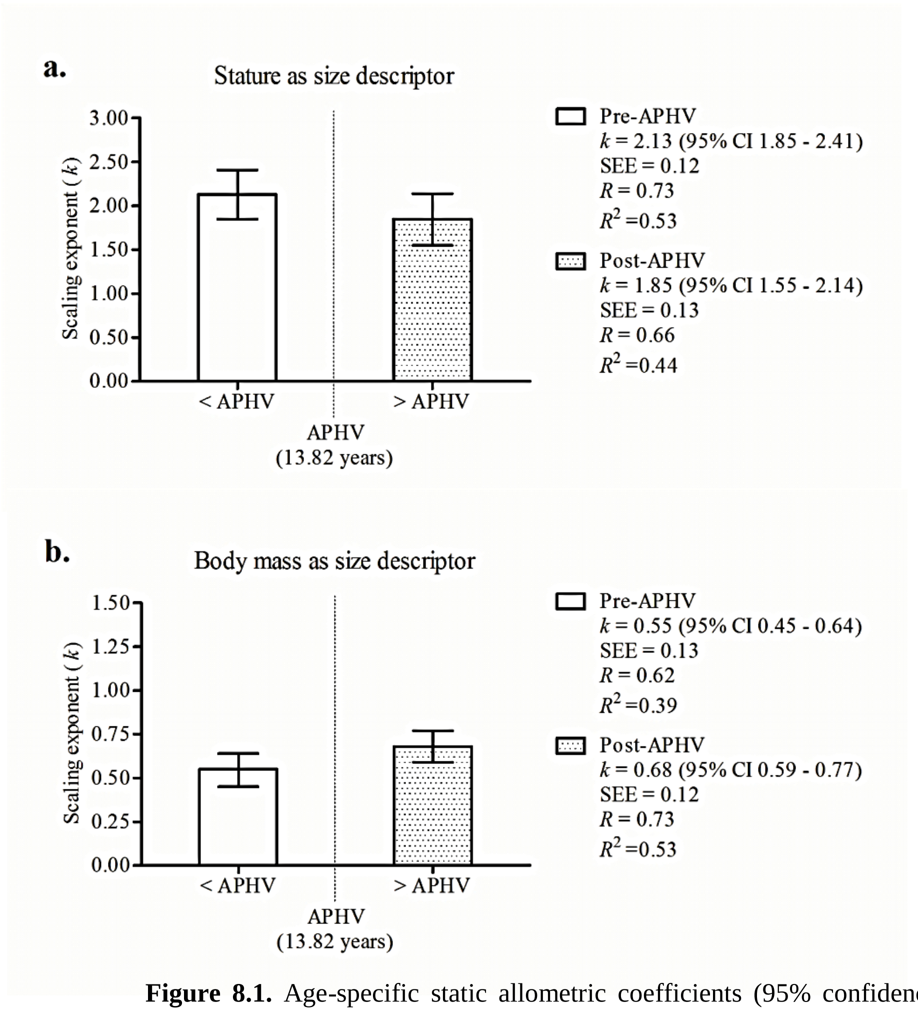

Figure 8.1. Age-specific static allometric coefficients (95% confidence intervals) of left ventricular mass (LVM) for stature (panel **a**) and body mass (panel b), before (*n* = 203) and after (*n* = 198) age at peak height velocity (APHV), based on simple models [ln (LVM) = ln *a* + *k* · ln (size descriptor) + ln *ε*]: scaling coefficients (*k*), standard error of estimate (SEE), model correlation coefficients (*R*) and coefficients of determination (R^2).

Ontogenetic allometric analysis

For ontogenetic (intra-individual) allometric coefficients (*k'*), the linear regression of natural logarithmic transformations of LVM on stature (R^2 = 0.61) and body mass (R^2 = 0.61) shows a similar statistical fit (Table 8.2). Coefficients of determination exceed 0.50 in about two-thirds of cases (stature: 65%; body mass: 65%), and are less than 0.10 in small percentages of boys (stature: 3%; body mass: 4%). Intraindividual allometric coefficients of LVM vary considerably for stature (*k*' = 0.80 to 7.84) and body mass (*k*' = 0.23 to 4.68); mean coefficients are *k*' = 2.76 and *k*' = 1.02, respectively.

Table 8.2. Ontogenetic allometric coefficients of left ventricular mass (LVM) considering stature and body mass as size descriptors based on simple models [ln (LVM) = ln a + k' × ln (size descriptor) + ln ε].

Size Descriptor	Models summary	Minimum	Maximum	Mean	SD
Stature	*k'*	0.80	7.84	2.76	1.69
	SEE	0.01	0.18	0.07	0.04
	R	0.21	0.99	0.76	0.20
	R^2	0.05	0.99	0.61	0.27
Body mass	*k'*	0.23	4.68	1.02	0.74
	SEE	0.01	0.18	0.07	0.04
	R	0.17	0.99	0.76	0.20
	R^2	0.03	0.99	0.61	0.28

Note: Only *k*' coefficients with significant slopes (n = 77 × 4 time moments) were included. Results are expressed as scaling coefficients (*k*'), standard error of estimate (SEE), model correlation coefficients (*R*), and coefficients of determination (R^2).

Multilevel allometric analysis

Significant multilevel allometric models combining more than one size descriptor in addition to CA or maturity offset are summarized in Table 8.3. The following are significant predictors in the multilevel allometric models:

Model A: ln stature (tolerance = 0.30; VIF = 3.31),
ln body mass (tolerance = 0.38; VIF = 2.63),
CA (tolerance = 0.62; VIF = 1.61);

Model B: ln stature (tolerance = 0.21; VIF = 4.84),
ln body mass (tolerance = 0.36; VIF = 2.77),
maturity offset (tolerance = 0.23; VIF = 4.27);

Model C: ln body mass (tolerance = 1.32; VIF = 0.76),
ln sum of the four skinfolds (tolerance = 1.32; VIF = 0.76).

According to the fixed part of the basic LVM-size-age model (Model A), once CA (i.e., centered age, which equals CA minus 13.19 years) is controlled (b = 0.023), the longitudinal allometric coefficient for stature is k_1 = 0.997 (antilog: 1 cm predicts ~ 2.710 g of LVM) and for body mass is k_2 = 0.353 (antilog: 1 kg predicts 1.423 g of LVM). Similar results are apparent in the LVM-size-maturity model (Model B) with slight decreases in body size longitudinal allometric coefficients (stature: k_1 = 0.865; body mass: k_2 = 0.353) due the increase in the exponential term (maturity offset: b = 0.032). The random effects coefficients of both Models indicates significant variance at level 1 (i.e., LVM increased significantly within individuals at each observation). The between-individuals variance matrix (level 2) indicates significantly different LVM growth curves among individuals as evident in the intercepts (constant/constant, $P < 0.05$).

The significance of considering adiposity as an indicator in addition to body size is suggested in Model C. Compared with the LVM-size-age and the LVM-size-maturity models, the exponential terms (CA or maturity offset) are no longer significant. The between-individuals variance observed in the previous models is also no longer significant. The sum of the four skinfolds reveals a strong, negative effect of adiposity on LVM (k_2 = 0.353).

Table 8.3. Allometric multilevel regression analyses of log-transformed left ventricular mass (LVM) adjusted for body size and age or maturity [a] (n = 401).

Multilevel models	*Estimate*	SE	Value
Model A			
Fixed parameters			
ln stature (k_1)	0.997	0.209	
ln body mass (k_2)	0.353	0.058	
Age (b)	0.023	0.009	
Within-individuals variance			
Constant (a_{ij})	0.00527	0.00044	
Between-individuals variance / covariance			
Constant (a_j) / Constant (a)	0.00822	0.00132	
IGLS deviance from the null model			582.42
Log likelihood statistics			– 379.21
Model B			
Fixed parameters			
ln stature (k_1)	0.865	0.230	
ln body mass (k_2)	0.312	0.061	
Maturity offset (b)	0.032	0.011	
Within-individuals variance			
Constant (a_{ij})	0.00529	0.00044	
Between-individuals variance / covariance			
Constant (a_j) / Constant (a)	0.00804	0.00129	
IGLS deviance from the null model			583.52
Log likelihood statistics			– 379.76
Model C			
Fixed parameters			
Constant (a)	2.114	0.128	
ln body mass (k_1)	0.743	0.032	
ln sum of skinfolds (k_2)	– 0.035	0.004	
Within-individuals variance			
Constant (a_{ij})	0.00501	0.00043	
IGLS deviance from the null model			596.63
Log likelihood statistics			– 386.31

[a] Equation 8.3: ln (LVM) = $k_1 \cdot$ ln (size descriptor$_1$) + $k_2 \cdot$ ln (size descriptor$_2$) + a_i + $b_j \cdot$ (age or maturity offset) + ln ε_{ij}.

Predicted development of LVM

Using the antilog function, developmental variation in LVM derived from the multilevel allometric models in Table 8.3 were plotted by CA and maturity offset (Figure 8.2: panel a for Model A; panel b for Model B). Predicted LVM increases substantially from 12 to 15 years (31.4 g; 33.5%) with constant annual gains (~10.5 g·year^{-1}). Predicted LVM aligned by estimated time relative to APHV) shows more variability and a linear increase in LVM from –3.0 years before to 1.0 year after APHV. LVM increases ~70% (53.4 g) at a continuous rate of growth (~13.4 g·year^{-1}).

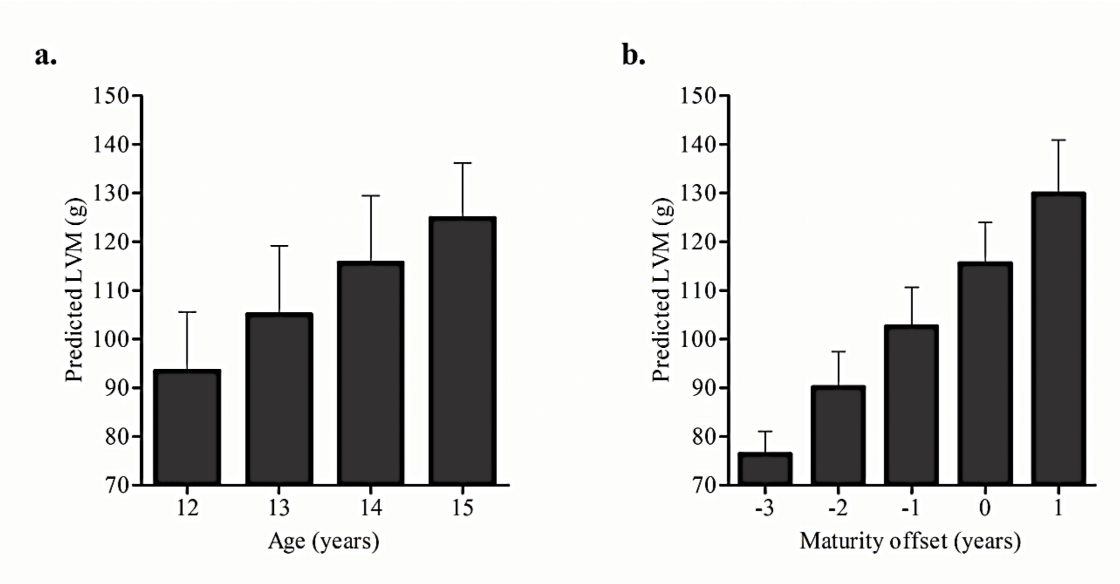

Figure 8.2. Predicted left ventricular mass (LVM) in boys aligned by chronological age (panel a, Model A) time before and after age at peak height velocity (panel b, Model B). Data are predicted from the multilevel allometric models in Table 8.3.

8.5. Discussion

The contributions of body size, adiposity, CA and biological maturity to developmental changes in LVM among adolescent boys aged 12-15 years were examined with allometric modelling. Average LVM values were within ranges reported for adolescent boys of the same CA (Foster et al., 2013; Hietalampi et al., 2012; Janz et al., 1995). Stature, body mass and/or body fatness were shown to be independent determinants of LVM, a finding previously supported (Dai et al., 2009; Daniels et al., 1995; de Simone et al., 1992; de Simone et al., 1998; Foster et al., 2013; Lang et al., 2005; Lauer et al., 1991). Chronological age and predicted maturity status were additional and independent sources of inter-individual variability in LVM in this sample of adolescent boys. The analysis also indicated no linear relationship between body descriptors and cardiac dimensions, which precludes the use of simple ratio scaling for normalization.

Static allometric coefficients provide information about the association between LVM and body size at a given CA or maturity level. Relationships among lengths, surface area and volume have implications for metabolism and thermoregulation. Linear anthropometric dimensions, such as stature, segment lengths and breadths have the dimension *d*. Areas including body surface area and muscle cross-sectional area have the dimension d^2. Total body volume and other volumes (lungs, heart, lower limbs) have the dimension d^3. In isometric bodies, scaling coefficients confirm geometric similarity. Therefore, LVM would linearly relate to body mass (dimension ratio: 3/3) and the third power function would be expected for stature (dimension ratio: 3/1). Different *k* coefficients have been proposed for LVM based on different models, e.g., $stature^{2.7}$, $stature^{2.3}$ and $stature^{2.0}$ (Dai et al., 2009; Daniels et al., 1995; de Simone et al., 1992; Khoury et al., 2009; Urbina et al., 1995). The *k* coefficients noted in Portuguese adolescent boys (Table 8.1, Figure 8.1) indicated substantial explained variance in LVM, but they were not consistent with the theoretical assumptions of geometric similarity or suggested scaling models. Differential growth in linear and mass dimensions during adolescence in addition to differential timing of growth spurts in stature, leg length, sitting stature and body mass (Malina et al., 2004) contribute to problems in the derivation of a unique scaling coefficient.

$LVM/stature^{2.7}$ is the most widely accepted indexing value in older children and adolescents (Dai et al., 2009; de Simone et al., 1992; Khoury et al., 2009), although, results of the present study and others (Foster et al., 2008; Foster et al., 2013; Valente-dos-Santos et al., 2014) indicated that $LVM/stature^{2.7}$ did not adequately normalize LVM for stature in boys 12-15 years. The power function $LVM/stature^{2.7}$, however, appeared acceptable at 12 years, but elastic similarity was suggested for log transformed LVM and stature as scaling coefficients decreased from $k = 2.47$ at 12 years to $k = 1.54$ at 15 years. These and other observations in the present study suggested that relationships between LVM and body descriptors varied with CA. LVM was proportionally higher at 12 years and proportionally lower at 15 years than theoretically expected from stature. The coefficients showed wide confidence intervals and high residual error, and the variance explained by stature decreased by about 48% from 12 to 15 years.

Previous research has shown that scaling coefficients obtained for a particular size descriptor may not be appropriate for all ages (Thomis et al., 2000) and that allometric coefficients should be sample-specific (Balasekaran et al., 2005; Rowland, 1998). These trends were observed for body mass (12 years: k = 0.61; 15 years: k = 0.49), while the relationship between LVM and body mass (12 to 14 years, R^2 = 0.49-0.54) was the most stable across the CA categories (Table 8.1). In contrast, previous research has suggested that a body mass-based scaling approach may be misleading from late childhood through adolescence (Foster et al., 2013). Body mass is confounded by fat mass, resulting in an underestimation of LVM in youth with higher levels of fatness (Dai et al., 2009; de Simone et al., 1992; Valente-dos-Santos et al., 2013). Overall, coefficients varied with CA but also with predicted biological maturity status. The coefficient for stature (k = 2.13) explained a substantial amount of variance in LVM among pre-APHV boys (R^2 = 0.53). On the other hand, the coefficient (k = 0.55) for log transformed LVM and body mass in post-APHV boys accounted for a similar amount of variance (R^2 = 0.53) to that of the LVM-stature model for pre-APHV boys. Studies relating scaling LVM in adolescent boys are limited. Skeletal maturity was a relevant contributor to explained inter-individual variance in LVM among late adolescent elite roller hockey athletes (Valente-dos-Santos et al., 2013).

Individual ontogenetic coefficients for stature and body mass differed compared to static coefficients, ranging from k' = 0.80 to 7.84 and k' = 0.23 to 4.68, respectively. This probably reflected individual differences in timing and tempo of the adolescent growth spurt in stature, body mass and cardiac dimensions. Another constraint for ontogenetic allometric modelling is the asymptotic nature of the growth curves for stature and body mass (Kuczmarski et al., 2002), and for LVM (de Simone et al., 1992; de Simone et al., 1995; Foster et al., 2013; Lauer et al., 1991). Ontogenetic allometry coefficients were previously used to scale aerobic fitness to body mass and substantially varied between individuals, especially between early and average, and late maturing boys (Beunen et al., 1997). In pubertal boys mean LVM ontogenetic allometry coefficients were close (k' = 2.76 and k' = 1.02, respectively) to those expected from stature (*k* = 2.7; Dai et al., 2009; de Simone et al., 1992; Khoury et al., 2009) or body mass (*k* = 1.0; McMahon, 1973). Nevertheless, increments in LVM during adolescents are strongly correlated with increases in body mass which, among other factors, are associated with growth related changes in skeletal muscle mass (Malina et al., 2004). Similar to aerobic fitness (Rowland, 2005), LVM and cardiac output is strongly determined by the demands of metabolically active tissues (Daniels et al., 1995). Even though stature is strongly correlated with lean body mass, the relationship is not perfect. Moreover, it should be noted that most studies are limited by sample sizes (Batterham and George, 1998; Foster et al., 2013; George et al., 2009; Janz et al., 1995) and more than four observations per subject are recommended. When data from fewer observations are used in linear regressions, deviations from the line of identity related in part to measurement error become critical and eventually result in non-significant allometric coefficients (Beunen et al., 1997).

By adopting the multiplicative allometric model within a multilevel structure, a one-stage allometric analysis that simultaneously incorporates covariates is achieved (Table 8.3). It also allows each individual in the sample to have his own individual stature or body mass coefficient. In the present analysis, the confounding effects of CA and estimated maturity status were controlled by aligning LVM on CA and maturity offset. When stature, body mass and CA (Model A), or stature, body mass and maturity offset (Model B) were included in the fixed part of the multilevel allometric regression model, a significant independent effect of the time-related variables was apparent. The multiplicative approach also reduced the stature coefficients to *k*' = 0.997 (Model A) or 0.865 (Model B) which were both close to linearity. Compared to simple allometric models, the body mass coefficient decreased in multiplicative models (*k*' = 0.353 in Model A or *k*' = 0.312 in Model B), corresponding to a more evident departure from linearity. The coefficients were significantly different from the static and ontogenetic coefficients and also from previously proposed models and theoretical assumptions. Allowing for the limitations of the prediction (Malina and Kozieł, 2014; Mirwald et al., 2002) estimated years before or after APHV provided a continuous indicator of maturational timing in this sample of Portuguese adolescent boys 12-15 years of age.

Accordingly, the results suggested synchronization of the changes in LVM than stages of secondary characteristics in boys (Daniels et al., 1995). LVM showed greater variability aligned relative to time before/after APHV (panel b, Figure 8.2) compared to alignment on CA (panel a, Figure 8.2). When stature and body mass were statistically controlled (Model B), a continuous increase in LVM (~13.4 $g.year^{-1}$) was noted before and after APHV (~70%; 53.4 g).

The results of the multilevel analysis also indicated an effect of adiposity on LVM that was largely dependent on the particular combination of body size and fatness predictors chosen for the models. A cross-sectional study of healthy children 6-17 years of age (n = 201) showed that a 10 kg increase in fat mass corresponded to 5 g increase in LVM after adjusting for lean body mass and systolic blood pressure (Daniels et al., 1995). Longitudinal echocardiographic data for youth are limited (Dai et al., 2009; Gardin et al., 2002; Urbina et al., 1995). A 5-year follow-up of biracial cohorts of the Bogalusa Heart (Urbina et al., 1995) and CARDIA Study (Gardin et al., 2002) noted that the onset of obesity predicted longitudinal changes in LVM in healthy children and young adults. Data from Project HeartBeat showed a mean change in LVM of 11–14 g for a 10 kg change in fat mass, when fat-free mass and other covariates (CA, sex or body size descriptors) were statistically controlled (Dai et al., 2009). Results of allometric multilevel Model C suggested that subcutaneous adiposity (sum of skinfolds) had an independent negative effect (k'= -0.035) on LVM when body mass was statistically controlled (10 kg of body mass predicted 27 g of LVM). Overall, the limited longitudinal observations emphasized the importance of interrelationships among CA, body size and composition, and LVM during adolescence.

Although the current investigation has several strengths (e.g., covers the period of the adolescent growth spurt; 4 time-moments of longitudinal data; a relatively large sample; multiple analytical procedures), it has several limitations. The method for estimating maturity used in the present study was based on an equation developed on two Canadian and one Belgian samples (Mirwald et al., 2002). In a recent longitudinal study of Polish boys, errors between predicted APHV and actual APHV based on modeled stature records for individual boys were associated with CA and maturity status based on actual APHV (Malina and Koziel, 2014). Applicability of the maturity-offset protocol was suggested as suitable for boys 12–15 years who are average or on time in APHV. Additional research on the validity of the maturity-offset protocol is needed. Nevertheless, the concordance of maturity status classifications based on predicted APHV and on skeletal age was relatively poor in Portuguese soccer players aged 11-14 years (Malina et al., 2012). Future research addressing maturity-associated variability in LVM needs to identify factors that could mediate (direct effects) or moderate (indirect effects) the relationship between biological maturation and developmental changes in echocardiographic parameters. Another limitation may be the marker of adiposity used in the current study (sum of four skinfold thicknesses). It would be relevant to include alternative measurements such as fat-free mass and fat mass derived from dual-energy X-ray absorptiometry or air displacement plethysmography. Lastly, the study included boys of Caucasian ancestry; the developmental models in this study need to be confirmed in other groups.

8.6. Conclusions

Although individual growth patterns in LVM showed substantial variation, scaling models based on a single descriptor for indexing LVM should be questioned. Developmental changes in LVM were better explained when the confounding effects of stature, body mass and CA or estimated biological maturation were simultaneously considered. Distinctions between longitudinal changes in LVM derived from variation in body size from changes due to intra-individual variability in body fatness were also apparent, where adiposity had a negative effect. Compared to simple static or ontogenetic allometric models, LVM-size-age, LVM-size-maturity, and LVM-size-adiposity models based on allometric multilevel modelling permitted more accurate quantification of LVM in adolescent boys longitudinally followed for two years. Allometric multilevel modelling is recommended for evaluating longitudinal changes in LVM as it allows for variation within and among individuals.

8.7. References

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Chapter IX

Study 7

Multilevel modelling of developmental changes in cardiorespiratory fitness using objective measurements of physical activity and time sedentary in adolescent boys

9. Multilevel modelling of developmental changes in cardiorespiratory fitness using objective measurements of physical activity and time sedentary in adolescent boys

Journal: Valente-dos-Santos J, et al. 2014. Multilevel modelling of developmental changes in cardiorespiratory fitness using objective measurements of physical activity and time sedentary in adolescent boys. (Being Submitted)

9.1. Abstract

Background: Physical activity (PA) and sedentary time are often considered correlates of cardiorespiratory fitness (CRF), although the longitudinal interrelationship among CRF, sedentary time and PA, in parallel to growth and maturation, remains to be understood.

Aim: The present study aimed to examine the independent and combined contribution of age, adiposity, biological maturation and objective measurements of moderate-to-vigorous PA (MVPA) and sedentary time to the developmental changes of CRF in male adolescents.

Methods: The sample was composed of 110 boys (11-14 at baseline) classified as fit according to FITNESSGRAM standards for CRF. Stature, body mass, skinfolds, PA and CRF were assessed biannually for 2 years. Current stature, expressed as a percentage of attained predicted mature stature (PMS) was used as an indicator of biological maturation. PA was measured using the Actigraph GT1M accelerometer. The 20-m shuttle-run test was used to estimate CRF. Multilevel regression modelling was used to analyse the longitudinal data.

Results: After adjustments for age and subcutaneous adiposity, the statistical model showed that a decrease of 100 $min \cdot d^{-1}$ in sedentary time (P = 0.029) predicted 4% of improvement in the CRF of pubertal boys. No longitudinal associations were observed between percentage of attained PMS expressed as a z-score and MVPA with CRF.

Conclusions: The longitudinal decrease of sedentary time and subcutaneous adiposity had a significant impact in maximizing CRF among male adolescents who were classified as fit.

Keywords: Adolescence · Fitness · Maturation · Adiposity · Accelerometry · Longitudinal Analysis

9.2. Introduction

Cardiorespiratory fitness (CRF) reflects the overall capacity of the cardiovascular and respiratory systems to supply oxygen during prolonged strenuous exercise, as well as the ability to perform such exercise (Ortega et al., 2008; Taylor et al., 1955). CRF is stable from childhood through adolescence in males (Malina et al., 2004), but data from a recent survey (Sandercock et al., 2010) indicates a larger decline in CRF in United Kingdom youth males (~7%) over a 10-year period. The secular decline in CRF is attributed, in part, to a reduction in habitual physical activity (PA) and the increased prevalence of overweight and obesity among youth (Malina and Katzmarzyk, 2006; Pate et al., 2006).

The positive influence of aerobic exercise and fitness on health is well documented. This is particularly important in children and adolescents, among whom the evidence from epidemiological studies suggest that CRF is associated with the clustering of cardiovascular diseases risk (Andersen et al., 2007, 2008; Eisenmann et al., 2005), which tends to track from adolescence through adulthood (Andersen et al., 2004). Moreover, it provides strong and independent prognostic information about the overall risk of illness and death, especially related to cardiovascular causes (LaMonte and Blair, 2006).

Allowing for limitations of secular comparisons, CRF is influenced by several factors, including body fatness (Hussey et al., 2007; McGavock et al., 2009; Suriano et al., 2010), growth, sexual maturation, age, sex, health status and genetics (Teran-Garcia et al., 2008), yet its principal modifiable determinants seems to be moderate-to-vigorous PA (MVPA) and sedentary behaviour (Machado-Rodrigues et al., 2011; Santos et al., 2013; Teran-Garcia et al., 2008). Furthermore, it is known that sedentary behaviour increases and MVPA decreases from childhood to adolescence (Nelson et al., 2006; Riddoch et al., 2004; Troiano et al., 2008). Recent research (Moore et al., 2013) have reported that sedentary time and vigorous PA explain a modest (28%) but significant proportion of the variance in CRF, after controlling for adiposity, sex, and age. Furthermore, it suggested that the negative impact of sedentary time can be mitigated by engaging in vigorous activity. On the other hand, findings from a representative sample of Portuguese children and adolescents aged 10-18 years suggested that MVPA levels may not overcome the deleterious influence of high-sedentary time, independently of the contribution of PA and sedentary time per se to CRF (Santos et al., 2013). The preceding evidence was based on cross-sectional data and it is not clear how PA and sedentary time impact CRF during childhood and adolescence in studies with different designs.

Longitudinal studies are recommended (Boddy et al., 2012; Moore et al., 2013) to assess changes in CRF although such kind of research is limited (Sorić et al., 2014). With appropriate techniques, longitudinal studies can identify changes in CRF and partition the relative contributions of PA and sedentary time from those associated with growth and maturation. Multilevel modelling is appropriate for the analysis of longitudinal observations, i.e., repeated measurements (Goldstein, 1995). Therefore, the present study evaluated longitudinal changes in CRF of adolescent boys classified as fit according to FITNESSGRAM standards for CRF (Welk and Meredith, 2008). Objectives were twofold: firstly, to document the characteristics of Portuguese boys aged 11-15 years, and secondly, to examine the independent and combined effects of age, biological maturity status, adiposity and objectively measured PA and sedentary time on the development of CRF.

9.3. Methods

Participants and design

The study was conducted in accordance with the ethical procedures of the Declaration of Helsinki for human studies by the World Medical Association (2008) and was approved by the Scientific Committee of the University of Coimbra and by the Portuguese Foundation for Science and Technology (SFRH/BD/64648). Participants were invited from five city-councils of the Portuguese Midlands to participate in this mixed-longitudinal study. Informed written consent was obtained from the parents. The present study is limited to boys who were measured at least on two occasions and to those belonging to the healthy zone or above (i.e., fit) according to the age-specific and sex-specific cut-off points of FITNESSGRAM criteria for CRF (Welk and Meredith, 2008). The sample included 110 boys in grades 6-8 aged (11-14 years) at baseline (~80% of the initial sample). Tests were performed at 6-month intervals for two years (four time moments) in the same indoor facility; central to the region. A total of 429 observations [average 3.95 (SD = 0.31, range 2 to 4) observations per subject; Table 9.1] were available for each variable.

Table 9.1. Number of subjects and number of measurements by age group.

Age	Number of measurements			
	2 occasions	3 occasions	4 occasions	Total
11 years	2	0	32	34
12 years	3	0	101	104
13 years	3	1	137	141
14 years	2	2	94	98
15 years	0	0	52	52
Total measurements	10	3	416	429
Number of subjects	5	1	104	110

Note: Chronological age is defined with the whole year as the midpoint of the range, i.e., 11 = 10.50–11.49 years.

Physical examination

Stature (Harpenden stadiometer, model 98.603, Holtain Ltd, Crosswell, UK), body mass (SECA balance, model 770, Hanover, MD, USA) and four skinfolds (triceps, subscapular, suprailiac and medial calf; Lange Caliper, Beta Technology, Ann Arbor, MI, USA) were measured by a single, experienced observer following standard procedures (Lohman et al., 1988). Technical errors of measurement for a subsample of participants (*n* = 22) measured one week apart were as follows: stature, 0.3 cm; body mass, 0.5 kg; skinfolds, 0.5 to 0.7 mm. The magnitude of errors was well within the range of several health surveys in the United States and a variety of field surveys (Malina, 1995a).

Chronological age and biological maturation

Chronological age (CA), stature, and body mass of each boy and midparent stature were used to predict mature (adult) stature using the Khamis–Roche protocol (Khamis and Roche, 1994). Parent heights were extracted from national identification cards which included height measured to the nearest centimeter. Measurements were taken by experienced, but not necessarily trained observers. A similar protocol was used in previous studies (Malina et al., 2012; Padez, 2003). The median error bound (median absolute deviation) between actual and predicted mature stature (PMS) at 18 years of age is 2.2 cm in males (Khamis and Roche, 1994). Percentage of PMS attained at a given age is positively related to skeletal and sexual maturation in boys (Bielicki et al., 1984). Percentage of attained PMS is a commonly used measure of biological maturity (e.g., Malina et al., 2012; Malina et al., 2007). Percentage of attained PMS expressed in the analyses as a *z*-score relative to age-specific (half-year intervals) means and standard deviations from the Berkeley Guidance Study (Bayley and Pinneau, 1952) was used as an the indicator of maturity status.

Physical activity and sedentary time

Physical activity and time spent sedentary were measured for seven consecutive days using ActiGraph GT1M accelerometer (ActiGraph^{TM}, LLC, Fort Walton Beach, FL, USA). Participants wore the accelerometer over the right hip and were instructed to remove the sensor while showering and performing aquatic activities. KineSoft program (version 3.3.20; Loughborough, UK) was used to reduce the 15-second epoch data to outcome variables. Non-wear was defined as 60 minutes of consecutive zeros, allowing for 2 minutes of non-zero interruptions. The criterion for a valid day was 600 wear minutes per day. All participants had at least three valid days (two weekdays and one weekend day) of monitoring. Minutes spent in each intensity were determined using age-specific regression equations (Trost et al., 2002). The threshold of sedentary activity was established at 100 counts·min^{-1} (Trost et al., 2011). Participants were classified as meeting current PA guidelines for youth (≥ 60 min/day in MVPA; active) or not meeting PA guidelines (< 60 min/day in MVPA; low active) (WHO, 2010).

Cardiorespiratory fitness

Cardiorespiratory fitness was measured using the 20-m multi-stage continuous shuttle endurance test (Léger et al., 1988). It corresponds to a standard field test included in the European fitness test battery (Council of Europe, 1988) and is part of the Portuguese physical education curriculum. In brief, 5–10 subjects performed a series of runs across a 20-m track, changing direction at the end of each run to coincide with an audio signal that was getting progressively faster. Participants started running at a speed of 8.5 km $\cdot$ h $^{-1}$, and speed increased at various stages (0.5 km $\cdot$ h $^{-1}$ every minute). Each stage was made up of several shuttle-runs, and subjects were instructed to keep pace with the signals as long as possible. Total number of completed laps was recorded. This test is considered valid (Castro-Piñero et al., 2010), reliable (Artero et al., 2011) and feasible (España-Romero et al., 2010) in adolescents. Based on the performance achieved in the shuttle-run test, maximal oxygen uptake (VO_{2max}) was estimated (Mahar et al., 2011) and adolescents were then classified according to the age-specific and sex-specific cut-off points of FITNESSGRAM criteria, as belonging to the healthy zone or above (i.e., fit), or under the healthy zone (i.e., unfit) (Welk and Meredith, 2008).

Statistical analyses

Descriptive statistics are presented by age group as means and standard deviations and percentages. For the longitudinal analysis, the multilevel regression analysis was performed using MLwiN 2.02 software to identify those factors associated with the development of CRF. Multilevel model technique allows the number of observations and temporal spacing between measurements to vary among subjects, thus using all available data. It is assumed that the probability of data being missing is independent of any of the random variables in the model. As long as a full information estimation procedure is used, such as maximum likelihood in MLwiN for normal data, the actual missing mechanism can be ignored (Rasbash et al., 1999). A detailed description of the multilevel modelling procedure has been previously reported (Valente-dos-Santos et al., 2012a) and complete details of this approach are presented elsewhere (Baxter-Jones and Mirwald, 2004). In brief, CRF were measured repeatedly in individuals (level 1 of hierarchy) and between individuals (level 2 of hierarchy). The following additive polynomial random-effects multilevel regression model was adopted to describe the developmental changes in CRF (Rasbash et al., 1999):

$$y_{ij} = \alpha + \beta_j x_{ij} + k_1 \zeta_{ij} + \cdots k_n \zeta_{ij} + \mu_j + \varepsilon_{ij} \qquad \textbf{(9.1)}$$

where y is the CRF parameter on measurement occasion i in the jth individual; α is a constant; $\beta_j x_{ij}$ is the slope of the CRF parameter with age for the jth individual; and k_1 to k_n are the coefficients of various explanatory variables at assessment occasion i in the jth individual. Both μ_j and ε_{ij} are random quantities, whose means are equal to zero; they form the random parameters in the model. They are assumed to be uncorrelated and follow a normal distribution; μ_j is the level 2 and ε_{ij} the level 1 residual for the ith assessment of CRF in the jth individual. The model was built in a stepwise procedure, i.e., predictor variables (k fixed effects) were added one at a time, and likelihood ratio statistics were used to judge the effects of including further variables (Baxter-Jones et al., 2004). If the retention criteria were not met (mean coefficient greater than 1.96 the standard error of the estimate at an alpha level of 0.05), the predictor variable was discarded. The final model included only variables that were significant independent predictors. Age, as explanatory random variable, was centered on its mean value (i.e., 13.1 years). To allow for the nonlinearity of the CRF development, age power function (i.e., age centered^2) was introduced into the linear model (Valente-dos-Santos et al., 2014). Subsequently, the inclusion of predictors in their raw measurements was tested to improve the statistical fit of the multilevel model. Multicollinearity was examined for the final multilevel model, using correlation matrix and diagnostic statistics (Schroeder, 1990). Variables with a variance inflation factor (VIF) > 10 and with small tolerance (1/VIF $\leq$ 0.10; corresponding to an R^2 of 0.90) were considered indicative of harmful multicollinearity (Slinker and Glantz, 1985). Magnitude of correlations was interpreted as follows: trivial ($r < 0.1$), small ($0.1 < r < 0.3$), moderate ($0.3 < r < 0.5$), large ($0.5 < r < 0.7$), very large ($0.7 < r < 0.9$), and nearly perfect ($r > 0.9$) (Hopkins et al., 2009). Alpha level was set at 0.05.

9.4. Results

Descriptive statistics

Descriptive characteristics of participants by age groups are summarized in Table 9.2. Stature and body mass increase with age. Mean stature falls between the 40-60th age specific percentiles, while mean body mass falls between 65-70th age specific percentiles of US reference data (Kuczmarski et al., 2002). The trend suggests elevated mass-for-stature, although subcutaneous adiposity declines with age. PA was monitored for an average of 5.1±1.5 days (median, 810.3 min·d^{-1}; 25th and 75th percentiles, 751.4 and 848.0 min·d^{-1}). PA decreased with increasing age. At baseline (11-14 years), there was, on average, 187 and 100 min·d^{-1} of light and MVPA, respectively. Corresponding values at the fourth observation (12-15 years) were 170 and 82 min·d^{-1}, respectively. About 95% and 76% of the adolescents were classified as active by current PA guidelines for youth, at baseline and at the fourth observation, respectively. Time spent sedentary increased by 4% from baseline to time moment four (WHO, 2010). CRF increased within the healthy zone (Welk and Meredith, 2008).

Table 9.2. Descriptive characteristics of the participants by age group.

	11 years (n = 34)	12 years (n = 104)	13 years (n = 141)	14 years (n = 98)	15 years (n = 52)
Chronological age (years)	11.21 ± 0.20	12.06 ± 0.29	13.01 ± 0.30	14.06 ± 0.28	15.01 ± 0.43
Predicted mature stature (%)	83.0 ± 2.2	85.8 ± 2.5	89.7 ± 2.9	93.4 ± 2.4	96.4 ± 1.9
Maturity status *z*-score	0.54 ± 1.03	0.70 ± 0.95	0.73 ± 0.82	0.64 ± 0.52	0.53 ± 0.44
Stature (cm)	144.1 ± 8.5	150.3 ± 9.1	158.4 ± 9.8	164.2 ± 8.1	168.7 ± 6.4
Body mass (kg)	40.5 ± 8.1	45.1 ± 9.5	50.9 ± 11.5	56.0 ± 12.4	61.5 ± 11.1
Body mass index ($kg \cdot m^{-2}$)	19.3 ± 2.5	19.8 ± 2.6	20.1 ± 3.2	20.7 ± 3.8	21.5 ± 3.3
Sum of skinfolds (mm)	68.9 ± 23.1	67.4 ± 25.2	61.7 ± 28.7	52.3 ± 27.5	48.9 ± 23.5
Total physical activity ($counts \cdot min^{-1}$)	474.9 ± 125.9	489.5 ± 147.0	469.9 ± 153.5	403.0 ± 112.8	390.1 ± 145.2
Accelerometer wear time ($min \cdot d^{-1}$)	831.6 ± 78.2	821.4 ± 55.7	799.5 ± 69.3	784.7 ± 74.0	773.1 ± 75.7
Sedentary time ($min \cdot d^{-1}$)	539.1 ± 54.7	527.2 ± 63.0	524.6 ± 78.7	545.9 ± 70.1	548.4 ± 53.1
Sedentary time (%)	64.8	64.2	65.5	69.6	70.9
Light physical activity ($min \cdot d^{-1}$)	179.4 ± 35.8	187.6 ± 36.9	183.4 ± 40.7	164.7 ± 38.0	159.5 ± 39.7
Light physical activity (%)	21.6	22.8	23.0	21.0	20.6
Moderate-to-vigorous physical activity ($min \cdot d^{-1}$)	113.1 ± 40.2	106.5 ± 32.0	91.6 ± 28.9	74.1 ± 26.0	65.3 ± 27.3
Moderate-to-vigorous physical activity (%)	13.6	13.0	11.5	9.4	8.5
Active, ≥ 60 minutes MVPA/day (%)	91.2	94.2	87.9	71.4	51.9
Low active, < 60 minutes MVPA/day (%)	8.8	5.8	12.1	28.6	48.1
20-m shuttle-run (laps)	35 ± 13	41 ± 16	49 ± 18	57 ± 19	65 ± 21
VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	45.4 ± 3.5	47.1 ± 3.7	49.4 ± 4.3	51.7 ± 4.6	53.6 ± 3.9

Note: Where applicable, results are expressed as percentages or mean ± SD. Chronological age is defined with the whole year as the midpoint of the range, i.e., 11 = 10.50–11.49 years.

Multilevel analysis

Results of the multilevel analysis are summarized in Table 9.3. The random-effects coefficients describe two levels of variance (within individuals: level 1 of the hierarchy; between individuals: level 2 of the hierarchy). The significant variances at level 1 indicated that CRF improved significantly at each measurement occasion within individuals (estimate > 1.96 · SE; $P < 0.05$). The between-individuals variance matrix (level 2) indicated that male adolescents had significantly different CRF growth curves in terms of their intercepts (constant/constant, $P < 0.05$) but not in terms of the slopes of their lines (CA/CA, $P > 0.05$). The positive covariance between intercepts and slopes of the CRF performance suggested a continuous rate of improvement during the pubertal years. After adjustments for CA (increment of 1 year predicts an increment of ~ 7 laps in the 20-m shuttle-run test; $P < 0.001$) and sum of skinfolds (increment of 10 mm predicts a decrease of ~ 3 laps in the 20-m shuttle-run test; $P < 0.001$), significant independent effects of sedentary time was noted (increment of 100 $min \cdot d^{-1}$ predicts a decrease of ~ 2 laps in the 20-m shuttle-run test; $P = 0.029$). Although showing small associations with CRF ($P < 0.05$), maturity status *z*-score ($r = – 0.21$), and MVPA ($r = 0.17$) were not significant longitudinal predictors.

Table 9.3. Multilevel regression analysis of cardiorespiratory fitness (CRF) adjusted for age, subcutaneous adiposity, maturation and physical activity level (429 measurements).

CRF (20-m shuttle-run, laps)						
Variance-covariance matrix of random variables		Constant			Chronological age	
Level 1 (within individuals)						
	Constant	48.449 (4.365)				
Level 2 (between individuals)						
	Constant	116.096 (18.276)			20.023 (6.939)	
	Chronological age	20.023 (6.939)			10.030 (5.804)	

Step	Fixed explanatory variables	*P*	*VIF*	*1/VIF*	Value at final step	
					k	SE
1	Intercept (constant)				77.861	5.246
2	Chronological age	< 0.001	1.172	0.853	7.220	0.678
3	Chronological age^2	NS				
4	Maturity status *z*-score	NS				
5	Sum of skinfolds	< 0.001	1.089	0.918	– 0.298	0.029
6	Sedentary time *	0.029	1.121	0.892	– 0.019	0.007
7	Moderate-to-vigorous physical activity *	NS				
	IGLS deviance from the null model				620.616	
	– 2 × log likelihood				3159.263	

Note: random-effects values are estimated mean variance ± SE; fixed-effect values (explanatory variables) are estimated mean coefficients ± SE; chronological age was adjusted about origin using mean age ± 13.1 years. *k* (mean coefficients of various explanatory variables); SE (standard error); NS (non-significant). Multicollinearity statistics: VIF (variance inflation factors); 1/VIF (tolerance).
* Adjusted for accelerometer wear time.

Predicted cardiorespiratory fitness

The measured and estimated curves for CRF development were plotted by age in Figure 9.1. Predicted CRF development (── solid line in Figure 9.1) fluctuated below and above measured CRF development (---- dashed line in Figure 9.1) without significant differences ($P > 0.05$). CRF improved in a continuous rate of growth from 11 to 15 years (~ 32 laps, 94.1 %).

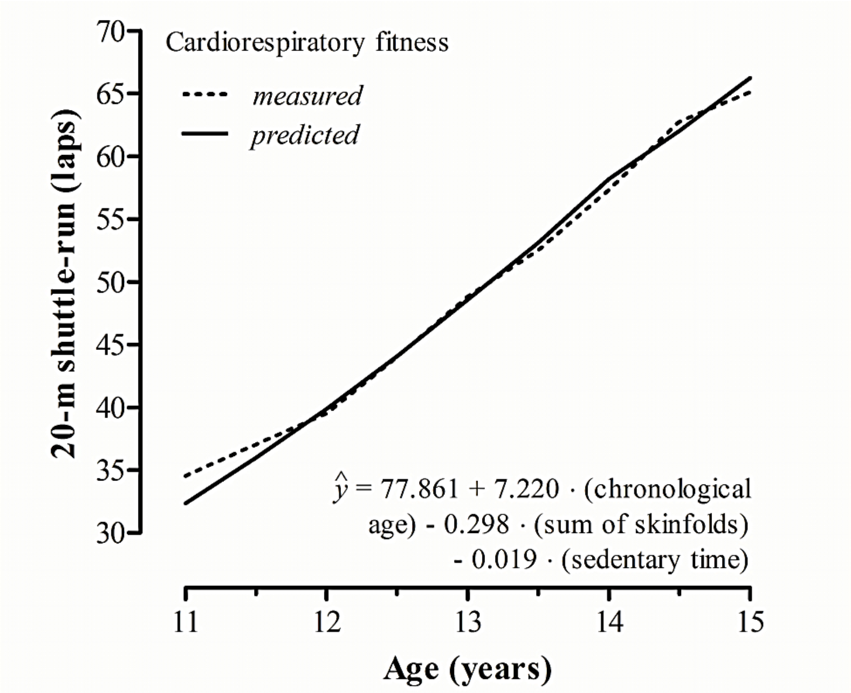

Figure 9.1. Real and estimated cardiorespiratory fitness aligned by chronological age. Data are predicted from the multilevel model in Table 9.3.

9.5. Discussion

The present study evaluates the contributions of CA, biological maturity status, subcutaneous adiposity, PA intensity and sedentary time to developmental changes in CRF among fit adolescent boys 11-15 years. Adolescence is often perceived as period of dramatic changes in health related behaviours and it is intuitively assumed that current adolescents are less fit and also less active compared to previous generations (Malina, 1995b). The literature is somewhat abundant in contributions to understand inter-individual variability in CRF, mostly derived from cross-sectional samples. Adolescents classified as fit tend to be viewed as more likely to be classified as fit in adulthood. However, the Zagreb Growth and Development Longitudinal Study provided data on long-term tracking based in directly measured CRF and fatness and only low-to-moderate correlations (r = 0.19-0.30), from late adolescence to middle adulthood, were found (Sorić et al., 2014). The most important findings of the present study are that CA (k = 7.220 ± 0.678), subcutaneous adiposity (k = –0.298 ± 0.029) and sedentary time (k = –0.019 ± 0.007) had significant longitudinal associations with CRF.

Recent research with Portuguese children and adolescents examined the combined association of objectively assessed MVPA and time sedentary and it was observed that children and adolescents with low-sedentary behaviour exhibited higher odds of being fit regardless of their PA level (Santos et al., 2013). PA level has been shown to have a very low stability between adolescence and adulthood (Telama, 2009). Malina (1990) summarized tracking studies in physical fitness and performance during growth and noted a similar trend from 11 to 18 years of age. However, the same author noted that over a shorter interval, 11 to 14 years of age, absolute VO_{2max} was moderately stable in active Polish boys. More recent research examined the stability of clustering cardiovascular disease risk factors over the course of childhood through adolescence and it was found that sex-specific z-scores for blood pressure, homeostatic model assessment, triglyceride, subcutaneous adiposity, high-density lipoprotein cholesterol and peak oxygen uptake significantly track at all 3-year time intervals (6-9 years: r = 0.51; 9-13 years: 0.56) but the coefficient was modest between 6 and 13 years (r = 0.38). The authors also suggested that tracking was higher for low-fit Danish children compared to their peers classified as fit. The sample of the present study was selected from a non-urban area of Portuguese Midlands and a substantial percentage of boys (~80% of the initial sample) were classified as fit in the 20-m shuttle-run test. By inference, it may be argued that among adolescent boys who are not at risk, time spent sedentary is a contributor to developmental changes in CRF scores.

Compared with previous studies that assessed CRF with the 20-m shuttle-run test, the overall prevalence of fit participants found in this study is similar to that reported for healthy Portuguese children and adolescents (~78%; Santos et al., 2013) and higher than that reported for other European adolescents (~59%; Ortega et al., 2011). In the present study both the measured and the estimated CRF, using multilevel modelling procedures, improved continuously from 11 to 15 years (Figure 9.1). In addition, 76% of boys from the current study accumulated more than 60 minutes per day of MVPA across all time moments. Note, however, that the current study adopted an age-specific threshold and future research may consider concurrent analyses using alternative cut-points that are constant across adolescence (e.g., Puyau et al., 2002).

Multilevel modelling allows the identification of longitudinal predictors of a particular component of physical fitness or performance (Valente-dos-Santos et al., 2012b). Such studies are more abundant in athletes compared to healthy school boys and do not systematically consider PA. The longitudinal development of the 20-m shuttle-run performance of youth soccer players aged 10 to 18 years showed that this trait was substantially related to annual volume of training (Valente-dos-Santos et al., 2012a). On the other hand, results from the Training of Young Athletes (TOYA) study indicated that the VO_{2max} adjusted for age and body dimensions, increased with pubertal status in male athletes from different sports (Baxter-Jones et al., 1993). An additional investigation of British youngsters used a multilevel approach to examine peak oxygen uptake from 11 to 17 years, and both CA and pubertal status were identified as explanatory variables independent of body size and subcutaneous adiposity (Armstrong and Welsman, 2001). However, the aforementioned literature did not systematically consider growth, maturation and objective measurements of PA intensity levels and sedentary behaviour within longitudinal designs. In the current study, the increment of total PA, especially MVPA, in addition to decreases in sedentary time, seemed to be a suggestive insight for public health policies. In other words, MVPA may be especially relevant for adolescents at risks (Strong et al., 2005) and prevention of sedentary behaviour associated with more time in light PA (such as walking or other options in active transportation) may be a valid recommendation for all adolescents (Mota et al., 2007).

A model for adolescent involvement in PA that incorporates individual differences in biological maturation was recently presented (Cumming et al., 2012). This biocultural model of maturity-associated variance in PA recognizes it as a complex and multifaceted behaviour that exists in multiple contexts and can be viewed from multiple perspectives. The model established that biological maturation can exert both direct and indirect effects on PA during adolescence (direct effects imply a direct and unmediated effect of individual differences in maturation on PA, while indirect effects imply influences of individual differences in maturation on PA that are mediated by psychological constructs such as self-perceptions associated with maturation). The potential interaction between biological maturation, PA and sedentary behaviour was also central in a study that evaluated 302 Portuguese adolescents aged 13-16 years that initially suggested boys as spending more time in MVPA and less time sedentary than females, but sex differences were attenuated when maturation was controlled; thus suggesting that biological maturation played an important role in adolescent behaviours (Machado-Rodrigues et al., 2010). The multilevel model of the present study did not identified biological maturation as a significant predictor of CRF. Recognizing that maturity status was based on current stature of the boys as a percentage of his PMS and that stature, per se, does not seems to influence CRF a more sensitive indicator is needed. Skeletal age is considered the best maturity indicator as it is a continuous variable that spans childhood through adolescence (Malina et al., 2004) and was recently identified as a relevant contributor to explain intra- and inter-individual variance in several physical fitness components of adolescent athletes (Valente-dos-Santos et al., 2012b).

Although the independent effects of biological maturation on CRF remains debatable, the prospective part of the present study revealed that a decrease of 10 mm of subcutaneous adiposity predicts an improvement of ~ 3 laps in the 20-m shuttle-run test ($P < 0.001$). In fact, the negative influence of body fatness on CRF has been systematically noted (Hussey et al., 2007; McGavock et al., 2009; Suriano et al., 2010); furthermore, intervention studies, with obese children aged 8-12 years also reported that targeting decreased sedentary behaviour led to increases in both PA and non-targeted sedentary behaviours, which, in turn, led to increases in CRF and decreases in body fatness (Epstein et al., 2000). In the context of the preceding results it is possible to infer that a decrease in sedentary time could lead to an increase in PA levels. In the present study, the average time spent sedentary, adjusted for accelerometer wear time, was ~9 $h \cdot d^{-1}$. Similar values were reported for Portuguese (Santos et al., 2013) and European adolescents (Martinez-Gomez et al., 2011). Results of the multilevel model (Table 9.3) also suggested that a reduction of 100 $min \cdot d^{-1}$ predicts an improvement of ~ 2 laps in the 20-m shuttle-run test ($P = 0.029$) when age and subcutaneous adiposity were simultaneously controlled. This observation emphasizes the importance of inter-relationships among age, body composition, time sedentary and CRF during male adolescence. Daily PA did not independently predict CRF after adjustments for age, subcutaneous adiposity and sedentary time. In addition, PA intensity levels of the boys were well within ranges reported in paediatric epidemiological studies (Riddoch et al., 2004; Troiano et al., 2008). It is likely that more vigorous PA as in intensive training is needed to influence CRF over and beyond changes occurring with normal growth and development in fit adolescents. Additionally, present results might be a reflection of the homogeneity of the boy's PA. Nevertheless, overall results compares favourably with recent cross-sectional literature that suggested that MVPA levels may not overcome the deleterious influence of high-sedentary time on CRF (Santos et al., 2013). Beyond these implications, results suggest that strategies to discourage sedentary behaviour, even in fit adolescents, are necessary, given the adverse effects on health and health risk behaviours (Chinapaw et al., 2011; Nelson and Gordon-Larsen, 2006).

This study is not without limitations. Results suggest that this is a relatively active sample and they may not be representative of middle school Portuguese youth. Thus, the findings should be interpreted with caution. The method for estimating maturity status used in the present study was non-invasive but reference values were based on American youth in the Fels Longitudinal Study (Khamis and Roche, 1994) and Berkeley Guidance Study (Bayley and Pinneau, 1952). Validation of the prediction equations in Portuguese youth is needed. Another limitation may be the marker of adiposity used in the current study (sum of four skinfold thicknesses). It would be relevant to include alternative measurements such as fat-free mass and fat mass derived from dual-energy X-ray absorptiometry (DXA) or air displacement plethysmography. Finally, while objective measurements of PA and sedentary time are desirable, it does not allow identification of PA contexts, such as commuting to school, leisure time PA and sedentary behaviour (standing still, sitting or lying). This is an important limitation since, for example, the type of sedentary activity can modify the relationship with health-related factors (Leatherdale and Wong, 2008). Discussion about cut-points for PA intensity levels in longitudinal studies is an additional topic for future research. In light of these limitations, the present study has a number of strengths; it covers the period of the adolescent growth spurt; 4 time-moments of longitudinal data; the novelty of the analytical procedures; the use of a threshold of 100 counts·min^{-1} to identify sedentary behaviour, as this threshold has been shown to have an excellent classification accuracy (Trost et al., 2011); and, the use of a valid field test for CRF (Castro-Piñero et al., 2010), that offers a cost-effective alternative to the gold-standard laboratory-based assessments of VO_{2max} which are impractical at a large scale.

9.6. Conclusions

In conclusion, sedentary time had a significant negative association with CRF in a sample of healthy adolescent boys followed longitudinally for two years, after controlling for age and adiposity. This supports previous literature that noted a substantial contribution of PA in health outputs of adolescent samples classified at risk for cardiovascular disease. However, our findings suggest a pathway for promoting healthier levels of CRF during puberty, through the reduction of sedentary time and by inference through the increase of time spent in light PA. Long-term longitudinal studies are advocated to better understand the tracking of CRF during adolescent years, with the inclusion of invasive assessments of body composition (such as DXA), biological maturation (such as skeletal age) and concurrent cut-points in the determination of PA intensity levels.

What are the new findings?

- This is perhaps the first study to investigate the longitudinal associations between objectively measured PA and sedentary time with CRF in pubertal boys, while controlling for adiposity and biological maturity.
- PA and sedentary time act independently in their longitudinal relation with CRF.

- Habitual free-living PA from late childhood through adolescence was not associated with CRF. Habitual PA levels are probably not sufficient to influence the pattern of normal growth and development of CRF in the present sample of fit youth.

How might it impact on clinical practice in the near future?

- The approach used in the present study offers a different statistical and biological approach to understanding CRF.
- For CRF, in fit early adolescent boys, it seems important to reduce sedentary behaviour.

9.7. References

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Chapter X

Study 8

Modelling developmental changes in repeated-sprint ability by chronological and skeletal ages in young soccer players

10. Modelling developmental changes in repeated-sprint ability by chronological and skeletal ages in young soccer players

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Journal: Valente-dos-Santos J, et al. 2012. Modelling developmental changes in repeated-sprint ability by chronological and skeletal ages in young soccer players. Int J Sports Med 33:773–780. doi: 10.1055/s-0032-1308996

10.1. Abstract

This study investigated the influence of chronological (CA) and skeletal ages (SA), anthropometry, aerobic endurance and lower limb explosive strength on developmental changes in repeated-sprint ability (RSA) in soccer players aged 11-17 years. Participants were annually followed over 5 years, resulting in 366 measurements. Multilevel regression modelling analysed longitudinal data aligned by CA and SA (Model 1 and 2, respectively). After diagnosing for multicollinearity, it was possible to predict RSA with two-level hierarchical models [Model 1 (CA as Level 2 predictor): Log-Likelihood=1515.29, $p<0.01$; Model 2 (SA as Level 2 predictor): Log-Likelihood=1513.89, $p<0.01$]. Estimating sum of sprints for young soccer players are given by equations: sum of sprints = 84.47 – 1.82 x CA + 0.03 · CA2 – 0.05 · aerobic endurance – 0.10 · lower limb explosive strength – 0.09 · fat-free mass + 0.13 · fat mass (Model 1); 73.58 – 0.43 · SA – 0.05 · aerobic endurance – 0.10 · lower limb explosive strength – 0.08 · fat-free mass – 0.45 · training experience + 0.13 · fat mass (Model 2). The models produced performance curves that may be used to estimate individual performance across adolescent years. Finally, the validity of each model was confirmed based on corresponding measurements taken on an independent cross-sectional sample.

Keywords: Youth Soccer · Longitudinal Analysis · Multilevel Modelling · Growth · Skeletal Maturation

10.2. Introduction

Heights and weights of early- and mid-adolescent male soccer players tend to fluctuate, on average, above and below reference medians for the general population, but in late adolescence and young adulthood players tend to have, on average, more body mass-for-stature (Malina et al., 2000). The trend may reflect late adolescent growth in muscle mass. The inter-individual variation in body size is related, in part, to individual differences in biological maturation during adolescence (Figueiredo et al., 2009b; Malina et al., 2004b). Interrelationships among growth, maturation and functional capacities and their potential implications for talent development in youth soccer are often discussed in the context of sport selection and attrition (Coelho-e-Silva et al., 2010; Figueiredo et al., 2009a). Within a given chronological age (CA) group, skeletally advanced soccer players tend to be, on average, taller, heavier and more powerful than peers classified as delayed (Figueiredo et al., 2009b; Malina et al., 2004b). These observations are largely based on cross-sectional data, while longitudinal changes in relationships between growth and functional characteristics of young athletes are limited. For example, longitudinal changes in dribbling and sprinting abilities of Dutch talented soccer players aged 12-19 years were followed annually for 7 years, but interactions with growth and maturity status were not considered (Huijgen et al., 2010).

Short, high-intensity sprints in the range of 10–30 m are common in soccer and often precede decisive moments in a match (Impellizzeri et al., 2008; Malina et al., 2010; Rampinini et al., 2007; Reilly et al., 2000). The ability to repeat sprinting efforts with brief recovery periods has been labelled ‘repeated-sprint ability’ (RSA) and has received particular attention in the literature (Buchheit et al., 2010a, 2010c). Field tests have been developed and construct validity of the tests as indicators of match-related physical performance in soccer have been evaluated among professional players using a video-computerized image system (Rampinini et al., 2007). Mean RSA times were significantly correlated with high and very high intensity running, sprinting distance and total distance covered during a match.

Although there is an increasing interest in RSA tests, the literature has not considered developmental changes in sprinting performances.

Available data indicate positive associations between RSA and lower limb explosive strength (Buchheit et al., 2010a), and among stature, body mass and fat-free mass and a 10x30-m sprint protocol (Mendez-Villanueva et al., 2011; Mujika et al., 2009). Among soccer players 13–14, training history, stature, body proportions (sitting stature ratio) and adiposity explained 48% of the variance in the fastest sprint of seven trials (Figueiredo et al., 2011). Boys advanced in biological maturation compared to age peers tend to perform better in tests that place a premium on strength, power and speed during adolescence (Beunen and Malina, 1988; Figueiredo et al., 2009a; Lefevre et al., 1990; Malina et al., 2004a, 2004b). Differences are especially marked between 13 and 15 years that correspond to maximal rate of growth in stature and body mass (Philippaerts et al., 2006). Of relevance to talent development programs, maturity-related differences in strength, speed and power during the interval of the growth spurt in stature are attenuated in later adolescence and young adulthood (Lefevre et al., 1990), thus highlighting the transient nature of maturity-associated variation in performance during adolescence.

Longitudinal observations for youth soccer players are limited to running speed, aerobic endurance and lower limb explosive strength, which, on average, tended to demonstrate maximal gains close to peak height velocity (Philippaerts et al., 2006). In addition, development of intermittent endurance capacity in youth soccer players aged 14-18 years differed between players who attained professional status or amateur level (Roescher et al., 2010). Those who attained a professional position evidenced faster development of intermittent endurance capacity after 15 years of age than peers who continued playing at amateur level. Given relatively limited data for youth soccer players, especially covering early, middle and late pubertal development of functional capacities, the longitudinal changes of RSA in young soccer players was considered from 11 to 17 years of age using multilevel modelling designs initially aligned for chronological age and afterwards by skeletal age.

10.3. Methods

Participants

The study fits the established ethical standards for sports medicine (Harriss and Atkinson, 2009). The research proposal was approved by the Scientific Committee of the University of Coimbra and also by the Portuguese Foundation for Science and Technology [PTDC/DES/121772/2010]. The Portuguese Soccer Federation and clubs were contacted and institutional agreements were signed between the University of Coimbra and the sport organizations to assure 5-year data collection. The guardians of the young athletes provided informed consent and players provided assent. Players were also informed that participation was voluntary and that they could withdraw at any time. Goalkeepers were not included.

The study included 135 players from the Midlands of Portugal, divided into two groups (Table 10.1). A subsample of 83 players aged 11-13 years at baseline was annually measured on 3 to 5 occasions. A total of 366 observations (average 4.4 observations per player) were available. An additional cross-sectional subsample of 52 soccer players aged 11-17 years was tested with the same protocol in order to examine the fitness of the predictive model.

The clubs were involved in a 9-month competitive season (September-May) regulated by the Portuguese Soccer Federation. Teams had 3-5 training sessions per week (90-120 $min.session^{-1}$) and one game, usually on Saturday. Training experience (years) of formal participation in soccer was obtained from each player and verified by club records. The Portuguese Soccer Federation records the training history of players and data were publicly available.

Table 10.1. Number of players and number of measurements per age group in the longitudinal data set and in the control group.

Age	Longitudinal data set (number of measurements) 3	4	5	Total	Control group
11 years	3	13	24	40	8
12 years	4	18	35	57	9
13 years	11	27	45	83	10
14 years	8	27	45	80	10
15 years	7	14	45	66	8
16 years	0	9	21	30	4
17 years	0	0	10	10	3
Total measurements	33	108	225	366	52
Number of players	11	27	45	83	52

Chronological age, skeletal age and biological maturity

Chronological age (CA) was calculated as the difference between date of birth and of the hand-wrist radiograph used to assess skeletal age (SA). Posterior-anterior radiographs of the left hand-wrist were taken. All films were rated using the Fels method for assessing SA (Roche et al., 1988). This method assigns grades to specific maturity indicators for the radius, ulna, carpals, metacarpals plus phalanges of the first, third and fifth rays, and utilizes ratios of linear measurements of the widths of the epiphysis and metaphysis of the long bone. The protocol also notes the presence (ossification) or absence of the pisiform and addutor sesamoid bones. Ratings are entered into a program (Felshw 1.0 Software, Lifespan Health Research Center, Departments of Community Health and Pediatrics, Boonshoft School of Medicine, Wright State University, Dayton, Ohio). The treatment weights the contributions of the specific indicators, depending on CA and sex in calculating a SA and standard error of estimate (a confidence interval for the assessment). The difference between SA and CA (SA minus CA) was used to classify players into maturity categories: late (delayed), SA younger than CA by > 1.0 yr; average (on time), SA ± 1.0 yr CA; early (advanced), SA older than CA by > 1.0 yr; a SA was not assigned if the individual had attained skeletal maturity. The band of 1.0 year is consistent with age-specific standard deviations for SAs in adolescent boys and allows for errors associated with assessments (Malina et al., 2010).

Anthropometry

A single anthropometrist measured body mass, stature and four skinfolds (triceps, subscapular, suprailiac, medial calf) following standard procedures (Lohman et al., 1988). Statures were measured to the nearest 0.1 cm with a Harpenden stadiometer (model 98.603, Holtain Ltd, Crosswell, UK) and body mass was measured to the nearest 0.1 kg with a SECA balance (model 770, Hanover, MD, USA). Skinfolds were measured to the nearest mm using a Lange caliper (Beta Technology, Ann Arbor, MI, USA). Technical errors of measurement for body mass (0.47 kg), stature (0.27 cm) and skinfolds (0.47-0.72 mm) were well within the range of several health surveys in the United States and a variety of field surveys (Malina et al., 2004a). Body fat was estimated from triceps and subscapular skinfold thicknesses (Slaughter et al., 1988). Fat-free mass (FFM) was derived in kg.

Repeated-sprint ability

The RSA test is soccer-specific and also called the Bangsbo Sprint Test (Bangsbo, 1994). The protocol includes seven, 34.2-m maximal sprints (including a slalom) with a 25-second recovery interval between runs. The time for each sprint was recorded with a digital chronometer connected to photoelectric cells (Globus Ergo Timer Timing System, Codogné, Italy). Performance was expressed as the sum of the seven sprints. The ideal time corresponds to the best sprint time (which usually occurs in the first or second trials) multiplied by seven. Decrement among the seven sprints relative to the ideal time were also calculated as [(mean sprint time / best sprint time × 100) – 100]. Coefficients of reliability for replicate tests of 32 players within 1 week were 0.86 for ideal time and 0.91 for total time.

Aerobic endurance

The 20-meter shuttle-run test was the measure of aerobic endurance (Léger et al., 1988). The test includes a series of runs following a cadence set by an audio metronome. The time between runs is systematically reduced, thus increasing the effort required to keep the pace. The objective of the test is to perform as many shuttles as possible following the cadence; the test stops when the athlete is no longer able to maintain the required pace. The number of fully completed 20-m runs was recorded and used in the analysis. Based on a test-retest protocol (one week apart) with 21 players, the coefficient of reliability was 0.86.

Lower limb explosive strength

Participants were instructed to keep the hands on hips from the starting position through counter movement phase, jump and end of the flight trajectory (Bosco et al., 1983). Three trials were performed and the best score was retained for analysis. The reliability for replicate tests derived from 32 players evaluated within 1 week was 0.86.

Statistical analysis

Age-related mean scores and standard deviations were calculated for the longitudinal series of training experience, anthropometric variables, RSA, aerobic endurance and lower limb explosive strength. Developmental changes in total sprint time were investigated using multilevel additive polynomial modelling (Nevill et al., 1988; Rasbash et al., 1999) (MLwiN 2.02). The technique is an extension of multiple regression analysis and is appropriate for analysing hierarchically structured data. Although measures were assessed 3 to 5 occasions, the hierarchical linear model permitted construction of the developmental model of RSA from 11 to 17 years because age ranged 11-13 years and 13-17 years, respectively at baseline and ending moment (see Table 10.1).

Multicollinearity was examined using correlation matrix and diagnostic statistics (Schroeder, 1990). Severity of multivariate multicollinearity was checked using auxiliary regression in order to compute the proportion of variability in an independent variable that was not explained by the other independent variables (tolerance). The Variance Inflation Factor (VIF) was also determined. Variables with small tolerance (≤ 0.10) and a VIF > 10 (corresponding to an R^2 of 0.90) are generally considered indicative of harmful multicollinearity, although values are somewhat arbitrary (Slinker and Glantz, 1985). In a first attempt, a regression model explored CA, SA, anthropometry, aerobic endurance and lower limb explosive strength as predictors. The incidence of a nearly perfect bivariate correlation between body mass and FFM (r = 0.96) and a very large bivariate correlation between CA and SA (r = 0.83) suggested an unacceptable multicollinearity occurrence. To avoid harmful multicollinearity body mass was discarded by the auxiliary regression and two Models of predictors were considered (Table 10.2). The Model 1 adopted CA, which was used as level 2 variance component and the Model 2 considered SA. Additionally, Pearson product moment correlation coefficients were used to control the relationship between the dependent variable and the possible explanatory variables, establishing the order of entrance in the multilevel analysis. The magnitude of correlations was interpreted as follows (Hopkins, 2000): trivial (r < 0.1), small (0.1 < r < 0.3), moderate (0.3 < r < 0.5), large (0.5 < r < 0.7), very large (0.7 < r < 0.9), and nearly perfect (r > 0.9).

Table 10.2. Diagnostic tests for multicollinearity between predictor variables.

	Model 1			Model 2	
	Tolerance	VIF		Tolerance	VIF
Chronological age	0.17	5.76	Skeletal age	0.23	4.37
Training experience	0.34	2.94	Training experience	0.48	2.09
Stature	0.13	7.85	Stature	0.13	7.65
Fat-free mass	0.11	8.96	Fat-free mass	0.11	8.40
Fat mass	0.49	2.05	Fat mass	0.47	2.12
Aerobic endurance	0.50	2.00	Aerobic endurance	0.54	1.84
Lower limb explosive strength	0.53	1.89	Lower limb explosive strength	0.53	1.90

VIF, Variance inflation factors.

Simple two-level models were defined with the repeated measures, i.e., training experience, FFM, fat mass (FM), stature, aerobic endurance and lower limb explosive strength (level 1 fixed parameters) nested within the differences between individual players (level 2). The first step was to obtain a model that fit the non-linear age-related changes. The constant and CA (Model 1) and the constant and SA (Model 2) were allowed to vary randomly between individuals (level 2). The second step in the multilevel modelling consisted of testing the inclusion a step at a time of potential explanatory variables (i.e., CA^2, anthropometry, aerobic endurance, lower limb explosive strength, and soccer experience) in order to improve the fit of the model. CA^2 and SA^2 were used because it was hypothesized that the RSA would increase most rapidly at younger ages and decelerate at older ages of the range considered; thus CA^2 and SA^2 were entered in the models to examine if the data fit a linear or quadratic pattern. Changes in the – 2 Log Likelihood (deviance) statistic indicates whether the improvement or reduction in the goodness of fit of the model was significant after the inclusion or exclusion of a predictor variable. Predictor variables were accepted as significant if the estimated mean coefficient was greater than twice the standard error of the estimate ($p<0.05$). If the retention criteria were not met, the predictor variable was discarded. The final model includes only variables that were significant independent predictors.

The validity of the RSA multilevel regression models were investigated using the correspondence of predicted and actual RSA scores of the cross-sectional independent subsample of 52 players. Paired samples t-tests were used to evaluate differences between the original and predicted RSA scores. Alpha level was set at 0.05.

10.4. Results

Descriptive data for CA, soccer experience, anthropometric characteristics and functional capacities, by age group, are described in Table 10.3. As expected, soccer experience, stature, body mass, FFM, FM and performance scores generally improved with age (lower score for RSA indicates better performance).

It was possible to obtain two significant models of contributors to explain longitudinal changes in RSA (Table 10.4). After 10 steps, the following effects were selected as significant predictors of the Model 1 (Log Likelihood = 1515.29): CA ($p<0.01$), CA^2 ($p<0.01$), aerobic endurance ($p<0.01$), lower limb explosive strength ($p<0.01$), FFM ($p=0.01$) and FM ($p<0.05$). The random slopes also improved the model ($p<0.01$) suggesting that the relationship among CA and RSA performance was not identical for all players. It was possible to obtain an equation to obtain estimates of the RSA score of young soccer players through the use of multilevel Model 1: $84.47 – 1.82 \cdot CA + 0.03 \cdot CA^2 – 0.05 \cdot$ aerobic endurance $– 0.10 \cdot$ lower limb explosive strength $– 0.09 \cdot FFM + 0.13 \cdot FM$. Improvements over time may also be predicted using multilevel modelling. By the interpretation of Model 1, soccer players' repeated-sprint time decreased by about 1.82 seconds per year (i.e., RSA improved) from 11 to 17 years of age. The multilevel Model 2 (Log Likelihood = 1513.89) included SA (<0.01), aerobic endurance ($p<0.01$), lower limb explosive strength ($p<0.01$), FFM ($p<0.01$), training experience ($p<0.01$) and FM ($p<0.05$). The Model 2 produced the following equation: $73.58 – 0.43 \cdot SA – 0.05 \cdot$ aerobic endurance $– 0.10 \cdot$ lower limb explosive strength $– 0.08 \cdot FFM – 0.45 \cdot$ training experience $+ 0.13 \cdot FM$.

Table 10.3. Mean scores (± *SD*) for chronological age, soccer experience, anthropometric characteristics, repeated-sprint ability, aerobic endurance and lower limb explosive strength and frequencies of skeletal maturity status.

	units	11 years (n = 40)	12 years (n = 57)	13 years (n = 83)	14 years (n = 80)	15 years (n = 66)	16 years (n = 30)	17 years (n = 10)
Chronological age	years	11.58 ± 0.29	12.56 ± 0.30	13.66 ± 0.32	14.66 ± 0.3	15.67 ± 0.34	16.74 ± 0.33	17.64 ± 0.33
Training experience	years	2.50 ± 0.85	3.46 ± 1.15	4.46 ± 1.07	5.49 ± 1.08	6.55 ± 1.14	7.30 ± 1.39	8.20 ± 1.14
Stature	cm	143.1 ± 6.0	149.3 ± 7.1	158.0 ± 8.1	164.9 ± 7.6	169.9 ± 6.4	172.5 ± 5.2	173.7 ± 4.2
Body mass	kg	36.4 ± 5.3	40.5 ± 6.4	47.4 ± 8.6	53.7 ± 8.5	59.9 ± 8.6	64.4 ± 9.6	68.0 ± 9.4
Fat-free mass	kg	32.2 ± 3.8	35.6 ± 4.9	41.4 ± 6.5	46.7 ± 6.4	51.4 ± 5.4	54.2 ± 5.6	56.8 ± 6.1
Fat mass	kg	4.2 ± 2.4	4.9 ± 2.7	6.1 ± 3.5	7.0 ± 3.6	8.6 ± 4.3	10.2 ± 5.3	11.3 ± 4.5
Sum of 7 sprints	s	62.11 ± 3.41	59.18 ± 3.13	57.63 ± 3.12	55.11 ± 2.32	53.74 ± 2.19	52.02 ± 2.44	51.41 ± 2.19
Sprint decrement	%	5.54 ± 2.87	4.09 ± 1.75	3.85 ± 1.93	3.49 ± 1.62	3.74 ± 1.89	3.83 ± 2.08	2.83 ± 1.13
Aerobic endurance	m	680 ± 360	960 ± 360	1140 ± 320	1320 ± 380	1520 ± 320	1620 ± 220	1720 ± 120
Lower limb explosive strength	cm	25.6 ± 4.2	27.8 ± 5.0	30.6 ± 5.3	32.9 ± 5.0	35.3 ± 4.7	37.3 ± 5.6	35.9 ± 2.6
Skeletal maturity status								
Late	f	6	9	11	11	7	4	0
On Time	f	23	33	48	45	38	16	6
Early	f	11	15	24	24	21	10	4

Table 10.4. Multilevel regression models for the repeated-sprint ability aligned by chronological age and by skeletal age.

Step	Fixed Effects	Model 1 (aligned by CA)				Model 2 (aligned by SA)			
		Log Likelihood	p	At final step: Coefficient	At final step: SE	Log Likelihood	p	At final step: Coefficient	At final step: SE
1	Intercept (constant)	2065.16		84.47	6.62	2065.16		73.58	1.18
2	Chronological age	1604.11	< 0.01	– 1.82	1.22				
3	Chronological age^2	1595.37	< 0.01	0.03	0.03				
2	Skeletal age					1661.47	< 0.01	– 0.43	0.13
3	Skeletal age^2					1661.07	0.53	NE	
5	Aerobic endurance	1551.29	< 0.01	– 0.05	0.01	1569.20	< 0.01	– 0.05	0.01
6	Lower limb explosive strength	1529.26	< 0.01	– 0.10	0.03	1539.43	< 0.01	– 0.10	0.03
7	Fat-free mass	1522.64	= 0.01	– 0.09	0.03	1532.46	< 0.01	– 0.08	0.03
8	Training experience	1521.46	0.28	NE		1525.71	< 0.01	– 0.45	0.13
9	Stature	1521.27	0.24	NE		1525.49	0.64	NE	
10	Fat mass	1515.29	< 0.05	0.13	0.05	1513.89	< 0.01	0.16	0.05
	Random Effects			Variance	SE			Variance	SE
	Residual variance (level 1)			2.22	0.21			2.37	0.22
	Intercept slope variance (level 2)			35.79	15.04			12.73	9.10
	Deviance from the empty model			549.87				551.27	
	p < 0.01								

SE, standard error; NE, not entered.

Real measured (RSAr) and predicted (RSAp) mean scores were plotted by CA and SA in Figure 10.1. RSAr fluctuates above and below predicted values, improving from 11 to 17 years. However, after 15 years of age small improvements were noted (panel a, left part). By plotting mean scores of RSAp across age by skeletal maturity status (Figure 10.2), it was possible to identify significant differences between contrasting maturity groups: 13 years (early > on time > late, $p<0.05$) and 14 years (early = on time > late, $p<0.05$).

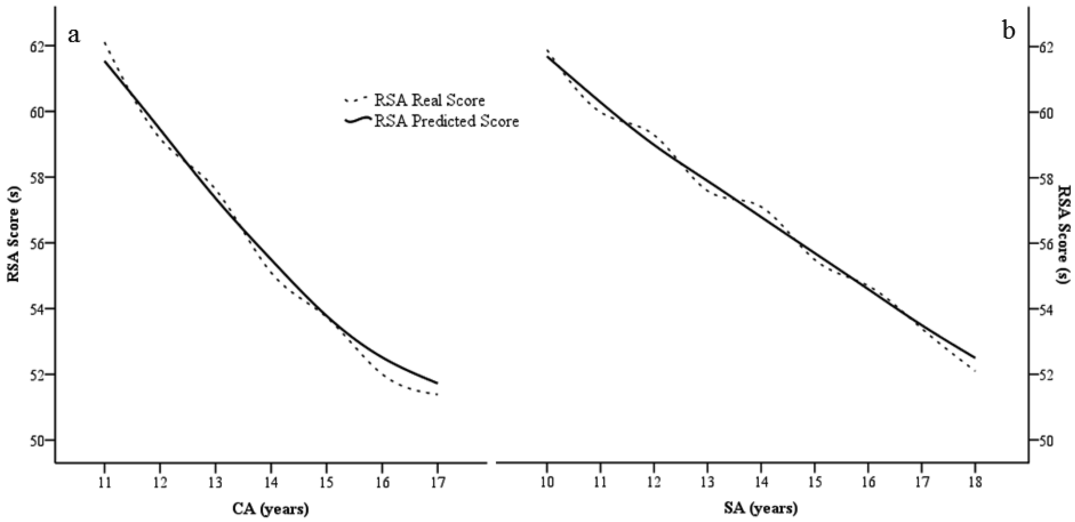

Figure 10.1. Real and estimated RSA scores aligned by chronological age (panel a) and skeletal age (panel b) in young soccer players.

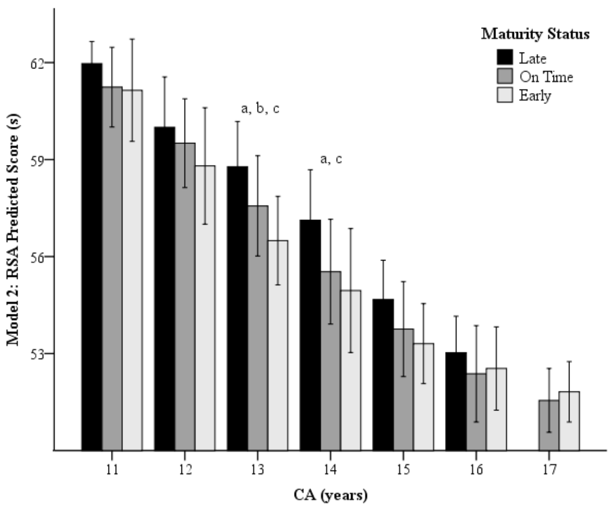

Figure 10.2. Model 2 estimated RSA scores (± *SD*) by maturity groups aligned by chronological age. a: significant difference Late vs. On Time ($p<0.05$), b: On Time vs. Early, c: Early vs. Late maturing players.

Cross-validation of the RSA equations in the independent control group is summarized in Table 10.5. Allowing for variation in sample sizes within CA groups, RSAr and RSAp of players did not significantly differ for the independent 52-players subsample.

Table 10.5. Validity of the multilevel regression models based on the difference between real scores and predicted scores for the repeated-sprint ability of the control group.

	Control group		Mean difference (s)	t (p)
	RSAr (s)	RSAp (s)		
Model 1 : aligned by CA	56.83 ± 3.52	56.81 ± 3.54	0.02 ± 0.56	0.23 (n.s.)
Model 2 : aligned by SA	56.83 ± 3.52	56.93 ± 3.64	– 0.11 ± 0.62	– 1.27 (n.s.)

RSAr, Repeated-sprint ability real measured mean scores; RSAp, Repeated-sprint ability predicted mean scores; n.s., not significant.

10.5. Discussion

Studies of youth athletes often characterize samples as prepubertal, pubertal and postpubertal. Stages of puberty, though useful, have limitations (Sherar et al., 2004). Secondary sex staging does not inform when or for how long the youngster entered the stage. Although biological maturation is a continuum process, stages are discrete categories and, not surprisingly, age at transition from one stage to another is difficult to determine. Prepubertal simply indicates that the players did not present manifestations of pubic hair (the most popular indicator in the literature) at the time of observation. Prepubertal boys, however, vary in biological maturation and consequently skeletal is claimed as the best indicator. To our knowledge the present study provides a singular contribution in the analysis of development changes of repeated-sprint ability taking into account skeletal maturation.

Previous longitudinal studies with circumpubertal school boys have highlighted the influence of changes in stature and body mass on mean and peak power assessed by the Wingate test (Armstrong and Welsman, 2000; Falk and Bar-Or, 1993). A subsequent study (De Ste Croix et al., 2001) confirmed the influence of stature and mass in the development of mean and peak power in 15 males and 19 females (aged 10-12 years) who were measured twice. However, when sum of skinfolds was considered to explain changes in peak and mean power, the effect of age remained significant, the effect of stature was negated but the mass term increased. In the present study, stature was not selected to enter in the multilevel models in contrast to estimated FFM and FM (Table 10.4). In addition, upper body peak anaerobic power and mean anaerobic power did not consistently differ among early-maturing, average-maturing and late-maturing girls followed from 11 to 14 years of age (Little et al., 1997). However, when expressed per unit body mass, peak and mean power tended to be slightly greater in late-maturing girls from 11 to 13 years of age. Given the importance of body size and muscle mass in short-term power outputs, the maturity effects are probably mediated through the influence of maturity status on body size and muscle mass. The mentioned studies reported healthy non sporting samples.

Comparisons of players classified late, on time or early maturing on the basis of skeletal maturation (difference between SA and CA) provide insights into the implications of maturity-associated variation in body size and performance among adolescent soccer players. Portuguese adolescent players aged 13-14 years attained significantly better mean scores than players 11-12 years on the seven sprints, 8.06 and 8.79 s, respectively (Figueiredo et al., 2009b). This research in youth soccer was based on cross-sectional data. An overview of the timing in strength and motor performance relative to peak height velocity (PHV) and peak weight velocity (PWV) is given elsewhere (Malina et al., 2004a). Because PWV follows PHV, it is obvious that maximum velocities in running speed (shuttle-run), speed of limb movement (plate tapping), and flexibility (sit-and-reach) also precede PWV. Longitudinal observations for Belgian youth soccer players indicated peak gains in explosive strength and running speed close to the age at peak height velocity (Philippaerts et al., 2006). Of interest, estimated annual increments in tests of explosive strength and running speed in the soccer players decline immediately after PHV but then maintained at a plateau in contrast to declining increments noted in vertical jump and shuttle-run increments observed in an earlier longitudinal study of Belgian boys using a similar modelling protocol (Beunen and Malina, 1988). Although the tests were not identical, the plateau observed after PHV in soccer players probably reflects the influence of systematic training in the sport.

The overall trend for the body size of young soccer players suggested an appropriate body mass-for-stature during childhood and early adolescence, but more body mass-for-stature in later adolescence. The proportionally high body mass-for-stature probably reflects a larger fat-free mass (FFM), specifically muscle mass. Mean relative fatness (densitometry, total body water) of elite young adult soccer players in four studies range from 6.2±1.9% to 9.7±3.0% (Malina, 2007). Players who attained better performances in sprinting tests tended to possess lower percentage of body fat (Reilly et al., 2000), and simultaneously more percentage lean body mass. Greater lean body mass also means more muscle mass, and most likely more strength, which has an influence on change-of-direction speed over short distances (Negrete and Brophy, 2000). The present study illustrated that the estimated amounts of FFM and FM significantly improved the fitness of the models. From 11 to 17 years, each unit of FFM (in kg) corresponds to an improvement of 0.09 and 0.08 seconds in RSA, considering the Model 1 and 2, respectively. Age and skeletal age seemed to be other relevant predictors of RSA. They appear independent from the effect of body size in contrast with other cross-sectional evidences that emphasized stature and body mass as predictors of inter-individual variability in RSA (Mujika et al., 2009).

Time-motion analyses using a global positioning system were used on trained young soccer players during international games (Buchheit et al., 2010c). Sprint activities were defined as at least a 1-s run at intensities higher than 61% of the individual peak running velocity and repeated-sprint sequences (RSS), as a minimum of two consecutive sprints interspersed with a maximum of 60 seconds. RSS occurrence varied by playing position and tended to decrease throughout the game. A trend for higher frequencies of RSS activities was noted in under-13 compared to under-17 and under-18. A recent study on sport selection classified under-14 soccer players as local and regional elite (Coelho-e-Silva et al., 2010). Top players classified as advanced in skeletal maturation were also heavier, taller, performed better in explosive lower limb strength and in the repeated-sprint test. Another study did a 2-year follow-up analysis and compared the growth, maturity status, functional capacities and sport specific skills of adolescent players who either discontinued competitive soccer, or continued and moved up to a higher level (Figueiredo et al., 2009a). At baseline, differences between groups were significant for most functional capacities (speed, sum of seven sprints, agility shuttle-run, intermittent endurance run and vertical jumps) in both 11-to 12-years-old and 13-to 14- years-old age groups. In the RSA, the best scores were obtained by the players who moved up. Longitudinal changes in RSA considering skeletal age is lacking and the underlying mechanisms involved in improvements are unclear. After obtaining two multilevel models to predict developmental changes in the RSA, the current study illustrated that when data was aligned by chronological age, the most marked improvements occurred from ages 11 to 15 years (14.6%). After that age, the improvement rate was substantially reduced (about 2%).

The distinction between age and training effects on functional capacities is complex. Based on a small sample (7 players), the effect of a 10-week specific training with one session per week of 2-3 sets of 5-6 x 15-20-m shuttle sprints interspersed with 14-23 seconds recovery produced a beneficial impact on maximal sprinting speed and repeated shuttle sprint performance (Buchheit et al., 2010a). Research considering additional aspects that contribute to explain development changes of repeated-sprint ability is needed. Among potential predictors are training volume and intensity, playing time, type of conditioning methods, and habitual physical activity in the daily life of athletes. The importance of endurance training programs on repeated-sprint performance and post-sprints muscle re-oxygenation rate was confirmed in a eight-week intervention comprising 18 moderately trained adult males (Buchheit and Ufland, 2011). Another interventional study conducted with elite adolescent soccer players (Buchheit et al., 2010a) exposed two independent groups to explosive strength (EST) training and repeated shuttle sprint training (RSST), respectively. RSST and EST were equally efficient at improving the best of twelve 15-m sprint protocols departing every 20 seconds, while RSST tended to enhance the total time. Therefore, repeated-sprint ability is a compound quality believed to be affected by factors such as maturation, neuromuscular determinants and metabolic efficiency (Glaister, 2005).

The current study confirmed the trend for under-representation of boys classified as delayed boys in late adolescence as previously noted in a larger cross sectional sample (Malina et al., 2000) and suggested the disappearance of differences between soccer players classified as on time and advanced at ages 15-17 years. The literature often suggest that late maturing boys may be encouraged to participate in others sports and in the context of soccer they may receive less opportunities to practice and play (Malina et al., 2000). Relative age effect (RAE) presents a similar trend (Vaeyens et al., 2005). Athletes, families and coaches often ignore that the effect of maturation and RAE correspond to transitory advantages at the initial phase of the long-term preparation. Data from the Football Association’s School of Excellence showed that the distribution of 103 boys recruited over a period of 6 years revealed 67% of the participants born in the first quarter and 89% in the first half. This unequal distribution persisted into seniors (Armstrong and McManus, 2011). A comprehensive approach to talent identification and selection in youth soccer is offered by the Ghent Youth Soccer Project (Vaeyens et al., 2006). The study considered the contribution of body size, adiposity, EUROFIT tests, sprinting ability and soccer-specific skills to discriminate youth players by competitive level. Discriminating variables varied between early (U-13, U-14) and late (U-15, U-16) adolescents. Skeletal and chronological ages (SA, CA, respectively) of youth players 11-12 years overlap considerably, whereas SA is, on average, in advance of CA in adolescent players 13-16 years. Moreover, during late adolescent years, players who were delayed in SA were under-represented while players classified as advanced were over-represented (Malina et al., 2004b). The potential interactions among growth, functional and sport-specific skill characteristics on one hand, and biological maturation on the other hand, among adolescent soccer players in talent development and selection programs needs further investigation. Available data are mostly cross-sectional and have limitations for long-term developmental programs.

The present study assessed the development of individual players, thus improving our understanding of the factors that contribute to performance development. The study has a substantial number of measurements (on average 4.4

measurements per player) and gives a satisfactory illustration of the development of RSA considering biological maturation. Multilevel modelling provided performance curves that allow coaches to evaluate annual progresses relative to estimated improvements (given by the curves). Additionally, it was also possible to assess the validity of the multilevel model by checking the difference between the predicted and real RSA scores (using an independent group). Another recent study examined the longitudinal development of endurance capacity in a sample of 130 talented Dutch soccer players aged 14-18 who attained professional and amateur level in adulthood (Roescher et al., 2010). Players who reached the professional league showed a differential development pattern compared to their counterparts. The study also noted a direct relationship between endurance capacity and time spent in soccer activities (training plus independent practice).

10.6. Conclusions

In summary, the present study identified the importance of chronological age, skeletal maturation, two-component of body mass in combination with aerobic endurance and lower limb explosive strength as predictors of developmental changes in total time derived from a 7-sprint protocol. The equations are useful analytical tools in the interpretation of progresses across adolescent years. Assessment of deviances between expected and real performances estimated by CA and SA may provide a better understanding of individual responsiveness to training in youth soccer.

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Chapter XI

Study 9

Modelling developmental changes in functional capacities and soccer-specific skills in male players aged 11-17 years

11. Modelling developmental changes in functional capacities and soccer-specific skills in male players aged 11-17 years

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Journal: Valente-dos-Santos J, et al. 2012. Modelling developmental changes in functional capacities and soccer-specific skills in male players aged 11-17 years. Pediatr Exerc Sci 24:603–621.
PMID: 23196767

11.1. Abstract

This study evaluates the contributions of age, growth, skeletal maturation, playing position and training to longitudinal changes in functional and skill performance in male youth soccer. Players were annually followed over 5 years (*n* = 83, 4.4 measurements per player). Composite scores for functional and skill domains were calculated in order to provide an overall estimate of performance. Players were also classified by maturity status and playing position at baseline. After testing for multi-collinearity, two-level multilevel (longitudinal) regression models were obtained for functional and skill composite scores. The scores improved with age and training. Body mass was an additional predictor in both models [functional (late maturing): 13.48 + 1.05 x centered on chronological age (CA) – 0.01 · centered CA^2 – 0.19 · fat mass (FM) + 0.004 · annual volume training – 1.04 · dribbling speed; skills (defenders): 7.62 + 0.62 · centered CA – 0.06 · centered CA^2 + 0.04 · fat-free mass – 0.03 · FM + 0.005 · annual volume training – 0.19 · repeated-sprint ability + 0.02 · aerobic endurance]. Skeletal maturity status was a significant predictor of functional capacities and playing position of skill performance. Sound accuracy of each multilevel model was demonstrated on an independent cross-sectional sample (n=52).

Keywords: Maturation · Skeletal Age · Playing Position · Multilevel Modelling

11.2. Introduction

Soccer combines running activities with ball skills such as passing, dribbling, heading and shooting. Match-performance analyses in youth soccer demonstrate position-associated variation in covered distance and duration and frequency of high-intensity running (Buchheit et al., 2010; Stroyer et al., 2004). Prediction of performance is of prime interest to many of those involved in the development of young athletes. Talent identification often emerged from the observation of games, in which young soccer players of similar age compete. This strategy can be misleading and does not take into account the inter-individual variability in biological maturation and its potential impact on performance. During adolescence, there is considerable variation in size and performance due to inter-individual differences in biological maturation. Players who were classified 'advanced' in maturation tended to be taller, heavier, stronger, more powerful and faster than 'delayed' players (Coelho-e-Silva et al., 2010; Figueiredo et al., 2011; Malina, 2011). Some advantages such as body size are only temporary (Malina et al., 2005). The effect of maturation and body size on soccer-specific skills are less documented and the trend is not as consistent as it is for studies devoted to functional capacities (Figueiredo et al., 2009a, Malina et al., 2007).

Among under-14 (U-14) Portuguese male soccer players (Coelho-e-Silva et al., 2010), playing position (i.e., defenders, midfielders and forwards) was not a relevant source of variability. However, dribbling performance was a relevant variable to identify the best Dutch players during adolescent years (Huijgen et al., 2009). In contrast to the lack of variation by playing position in the skill field tests, functional capacities tend to vary among different playing positions in youth soccer (Reilly et al., 2000). Goalkeepers and central defenders are taller and heavier than full-backs, midfielders and forwards, but midfielders attained the highest values in maximal oxygen uptake and the best performance in the intermittent shuttle-run (Reilly et al., 2000). The preceding evidence was based on cross-sectional data and it is not clear whether positional differences in functional capacities are due to selection or emerged from training and playing in a specific position.

Developmental changes in functional and skill characteristics are still limited in the literature that typically does not consider variation by playing position and the interaction between growth, biological maturation, fitness and skills. Accordingly, this study evaluates the longitudinal changes in several functional and soccer-specific skills of male youth soccer players that can be described as a developmental pool for potential talent identification. The aims of the current study are: First, to document the characteristics of young soccer players aged 11-17 years. Second, to identify the longitudinal predictors of functional capacities and skills. Finally, to examine developmental curves by maturity status and playing position.

11.3. Methods

Participants

The research proposal was approved by the Scientific Committee of the University of Coimbra and also by the Portuguese Foundation for Science and Technology [PTDC/DES/121772]. The Portuguese Soccer Federation and clubs were contacted and institutional agreements were signed between the University of Coimbra and the sport organizations to assure 5-year data collection. The guardians of the young athletes provided informed consent and players provided their assent.

Goalkeepers were not included since they complete different tasks compared to outfield players who were classified at baseline as defenders, midfielders and forwards (Coelho-e-Silva et al., 2010; Gil et al., 2007; Huijgen et al., 2010). The study included 135 players from the Midlands of Portugal, divided into two groups. A subsample of 83 players [defenders, n = 35; midfielders, n = 27; forwards, n = 21 (playing position); late, n = 11; on time, n = 48; early, n = 24 (maturity groups)] aged 11-13 years at baseline who were followed on an annual basis over 3 to 5 years (mixed-longitudinal). A total of 366 observations (4.4 observations per player) were available. An independent cross-sectional sample of 52 players aged 11-17 years was also measured on one occasion to test the statistical fit of the model derived from the mixed-longitudinal data.

Training history

The clubs were involved in a 9-month competitive season (September-May) regulated by the Portuguese Soccer Federation. Under 13 (11-12 years) and Under 15 players (13-14 years) had ~ 3 training sessions per week (~ 90 $min \cdot session^{-1}$) and one game, usually on Saturdays. Under 17 (15-16 years) and Under 18 players participated in 3-5 training sessions per week (90-120 $min \cdot session^{-1}$) and one game per week. All players competed in national level and had a minimum of 2 years of prior soccer-specific training, at baseline. Years of formal participation in the sport was obtained through the publically available Portuguese Soccer Federation records and confirmed by interview at clubs. Volume of training (number of session and minutes) was individually recorded on a weekly basis.

Chronological age, skeletal age and skeletal maturity

Chronological age (CA) was calculated as the difference between date of birth and date of the hand-wrist radiograph. The posterior-anterior x-ray exam of the left hand-wrist were taken. All films were assessed by a single observer following the Fels method for skeletal age (SA) determination (Roche et al., 1988). The protocol assigns grades to specific maturity indicators for the radius, ulna, carpals, metacarpals plus phalanges of the first, third and fifth rays (metacarpal and phalanges) and utilizes ratios of linear measurements of the widths of the epiphysis and metaphysis of the long bones. The presence (ossification) or absence of the pisiform and adductor sesamoid is also rated. Grades and ratios are entered into a program (Felshw 1.0 Software, Lifespan Health Research Center, Departments of Community Health and Pediatrics, Boonshoft School of Medicine, Wright State University, Dayton, Ohio) to derive a SA. The statistical protocol weighs the contributions of specific indicators, depending on CA and sex, in calculating a SA and its standard error of estimate (a confidence interval for the assessment). The standard error is an unique feature of this protocol. Standard errors slightly increased with age (11–12 years: 0.27–0.32; 13–14 years: 0.27–0.49; 15–17 years: 0.28–0.72) because at later ages the assessment is based on less indicators (Malina et al., 2010; Roche et al., 1988).

Twenty radiographs were independently assessed by the single observer who was trained by an experienced assessor. The mean difference between SA assessments of the two observers and the inter-observer technical error of measurement were small, respectively 0.03 ± 0.99 years. The total number of indicators involved in the assessment of the 20 films was 988 and disagreement between assessors occurred on 48 occasions (all disagreements were by one grade or by 0.5 mm for metaphyseal and epiphyseal widths).

At baseline, difference between SA and CA (SA-CA) was used to classify players similarly to previous studies (Malina, 2011; Malina et al., 2012): late (or delayed) when SA younger than CA by > 1.0 yr; average (or 'on time') when SA ± 1.0 yr CA; and early (or advanced) when SA older than CA by > 1.0 yr.

Anthropometry

A single observer measured stature, body mass, and four skinfolds (triceps, subscapular, suprailiac, medial calf) following standard procedures (Lohman et al., 1988). Stature was measured to the nearest 0.1 cm with a Harpenden stadiometer (model 98.603, Holtain Ltd, Crosswell, UK) and body mass was measured to the nearest 0.1 kg with a SECA balance (model 770, Hanover, MD, USA). Skinfolds were measured to the nearest mm using a Lange caliper (Beta Technology, Ann Arbor, MI, USA). Technical errors of measurement for stature (0.3 cm), body mass (0.5 kg), and skinfolds (0.5-0.7 mm) were well within the range of several health surveys in the United States and a variety of field surveys (Malina et al., 2004a). Body fat (FM) was estimated from triceps and subscapular skinfold thicknesses (Slinker and Glantz, 1985). Fat-free mass (FFM) was derived in kg.

Functional capacities

The repeated-sprint ability was assessed using the 7-sprint protocol, also called the Bangsbo Sprint Test (Bangsbo, 1994). The time for each sprint was recorded with a digital chronometer connected to photoelectric cells (Globus Ergo Timer Timing System, Codogné, Italy) to a resolution of 0.01 s. The photocells were positioned 0.8 m above the floor, which typically corresponded to the hip level. The first pair was positioned along the starting line and the second pair along the finish line. The trials were initiated after the exact end of the recovery interval with the subject positioning the lead foot 0.3 m behind the starting line. Performance was expressed as the sum of the seven sprints.

Agility was assessed using the 10 x 5 agility shuttle-run test (Council of Europe, 1988). The time for each shuttle was also recorded with a digital chronometer connected to photoelectric cells following the same procedures of the repeated-sprint ability test. Time was recorded by the split gate placed on the baseline where athletes changed directions in the shuttle-runs.

The lower limb explosive strength was assessed with the vertical counter-movement jump using the ergo-jump protocol (Bosco et al., 1983). Two trials were administered for the agility and jump tests and the best score was retained for analysis.

Aerobic endurance was measured with the 20-m multi-stage continuous shuttle endurance test (Léger et al., 1988). Participants were familiar with the protocol because the test is included in the Portuguese physical education curriculum as part of the FITNESSGRAM battery.

Test-retest protocols of the functional capacities (one week apart) were performed with 32 players. The 95% limits of agreement (LOA) where adopted to examine the test-retest agreement (Bland and Altman, 1986; Nevill and Atkinson, 1997). A visual inspection of Bland-Altman plots (Bland and Altman, 1986) showed that measurement differences (error) against the respective means where homoscedastic (Nevill and Atkinson, 1997). This was confirmed by the correlation coefficients between the absolute differences and the corresponding means (Nevill and Atkinson, 1997). The test-retest LOA were –5.04 to 6.01 s (r = 0.10, p = 0.60) for repeated-sprint ability, –1.46 to 2.25 s (r = 0.05, p = 0.78) for agility shuttle-run, –6.42 to 3.56 cm (r = –0.08, p = 0.66) for lower limb explosive strength and –12.14 to 8.52 (r = 0.18, p = 0.32) for number of laps (i.e. aerobic endurance).

Soccer-specific skills

Four tests of soccer-specific skills were administrated: ball control with the body, without using the arms or hands, dribbling speed, shooting accuracy (Federação Portuguesa de Futebol, 1986) and wall pass (Kirkendall et al., 1987).

In brief, ball control (Federação Portuguesa de Futebol, 1986) was tested within a 9 x 9 m square. The player had to keep the ball in the air without using the arms or hands. The score was the number of hits of the ball before it fell to the floor.

For the dribbling speed test (Federação Portuguesa de Futebol, 1986), a cone was placed on each corner of the 9 x 9 m square. A fifth cone was placed midway (4.5 m) on the line of the square where the test began. Beginning at one corner, the participant had to move the ball with the feet (dribble) around the three cones (corner directly opposite the starting cone, the cone placed midway and the cone diagonally opposite the starting cone) in slalom fashion, and then dribble the ball into the fifth cone (i.e., not with a pass). The objective was to complete the drill in the fastest time possible by controlling the ball only with the feet. The time for each trial was recorded with the photoelectric cells. The first pair of the photocells was positioned along the starting line and the second pair along the finish line.

Shooting accuracy (Federação Portuguesa de Futebol, 1986) was measured in five attempts at kicking the ball at a 2 x 3 m goal located at the end line of a 9 x 9 m square. The target was divided by ropes into six sections. One rope was placed horizontally between the posts at a height of 1.5 m. Two ropes were dropped from the crossbar, 0.5m from each post. Five points were allocated for the upper right and left sections, and two for the upper middle section. Three points were allocated for the lower right and left sections, and one for the lower middle section. Kicks were attempted with the player standing outside of the square at the line opposite the goal. The maximum score was 25 points. The test was recorded and scored subsequently.

The wall pass test (Kirkendall et al., 1987), involved a 1.22m high (starting from the floor) and 2.44m wide target drawn on a flat wall. At a distance of 1.83m from the marked wall, an area of 1.83m in length and 4.23m in width was marked on the floor. The player had to remain in this area. The test consisted of making as many passes to the wall in 20 s. Players could use all body parts except the hands to make a pass to the wall. The best of two trials of each test was retained for analysis.

Replicates were obtained from 32 players within 1 week and the test-retest agreement for the soccer-specific skills were determined (Bland and Altman, 1986; Nevill AM and Atkinson, 1997). The test-retest LOA were –10.28 to 5.78 **(**r = 0.29, p = 0.11) for number of hits (i.e. ball control), –0.64 to 2.16 s (r = 0.15, p = 0.40) for dribbling speed, –4.79 to 2.42 points (r = –0.21, p = 0.26) for shooting accuracy and –4.19 to 3.06 (r = –0.17, p = 0.36) for number of passes.

Statistical analysis

Means and standard deviations were calculated for the repeated measures CA, SA, of training history, anthropometrics, functional capacities and soccer-specific skills. Frequencies of players in each skeletal maturity status category (late, on time, early) and playing position (defenders, midfielders and forwards) were summarized in each age group. Each functional and skill test was converted to a z-score; z-scores were reversed for timed items, since lower scores correspond to better performances. The respective z-scores were summed to provide composite scores of the functional capacity and skill of each player.

Multi-collinearity was examined for the two Models of predictors (Model 1: potential predictors of the functional composite scores; Model 2: potential predictors of the skill composite scores), using correlation matrix and diagnostic statistics (Schroeder, 1990). Variables with small tolerance (≤ 0.10) and a VIF > 10 (corresponding to an R^2 of 0.90) are generally considered indicative of harmful multi-collinearity (Slinker and Glantz, 1985). The incidence of a nearly perfect bivariate correlation between body mass and FFM (r=0.96) suggested an unacceptable multi-collinearity occurrence. To avoid harmful multi-collinearity body mass was discarded by the auxiliary regression.

For the longitudinal analyses, hierarchical (multilevel) random-effects model was constructed using a multilevel modelling approach (MLwiN 2.02). Multilevel modelling effectively captures the feature that the variance of the observations increases with time, and permits individual slopes and intercepts. It thus provides the opportunity to determine the effects of each predictor variable on the slope and intercept and its significance can be determined by relating the observed effects to the respective change in standard errors (Baxter-Jones et al., 1993). Thus group effects larger than within-individual variation can be identified.

Longitudinal studies often deal with missing data and experience dropout occurrences. In the multilevel model technique, the number of observations and temporal spacing between measurements can vary among subjects. All available data can thus be incorporated into the analysis. It is assumed that the probability of being missing is independent of any of the random variables in the model. As long as a full information estimation procedure is used, such as maximum likelihood in *MLwiN* for Normal data, the actual missing mechanism can be ignored (Rasbash et al., 1999).

Detailed description of multilevel modelling and complete details of this approach are presented elsewhere (Baxter-Jones and Mirwald, 2004). Functional and skill composite scores were modeled within an additive polynomial model (Rasbash et al., 1999). In a first attempt, the constant and CA were allowed to vary randomly between individuals (Level 2). However, CA dramatically increased the parameter estimate of variance at Level 2, around the between-individuals intercept. This is because all individuals have different developmental performance trajectories. The intercept for each individual's line is the height of that line at $x = 0$. Since individuals were not measured at CA = 0 the model extrapolated the interceptions of developmental trajectories with y axis. Since participants were measured between the 11 and 17 years and taking into account a substantial inter-individual variability in the trajectories, extrapolated lines at CA=0 may reflect excessive variance (Baxter-Jones and Mirwald, 2004). Consequently, the technique would be estimating the variance of the intercepts at an age that never occurred in the sample. To overcome this problem, it was decided to shift the origin of the explanatory random variable (CA) by centering on its mean value (i.e., 14.21 years).

Subsequently, the inclusion of predictors in their raw measurements was tested in order to improve the statistical fit of the multilevel models. A predictor is included in the model if the respective estimated mean coefficient is greater than twice the standard error of the estimate. The final model only includes significant independent predictors. To allow for the nonlinearity of the functional and skill performance development, age power functions (i.e., centered CA^2) were introduced into the linear model (Baxter-Jones et al., 1993).

Agreement between real and predicted scores was tested using the cross-sectional sample of 52 players (Bland and Altman, 1986; Nevill and Atkinson, 1997). First, statistical differences between real and predicted scores were examined using dependent *t*-tests. Second, data was visually inspected by plotting the errors against the mean calculated from real and estimated values using the Bland–Altman plot (Bland and Altman, 1986). Third, if errors were associated with the magnitude of the values, heteroscedasticity was examined by calculating the correlation coefficients between the absolute differences and the corresponding mean. Finally, if measurement differences were normally distributed, reliability was assessed on the original scale using the standard error of measurement (SEM) and 95% LOA (Bland and Altman, 1986; Nevill and Atkinson, 1997). This procedure was performed using GraphPad Prism software (GraphPad Software, Inc.; La Jolla, CA, USA). Alpha level was set at 0.05.

11.4. Results

Age, training history, anthropometry, functional, skills, maturity and playing position-related characteristics, by age group, are described in Table 11.1. As expected, all scores generally improved with age (lower scores for time elapsed tests correspond to better performances). The majority of players in all age groups were classified as “on time” (or average) in skeletal maturation. The proportion of players classified as advanced (early) increased from early adolescent to older age groups.

In contrast, the proportion of players classified as delayed (late) decreased with age. Classification by playing position show a slightly higher frequency of defenders compared to midfielders and forwards that tends to diminish with increasing age.

Table 11.1. Mean scores (± *SD*) for chronological age, skeletal age, training history, anthropometric characteristics, functional capacities and soccer-specific skills and frequencies of skeletal maturity status and playing position.

	Units	11 years (*n* = 40)	12 years (*n* = 57)	13 years (*n* = 83)	14 years (*n* = 80)	15 years (*n* = 66)	16 years (*n* = 30)	17 years (*n* = 10)
Chronological age	y	11.6 (0.3)	12.6 (0.3)	13.7 (0.3)	14.7 (0.3)	15.7 (0.3)	16.7 (0.3)	17.6 (0.3)
Skeletal age	y	11.8 (1.0)	12.7 (1.2)	14.0 (1.1)	15.0 (1.1)	16.3 (0.7)	17.1 (0.3)	17.7 (0.2)
Skeletal maturity status								
Late	f	6	9	11	11	7	4	0
On Time	f	23	33	48	45	38	16	6
Early	f	11	15	24	24	21	10	4
Playing position								
Defenders	f	19	24	35	35	28	12	4
Midfielders	f	12	19	27	26	23	10	3
Forwards	f	9	14	21	19	15	8	3
Years of practice	y	2.5 (0.9)	3.5 (1.2)	4.5 (1.1)	5.5 (1.1)	6.6 (1.1)	7.3 (1.4)	8.2 (1.1)
Annual volume training	h	111.7 (25.8)	141.7 (33.2)	147.7 (52.5)	167.1 (49.2)	158.8 (48.7)	185.2 (46.8)	177.8 (40.8)
Stature	m	1.43 (0.06)	1.49 (0.07)	1.58 (0.08)	1.65 (0.08)	1.70 (0.06)	1.73 (0.05)	1.74 (0.04)
Body mass	kg	36.4 (5.3)	40.5 (6.4)	47.4 (8.6)	53.7 (8.5)	59.9 (8.6)	64.4 (9.6)	68.0 (9.4)
Fat-free mass	kg	32.2 (3.8)	35.6 (4.9)	41.4 (6.5)	46.7 (6.4)	51.4 (5.4)	54.2 (5.6)	56.8 (6.1)
Fat mass	kg	4.2 (2.4)	4.9 (2.7)	6.1 (3.5)	7.0 (3.6)	8.6 (4.3)	10.2 (5.3)	11.3 (4.5)
Repeated-sprint ability	s	62.11 (3.41)	59.18 (3.13)	57.63 (3.12)	55.11 (2.32)	53.74 (2.19)	52.02 (2.44)	51.41 (2.19)
Agility shuttle-run	s	20.76 (1.02)	20.24 (1.11)	19.25 (1.01)	18.57 (0.81)	18.28 (0.88)	17.69 (1.02)	18.18 (0.98)
Lower limb explosive strength	cm	25.6 (4.2)	27.8 (5.0)	30.6 (5.3)	32.9 (5.0)	35.3 (4.7)	37.3 (5.6)	35.9 (2.6)
Aerobic endurance	m	680 (360)	960 (360)	1140 (320)	1320 (380)	1520 (320)	1620 (220)	1720 (120)
Functional composite		–4.89 (2.39)	–2.76 (2.40)	–0.76 (2.39)	1.15 (2.17)	2.54 (1.19)	3.98 (1.84)	3.77 (1.34)
Ball control, juggling	# hits	17 (16)	32 (23)	47 (49)	79 (71)	129 (116)	176 (138)	163 (151)
Dribbling speed	s	15.97 (1.99)	14.59 (1.26)	13.59 (1.14)	12.90 (0.60)	12.48 (0.62)	11.96 (0.51)	11.88 (0.67)
Shooting accuracy	points	6 (2)	8 (2)	9 (3)	9 (3)	10 (4)	10 (3)	10 (5)
Passing	# passes	18 (3)	20 (3)	21 (2)	22 (3)	23 (2)	24 (2)	24 (2)
Skill composite		–4.19 (2.48)	–2.03 (1.80)	–0.40 (1.86)	0.54 (2.14)	2.22 (2.10)	3.18 (2.24)	3.17 (2.63)

Figure 11.1 presents the developmental curves for the functional (panel a) and skill z-scores (panel b). The developmental curve for shooting accuracy is flatter compared to ball control, which means that shooting ability is less explained by CA. Repeated-sprint ability was the functional capacity with largest magnitude of age changes, in contrast to jumping performance which presented a flatter developmental curve.

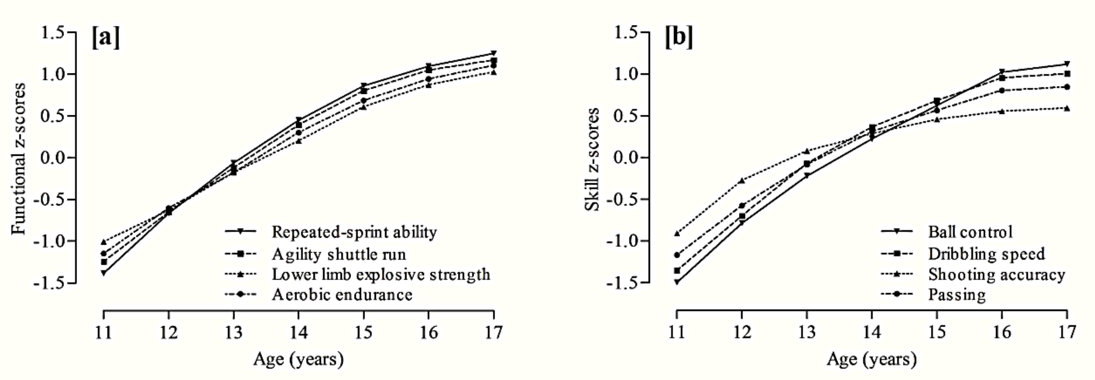

Figure 11.1. Development of the functional (panel a) and skill z-scores (panel b) in young soccer players aligned by chronological age. Functional and skill z-scores were determined using the mean and standard deviation for the total sample.

Predicted functional and skill composite scores from the multilevel model are summarized in Table 11.2. The random-effects coefficients describe the two levels of variance (within individuals: level 1 of the hierarchy; between individuals: level 2 of the hierarchy). The significant variances at level 1 indicated that performances in both functional and skill tests improved significantly at each measurement occasion within individuals (estimate $> 2 \cdot$ SE; $p<0.05$). The between-individuals variance matrix (level 2) indicated that individuals had significantly different functional and skills growth curves in terms of their intercepts (constant/constant, $p<0.05$) and slopes of their lines (age/age, $p<0.05$). The negative covariance between intercepts and slopes of the functional performance suggested that at the end of adolescent years the rate of improvement decreases. The following effects were selected as significant predictors of the Model 1 [functional composite (deviance from the intercept only model = 530.15)]: centered CA ($p<0.01$), centered CA^2 ($p<0.01$), maturity status ($p<0.01$), fat mass ($p<0.01$), annual volume of training ($p<0.01$) and dribbling speed ($p<0.01$). Equations for the three skeletal maturity groups were also derived. The best fitting model for 'late' maturing athletes on the functional composite score could be expressed in the following equation: 13.48 + 1.05 · centered CA – 0.01 · centered CA^2 – 0.19 · FM + 0.004 · annual volume training – 1.04 · dribbling speed. The best models for players 'on time' and 'early' in maturity status were the same as for 'late' maturing players plus 1.26 and 1.82, respectively. The multilevel Model 2 [skill composite (deviance from the intercept only model = 354.86)] included: centered CA ($p<0.01$), centered CA^2 ($p=0.01$), playing position ($p=0.01$), fat-free mass ($p<0.05$), FM ($p<0.01$), annual volume of training ($p<0.01$), repeated-sprint ability ($p<0.01$) and aerobic endurance ($p<0.01$). The Model 2 produced the following equation for 'defenders': 7.62 + 0.62 · centered CA – 0.06 · centered CA^2 + 0.04 · FFM – 0.03 · FM + 0.005 · annual volume training – 0.19 · repeated-sprint ability + 0.02 · aerobic endurance. For midfielder players, the best model was the same as for defenders plus 0.68, while that for forwards was the same as for defenders less 0.11.

Table 11.2. Multilevel regression models for the functional and skill composite (366 measurements).

Random effects	Model 1 (functional composite)		Model 2 (skill composite)	
	Constant	Centered chronological age	Constant	Centered chronological age
Level 1 (within individuals)				
Constant	0.03 (0.01)		0.04 (0.01)	
Level 2 (between individuals)				
Constant	2.71 (0.26)		3.08 (0.32)	
Centered chronological age	–0.15 (0.06)	0.04 (0.01)	0.23 (0.09)	0.15 (0.04)

		Model 1				Model 2				
Step	Explanatory variables	Log Likelihood	p	At final step: Coefficient	SE	Step	Log Likelihood	p	At final step: Coefficient	SE
1	Intercept (constant)	1941.87		13.48	1.30	1	1839.53		7.62	2.86
2	Centered chronological age	1635.43	< 0.01	1.05	0.09	2	1578.23	< 0.01	0.62	0.12
3	Centered chronological age^2	1625.11	< 0.01	–0.01	0.03	3	1571.98	= 0.01	–0.06	0.04
4	On time vs late			1.26	0.27	4				
	Early vs late	1604.62	< 0.01	1.82	0.31		1569.60	0.30	N.E.	
5	Midfielder vs defender					5			0.68	0.23
	Forward vs defender	1600.72	0.14	N.E.			1562.73	= 0.01	–0.11	0.25
6	Fat-free mass	1602.72	0.17	N.E.		6	1557.16	< 0.05	0.04	0.02
7	Stature	1602.41	0.14	N.E.		7	1557.02	0.71	N.E.	
8	Years of practice	1604.40	0.64	N.E.		8	1557.14	0.89	N.E.	
9	Fat mass	1534.06	< 0.01	–0.19	0.02	9	1543.23	< 0.01	–0.03	0.03
10	Annual volume training	1523.62	< 0.01	0.004	0.002	10	1529.97	< 0.01	0.005	0.002
11	Dribbling speed	1411.72	< 0.01	–1.04	0.09					
12	Passing	1411.21	0.48	N.E.						
13	Ball control, juggling	1410.70	0.31	N.E.						
14	Shooting accuracy	1410.58	0.29	N.E.						
	Repeated-sprint ability					11	1492.06	< 0.01	–0.19	0.04
	Agility shuttle-run					12	1491.81	0.62	N.E.	
	Aerobic endurance					13	1484.91	< 0.01	0.02	0.01
	Lower limb explosive strength					14	1484.67	0.62	N.E.	
	Deviance from the intercept only model	530.15	< 0.01				354.86	< 0.01		

SE, standard error; N.E., not entered; random-effects values are estimated mean variance ± SE; fixed-effect values (explanatory variables) are estimated mean coefficients ± SE. All potential predictors are entered in their raw measurements.

The estimated curves for the functional (panel a) and skill (panel b) composite scores derived from the respective multilevel models aligned by CA were plotted in Figure 11.2. Functional performance improved from 11 to 17 years but significant differences among maturity groups were found at 13 to 15 years of age [13 to 14 years: early = on time > late (p<0.01); 15 years: early > late (p<0.05)]. In the predicted skill performance, significant differences were found among playing positions from 15 to 17 years of age [15 years: midfielders > defenders (p< 0.01); 16 to 17 years: midfielders > defenders = forwards (p<0.01)].

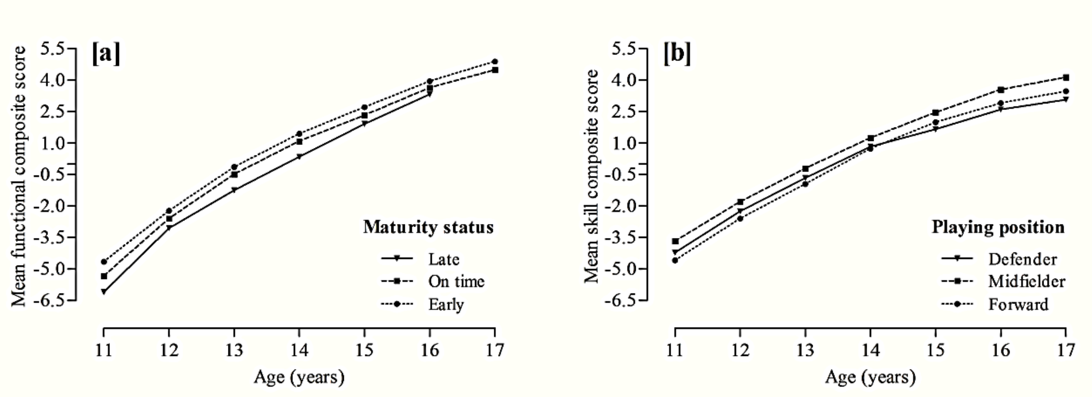

Figure 11.2. Predicted functional (panel a) and skill composite scores (panel b) from the multilevel regressions (Table 11.2) by skeletal maturity and playing position groups, respectively.

The cross-validation of estimates are summarized in Table 11.3. Allowing for variation in sample sizes within age groups, real and predicted scores did not significantly differ [mean difference (± *SD*): functional composite score: 0.15 (0.58); 0.04 (1.08)]. A visual inspection of Bland-Altman plots (Bland and Altman, 1986) showed no heteroscedasticity, which was confirmed by the correlation coefficients. The differences were normally distributed. The SEM values for functional and skill composite scores were 0.40 and 0.68, respectively. The test-retest limits of agreement were –0.98 to 1.28 (functional composite) and – 2.09 to 2.17 (skill composite).

Table 11.3. Cross-validation of the functional and skill composite multilevel regression models (control group).

	Functional composite (*n* = 52)	Skill composite (*n* = 52)
Real mean scores (± *SD*)	–0.49 (3.71)	–0.14 (2.52)
Predicted mean scores (± *SD*)	–0.64 (3.87)	–0.18 (2.64)
Mean difference (± *SD*)	0.15 (0.58)	0.04 (1.08)
t-test (*p*)	1.86 (0.07)	0.28 (0.79)
Correlation, absolute difference vs mean (*p*)	–0.13 (0.38) [a]	–0.21 (0.14) [a]
Standard error of measurement	0.40	0.68
Absolute limits of agreement	–0.98 to 1.28	–2.09 to 2.17

[a] Heteroscedasticity is not present.

11.5. Discussion

This study investigated the contribution of CA, skeletal maturity, body size and composition and annual volume of training to developmental changes in functional capacities and soccer-specific skills among male soccer players aged 11-17 years. Additionally, variation by playing position and skeletal maturity status were also considered in the development of the models. Youth talent identification and development in many sports takes place during athletes' adolescent growth spurt (Elferink-Gemser et al., 2011). Studies on the relative age effect (Helsen et al., 2005) support the notion that in reality many youth athletes are still identified as being talented on the basis of their 'current' rather than their 'future' level of performance. Thus current talent identification systems, systematically and mistakenly fail to notice the talented athletes who may not be the best performers at the current time, but who have the potential to be so in the future (Elferink-Gemser et al., 2011).

Results from the present study showed a progressive improvement in functional and skill composite scores with age. A similar finding, using different statistics and predictors, was found in a sample of Spanish players (Feliu Rovira et al, 1991). CA explained 54% of the variance in a 500-m run test and 59% of the variance in the 60-m dash. When maturation and adiposity were taken into account, the explained variance raised to 72% in 500-m run and 75% in velocity (Feliu Rovira et al, 1991). Likewise, in a cross-sectional sample of Portuguese players (Figueiredo et al., 2011), CA positively influenced three functional capacities, three soccer skills and the respective composite scores in male players aged 11–12 years. Follow-up of the players at 13- 14 years showed that CA influenced functional capacities more than soccer skills (Figueiredo et al., 2011). In contrast, another study did not confirm the contribution of age to explain inter-individual variance in a composite skill score derived from six soccer-specific tests (i.e., ball control with the body, ball control with the head, slalom dribbling with a pass, dribbling speed, passing and shooting accuracy) in adolescent soccer players aged 13.2-15.1 years (Malina et al., 2007). However, this result could be explained by the narrow age range of the sample.

Skeletal maturity status, established at baseline, was also considered as a longitudinal predictor of functional capacity. The composite score showed an expected gradient of early > on time > late (see Figure 11.2, panel a). In the present study 'late' and 'early' maturing boys were equally represented among youth players at 11 and 12 years. However, with increasing age through adolescence, players who were classified as 'on time' (average) and/or 'advanced' in SA were more likely to be represented, whereas younger players identified as 'late' in SA were underrepresented. This age-related trend of preferential selection of 'early' in contrast to 'late' maturing male players is consistent with previous research in youth soccer (Carling et al., 2012; Hirose, 2009; Huijgen et al., 2010), and also in other sports with the exception of gymnastics (Malina, 2011). In the past decade, stature and body mass of professional players in the top English Football League increased by approximately 2 cm and 1.5 kg, respectively (Nevill et al., 2009). It is possible that youth sport selection is retaining players with larger adolescent body size.

Results from previous cross-sectional youth soccer studies (Figueiredo et al., 2009a, Malina et al., 2007), suggested that size and maturity status are important contributors of soccer-specific skills. However, size and maturation were not significant predictors of the composite skill score in the current study. Skill is likely more difficult to be accurately measured when compared to functional fitness (i.e., aerobic endurance, speed, agility and muscle power). It is well known that maturation affects power, speed and the aerobic endurance of adolescent boys (Malina et al., 2004a; 2004b). By inference, maturation exerts its effect via body size and functional capacities (like in the repeated-sprint ability and aerobic endurance) on skills. The mentioned capacities were identified as significant longitudinal predictors in the composite skill model. Many factors, other than body size and maturity status (e.g., neural control of movement and perceptual-cognitive skills such as anticipation and visual search strategies) influence performances on sport-specific skill tests (Williams and Reilly, 2000). Thus it is expected that more players reached the 'ceiling' in the skills tests in comparison with the functional tests. Several studies reported time spent in practice as a strong discriminator across skill levels (Helsen et al., 2000) and not surprisingly the annual volume training was a significant predictor of skill and functional capacity in the current study. These findings may support the conjecture that CA is more closely related to skill (i.e., ball control, dribbling speed, shooting and passing) and SA to the functional performance (i.e., repeated-sprint ability, agility, lower limb explosive strength and aerobic endurance).

Soccer players who are advanced in SA relative to CA, on average, tend to be taller, heavier, stronger, more powerful and faster than 'late' maturing players (Coelho-e-Silva et al., 2010; Hirose, 2009; Malina et al., 2004b; Malina et al., 2000; Philippaerts et al., 2006). Evidence suggests that variation in size and performances among youth players may be factors in career success (Figueiredo et al., 2009b; Roescher et al., 2010). The characteristics by position (i.e., defenders and forwards taller, heavier and stronger than midfielders) among Portuguese players ages 13–15 years (Malina et al., 2004b) and 15–16 years (Malina et al., 2000) may overlap with age- and maturity-associated variation, and perhaps demands of specific positions as the level of competition increases. Previous literature shows the performance advantages associated with 'early' maturation are short lived (Malina et al., 2004a). The drop-out of 'late' maturing players is documented (Malina, 2011) and is suggested in this sample. However, no size, maturity and functional differences were found between elite youth French football players who played professionally and those that dropped out of the sport (Carling et al., 2012).

The current study suggested an appropriate body mass -for-stature of players during childhood and early adolescence, but more body mass-for-stature in later adolescence. The higher body mass-for-stature probably reflects a larger fat free mass (FFM), specifically muscle mass, in later adolescence. Mean relative fatness (densitometry, total body water) of elite young adult soccer players in four studies range from 6.2±1.9% to 9.7±3.0% (Malina, 2007). Players who attained better performances in functional tests tended to possess less percentage of body fat (Reilly et al., 2000). Greater FFM is likely indicative of greater muscle mass, and likely greater strength, which has an influence on change-of-direction speed over short distances (Negrete and Brophy, 2000). The present study showed that the estimated amounts of FFM and FM significantly improved the statistical fit of the functional and skill composite models.

Playing position was a significant developmental predictor of skill performance, with midfielders being the most skilled players across time (see Figure 11.2, panel b). Forwards and midfielders presented a cross-over around the 14 years of age. Coaches might assign the specialization of midfielders at an early phase of long term preparation. Conversely, the specialization of defenders and forwards might occur at latter ages. It is also possible that variation in body size and maturation (Coelho-e-Silva et al., 2010; Figueiredo et al., 2009a; Malina et al., 2004b) interacts with sport orientation at younger ages of sport participation. By inference, maturity-associated variation of functional composite score may interact with sport orientation.

A shortcoming of the current study is that does not extend beyond 17 years of CA so that continued improvements in both composite scores from late adolescence into young adulthood cannot be addressed. The curve trends of Figure 11.2 suggest a more curvilinear development with increasing age but with a higher rate of development of the functional capacity after the age of 14, compared to skill ability. This trend is somewhat expected and consistent with the longitudinal perspective provided by Philippaerts and colleagues (2006), where peak weight velocity (PWV) and many physical performance characteristics showed peak developments at or slightly after peak height velocity (PHV). Skill is likely closer related to the development of motor coordination in children and adolescents, rather than growth and maturation factors (Skinner and Piek, 2001).

The small sample size should be recognized as a limitation of the current study, but it should be noted that the models were tested using an independent sample. The SEM and LOA expressed the reliability of the multilevel models using the independent group. Results showed that the new models predict functional and skill composite scores with a sound degree of accuracy (Table 11.3). Note however, that the present study does not cover all functional and skill components of soccer performance. Ideally, future longitudinal approaches should explore a fitness battery that corresponds better to match performance and incorporates the characteristics that appeared relevant in sport selection and sport orientation. Lastly, the soccer-specific skills in the present study may be more valid during early rather than late adolescent years.

When adjusted for age and individual playing time, distance covered in very high-intensity speed (i.e., >16.1 km·h $^{-1}$) was lower for defenders compared with all other positions (Buchheit et al., 2010). Relationships between match running performance and physical capacities were also position-dependent (Buchheit et al., 2010) with large associations among forwards (i.e., very high-intensity activities vs. peak running speed during an incremental field test: *r*=0.70). As in the present study, causal relationships should not be assumed as fitness level may be simultaneously a predictor and a consequence of match running performance which is a function of playing position. Distances covered at specific bands expressed as percentages of estimated hear rate showed a position-related variation (Stroyer et al., 2004).

11.6. Conclusions

In summary, the two multilevel models were explained by different predictors (i.e., changes in functional composite score were partially explained by age, maturation, fat mass, annual volume of training and dribbling speed; developmental changes in skills appeared to be, in part, explained by age, playing position, fat and fat-free masses, annual volume of training, repeated-sprint ability and aerobic endurance). Multilevel modelling is a promising statistical technique for analyzing the longitudinal changes of functional and skill capacities in a particular sport. It has the potential to provide useful information both for coaches and conditioning trainers in the explanation of inter-individual differences at a certain age and probably more important to predict and explain changes over time.

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Chapter XII

Study 10

Allometric multilevel modelling of agility and dribbling speed by skeletal age and playing position in youth soccer players

12. Allometric multilevel modelling of agility and dribbling speed by skeletal age and playing position in youth soccer players

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Journal: Valente-dos-Santos J, et al. 2014. Allometric multilevel modelling of agility and dribbling speed by skeletal age and playing position in young soccer players. Int J Sports Med. doi: 10.1055/s-0033-1358469

12.1. Abstract

This study evaluates the contributions of age, skeletal maturation, body size and composition, training and playing position to the development of agility and dribbling speed in young male soccer players (10-18 years) followed longitudinally. Eighty-three players [defenders (*n* = 35), midfielders (*n* = 27), forwards (*n* = 21)] were followed annually over five years (average: 4.4 observations per player). Skeletal age (SA), stature, body mass, triceps and subscapular skinfolds, agility and dribbling speed were measured annually. Body composition was estimated from the two skinfolds. Annual training volume was estimated from weekly participation forms completed by coaches. The multiplicative allometric models with the best statistical fit showed that statural growth of 1 cm predicts 1.334 s and 1.927 s of improvement in agility and dribbling speed, respectively. Significant independent effects of fat-free mass and annual volume training were found for agility and dribbling speed, respectively (P<0.05). Predicted agility (from 12 to 18 years of SA) and dribbling speed (from 13 to 18 years of SA) differed significantly among players by playing positions (midfielders > forwards > defenders). The present results provide developmental models for the interpretation of intra- and inter-individual variability in agility and dribbling speed among youth soccer players across adolescence, and may provide a framework for trainers and coaches to develop and evaluate individualized training protocols.

Keywords: Youth Sport · Adolescence · Longitudinal Analysis · Allometry · Maturation · Skills

12.2. Introduction

Agility (ability of rapidly changing body direction and position in the horizontal plane) and dribbling speed (sprinting while controlling the ball) are central components in the development of talented youth soccer players (Huijgen et al., 2010; Malina et al., 2005; Reilly et al., 2000). The contributions of agility and dribbling speed to the discrimination of soccer players by age, skill and competitive levels have been addressed (Coelho-e-Silva et al., 2010; Figueiredo et al., 2009; Huijgen et al., 2010; Kaplan et al., 2009; Malina et al., 2007; Sporis et al., 2010), but the protocols for the measurement of dribbling speed (Federação Portuguesa de Futebol, 1986; Huijgen et al., 2010; Kirkendall et al., 1987) and agility (Council of Europe, 1988) vary considerably.

The Ghent Youth Soccer Project (Vaeyens et al., 2006), for example, used the agility shuttle-run test (SHR) of the EUROFIT protocol. Elite players performed significantly better in the SHR and group differences were most apparent in U-13 and U-14 players. Among Portuguese soccer players, chronological age (CA), stature and adiposity at 11–12 years, and training history and adiposity at 13–14 years explained 34% and 24 % of the variance in the SHR, respectively (Figueiredo et al., 2011). Several cross-sectional analyses of soccer players 11–15 years (Coelho-e-Silva et al., 2010; Figueiredo et al., 2011; Malina et al., 2005, 2007) did not identify significant predictors for a dribbling speed protocol that included three changes of direction, but dribbling performance over a 30-m course with 12 changes of direction was relevant to the differentiation of the best adolescent Dutch player (Huijgen et al., 2010). Factors associated with excellent performance in this test were advanced CA, fat-free mass (FFM) and hours of practice (soccer and additional). Playing position also impacts performance in youth soccer match play (Buchheit et al., 2010). The limited results suggest a need for further research into variation in the agility and dribbling speed characteristics of youth soccer players in general and by playing position. There is also a need for normative data across adolescence for youth players by position; such data are potentially valuable for screening and monitoring development among individuals.

Inter-individual variation in growth and maturation impacts in body size and performance (Malina et al., 2004a). The literature suggests that the elite adolescent male athletes in team sports, including soccer, tend to be advanced in skeletal maturation especially after 14 years age (Malina, 1968). Moreover, the relative age effect evident in many sports represents age and possibly maturational advantages compared to peers (Baxter-Jones, 1995). Soccer players who are ‘advanced’ in maturation tend to be taller, heavier, stronger, more powerful and faster than players who are ‘later’ in maturation (Coelho-e-Silva et al., 2010; Figueiredo et al., 2011; Malina, 2011).

The influence of variation in body size and maturation on soccer-specific skills is less documented and trends are not as consistent as for functional capacities (Figueiredo et al., 2009; Malina, 2007). Use of a longitudinal design has been recommended for the evaluating and profiling the development of sport-specific skills in talented players (Reilly et al., 2000a, 2000b), although such research is limited. With appropriate statistical techniques, a longitudinal design can identify changes in specific skills and partition the relative contributions of training from those associated with growth and maturation. Multilevel modelling (Goldstein, 1995) is appropriate for the analysis of longitudinal observations, i.e., repeated measurements. It is suggested that multiplicative allometric rather than additive models would provide a superior fit and more plausible interpretation of such longitudinal data (Nevill and Holder, 1995; Nevill et al., 1998).

The purposes of this study are twofold: (1) to estimate the longitudinal contributions of CA, training history, body size and composition, and skeletal age (SA) to developmental changes in the agility and dribbling performances of youth soccer players between 10 and 18 years of age considering playing position as an additional source of variation; and (2) to explore the utility of multiplicative allometric model structures for the derivation of developmental curves for agility and dribbling performance. It is hypothesized that the prediction of longitudinal changes in protocols without the ball (agility run) and with the ball (dribbling test) would require different models.

12.3. Methods

Participants

The research was approved by the Scientific Committee of the University of Coimbra and by the Portuguese Foundation for Science and Technology [PTDC/DES/121772]. The Portuguese Soccer Federation and clubs were contacted and institutional agreements were signed between the University and sport organizations to assure data collection over five years. Parents/guardians provided informed consent and players provided their assent. All procedures adhered to established ethical standards for sports medicine (Harriss and Atkinson, 2011).

The sample included 83 boys, aged 10-14 years at baseline, recruited from five soccer clubs in the midlands of Portugal which regularly participated at national level competitions. The sample was a group from which potentially talented players could be identified. All players had a minimum of 2 years of prior soccer-specific training at baseline. The small number of goalkeepers and players who played in multiple positions was not retained for analysis. Players were classified as defenders (DF, n = 35), midfielders (MF, n = 27) and forwards (FW, n = 21) (Coelho-e-Silva et al., 2010; Huijgen et al., 2010). Positions were designated by the coaches. They were followed annually for 3 to 5 years. The number of observations was 366 (DF, 156; MF, 120; FW, 90). Mean numbers of observations per player by position were 4.5, 4.4 and 4.3, respectively. Drop out was, on average, 20% over the duration of the study (DF, 14%; MF, 23%; FW, 23%) and 22% at observation four and five (DF, 34%; MF, 22%; FW, 10%), respectively.

All data were collected within a 2-week period during Easter break, an official school holiday, and were done at the same hours (6:00 to 7:00 PM). Field testing was done under standard conditions in an indoor facility with a flat non-slip wood surface at the University of Coimbra. Testing was completed within one week of the hand-wrist radiograph and with at least 48 hours between sessions.

Variables

Variables considered in the study were included among the generally accepted components of "soccer talent" (Reilly et al., 2000b). Specific details for the measurement and testing protocols have been previously reported (Valente-dos-Santos et al., 2012a, 2012b) but are briefly described.

Training history

Clubs were involved in a 9-month competitive season (September-May) organized by the Portuguese Soccer Federation. Under 13 (11-12 years) and Under 15 players (13-14 years) had ~ 3 training sessions per week (~ 90 $min \cdot session^{-1}$) and one game, usually on Saturdays. Under 17 (15-16 years) and Under 18 players (17 years) participated in 3-5 training sessions per week (90-120 $min \cdot session^{-1}$) and one game per week. Years of formal participation in the sport was obtained through the publically available Portuguese Soccer Federation records and confirmed by an interview at clubs. Training participation (number of sessions; minutes / hours per session) for each player was recorded daily by the head coaches of the respective clubs using standard reporting forms. Modification of usual training volume associated with injury, illness and competition were noted and accounted in final calculations.

Skeletal age

The posterior-anterior radiograph of the left hand-wrist was taken annually on the first day of testing. Chronological age (CA) was calculated as the difference between date of the hand-wrist radiograph and date of birth. The Fels method of assessment (Roche et al., 1988) was used to estimate SA. The method utilizes specific criteria for each bone of the hand-wrist and ratios of linear measurements of epiphyseal and metaphyseal widths. Ratings were entered into a program (Felshw 1.0 Software, Lifespan Health Research Center, Departments of Community Health and Pediatrics, Boonshoft School of Medicine, Wright State University, Dayton, Ohio) to derive a

SA and associated standard error. Twenty radiographs were independently assessed by a single observer who was trained by an experienced assessor. The mean difference between SA assessments of the two observers and the inter-observer technical error of measurement were small, respectively 0.03 ± 0.04 and 0.12 years.

Anthropometry

The same trained technician measured stature, body mass, and the triceps and subscapular skinfold thicknesses following standard procedures (Lohman et al., 1988). Players wore shorts and a t-shirt; shoes were removed. Intra-observer technical errors of measurement for stature (0.3 cm), body mass (0.5 kg), and skinfolds (0.5-0.7 mm) were within the range of several health surveys in the United States and a variety of field surveys, including studies of young athletes (Malina et al., 2004a). Percentage of body fat was estimated from age and gender specific anthropometric formulas (Slaughter et al., 1988). Fat mass (FM) and fat-free mass (FFM) were derived.

Agility: Shuttle-run test (SHR)

The SHR was assessed according to the EUROFIT test battery (Council of Europe, 1988). Players wore indoor shoes and were tested individually. The player started the test with the feet on the starting line. On the command “Go”, subjects ran 10 consecutive shuttle sprints of 5 m (total 50 m). Both feet had to fully cross the line at each end of the shuttle course. The time to complete the 10 shuttles was recorded with a digital chronometer connected to photoelectric cells placed on the start/finish line (Globus Ergo Timer Timing System, Codogné, Italy). Times were recorded to a resolution of 0.01 s (Figure 12.1, panel a). Two trials were performed and the faster of the two was retained for analysis.

The SHR was tested after one week in 32 randomly selected players. The 95% limits of agreement where adopted to examine test-retest agreement (Bland and Altman, 1986; Nevill and Atkinson, 1997). Visual inspection of Bland-Altman plots (Bland and Altman, 1986) showed that measurement differences (error) against the respective means were homoscedastic (Nevill and Atkinson, 1997). The test-retest limits of agreement was –1.46 to 2.25 s (r = 0.05, P = 0.78). The SHR has been used in both youth (Coelho-e-Silva et al., 2010; Figueiredo et al., 2011; Figueiredo et al., 2009) and adult (Kaplan et al., 2009) players, and is part of the Portuguese physical education curriculum.

Dribbling speed

The dribbling speed test (DST) recommended by the Portuguese Football Federation was used (Federação Portuguesa de Futebol, 1986). A cone was placed on each corner of the 9 x 9 m square; a fifth cone was placed midway (4.5 m) on the line where the test started. The overall slalom distance was approximately 40 m. Beginning at one corner, the participant was instructed to move the ball with the feet (i.e., dribble) around the three cones in slalom fashion, and then dribble the ball to the fifth cone (Figure 12.1, panel b). Two trials were administered and the faster of the two was retained for analysis. Replicates were obtained from the 32 players within 1 week; test-retest limits of agreement for DST was –0.64 to 2.16 s (r = 0.15, P = 0.40). Based on the ratio of within-subject and inter-subjects variances, reasonable reliability (R = 0.74) has been reported for the dribbling speed test (Figueiredo et al., 2009). Validity coefficients between performances on a slalom dribble and ratings of soccer playing ability ranged from 0.53 to 0.94 (Kirkendall et al., 1987). Face validity can be assumed since the tests assess specific elements of the sport.

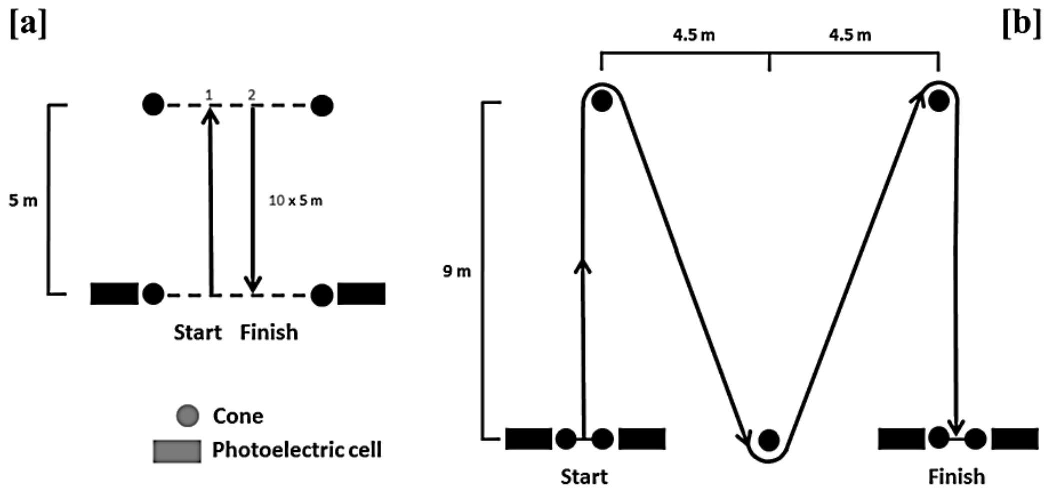

Figure 12.1. Course of the shuttle-run (10 x 5 m; panel a) and the dribbling speed test (panel b).

Statistical analysis

Means and standard deviations were calculated for CA, SA, training history, body size and estimate composition, and agility and dribbling speed performances. Differences between age groups for all variables were tested with multivariate analysis of variance (MANOVA). MANCOVA, with CA as covariate, was used as a preliminary cross-sectional analysis to compare SA, training history and performance variables by playing position at baseline. If a comparison was significant, pairwise comparisons were used to identify differences between specific pairs. The effect size correlations (ES-*r*) were estimated using the square root of the ratio of the F-value squared and the difference between the F-value squared and degrees of freedom (Rosnow and Rosenthal, 1986). Coefficients were interpreted as follows: trivial ($r < 0.1$), small ($0.1 < r < 0.3$) moderate ($0.3 < r < 0.5$), large ($0.5 < r < 0.7$), very large ($0.7 < r < 0.9$), nearly perfect ($r < 0.9$) and perfect ($r = 1$) (Hopkins et al., 2009).

Multi-collinearity was examined for the four models (Model 1 and 2: potential predictors of agility; Model 3 and 4: potential predictors of dribbling speed), using correlation matrix and diagnostic statistics (Schroeder, 1990). Variables with small tolerance (≤ 0.10) and a variance inflation factor > 10 (corresponding to an R^2 of 0.90) were considered indicative of harmful multi-collinearity (Slinker and Glantz, 1985). For the longitudinal analyses, a multilevel regression analysis was performed using MLwiN 2.02 software to identify playing position differences associated with the development of agility and dribbling speed, after adjustments for body size, body composition, SA, CA and training history. Multilevel model technique allows the number of observations and temporal spacing between measurements to vary among subjects, thus using all available data. It is assumed that the probability of data being missing is independent of any of the random variables in the model. As long as a full information estimation procedure is used, such as maximum likelihood in *MLwiN* for normal data, the actual missing mechanism can be ignored (Rasbash et al., 1999).

The multilevel regression analysis were performed using the multiplicative allometric models (y = agility or dribbling speed) based on the work of Nevill and colleagues (Nevill et al., 1998), as follows:

$$y = \text{stature}^{k1} \cdot \text{fat-free mass}^{k2} \cdot \text{fat mass}^{k3} \cdot \exp(a_{ij} + b_j \cdot \text{skeletal or chronological age} + c \cdot \text{skeletal or chronological age}^2 + d \cdot \text{annual volume training}) \cdot \varepsilon_{ij} \qquad \textbf{(12.1)}$$

where all the parameters were fixed with the exception of the constant (a_{ij}) and SA or CA (b_j), which were allowed to vary randomly from player to player (level 2), and the multiplicative error ratio ε_{ij}, which was used to describe the error variance between visit occasions (level 1). The number of visits was also assumed to be a random over time. The two levels of random variation take into account the fact that growth characteristics of individual players, such as average rate of improvement in performance, vary around a population mean and also that observations for each player vary around his own improvement trajectory.

The model can be linearized with a logarithmic transformation, and a multilevel regression analysis on ln y can be used to estimate the unknown parameters (Nevill et al., 1998). The transformed log-linear multilevel becomes:

$$\ln y = k_1 \cdot \ln \text{stature} + k_2 \cdot \ln \text{fat-free mass} + k_3 \cdot \ln \text{fat mass} + a_{ij} + b_j \cdot \text{skeletal or chronological age} + c \cdot \text{skeletal or chronological age}^2 + d \cdot \text{annual volume training} + \ln \varepsilon_{ij} \quad \textbf{(12.2)}$$

Playing position (DF, MF, FW) was incorporated into a subsequent analysis by introducing it as a fixed dummy coded variable with DF as the reference category. Predictor variables were accepted as significant if the estimated mean coefficient was greater than 1.96 (the standard error of the estimate at an alpha level of 0.05; Baxter-Jones and Mirwald, 2004). If the retention criteria were not met, the predictor variable was discarded. The final model included only variables that were significant independent predictors.

The multilevel allometric model structures were compared based on the Akaike information criterion (Akaike, 1974): – 2 · log likelihood + 2 · number of parameters fitted. When the performance of competing models is assessed, the model that fits the data best is the one with the minimum Akaike information criterion (AIC) value.

12.4. Results

Age, training history, anthropometry, agility and dribbling speed by age groups are described in Table 12.1. As expected, all scores generally improved with age (lower scores for time elapsed tests correspond to better performances). Stature (ES-r = 0.81, P<0.05), body mass (ES-r = 0.75, P<0.05) and FFM (ES-r = 0.79, P<0.05) increased largely between SAs of 11–15 years. Agility (ES-r = 0.70, P<0.05) and dribbling speed (ES-r = 0.74, P<0.05) increased largely between SAs of 11–14 years.

Table 12.1. Mean and standard deviations for chronological and skeletal ages, anthropometry, agility and dribbling speed, frequencies of playing positions by chronological age groups (infantiles, initiates, juveniles, juniors)* and results of MANOVAs.

	Age group (years)				Effect size *r*
	11–12 years [infantiles] (*n* = 97)	13–14 years [initiates] (*n* = 163)	15–16 years [juveniles] (*n* = 96)	17 years [juniors] (*n* = 10)	
Chronological age (years)	12.2 ± 0.6 [a]	14.2 ± 0.6	16.0 ± 0.6	17.6 ± 0.3	0.93
Skeletal age (years)	12.3 ± 1.3 [a]	14.5 ± 1.2	16.6 ± 1.3	17.7 ± 0.2	0.80
Soccer experience (years)	3.1 ± 1.1 [a]	5.0 ± 1.2	6.8 ± 1.3	8.2 ± 1.1	0.77
Annual volume training (hours)	129.3 ± 33.6 [c]	157.2 ± 51.7	167.1 ± 49.4	177.8 ± 40.8	0.31
Stature (cm)	146.8 ± 7.3 [b]	161.4 ± 8.6	170.7 ± 6.2	173.7 ± 4.2	0.77
Body mass (kg)	38.8 ± 6.3 [b]	50.5 ± 9.1	61.3 ± 9.1	68.0 ± 9.4	0.72
Percentage of fat mass (%)	13.3 ± 6.8	14.7 ± 7.3	17.0 ± 7.6	19.6 ± 6.3	0.21
Fat mass (kg)	4.6 ± 2.6 [b]	6.5 ± 3.6	9.1 ± 4.7	11.3 ± 4.5	0.44
Fat-free mass (kg)	34.2 ± 4.7 [b]	44.0 ± 7.0	52.3 ± 5.6	56.8 ± 6.1	0.75
Agility (seconds)	20.45 ± 1.10 [b]	18.91 ± 1.00	18.10 ± 1.00	18.18 ± 0.98	0.70
Dribbling speed (seconds)	15.16 ± 1.73 [b]	13.25 ± 0.98	12.32 ± 0.63	11.88 ± 0.67	0.74
Playing position (f)					
Defenders	43	70	40	4	
Midfielders	31	53	33	3	
Forwards	23	40	23	3	

[*] 11-year old player was defined as a player tested within the age range 11.00 – 11.99 years.

[a] Scores significantly improved from 11 to 17 years ($P<0.05$); [b] Scores significantly improved from 11 to 16 years ($P<0.05$); [c] Scores significantly improved from 11 to 14 years ($P<0.05$).

Characteristics of players by playing positions at baseline are summarized in Table 12.2. After controlling for CA, the cross-sectional analysis revealed position-related variation at baseline in stature, body mass and composition ($P<0.05$). DF and FW are moderately taller than MF (ES-r = 0.35, $P<0.05$). DF are also moderately heavier than MF [body mass: ES-r = 0.32 ($P<0.05$); FFM: ES-r = 0.30 ($P<0.05$)].

Table 12.2. Adjusted means and standard errors at baseline by playing position and results of MANCOVAs with chronological age as the covariate.

	Defenders (n = 35)	Midfielders (n = 27)	Forwards (n = 21)	Effect size r
Skeletal age (years)	12.71 ± 0.21	12.41 ± 0.25	12.98 ± 0.26	0.17
Soccer experience (years)	3.4 ± 0.2	3.5 ± 0.2	2.9 ± 0.2	0.23
Annual volume training (hours)	132.8 ± 6.5	143.0 ± 7.7	127.6 ± 8.1	0.16
Stature (cm)	151.1 ± 1.2 [a]	146.1 ± 1.4 [c]	151.8 ± 1.4	0.35
Body mass (kg)	43.5 ± 1.2 [a]	38.1 ± 1.4	42.0 ± 1.4	0.32
Percentage of fat mass (%)	14.7 ± 1.3	11.0 ± 1.5	12.2 ± 1.6	0.22
Fat mass (kg)	5.8 ± 0.6	3.7 ± 0.7	4.6 ± 0.7	0.27
Fat-free mass (kg)	37.7 ± 0.8 [a]	34.4 ± 1.0	37.4 ± 1.0	0.30
Agility (seconds)	20.04 ± 0.17	20.04 ± 0.20	19.61 ± 0.21	0.19
Dribbling speed (seconds)	15.06 ± 0.25	14.75 ± 0.29	14.60 ± 0.31	0.14

[a] Significant difference Defenders vs. Midfielders ($P<0.05$); [b] Defenders vs. Forwards, [c] Midfielders vs. Forwards.

Predicted agility derived from the multiplicative allometric models (Model 1: aligned by SA; Model 2: aligned by CA) is summarized in Table 12.3. The following were significant predictors in the multiplicative allometric models: Model 1 [ln agility aligned by SA (IGLS deviance from the null model = 310.62)]: ln stature ($P<0.01$), ln FFM ($P<0.05$), SA ($P<0.01$) and playing position ($P<0.05$). Model 2 [ln agility aligned by CA (IGLS deviance from the null model = 342.62)] incorporated ln stature ($P<0.01$), ln FFM ($P<0.05$), CA ($P<0.01$), CA^2 ($P<0.01$), annual volume training ($P<0.05$) and playing position ($P<0.05$). Model 1 required fewer parameters (n = 10) than Model 2 (n = 12). The AIC criterion provided stronger support for Model 1 [AIC = $(-2 \cdot -613.20) + (2 \cdot 10) = 1246.40$] compared to Model 2 [AIC = $(-2 \cdot -629.07) + (2 \cdot 12) = 1282.14$]. The best fitting model (Model 1) for DF is: $-0.288 \cdot \ln \text{stature} - 0.077 \cdot \ln \text{FFM} + 4.716 - 0.007 \cdot \text{SA}$. The best models for MF

and FW were the same as for DF minus 0.025 and 0.016, respectively. Overall, stature and FFM explained the largest portion (~ 95 %) of the within individuals variance in the improvement of agility. Taking the antilogs, the Model for players' agility indicates, for example, that once stature is controlled [1 cm predicts 1.334 s (6%) of improvement in the agility test], a significant independent effect of FFM effect is apparent [1 kg predicts 1.080 s (5%) of improvement in the agility test].
SA was also added as a random coefficient.

The random effects coefficients describe the two levels of variance [within individuals (level 1 of the hierarchy) and between individuals (level 2 of the hierarchy)]. The significant variances at level 1 indicate that agility performance improved significantly at each measurement occasion within individuals (estimate > 1.96 · SE; P<0.05). The mean rate of improvement per year is 2.2 % (0.46 s). The between-individuals variance matrix (level 2) indicates that growth curves for agility differed significantly among individuals in terms of (constant/constant, P<0.05) and slopes (SA/SA, P<0.05). The positive and significant covariance between intercepts and slopes (constant/SA) suggests that the rate of improvement at the end of adolescent years is similar to that of early adolescent years.

Table 12.3. Allometric multilevel regression analysis of log-transformed agility, adjusted for body size, body composition, skeletal age, chronological age and annual training volume (n = 366).

Model 1: ln agility aligned by skeletal age		
Fixed explanatory variables	*P*	Value at final step
Constant (a)	<0.01	4.7159 ± 0.5219
ln stature (k_1)	<0.01	– 0.2884 ± 0.1080
ln fat-free mass (k_2)	<0.05	– 0.0770 ± 0.0340
ln fat mass (k_3)	NS	
Skeletal age (b)	<0.01	– 0.0070 ± 0.0026
Skeletal age^2 (c)	NS	
Annual volume training (d)	NS	
Midfielders *vs* Defenders (Δa)		– 0.0245 ± 0.0101
Forwards *vs* Defenders (Δa)	<0.05	– 0.0164 ± 0.0071
Interaction		
Midfielders × Skeletal age (Δb)		
Forwards × Skeletal age (Δb)	NS	
Variance-covariance matrix of random variables	Constant (a)	Skeletal age (b)
Level 1 (within individuals)		
Constant (a_{ij})	0.0014 ± 0.0001	
Level 2 (between individuals)		
Constant (a_j)	0.0015 ± 0.0003	
Skeletal age (b_j)	0.000061 ± 0.000005	0.0000196 ± 0.0000085
IGLS deviance from the null model = 310.62		
Log likelihood statistics = – 613.20		

Model 2: ln agility aligned by chronological age		
Fixed explanatory variables	*P*	Value at final step
Constant (a)	<0.01	3.7818 ± 0.4899
ln stature (k_1)	<0.01	0.0679 ± 0.0133
ln fat-free mass (k_2)	<0.05	– 0.0898 ± 0.0414
ln fat mass (k_3)	NS	
Chronological age (b)	<0.01	– 0.0962 ± 0.0237
Chronological age^2 (c)	<0.01	– 0.0015 ± 0.0006
Annual volume training (d)	<0.05	– 0.00006 ± 0.00003
Midfielders *vs* Defenders (Δa)		– 0.0213 ± 0.0102
Forwards *vs* Defenders (Δa)	<0.05	– 0.0132 ± 0.0051
Interaction		
Midfielders × Chronological age (Δb)		
Forwards × Chronological age (Δb)	NS	
Variance-covariance matrix of random variables	Constant (a)	Skeletal age (b)
Level 1 (within individuals)		
Constant (a_{ij})	0.0013 ± 0.0001	
Level 2 (between individuals)		
Constant (a_j)	0.0048 ± 0.0006	
Skeletal age (b_j)	– 0.000297 ± 0.000042	0.0000233 ± 0.0000030
IGLS deviance from the null model = 342.62		
Log likelihood statistics = – 629.07		

Values are means ± SE; NS, non-significant; random-effects values are estimated mean variance ± SE; fixed-effect values (explanatory variables) are estimated mean coefficients ± SE. Skeletal and chronological ages were adjusted about origin using mean age ± 14 years, respectively. Defenders was used as baseline measure (a), and other field positions were compared with it, indicated by (Δa).

Table 12.4 summarizes the results of Models 3 [ln dribbling speed aligned by SA (IGLS deviance from the null model = 452.62)] and Model 4 [ln agility aligned by CA (IGLS deviance from the null model = 532.06)]. The AIC criterion provides strong support [AIC = (– 2 · – 518.83) + (2 · 10) = 1057.66] for the allometric multilevel Model 3 compared to Model 4 [AIC = (– 2 · – 558.55) + (2 · 11) = 1139.10]. According to Model 3 for dribbling speed among DF: – 0.657 · ln stature + 5.959 – 0.019 · SA – 0.002 · SA^2 – 0.013· annual volume training. The fixed effects indicate that stature was responsible for the highest rate of improvement in dribbling speed within individuals [antilog: 1.927 s (11%) of improvement per cm of growth]. Also, 1 h of annual training per week during the competitive period (~ 40-weeks) predicts ~ 0.524 s (3%) of improvement in dribbling speed. Coefficients for interactions of SA and playing position were not significant. The significant variances at level 1 indicates that dribbling performance improved significantly at each measurement occasion within individuals (estimate > 1.96 x SE; $P<0.05$). The estimated mean rate of improvement per year was 3.7 % (0.62 s). The between-individuals variance matrix (level 2) indicates that individuals growth curves were significantly different only in their intercepts (constant/constant, $P<0.05$). The negative and significant covariance between intercepts and slopes (SA/constant) of dribbling speed performance suggests that rate of improvement decreases in late adolescence.

Table 12.4. Allometric multilevel regression analysis of log-transformed dribbling speed, adjusted for body size, body composition, skeletal age, chronological age and annual training volume (n = 366).

Model 3: ln dribbling speed aligned by skeletal age		
Fixed explanatory variables	*P*	Value at final step
Constant (a)	<0.01	5.9589 ± 0.5294
ln stature (k_1)	<0.01	– 0.6565 ± 0.1040
ln fat-free mass (k_2)	NS	
ln fat mass (k_3)	NS	
Skeletal age (b)	<0.01	– 0.0193 ± 0.5294
Skeletal age^2 (c)	<0.05	– 0.0015 ± 0.0006
Annual volume training (d)	<0.05	– 0.0131 ± 0.0055
Midfielders *vs* Defenders (Δa)		– 0.0284 ± 0.0109
Forwards *vs* Defenders (Δa)	<0.05	– 0.0031 ± 0.0014
Interaction		
Midfielders × Skeletal age (Δb)		
Forwards × Skeletal age (Δb)	NS	
Variance-covariance matrix of random variables	Constant (a)	Skeletal age (b)
Level 1 (within individuals)		
Constant (a_{ij})	0.0020 ± 0.0002	
Level 2 (between individuals)		
Constant (a_j)	0.0036 ± 0.0007	
Skeletal age (b_j)	– 0.000669 ± 0.000155	0.0000765 ± 0.0000578
IGLS deviance from the null model = 452.62		
Log likelihood statistics = – 518.83		

Model 4: ln dribbling speed aligned by chronological age		
Fixed explanatory variables	*P*	Value at final step
Constant (a)	<0.01	4.7819 ± 0.4322
ln stature (k_1)	<0.01	– 0.0785 ± 0.0104
ln fat-free mass (k_2)	NS	
ln fat mass (k_3)	NS	
Chronological age (b)	<0.01	– 0. 2047 ± 0.0289
Chronological age^2 (c)	<0.05	0.0056 ± 0.0009
Annual volume training (d)	<0.05	– 0.00013 ± 0.00006
Midfielders *vs* Defenders (Δa)		– 0.0101 ± 0.0012
Forwards *vs* Defenders (Δa)	<0.05	– 0.0058 ± 0.0011
Interaction		
Midfielders × Chronological age (Δb)		
Forwards × Chronological age (Δb)	NS	
Variance-covariance matrix of random variables	Constant (a)	Skeletal age (b)
Level 1 (within individuals)		
Constant (a_{ij})	0.0017 ± 0.0002	
Level 2 (between individuals)		
Constant (a_j)	0.0562 ± 0.0158	
Skeletal age (b_j)	– 0.003269 ± 0.001001	0.000192 ± 0.000064
IGLS deviance from the null model = 532.06		
Log likelihood statistics = – 558.55		

Values are means ± SE; NS, non-significant; random-effects values are estimated mean variance ± SE; fixed-effect values (explanatory variables) are estimated mean coefficients ± SE. Skeletal and chronological ages were adjusted about origin using mean age ± 14 years, respectively. Defenders was used as baseline measure (a), and other field positions were compared with it, indicated by (Δa).

Using the antilog function, the estimated curves for agility, aligned by SA and CA based upon the respective multiplicative allometric models are plotted in Figure 12.2 (panel a and b, respectively). Predicted times improved substantially from 10 to 18 years of SA (3.72 s / 17.4 %; panel a) and from 11 to 17 of CA (3.03 s / 14.6 %; panel b). Predicted agility time differed significantly among playing positions from 12 to 18 years of SA and from 12 to 17 years of CA.

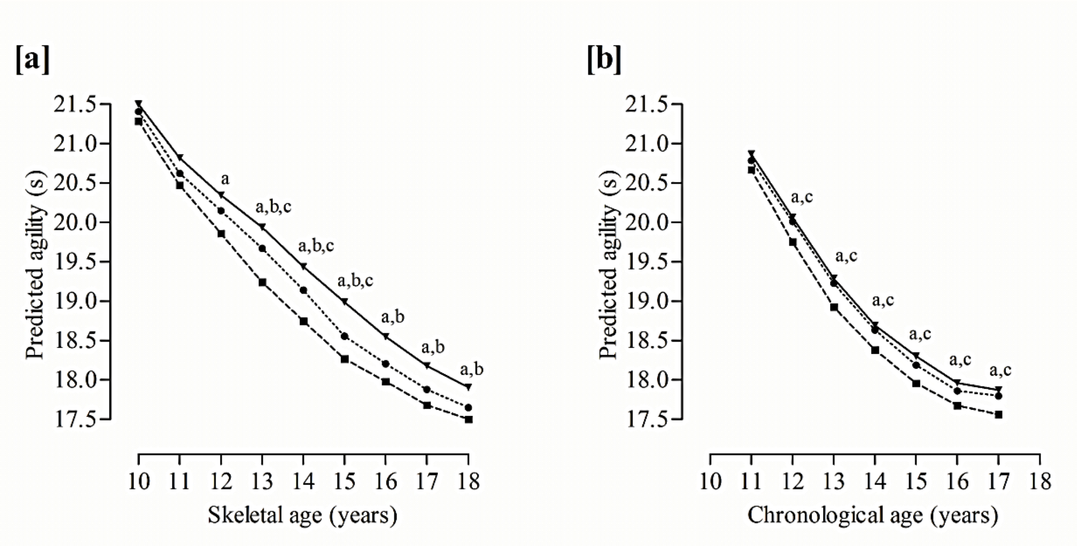

Figure 12.2. Predicted agility mean scores, by playing position [(▼) Defenders, DF; (■) Midfielders, MF; (●) Forwards, FW], aligned by skeletal (SA, panel a) and chronological age (CA, panel b). a significant difference DF vs. MF (*P*<0.05), b DF vs. FW, c MF vs. FW. Significant differences from 12 to 18 years of SA [12 years: MF were 0.49 s (2.4 %) faster than DF (*P*<0.05). 13 to 15 years: MF were, on average, 0.70 s (3.6 %) and 0.37 s (1.9 %) faster than DF and FW, respectively (*P*<0.05). Also, FW were, on average, 0.33 s (1.7 %) faster than DF (*P*<0.05). 16 to 18 years: MF and FW were, on average, 0.49 s (2.7 %) and 0.30 s (1.6 %) faster than DF (*P*<0.05), respectively] and from 12 to 17 years of CA [12 to 17 years: MF were, on average, 0.32 s (1.8 %) and 0.24 s (1.3 %) faster than DF and FW, respectively (*P*<0.05)].

Figure 12.3 (panel a and b, respectively) indicates significant improvement in dribbling speed from 10 to 15 years of SA (4.05 s / 24.1 %) and 11 to 14 years of CA (2.89 s / 18.2 %) and more modest annual gains from 16 to 18 years of SA (0.89 s /

5.3 %) and from 15 to 17 years of CA (0.50 s / 3.2 %). Dribbling performances differ by playing positions from 13 to 18 years of SA and from 14 to 17 years of CA.

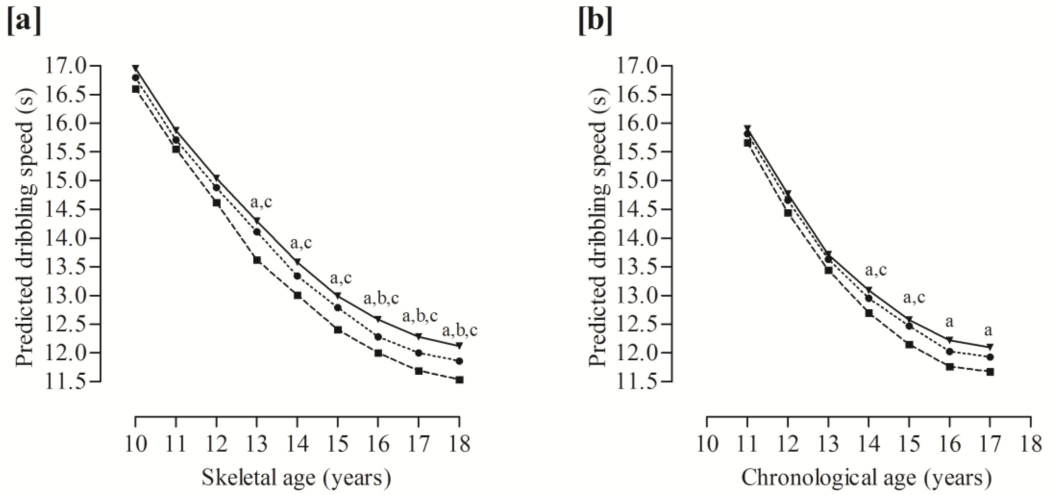

Figure 12.3. Predicted dribbling speed mean scores, by playing position [(▼) Defenders, DF; (■) Midfielders, MF; (●) Forwards, FW], aligned by skeletal (SA, panel a) and chronological age (CA, panel b). a significant difference DF vs. MF (*P*<0.05), b DF vs. FW, c MF vs. FW. Significant differences from 13 to 18 years of SA [13 to 15 years: MF were, on average, 0.61 s (4.5 %) and 0.40 s (3.0 %) faster than DF and FW, respectively (*P*<0.05). 16 to 18 years: MF and FW were, on average, 0.48 s (4.7 %) and 0.28 s (2.3 %) faster than DF (*P*<0.05), respectively. Also, MF were, on average, 0.30 s (2.5 %) faster than FW (*P*<0.05)] and from 14 to 17 years of CA [14 to 17 years: MF were, on average, 0.43 s (3.5 %) faster than DF (*P*<0.05). 14 to 15 years: MF were, on average, 0.29 s (2.3 %) faster than FW (*P*<0.05)].

12.5. Discussion

This study investigated the contribution of CA, SA, body size and estimated composition and annual training volume to developmental changes in agility and dribbling speed performances of male soccer players between 10 and 18 years through the use of allometric multilevel models. Variation by playing position was also considered. Based on the multilevel models with best statistical fit, the results suggest that an increase in 1 kg in FFM was associated with improvement in agility performance by 5%, when body size, body composition and SA are controlled, regardless of playing position. There also appears to be an independent training effect; each 1 h of additional training per week over the competitive season (~ 40-weeks) was associated with 3% improvement in dribbling speed performance, when stature and SA are controlled. Further, the estimated performance curves for agility and dribbling speed derived from the respective multiplicative models demonstrate significant variation by position. The predicted mean agility scores aligned by SA showed that MF were, on average, 2.7 % and 1.4 % faster than DF and FW, respectively. FW were, on average, 1.3 % faster than DF on the agility test. Results for dribbling speed aligned by SA indicated that MF were, on average, 3.7 % and 2.2 % faster than DF and FW, respectively. FW, were on average, 1.6 % faster than DF. The results emphasized the influence of inter-relationships among growth in body size and composition, skeletal maturation and training among soccer players across adolescence.

Comparisons of Portuguese adolescent soccer players 13-15 years by position indicated DF and FW were, on average, taller, heavier and more powerful than MF but the differences were not significant; MF in contrast had greater aerobic capacity (Malina et al., 2004b). On the other hand, players in the three positions did not differ in six soccer-specific skill tests (Malina et al., 2005). Generally similar results were noted in independent studies of Portuguese youth players 11–12 and 13–14 years (Figueiredo et al., 2009) and 15–16 years (Malina et al., 2000). All of the studies noted maturity-associated variation of body size and functional capacities, but not in soccer-specific skills. In general, youth players who were advanced in stage of puberty and SA tended to be taller, heavier, more powerful and faster than later maturing players of the same age (Coelho-e-Silva et al., 2010; Hirose, 2009; Malina et al., 2004b, 2000). The results highlight the need for awareness of the influence of individual differences in maturity status on body size and function among youth players of the same CA; however, the influence of maturity status on soccer-specific skills is negligible. By inference, inter-individual differences in biological maturation have the potential to confound talent identification during the adolescent years.

Players who attained better performances in functional tests tended to possess less percentage body fat (Reilly et al., 2000a). A similar trend was noted in U-20 Dutch soccer players, among whom FFM was a positive predictor of shuttle sprint performance but not of dribbling performance (Huijgen et al., 2010). The interrelationships between body composition, performance and sport-specific skills have been addressed more often in cross-sectional studies. Based on multiple linear regressions models, CA (positive), stature (positive) and adiposity (negative) explained 34% of the variance in the SHR in players aged 11–12 years, while the training history (positive) and adiposity (negative) explained 24% of the variance in the SHR in players aged 13–14 years (Figueiredo et al., 2011). CA and pubertal status accounted for 19% of the variance in a dribbling test that involved a pass (in contrast to a continuous slalom test of speed) in players 13-15 years, but CA, size, pubertal status and years of training were not significant predictors of dribbling speed in this sample (Malina et al., 2005). In the present study, stature and estimated FFM were the main determinants of improvement in the predicted agility performance across adolescence, once skeletal maturity was controlled. On the other hand, dribbling speed was not affected by longitudinal variability in estimated body composition, after adjustments for body size and skeletal maturity.

In many sports, size per se may be a significant factor affecting success at young ages by attracting the attention of coaches, which in turn may contribute to enhanced opportunities for selective advancement, better coaching, and so on. Adults involved in the development of talented youth soccer players should be aware of the contributions of individual differences in growth and maturation to the functional and skill demands of the sport. Note, however, the physical advantages afforded by advanced maturity status during adolescence are largely transient as all youth eventually attain physical maturity (Malina et al., 2004a). Data from a single longitudinal study of the general population of youth suggest that some of the maturity differences in performance during adolescence are reduced or reversed in young adulthood (Lefevre et al., 1990).

Annual training volume had a modest contribution to dribbling speed during adolescence. Note, however, the amount of training expressed in hours is probably not a sufficiently sensitive indicator. There is need for better quantification of the details of "time training "; e.g., warm-up, fitness activities, skill and game instruction, specific drills, small-sided activities, ratio of training activities to rest/recovery, scrimmages, and perhaps other activities. It is also important to document the intensity of specific activities.

Playing position was a significant predictor of the development of agility and dribbling speed; MF performed better on both tests across adolescence (see Figures 12.2 and 12.3). FW were significantly faster than DF in the agility and dribbling speed tests after 14 years of SA. Variation in body size, maturation and fitness by playing position at late adolescence may be a consequence of sport specialization and career orientation (Coelho-e-Silva et al., 2010; Figueiredo et al., 2009; Malina et al., 2004b). Coaches often tend to retain physically larger boys as DF and to assign faster players as FW. Such assignments in combination with specific training protocols to improve muscle mass, strength and speed probably contribute to variation among players by position. Nevertheless, causal relationships should not be assumed as fitness and skill levels may be simultaneously a predictor and a consequence of the physical demands of the game which vary by playing position.

The developmental pattern of predicted dribbling speed is curvilinear with increasing SA (Figure 12.3, panel a), but the rate of improvement declines as skeletal maturity is approached in late adolescence. The models suggested that the development of dribbling speed was partly determined by the amount of training, after growth and maturation were statistically controlled. As noted, there is a need to document specific components of "time training". Nevertheless, specific training methods to improve dribbling speed may be required to overcome the apparent plateau in late adolescence.

The relatively small size and perhaps limited representativeness of those players who agreed to participate are limitations of this study. The inclusion of players who were and were not selected by trainers for the regional team may better inform the characteristics of the performance curves of agility and dribbling speed at more elite levels. Additional research should examine performance curves in young soccer players by competitive level taking into account variation by playing position. However, there is a need to accommodate players who in fact change positions as they progress through adolescence.

Body composition was estimated from two skinfolds and prediction equations specific for adolescent males of a similar CA, pubertal stage and ethnicity (European or White ancestry). All prediction equations have associated errors. The standard error of the estimate for FFM using the Slaughter equations is 1.8 kg for males when compared to estimates based on densitometry (Janz et al., 1993). The validity correlation for Slaughter equations are $r = 0.99$ for males (Janz et al., 1993).

Although SA is considered the best maturity indicator as it is a continuous variable that spans childhood through adolescence, radiographs are costly and require experienced interpretation. Nevertheless, when SA is not available, agility and dribbling speed can be expressed relative to CA and the quadratic term of CA. The current dribbling test has only three changes of direction and seems an efficient solution to assessing the playing position-associated developmental changes. A dribbling test with 12 changes of direction was proposed but failed to differentiate among FW, MF and DF in the same age category (Huijgen et al., 2010).

The present study is limited to one functional test (agility) and one soccer skill (dribbling speed). Further, interrelationship between tests and match performances and sensitivity of a particular test also vary among different competitive soccer age groups, e.g., 11-12, 13-14, 15-16, and 17-18 years. Future research is needed to examine battery properties in relation to match performance, selection and orientation by playing position.

12.6. Conclusions

In summary, the present study assessed the development of agility and dribbling speed in individual players and provides insights into factors that contributing to improvements in performance across adolescence. The allometric multilevel models included different significant predictors for agility and dribbling speed relative to skeletal maturity or CA. Accordingly, the primary hypothesis of the study was supported. Allometric multilevel modelling is recommended for analysing longitudinal changes of functional and skill capacities, controlling for SA or CA and playing position. The approach offers an analytical framework that provides more plausible estimates of the dependent variables, allowing a critical interpretation of variation within and between individuals. The equations are also useful analytical tools for the interpretation of developmental progress of function and skill across adolescence in youth players. Assessment of deviance between expected and actual performances based on SA or CA may provide a better understanding of individual responsiveness to training in youth soccer. Coaches may also use the agility and dribbling speed tests to assist in profiling athletes by playing position.

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Part IV

General Discussion and Conclusions

Chapter XIII

General discussion and conclusions

13. General discussion and conclusions

An extensive discussion of each of the ten studies' main findings was included in the respective chapters. The rationale of this section was to gather and integrate the contributions of the ten studies, by summarizing the main results and globally reflecting on the implications for future research and practical applications.

13.1. Size descriptors and allometric models for physiological functions

The study in **Chapter 3** investigated the influence of biological maturation and several indicators of body size and estimated body composition on left ventricular mass (LVM) in boys 11-15 years of age using proportional allometric modelling. LVM/stature$^{2.7}$ is the most widely accepted indexing value in older children and adolescents (Dai et al., 2009; de Simone et al., 1992; Khoury et al., 2009), although, results of the present thesis (**Chapter 3, 4 and 6**) and others (Foster et al., 2008, 2013) indicated that LVM/stature$^{2.7}$ did not adequately normalize LVM for stature in adolescent boys. The results of **Chapter 3** showed that after adjusting for the effects of stature, it was also necessary to consider age at peak height velocity (APHV), body mass or fat mass and fat-free mass as simultaneous covariates. Additional variables were considered in the regression models used to normalize LVM in the study of **Chapter 3**. Sitting stature and APHV as combined predictors were important determinants of LVM among the explanatory variables evaluated. This was consistent with observations on late adolescent athletes **(Chapter 4)** among whom sitting stature and skeletal maturation had a major influence on LVM. Nevertheless, the interpretation of LVM in adolescent boys may also be expressed relative body mass if indicators of body composition or biological maturation are not available. The longitudinal contributions of body size, adiposity, chronological age (CA) and biological maturity to developmental changes in LVM among adolescent boys aged 12-15 years were examined in **Chapter 8**. LVM was proportionally higher at 12 years and proportionally lower at 15 years than theoretically expected from stature. The coefficients showed wide confidence intervals and high residual error, and the variance explained by stature decreased by about 48% from 12 to 15 years. By adopting multiplicative allometric models within a multilevel structure, the stature coefficient was isometric or near isometry (k' = 1 or 0.9), when body mass and CA or body mass and maturity offset were respectively encorporated as simultaneous covariates.

Correspondingly, it is expected that absolute peak oxygen uptake (VO_{2peak}) increases as a function of body size during childhood and adolescence, regardless of whether youth are engaged in organized sports. Theoretical allometric coefficients of $k = 0.66$ or $k \geq 0.75$ for body mass have been proposed as appropriate for the expression of oxygen consumption (e.g., Armstrong and Welsman, 1994; Batterham and Jackson, 2003; Cunha et al., 2011; Kleiber and Rogers, 1961; Nevill et al., 2005; Rowland, 2005; Schmidt-Nielsen, 1975). Studies with children and adolescents have noted coefficients ranging from $k = 0.37$ to $k = 1.17$ (Beunen et al., 2002; Cooper et al., 1984; Cunha et al., 2011; Eisenmann et al., 2001; Rowland, 2005; Sjodin and Svedenhag, 1992; Welsman et al., 1996). However, VO_{2peak} may be confounded by variability in total body composition (Dencker et al., 2011) and the individuality of timing and tempo of maturation and year-to-year changes in body mass or body mass components and VO_{2peak} may be masked by maturity effects (Eisenmann et al., 2001).

Chapter 4 and **5** investigate the independent and interactive contributions of maturity, stature, body mass, lean body mass and appendicular descriptors to the inter-individual variation in VO_{2peak} of adolescent roller hockey and soccer athletes. The studies in **Chapter 4** and **5** demonstrate that the relationship between body size dimensions and VO_{2peak} is not proportional. Thigh volume and biological age (i.e., skeletal age; SA) were identified as main contributors to explain the inter-individual variability in VO_{2peak} (**Chapter 4)**. This contribution of lower limb volume may be an effect of interactions among growth, maturation and systematic training (Nevill et al., 2004).

The proportional static allometric models adopted in the study of **Chapter 5**, were consistent with the research on **Chapter 4.** Statistical adjustments were provided to the size descriptors coefficients [8-12 years: k = 1 (lean body mass by anthropometry); 13-15 years: k = 0.7 (lean lower limbs mass); 16-18 years: k = 0.6 (body mass)] with the significant inclusion of the exponential term pubic hair (PH) stage. Allometric scaling of VO_{2peak} from early- to late-adolescence showed lean body mass and body mass as preferential size descriptors compared with appendicular lean mass. Body mass, among other factors, is influenced by growth and maturity related changes in size of the lungs, heart and skeletal muscle (Rowland, 2005), but lean body mass represents more closely the active metabolically cell mass than body mass (Sheng and Huggins, 1979).

Methodologically, **Chapter 5** demonstrates that the best model was obtained with an anthropometric estimate of lean body mass (Foster et al., 2012), that was noted as a non-biased assessment using dual energy X-ray absorptiometry (DXA) protocol as a reference (Bland and Altman, 2012; Holiday et al., 1995; Ludbrook, 2002; Ricker, 1973). By inference, lean body mass derived by anthropometry may be a reasonable and acceptable descriptor of the maximal metabolic capacity of muscles. The contribution of the muscle mass of the exercising lower limbs (Armstrong and Welsman, 2001; Nevill et al., 2004; Winter et al., 1991) and more specifically, the metabolically active tissue (McMurray et al., 2011), was an additional relevant element supported by the present study. Therefore, in **Chapter 7** a new model derived from anthropometry and somatic maturation was proposed for assessing lean lower limbs mass in research dealing with male pediatric samples. The equation was cross-validated using DXA measures as the reference method and shows a predictive power that compares favourably with those reported in the literature for similar purposes (Coelho-e-Silva et al., 2013; Fuller et al., 2002; Kim et al., 2006; Lee et al., 2000; Machado et al., 2013; Quitério et al., 2009; Welsman et al., 1997).

Overall, **Chapters 3 to 6 and 8** of the present thesis support the well-acknowledged limitations of ratio standards and theoretical allometric coefficients. Moreover, the differential growth in linear and mass dimensions during adolescence in addition to differential timing of growth spurts in stature, leg length, sitting stature and body mass (Malina et al., 2004a) seems to contribute to problems in the derivation of unique scaling coefficients. The aforementioned Chapters and previous research (e.g., Balasekaran et al., 2005; Rowland, 1998; Thomis et al., 2000) have shown that scaling coefficients obtained for a particular size descriptor may not be appropriate for all ages and that allometric coefficients should be sample-specific. Longitudinal studies spanning childhood and adolescence are needed to further understand curve trajectories of physiological functions with a focus on intra-individual or ontogenetic allometric coefficients (*k*') derived multilevel multiplicative models that may be more appropriate for the analysis of these traits during adolescence.

13.2. Modelling functional development during pubertal years

Through the use of multilevel modelling, we were able to model not only the within-subject variation of several functional capacities with time but also the variation between subjects while simultaneously accounting for other explanatory variables, such as age, biological maturation, body size, composition and training, of which have been shown to contribute substantially to within-subject variation (**Chapters 9 to 12**).

It is known, for example, that young adults typically show a 15–20% increase in aerobic fitness with training, although there may be large intra-individual variation due to genetic factors (Bouchard et al., 1992). Despite the arguments and research suggesting that prepubescent children are not capable of improving their aerobic fitness with training (Welsman et al., 1997), there is much evidence to suggest otherwise (Rowland and Boyajian, 1995; Valente-dos-Santos et al., 2012).

Longitudinal data on boys from the Leuven Longitudinal Twin Study, suggested a simultaneous regulation of the timing of maximum growth in body dimensions and aerobic fitness during adolescence (Geithner et al., 2004). Measured and the estimated cardiorespiratory fitness (CRF) among fit adolescent boys improved continuously from 11 to 15 years (**Chapter 9**). The age of 15, is consistent with the average age of end of peak growth velocity and the peak improvement in CRF (Philippaerts et al., 2006). Longitudinal studies that assess changes in CRF are more abundant in athletes compared to healthy school boys and did not systematically consider physical activity (PA). The study of **Chapter 9** showed that sedentary time had a significant negative association with CRF in healthy adolescent boys, after controlling for age and adiposity. Daily PA did not independently predicted CRF after adjustments for age, subcutaneous adiposity and sedentary time. It is likely that more vigorous PA as in intensive training is needed to influence CRF over and beyond changes occurring with normal growth and development in fit adolescents. Valente-dos-Santos et al. (2012) recently showed that the development of this trait in youth soccer players was substantially related to annual volume of training.

Longitudinal observations for Belgian youth soccer players indicated peak gains in explosive strength and running speed close to the APHV (Philippaerts et al., 2006). Of interest, estimated annual increments in tests of explosive strength and running speed in the soccer players decline immediately after APHV but then maintained at a plateau in contrast to declining increments noted in vertical jump and shuttle-run increments observed in an earlier longitudinal study of Belgian boys using a similar modelling protocol (Beunen and Malina, 1988). The **Chapter 10** study assessed two multilevel models to predict developmental changes in performance related fitness of short-term duration (i.e., repeated-sprint ability; RSA). The study highlighted biological age as one of the most relevant predictors of RSA. When data was aligned by CA, the most marked improvements occurred from ages 11 to 15 years (14.6%). After that age, the improvement rate was substantially reduced (about 2%). The study also showed that the estimated amounts of fat-free mass (FFM) and fat mass (FM) significantly improved the fitness of the models. From 11 to 17 years, each unit of FFM (in kg) corresponds to an improvement of ~0.1 seconds in RSA. The distinction between age and training effects on functional capacities is complex. Complementarily, the study in **Chapter 12** looked at the longitudinal changes in the of youth soccer players ability to rapidly changing body direction and position in the horizontal plane (agility run) and sprinting while controlling the ball. The longitudinal contributions training history, body size and composition, and SA where investigated through the use of multiplicative allometric model structures. Based on the multilevel models with best statistical fit, the results suggest that an increase in 1 kg in FFM was associated with improvement in agility performance by 5%, when body size, body composition and SA are controlled, regardless of playing position. There also appears to be an independent training effect; each 1 h of additional training per week over the competitive season (~ 40-weeks) was associated with 3% improvement in dribbling speed performance, when stature and SA are controlled. Generally similar results were independent studies of Portuguese youth players (Coelho-e-Silva et al., 2010; Hirose, 2009; Malina et al., 2004b, 2000). All of the studies noted cross-sectional maturity-associated variation of body size and functional capacities, but not in soccer-specific skills. The results highlight the need for awareness of conclusions drawn from cross-sectional studies that do not take into account the influence of individual differences in maturity status on body size and function among youth.

During adolescence, players develop themselves in numerous aspects of the multidimensional performance characteristics. In **Chapter 11**, attention was paid to the longitudinal changes in several functional and soccer-specific skills of male youth soccer players. The developmental curve trends suggest a more curvilinear development with increasing age but with a higher rate of development of the functional capacity after the age of 14, compared to skill ability. This trend is somewhat expected and consistent with the longitudinal perspective provided by Philippaerts and colleagues (2006), where peak weight velocity and many physical performance characteristics showed peak developments at or slightly after APHV. Skeletal maturity status, established at baseline, was considered as a longitudinal predictor of functional capacity. The composite score showed an expected gradient of early > on time > late. Playing position was a significant developmental predictor of skill performance, with midfielders being the most skilled players across time. These findings may support the conjecture that CA is more closely related to skill (i.e., ball control, dribbling speed, shooting and passing) and SA to the functional performance (i.e., repeated-sprint ability, agility, lower limb explosive strength and CRF). The characteristics by position may overlap with age- and maturity-associated variation, and perhaps demands of specific positions as the level of competition increases. Nevertheless, recent literature shows the performance advantages associated with 'early' maturation are short lived (Malina et al., 2004a; Valente-dos-Santos et al., 2014).

13.3. Directions for future research

Specific limitations of the ten studies included in this thesis were individually considered in previous **Chapters 3** to **12**. The purpose of this section is to point out more general concerns regarding the collective work in this thesis and to integrate these for future research.

The allometric models put forward in this paper need to be validated in different samples (e.g., in females, other ethnic groups, adults or specific populations of people with varying anthropometric characteristics) and sports contexts and a wider matrix of predictors should be explored.

Most of the allometric data presented in this thesis are cross-sectional. Thus, implications for understanding changes, for example, in aerobic fitness associated with growth and maturation are only suggestive. The data do not address longitudinal changes in VO_{2peak} during adolescent growth spurt and their associations with individual differences in biological maturity, which are discussed more in detail in **Chapter 9** relative to CRF.

After controlling for the primary sources of variation in the growing individual, substantial amount of variation in aerobic fitness remains. It is possibly related to structural, physiological, and biochemical factors associated with aerobic energy output. Longitudinal studies spanning childhood and adolescence are needed to further understand curve trajectories of VO_{2peak} with a focus on intra-individual or ontogenetic allometric coefficients derived from multilevel multiplicative models that may be more appropriate for the analysis of VO_{2peak} during adolescence.

Future research addressing maturity-associated variability in cardiac structure needs to identify factors that could mediate (direct effects) or moderate (indirect effects) the relationship between biological maturation and developmental changes in echocardiographic parameters. It would also be relevant to include alternative measurements such as lean body mass and FM derived from dual-energy X-ray absorptiometry, air displacement plethysmography or anthropometry.

Future studies should continue to consider the development and cross-validation of anthropometric equations for estimating total and regional size descriptors such as lean lower limbs mass using, for example, DXA assessments as a reference. This approach has the potential to provide reliable, inexpensive and useful information.

According to the important public health priority on promotion of active lifestyles among young people, and taking into account the related potential factors that influence aerobic fitness or CRF, PA and sedentary behaviour, further investigations are needed to better understand the role of sport participation in healthy lifestyles among youth. Research using built environment variables is also recommended to enhance the understanding of health-related behaviours and therefore define educational and clinical interventions.

Within sports context, more longitudinal data on growth, maturation, and performance of young athletes are obviously necessary to evaluate the importance of selective factors as well as potential effects of training. Ideally, future longitudinal approaches should explore a fitness battery that corresponds better to match performance and incorporates the characteristics that appeared relevant in sport selection and sport orientation.

Although this thesis revealed the some development characteristics of youth soccer players, it is still unclear to what extent and how these functional characteristics, for example, can be trained. If more insight can be given into this question, sports in general and soccer in particular can benefit enormously.

An important step to take is to measure current training programs and when necessary to develop, implement and evaluate new training programs with the goal to improve functional characteristics of adolescent athletes.

13.4. Conclusions and implications

The main purpose of this thesis was to gain more insight into the relationship between (the development of) cardiac dimensions and several functional capacities with body size, biological maturation, physical activity and/or sedentary time in adolescent male athletes and non-athletes, using allometric and multilevel modelling approaches. Allowing for the variation in methodology and sampling in studies presented on this thesis, it can be concluded that:

- *i.* Adolescents of the same CA vary considerably in biological maturity, and individual differences in maturity influence measures of physiological functions and functional performance.
- *ii.* Adolescent non-athletes grow in a manner similar to athletes. Much of the variation is most likely associated with selective or exclusion criteria's of some sports and with variation in rate of biological maturation.
- *iii.* During puberty, functional performance of boys, on average, show a marked improvement. High-intensity short-term, intermediate-term and long-term functional performance seems to improve during early- and mid-adolescent and then remains rather constant when aligned by CA. When aligned by SA the improvement, for example, in high-intensity short-term activities is rather constant through the adolescence period.
- *iv.* Body size and biological maturity account for a substantial part of variation in performance during adolescence, but a considerable variance is not accounted for by these variables.
- *v.* Lean body mass and lower limbs lean soft tissue derived by anthropometry may be a reasonable and acceptable descriptor of the maximal metabolic capacity of muscles. The equations to derive both descriptors were satisfactorily cross-validated in the present thesis at group and individual basis, ensuring its applicability to similar samples.

vi. Training is a significant factor affecting body composition, physiological parameters and functional performance. Again, this implies that some variation between athletes and non-athletes may represent an interaction between individual characteristics and selective considerations for sport participation.

vii. Given the association among maturity status, body size, and many functional parameters, the greater peak aerobic fitness and LVM is in part a function of maturity-associated variation in body size.

viii. Left ventricular mass do not grows proportionally to body mass as previously assumed in the literature. The heart progressively adapts to performing more work during puberty. Upper body length measured as sitting stature was the most robust individual determinant of LVM in the roller hockey players.

ix. Although regular PA is related to health related physical fitness, the relationship is not strong. Higher levels of CRF were significantly related with the longitudinal decrease of time sedentary and adiposity.

x. Allometric modelling procedures were statistically appropriate to account for the intra- and inter-individual variability in body dimensions, biological maturity, physiological and functional parameters. Nevertheless, the extensive use of allometric models based on single descriptors must be questioned.

xi. Multiplicative allometric modelling improves the statistical fit of regression models and provides plausible interpretations of cross-sectional and longitudinal data of cardiac morphology and functional capacity in adolescent boys. Multilevel modelling is a promising statistical technique for the derivation of developmental curves. It has the potential to provide useful information both for clinicians, coaches and conditioning trainers in the explanation of inter-individual differences at a certain age and probably more important to predict and explain changes over time, allowing a critical interpretation of variation within and between individuals.

From the studies in the current thesis practical implications for clinicians, physical education teachers, trainers, coaches, parents and other interested parties can be derived. The resulting equations permit to acquire this information in a safe, cost-effective and non-invasive manner. The studies focused in the cardiac structure may be relevant, for example, to the accuracy of clinical assessments of LVM in the context of preparticipation physical examinations of adolescent athletes, where the utility of echocardiography is still under discussion. The prospective part of the present thesis allowed quantifying the impact of the principal modifiable determinants of several functional parameters. The development curves on functional performance make it possible, for example, to compare the development of a youth player with these performance curves. This allows trainers and coaches to assess an individual's performance relative to these curves. Applying the curves, trainers and coaches can determine if a player is performing above or below average for his age. Since the studies also present possible underlying mechanisms of functional capacities, it may also be indicated which factors may be responsible for eventual poorer performance.

13.5. References

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Valente-dos-Santos J, Coelho-e-Silva MJ, Vaz V, Figueiredo AJ, Capranica L, Sherar LB, Elferink-Gemser MT, Malina RM. 2014. Maturity-associated variation in change of direction and dribbling speed in early pubertal years and 5-year developmental changes in young soccer players. J Sports Med Phys Fitness 54:307–316.

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Winter EM, Brookes FB, Hamley EJ. 1991. Maximal exercise performance and lean leg volume in men and women. J Sports Sci 9:3–13.

Appendix A

Curriculum Vitae

I.
PERSONAL AND PROFESSIONAL DATA

1.1. Personal data

Full name: João Alberto Valente dos Santos

National identity card: 12464667

Birth place and date: Salreu, 1983

Nationality: Portuguese

Institutional address: Universidade de Coimbra, Estadio Universitario, Pavilhao III, 3040-156 Coimbra, Portugal

Living address: Rua da Ladeira n.º 27, 3865-251 Salreu

Contact data: +351 916684596, j.valente-dos-santos@hotmail.com

1.2. Academic degrees

#4

Period: 2010-2014 (submitted and now waiting for public defence)

Academic degree: PhD in Sport Sciences in the branch of Sports Training

Institution: Faculty of Sport Sciences and Physical Education of the University of Coimbra, Portugal; Center for Human Movement Sciences da University of Groningen, The Netherlands; Department of Kinesiology and Health Education, University of Texas at Austin, United States.

Title: Body size, composition, cardiac morphology and functional capacities: Scaling and modelling developmental changes during the pubertal years

Classification: N.A.

Period: 2011-2013

Academic degree: Master in Management and Educational Administration: Educational Organizations and School Management

Institution: Faculty of Psychology and Educational Sciences of the University of Coimbra, Portugal.

Title: Socio-professional identity, participation in school life and interpersonal relationships between physical education teachers and the members of school community

Classification: 19 on a scale of 0 to 20

Period: 2007-2009

Academic degree: Master in Youth Sports Training

Institution: Faculty of Sport Sciences and Physical Education of the University of Coimbra, Portugal.

Title: Aerobic Power and cardiac dimensions in adolescent roller hockey players: Contribution of body size, skeletal age and training

Classification: Very good on a scale of unsatisfactory to very good

Period: 2002-2006

Academic degree: Bachelor in Sports Sciences and Physical Education

Institution: Faculty of Sport Sciences and Physical Education of the University of Coimbra, Portugal.

Classification: 17 on a scale of 0 to 20

1.3. Relevant professional formation

Period: 2006-2007

Position: Assistant Coach under the Leonardo da Vinci program

Institution: Football Club Barcelona

Classification: Very good on a scale of unsatisfactory to very good

1.4. Relevant scientific formation

#3

Period: 17 May to 07 June 2013
Designation: Advanced Course in Clinical and Translational Biostatistics
Institution: Faculty of Medicine of the University of Coimbra, Portugal
Classification: 17 on a scale of 0 to 20

Period: 23 February to 28 February 2012
Designation: Longitudinal Data Analysis
Institution: Canadian Society for Epidemiology and Biostatistics, Canada
Classification: Successfully Completed

Period: 07 February to 14 April 2011
Designation: Master Course of Multilevel Modelling
Institution: Faculty of Medical Sciences at the University of Groningen, The Netherlands.
Classification: Approved

1.5. Visiting positions

#3

04 January 2013 to 29 April 2013, in the Center for Human Movement Sciences da University of Groningen – The Netherlands, with a short period visit to the School of Sport, Exercise and Health Sciences, Loughborough University, United Kingdom. The working program was supervised by Prof. Dr. Lauren

Sherar, Prof. Dr. Dale Eslieger, Prof. Dr. Manuel João Coelho e Silva and Prof. Dr. Robert Malina.

06 January 2012 to 02 April 2012, in the Department of Kinesiology and Health Education, University of Texas at Austin with a short period visit to the University of Saskatchewan, College of Kinesiology, Canada. The working program was supervised by Prof. Dr. Robert Malina, Prof. Dr. Lauren Sherar, Prof. Dr. Adam Baxter-Jones and Prof. Dr. Manuel João Coelho e Silva.

07 February 2011 to 14 April 2011, in the Center for Human Movement Sciences da University of Groningen, The Netherlands. The working program was supervised by Prof. Dr. Marije Elferink-Gemser, Prof. Dr. Manuel João Coelho e Silva and Prof. Dr. Robert Malina.

1.6. Previous and current scientific and professional activities

Period: 2013-2014

Position or category: Scientific-pedagogical supervisor within the curricular units of Investments in Sports Careers and Initiation into Professional Practice in the MSc of Youth Sports Training

Institution: Faculty of Sport Sciences and Physical Education of the University of Coimbra, Portugal

Classification: N.A.

Period: 2012-2013

Position or category: Scientific-pedagogical supervisor within the curricular units of Investments in Sports Careers and Initiation into Professional Practice in the MSc of Youth Sports Training

Institution: Faculty of Sport Sciences and Physical Education of the University of Coimbra, Portugal

Classification: N.A.

Period: 2011-2012

Position or category: Invited Assistant Teacher: curricular units of Handball (Bachelor in Sport Sciences) and Readiness and Sports Talent (MSc of Youth Sports Training)

Institution: Faculty of Sport Sciences and Physical Education of the University of Coimbra, Portugal

Classification: N.A.

Period: 2010-2011

Position or category: Invited Assistant Teacher: curricular units of Handball (Bachelor in Sport Sciences) and Readiness and Sports Talent (MSc of Youth Sports Training)

Institution: Faculty of Sport Sciences and Physical Education of the University of Coimbra, Portugal

Classification: N.A.

Period: 2009-2010

Position or category: Physical Education Teacher

Institution: Agrupamento de Escolas da Serra da Gardunha, Fundão, Portugal

Classification: Excellent on a scale of unsatisfactory to excellent

Period: 2008-2009
Position or category: Physical Education Teacher
Institution: Agrupamento de Escolas de Finisterra, Cantanhede, Portugal
Classification: Excellent on a scale of unsatisfactory to excellent

Period: 2007-2008
Position or category: Physical Education Teacher
Institution: Agrupamento de S. Silvestre, Coimbra, Portugal
Classification: N.A.

Period: 2006-2007 (February to July)
Position or category: Physical Education Teacher
Institution: Agrupamento de Escolas de Vilarinho do Bairro, Anadia, Portugal
Classification: N.A.

Period: 2005-2006
Position or category: Physical Education Teacher
Institution: Agrupamento de Escolas Castro Matoso de Oliveirinha, Aveiro, Portugal
Classification: 19 on a scale of 0 to 20

1.7. Network / Main Coauthors

Adam Baxter-Jones: College of Kinesiology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

Amândio Cupido-dos-Santos: Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal

António J. Figueiredo: Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal

Analiza M. Silva: Exercise and Health Laboratory, Faculty of Human Kinetics, University of Lisbon, Lisbon, Portugal

André Seabra: Faculty of Sport, University of Porto, Porto, Portugal

Aristides M. Machado-Rodrigues: Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal

Chris Visscher: Center for Human Movement Sciences, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Claudia Minderico: Exercise and Health Laboratory, Faculty of Human Kinetics, University of Lisbon, Lisbon, Portugal

Daniel Courteix: Clermont Université, Université Blaise Pascal, Laboratoire des Adaptations Métaboliques à l'Exercice en Conditions Physiologiques et Pathologiques, Clermont-Ferrand, France

Édio L. Petroski: Research Centre for Kinanthropometry and Human Performance, Federal University of Santa Catarina, Florianopolis, Brazil

Joaquim Castanheira: School of Health and Technology, Coimbra, Portugal

Laura Capranica: Università degli Studi di Roma - Foro Italico, Italy

Lauren Sherar: School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, Leicestershire, United Kingdom

Luís B. Sardinha: Exercise and Health Laboratory, Faculty of Human Kinetics, University of Lisbon, Lisbon, Portugal

Manuel J. Coelho-e-Silva: Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal

Marije T. Elferink-Gemser: Center for Human Movement Sciences, University Medical Center Groningen, University of Groningen, Groningen; Institute for Studies in Sports and Exercise, The Netherlands; HAN University of Applied Sciences, Nijmegen, The Netherlands

Matthieu Lenoir: Department of Movement and Sports Sciences, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium

Raúl S. Martins: Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal

Renaat M. Philippaerts: Department of Movement and Sports Sciences, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium

Robert M. Malina: Department of Kinesiology and Health Education, University of Texas at Austin; Department of Kinesiology, Tarleton State University, Stephenville, United States

Roel Vaeyens: Department of Movement and Sports Sciences, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium

Sean P. Cumming: School of Health, University of Bath, United Kingdom

Vasco Vaz: Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal

II.
RESEARCH

2.1. Chapters in books

12. Valente-dos-Santos J, Coelho-e-Silva MJ, Castanheira J, Ronque E, Elferink-Gemser MT, Malina RM (2014). Modelling developmental changes in left ventricular mass using multiplicative allometric and additive polynomial multilevel modelling in boys aged 11-16 years. In M Coelho-e-Silva, A Cupido-dos-Santos, A Figueiredo, J Ferreira, N Armstrong (editors). *Children and Exercise XXVIII*. Abingdon, Oxon. Routledge. pp: 153-158.
11. Castanheira J, Valente-dos-Santos J, Duarte J, Pereira JR, Rebelo-Gonçalves R, Severino V, Machado-Rodrigues A, Vaz V, Figueiredo AJ, Coelho-e-Silva MJ, Sherar L, Elferink-Gemser MT, Malina RM (2014). Allometric scaling of left ventricular mass in relation to body size, fat-free mass and maturation in 13-year-old boys. In M Coelho-e-Silva, A Cupido-dos-Santos, A Figueiredo, J Ferreira, N Armstrong (editors). *Children and Exercise XXVIII*. Abingdon, Oxon. Routledge. pp: 133-137
10. Duarte J, Severino V, Pereira JR, Fernandes RA, Simões F, Rebelo-Gonçalves R, Valente-dos-Santos J, Vaz V, Seabra A, Coelho-e-Silva MJ (2014). Reproducibility of Repeated Sprint Ability. In M Coelho-e-Silva, A Cupido-dos-Santos, A Figueiredo, J Ferreira, N Armstrong (editors). Children and Exercise XXVIII. Abingdon, Oxon. Routledge. pp: 287-291
9. Wierike S, Yvonne-Tromp E, Valente-dos-Santos J, Elferink-Gemser M, Visscher C (2014). Coaches' judgment about current and future performance level of basketball players. Allometric scaling of left ventricular mass in relation to body size, fat-free mass and maturation in 13-year-old boys. In M Coelho-e-Silva, A Cupido-dos-Santos, A Figueiredo, J Ferreira, N Armstrong (editors). Children and Exercise XXVIII. Abingdon, Oxon. Routledge. pp: 243-246
8. Baptista R, Cupido-dos-Santos A, Duarte JP, Pereira JR, Rebelo-Gonçalves R, Severino V, Valente-dos-Santos J, Rego I, Coelho-e-Silva MJ, Coelho e Silva MJ, Fontes-Ribeiro CA, Capranica L, Armstrong N (2014). Allometric modelling of peak power output obtained from a force-velocity protocol in

prepubertal boys. In M Coelho-e-Silva, A Cupido-dos-Santos, A Figueiredo, J Ferreira, N Armstrong (editors). Children and Exercise XXVIII. Abingdon, Oxon. Routledge. pp: 293-296

7. Pereira J, Vaz V, Valente-dos-Santos J, Coelho e Silva MJ, Soles-Gonçalves R, Páscoa Pinheiro R, Areces A, Atkinson G (2014). Advancement in the interpretation of isokintic ratios derived from the harmstrings and quadriceps. In M Coelho-e-Silva, A Cupido-dos-Santos, A Figueiredo, J Ferreira, N Armstrong (editors). Children and Exercise XXVIII. Abingdon, Oxon. Routledge. pp: 125-129 [ISBN: 978-0-415-82972-4]

6. Rebelo-Gonçalves R, Figueiredo AJ, Duarte JP, Pereira JR, Fernandes RA, Simões F, Severino V, Valente-dos-Santos J, Cupido-dos-Santos A, Coellho-e-Silva MJ, Tessitore A, Armstrong N (2014). Agreement between peak power outputs obtained from the application of common braking force and the estimated optimal load in soccer goalkeepers. In M Coelho-e-Silva, A Cupido-dos-Santos, A Figueiredo, J Ferreira, N Armstrong (editors). Children and Exercise XXVIII. Abingdon, Oxon. Routledge. pp: 277-281.

5. Severino V, Coelho e Silva MJ, Duarte JP, Pereira JR, Rebelo-Gonçalves R, Valente-dos-Santos J, Castagna C, Figueiredo AJ (2014). Absolute and scaled peak power assessments in young male soccer players: variation by playing position. In M Coelho-e-Silva, A Cupido-dos-Santos, A Figueiredo, J Ferreira, N Armstrong (editors). Children and Exercise XXVIII. Abingdon, Oxon. Routledge. pp: 257-261 [ISBN: 978-0-415-82972-4]

4. Coelho e Silva MJ, Valente-dos-Santos J, Simões F, Pinho R, Abreu A, Figueiredo AJ, Pena-Reyes ME, Malina RM (2013). Sport selection in under-14 male soccer players. In PT Katzarzyk, MJ Coelho-e-Silva (editors). Growth and maturation in Human Biology and Sports: Festschrift honoring Robert M Malina by fellows and colleages. Imprensa da Universidade de Coimbra / Coimbra University Press.

3. Valente-dos-Santos J, Vaz V, Santos A, Figueiredo AJ, Elferink-Gemser MT, Malina RM, Coelho-e-Silva MJ (2012). Short-and long-term maximal protocols and their contribution to differentiate under-17 hockey players by

competitive level. In CA Williams, N Armstrong (editors). *Children and Exercise XXVII.* Routledge. 295-300 [ISBN: 978-0-415-57859-2]

2. Coelho e Silva MJ, Carvalho HM, Vaz V, Valente-dos-Santos, Figueiredo AJ, Elferink-Gemser MT, Malina RM (2012). Contribution of organized sports to estimated energy expenditure in female adolescents. In CA Williams, N Armstrong (editors). *Children and Exercise XXVII.* Routledge. 309-313 [ISBN: 978-0-415-57859-2]

1. Coelho e Silva MJ, Simões F, Valente-dos-Santos J, Vaz V, Figueiredo AJ, Peña Reyes ME, Malina RM (2011). Assessment of biological maturation in adolescent athletes – application of different methods. In MJ Coelho e Silva, AJ Figueiredo, MT ELferink-Gemser, RM Malina (editors). *Youth Sports – volume 2: growth, maturation and talent.* Coimbra University Press. [ISBN: 978-989-26-0005-5]

2.2. Articles in peer review international journals

28. Valente-dos-Santos J, Seabra A, Coelho-e-Silva MJ, Tavares OM, Rebelo A, Brito J, Sherar LB, Elferink-Gemser MT, Malina RM (Accepted, 2014). Allometric modelling of peak oxygen uptake in male soccer players 8-18 years of age. *Annals of Human Biology*.

27. Valente-Dos-Santos J, Coelho-E-Silva MJ, Castanheira J, Cyrino ES, Sherar LB, Esliger DW, Elferink-Gemser MT, Malina RM (Accepted, 2014). A longitudinal study of adolescent boys left ventricular mass development. *American Journal of Human Biology*.

26. Valente-dos-Santos J, Coelho-e-Silva MJ, Machado-Rodrigues AM, Elferink-Gemser MT, Malina RM, Petroski EL, Minderico C, Silva AM, Baptista F, Sardinha LB (Accepted, 2014). Determination of Lower Limbs Lean Soft Tissue in Circumpubertal Boys using Anthropometry and Biological Maturation. *Plos One*.

25. Courteix D, Valente-dos-Santos J, Coelho-e-Silva MJ, Burt L, Obert P, Lac G, Dutheil F (Under Review). Multilevel approach of a 1-year program of

diet and exercise interventions on bone mineral content and density in metabolic syndrome: Results from the RESOLVE randomized trial. *Journal of Bone Mineral Research*.

24. Vaz V, Dias G, Gama J, Rafael J, Valente-dos-Santos J, Areces-Gayo JA, Couceiro M (Under Review). Network – analysis of interpersonal interactions in sports teams. *Journal of Human Kinetics*.
23. Kramer T, Valente-dos-Santos J, Coelho-e-Silva MJ, Malina RM, Huijgen BCH, Smith J, Elferink-Gemser MT, Visscher C (Under Review). Modelling longitudinal changes in 5-m sprinting performance in youth male tennis. *International Journal of Sports Physiology and Performance*.
22. Martins R, Macedo RB, Sousa NF, Coelho-e-Silva MJ, Cumming SP, Valente-dos-Santos J, Machado-Rodrigues A, Gonçalves RS (Under Review). Quality of life, school backpack weight, and nonspecific lower back pain in children and adolescents *Jornal de Pediatria*.
21. Coelho-e-Silva MJ, Severino V, Martinho D, Pereira J, Duarte J, Valente-dos-Santos J, Machado-Rodrigues A, Cumming SP, Malina RM (Accepted, 2014). Reproducibility of peak power output during a 10-s sprint adopting different sampling rates. *Acta Physiologica Hungarica*.
20. Castanheira J, Valente-dos-Santos J, Duarte J, Vaz V, Figueiredo AJ, Leite N, Cyrino ES, Coelho-e-Silva MJ (Accepted, 2014). Youth left ventricular morphology: Comparison between elite athletes and non-athletes *Brazilian Journal of Sports Medicine*.
19. Valente-dos-Santos J, Coelho-e-Silva MJ, Vaz V, Figueiredo AJ, Capranica L, Sherar LB Elferink-Gemser MT, Malina RM (2014). Maturity-Associated Variation in Change of Direction and Dribbling Speed in Early Pubertal Years and 5-Year Developmental Changes in Young Soccer Players. *Journal of Sports Medicine and Physical Fitness*, 54(3):307-316.
18. Coelho e Silva MJ, Valente-dos-Santos J, Duarte J, Pindus DM, Lauren B Sherar, Malina RM (2013). Controlling Performance and Physiological Parameters for Body Size and Inter-individual Variability due to Biological Maturation during Adolescent Growth Spurt. *Journal of Sports medicine & Doping Studies*, 3(3): 2-3

17. Valente-dos-Santos J, Coelho-e-Silva MJ, Pereira J, Duarte J, Rebelo-Gonçalves R, Figueiredo A, Mazzuco M, Sherar L, Elferink-Gemser MT, Malina RM (2014). Allometric Multilevel Modelling of Agility and Dribbling Speed by Skeletal Age and Playing Position in Youth Soccer Players. *International Journal of Sports Medicine*. doi: 10.1055/s-0033-1358469

16. Machado-Rodrigues A, Leite N, Coelho-e-Silva M, Martins R, Valente-dos-Santos J, Padez C, Mascarenhas L, Malina RM (2014). Independent association of metabolic risk factors with cardiorespiratory fitness in boys 11-17 years. *Annals of Human Biology*. 41(3): 271–276.

15. Laureano MLM, Martins RA, Sousa NM, Machado-Rodrigues AM, Valente-Santos J, Coelho-e-Silva MJ (Accepted, 2014). Association between functional fitness with the annual cost of medicine consumption and mood states in elderly people. *Revista da Associação Médica Brasileira*.

14. Machado-Rodrigues AM, Neiva Leite, Manuel J. Coelho-e-Silva, Raul A. Martins, João Valente-dos-Santos, Luís PG Mascarenhas, Margaret C. S. Boguszewski, Cristina Padez, Robert M. Malina (Accepted, 2014). Metabolic syndrome, physical activity and sedentary behaviour in female adolescents. *Journal of Physical Activity and Health.*

13. Valente-Dos-Santos, J., Coelho, E. S. M. J., Ferraz, A., Castanheira, J., Ronque, E. R., Sherar, L. B., Elferink-Gemser MT, Malina, R. M. (2014). Scaling left ventricular mass in adolescent boys aged 11-15 years. *Annals of Human Biology*. doi: 10.3109/03014460.2013.866694

13. Deprez, D., Valente-Dos-Santos, J., Coelho, E. S. M., Lenoir, M., Philippaerts, R. M., & Vaeyens, R. (2014). Modelling Developmental Changes in Yo-Yo IR1 in Elite Pubertal Soccer Players. *International journal of sports physiology and performance*. doi: 10.1123/ijspp.2013-0368

12. Coelho-e-Silva MJ, Ronque E, Fernandes R, Valente-dos-Santos J, Machado-Rodrigues A, Martins R, Figueiredo AJ, Sanos R, Malina RM (2013). Weight status, biological maturation and cariorespiratory fitness in Azorean adolescents aged 11-15 years. *BMC Public Health.* 13: 495-505.

11. Coelho-e-Silva MJ, Valente-dos-Santos J, Simões F, Ronque E, Petroski E, Minderico C, Silva AM, Baptista F, Sardinha LB (2013). Determination of thigh volume in children: agreement between anthropometry and DXA assessments. *European Journal of Sport Science,* 13 (5): 527-533.

10. Valente-dos-Santos J, Coelho-e-Silva MJ, Vaz V, Castanheira J, Leite N, Sherar N, Elferink-Gemser MT, Baxter-Jones A, Malina RM (2013). Ventricular mass in relation to body size and skeletal in adolescent athletes. *Clinical Journal of Sports Medicine*, 23 (4): 293-299.

9. Valente-dos-Santos J, Sherar LB, Coelho-e-Silva MJ, Vaz V, Pereira J, Cupido-dos-Santos A, Baxter-Jones A, Visscher C, Elferink-Gemser MT, Malina RM (2013). Allometric scaling of peak aerobic fitness in Under-17 roller hockey players: The contribution of biological age and size-related characteristics. *Applied Physiology Nutrition and Metabolism.* 38: 390–395.

8. Coelho e Silva MJ, Valente-dos-Santos J, Figueiredo AJ, Lauren B Sherar, Malina RM (2013). Pubertal status – assessment, interpretation, analysis. *Journal of Sports medicine and Doping Studies*, 3(1): 2-3.

7. Valente-dos-Santos J, Coelho-e-Silva MJ, Simões F, Figueiredo A, Leite N, Elferink-Gemser MT, Malina RM, Sherar L (2012). Modeling developmental changes in functional capacities and soccer-specific skills in Male Players Aged 11-17 Years. *Pediatric Exercise Sciences*. 24 (4): 603-621.

6. Valente-dos-Santos J, Coelho-e-Silva MJ, Severino V, Duarte J, Martins R, Figueiredo A, Seabra AT, Philippaerts R, Cumming SP, Elferink-Gemser MT, Malina RM (2012). Longitudinal study of repeated sprints in youth soccer players of contrasting skeletal maturity status. *Journal of Sports Sciences and Medicine,* 11: 371-379.

5. Coelho e Silva MJ, Vaz V, Simões F, Valente-dos-Santos J, Figueiredo AJ, Pereira V, Vaeyens R, Philippaerts R, Elferink-Gemser MT, Malina RM (2012). Sport selection in under-17 male roller hockey. *Journal of Sports Sciences*. 30 (16): 1793-1802.

4. Valente-dos-Santos J, Coelho-e-Silva MJ, Martins R, Figueiredo A, Vaeyens R, Sherar L, Huijgen B, Elferink-Gemser MT, Malina RM (2012). Modeling

developmental changes in repeated sprints of young soccer players. *International Journal of Sports Medicine*. 33 (10): 773-780.

3. Coelho e Silva MJ, Valente-dos-Santos J, Figueiredo AJ, Malina RM (2012). Ages of youth sport participants – is verification a concern? *Journal of Sports medicine and Doping Studies*, 2(3): 2-3.
2. Carvalho HM, Coelho-e-Silva MJ, Valente-dos-Santos J, Gonçalves RS, Philippaerts R, Malina RM (2012). Scaling lower-limb isokinetic strength for biological maturation and body size in adolescent basketball players. *European Journal of Applied Physiology*, 112 (8): 2881-2889.
1. Valente-dos-Santos J, Coelho-e-Silva MJ, Duarte J, Figueiredo AJ, Liparotti J, Sherar LB, Elferink-Gemser MT, Robert M. Malina (2012). Longitudinal predictors of aerobic performance in adolescent soccer players. *Medicina (Kaunas)*, 48 (8): 410-416.

2.3. Articles in peer review national journals

4. Vaz V, Dias G, Gama J, Rafael J, Valente-dos-Santos J, Areces-Gayo JA, Couceiro M (2014, in press). [Network: analysis of the interaction and dynamics of a football match]. *Revista Portuguesa de Ciências do Desporto*.
3. Valente-dos-Santos, Coelho e Silva MJ, Figueiredo AJ, Castanheira J, Vaz V, Elferink-Gemser MT, Malina RM (2011). [Echocardiographic study of left ventricular dimension in young athletes: Concepts, methods, evidences and future research]. *Gymnasium – Revista de Educação Física Desporto e Saúde*, 3(4): 99-123.
2. Vaz V, Valente-dos-Santos J, Coelho e Silva MJ, Gayo A (2007). [Performance analysis in roller hockey]. *Revista Portuguesa de Ciências do Desporto*, 7(Supplement): 83-84.
1. Valente-dos-Santos J (2006). [Physical work of young roller hockey athletes]. *Revista de Patinagem*. 1(1): 55-66.

2.4. Abstracts in international conference proceedings

33. Valente-dos-Santos J, Coelho-e-Silva MJ, Castanheira J, Cyrino ES, Esliger D, Sherar LB, Elferink-Gemser MT, Malina RM (2014). Developmental changes of left ventricular mass during pubertal years using static and ontogenetic allometric exponents in boys aged 11-14 years. *Medicine and Science in Sports and Exercise*. Vol. 46 (5): S456-457.

32. Castanheira J, Coelho-e-Silva MJ, Valente-dos-Santos J, Ferraz A, Ronque E, Sherar LB, Elferink-Gemser MT, Malina RM (2014). Normalizing left ventricular mass for different size descriptors using allometric exponents in adolescent boys 11-15 years. *Medicine and Science in Sports and Exercise*. Vol. 46 (5):S456.

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27. Castanheira J, Valente-dos-Santos J, Duarte J, Pereira JR, Rebelo-Gonçalves R, Severino V, Machado-Rodrigues A, Vaz V, Figueiredo AJ, Coelho-e-Silva MJ, Sherar L, Elferink-Gemser MT, Malina RM (2013). Allometric scaling of left ventricular mass in relation to body size, fat-free mass and maturation in 13-year-old boys. In M Coelho-e-Silva, A Cupido-dos-Santos, A Figueiredo, J Ferreira, N Armstrong (editors). Book of abstracts of the 28th Pediatric Work Physiology Meeting. Anadia: University of Coimbra pp: 79
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25. Baptista R, Cupido-dos-Santos A, Duarte JP, Pereira JR, Rebelo-Gonçalves R, Severino V, Valente-dos-Santos J, Rego I, Coelho-e-Silva MJ, Coelho e Silva MJ, Fontes-Ribeiro CA, Capranica L, Armstrong N (2014). Allometric modelling of peak power output obtained from a force-velocity protocol in prepubertal boys. In M Coelho-e-Silva, A Cupido-dos-Santos, A Figueiredo, J Ferreira, N Armstrong (editors). Book of abstracts of the 28th Pediatric Work Physiology Meeting. Anadia: University of Coimbra pp: 108
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21. Severino V, Coelho-e-Silva MJ, Simões F, Duarte J Pereira J, Valente-dos-Santos, Rebelo-Gonçalves R, Castagna C, Figueiredo A (2013). Interpretation of peak power assessments in young soccer players. In N Balagué, C Torrents, A Vilanova, J Cadefeau, R Tarragó, E Tsolakidis (editors). Book of abstracts of the 18th annual Congress of the European College of Sport Science pp: 933
20. Severino V, Coelho-e-Silva MJ, Simões F, Duarte J Pereira J, Valente-dos-Santos, Rebelo-Gonçalves R, Castagna C, Figueiredo A (2013). Interpretation of peak power assessments in young soccer players. In N Balagué, C Torrents, A Vilanova, J Cadefeau, R Tarragó, E Tsolakidis (editors). Book of abstracts of the 18th annual Congress of the European College of Sport Science pp: 933
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17. Duarte J, Valente-dos-Santos J, Pereira J, Simões F, Rebelo-Gonçalves R, Severino V, Vaz V, Figueiredo A, Elferink-Gemser M, Malina RM (2013). Maturity-associated developmental changes in change of direction and dribbling speed in young soccer players. In N Balagué, C Torrents, A Vilanova, J Cadefeau, R Tarragó, E Tsolakidis (editors). Book of abstracts of the 18th annual Congress of the European College of Sport Science pp: 689 [ISBN 978-84-695-7786-8]
16. Pereira J, Vaz V, Valente-dos-Santos, Duarte J, Simões F, Rebelo-Gonçalves R, Severino V, Cupido-dos-Santos A, Elferink-Gemser MT, Malina RM, Coelho-e-Silva MJ (2013). Allometric scaling of peak oxygen uptake in under-17 male roller hockey players. In N Balagué, C Torrents, A Vilanova, J Cadefeau, R Tarragó, E Tsolakidis (editors). Book of abstracts of the 18th annual Congress of the European College of Sport Science pp: 512
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9. Simoes F, Rego I, Machado-Rodrigues A, Valente-dos-Santos J, Leite N, Ronque E, Cyrino ES, Rama L, Courteix D, Coelho-e-Silva MJ (2012). Agreement between estimated fat-mass by anthropometry and bioimpedance in girls aged 11-15 years. In R Meeusen, J Duchateau, B Roelands, M Klass, B De Geus, S Baudry, E Tsolakidis (editors). *Book of abstracts of the 17th annual Congress of the European College of Sport Science* pp: 540.

8. Valente-dos-Santos J, Pimenta R, Rêgo I, Gonçalves CE, Figueiredo AJ, Philippaerts RM, Malina RM, Coelho-e-Silva MJ (2011). Multivariate relationship between morphology, functional capacities and sport-specific skills in adolescent male basketball players. In N. Tim Cable, Keith Georg (Eds). *Book of Abstracts of the 16th Annual Congress of the. European College of Sport Science*. *Liverpool* pp: 413.
7. Valente-dos-Santos, J, Figueiredo AJ, Franco S, Huijgen BCH, Elferink-Gemser MT, Malina RM, Coelho-e-Silva MJ (2011). Repeated Sprint ability and skills in adolescent soccer players. In N. Tim Cable, Keith Georg (Eds). *Book of Abstracts of the 16th Annual Congress of the. European College of Sport Science*. *Liverpool.* pp: 137.
6. Coelho e Silva MJ, Francisco P, Valente-dos-Santos J, Figueiredo AJ, Ronque EV, Malina RM (2011). Application of the skinfold equations of Slaugher et al (1988) in Portuguese non-overweight adolescent males. In I Fragoso, H de Ridder, M Marfell Jones, A Stewart, JM Silva, F Esparza, S Mevaloo (Eds). Book of abstracts of the 2011 ISAK – International Society for the Advancement in Kinanthropometry. Lisboa. FMH – Universidade Técnica de Lisboa. pp: 47-48.
5. Coelho e Silva MJ, Moreira Carvalho H, Valente-dos-Santos J, Fonseca J, Figueiredo AJ, Malina RM (2011). Skeletal age and concurrent assessments of anaerobic fitness in adolscent basketball players. In I Fragoso, H de Ridder, M Marfell Jones, A Stewart, JM Silva, F Esparza, S Mevaloo (Eds). Book of abstracts of the 2011 ISAK – International Society for the Advancement in Kinanthropometry. Lisboa. FMH – Universidade Técnica de Lisboa. pp: 49-50.
4. Valente-dos-Santos J, Vaz V, Castanheira J, Figueiredo AJ, Santos A, Elferink-Gemser MT, Malina RM, Coelho-e-Silva M (2010). Maturity-associated variation of aerobic peak power and echocardiographic parameters in youth hockey players. In F Korkusuz, H Ertan, E Tsolakidis (Editors). Book of abstracts of the 15th Annual Congress of the European College of Sport Science, Antalaya-Turkey, p: 134.

3. Coelho-e-Silva M, Valente-dos-Santos J, Pinho R, Simões F, Figueiredo AJ, Malina RM, (2010). Discriminating U-14 soccer players by level and position: A biobehavioral approach. In F Korkusuz, H Ertan, E Tsolakidis (Editors). Book of abstracts of the 15th Annual Congress of the European College of Sport Science, Antalaya-Turkey, p: 163-164 [ISBN 978-605-61427-0-3].
2. Vaz V, Valente-dos-Santos J, Carvalho H, Gonçalves R, Pinheiro J e Coelho e Silva M (2009). Isokinetic profile of knee extensors and flexors among Portuguese adolescent roller-skate [RS] hockey players by level of practice. In: S Loland, K Bø, K Fasting, J Hallén, Y Ommundsen, G Roberts, E Tsolakidis (Eds.). Book of abstracts. 14th Annual Congress of the ECSS. 24-27 June. Oslo. Norway: PP-TT09 Training and Testing 09, p. 440.
1. Valente-dos-Santos J, Simões F, Vaz V, Santos A, Castanheira J, Figueiredo A, Coelho e Silva M, Ribeiro C, Elferink-Gemser M e Malina RM (2009). The effects of body size and maturation on aerobic power among Portuguese adolescent roller-skate [RS] hockey players. In: S Loland, K Bø, K Fasting, J Hallén, Y Ommundsen, G Roberts, E Tsolakidis (Eds.). Book of abstracts. 14th Annual Congress of the ECSS. 24-27 June. Oslo. Norway: PP-TT10 Training and Testing 10, p. 442.

2.5. Abstracts in national conference proceedings

3. Castanheira J, Valente-dos-Santos J, Figueiredo AJ, Coelho-e-Silva (2014). [Effect of biological maturation and body size on left ventricular mass in adolescent boys: an allometric approach]. *Revista Portuguesa de Cardiologia*. 33:150-151.
2. Cardoso-Rodrigues J, Coelho-Silva MJ, Proença J, Silva I, Valente-dos-Santos J, Martins RA, Machado-Rodrigues A (2012). [Application of Freedson cut off values to determine the prevalence of physically active adolescents evaluated by accelerometry]. *Revista Portuguesa de Cirurgia*.Suplemento:39.

1. Rêgo I, Ronque E, Cyrino E, Valente-dos-Santos J, Machado-Rodrigues A, Rêgo MA, Coelho-e-Silva MJ (2011). Prevalence of normal weight, overweight and obesity and physical fitness in Mondego Valley. *Revista Portuguesa de Cirurgia*. Suplemento: 58.

2.6. Reviewer in international journals

3. *Plos One* [May 2014; Manuscript ID: PONE-D-14-15571]
2. *European Journal of Sports Science* [August 2013; Manuscript ID: TEJS-2013-0366]
1. *European Physical Education Review* [February 2013; Manuscript ID ms-51-12]

2.7. Prizes, grants, distinctions

7. Young investigator poster award on the 28th Pediatric Work Physiology conference (2013): “Modelling Developmental Changes in Left Ventricular Mass Using Multiplicative Allometric and Additive Polynomial Multilevel in Boys Aged 11-16 Years”.
6. Hans Stoboy Award at the 28th Pediatric Work Physiology conference (2013).
5. Best poster award of the 2011 ISAK World Conference (2011): “Skeletal age and concurrent assessments of short-term power outputs in adolescent basketball players”.
4. Honourable Mention (2010). “School Alert: accessibility to all”. Lisbon: National Institute for Rehabilitation, Ministry of Labour and Social Solidarity and Ministry of Education.
3. Grant for the participation in the 2010 Annual Meeting of the European College of Sport Science. Antalya, Turkey. Portuguese Foundation for Science and Technology.

2. PhD Grant 2010-2014 (SFRH/BD/64648/2009/J527125E3Y95). Portuguese Foundation for Science and Technology.
1. Grant for Professional mobility in the branch of education and vocational training (2006). Leonardo da Vinci program. PROALV.

2.8. Participation as member of a jury of Master's thesis

7. João Coelho dos Santos (2013). [Performance analysis of the gate in sailing regattas to different types of interventions]. 8th MSc of Youth Sports Training. Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal. Jury: PhD Manuel João Coelho e Silva; PhD Gonçalo Dias, PhD Rui Manuel Sousa Mendes, MSc João Valente dos Santos [approved: 18/20; in 27/09/2013].
6. José Miguel Travassos Ventura Gama (2013). [Network – analysis of the interaction and dynamics of a football match]. 8th MSc of Youth Sports Training. Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal. Jury: PhD Manuel João Coelho e Silva, PhD Gonçalo Dias, PhD Vasco Vaz, MSc João Valente dos Santos, MSc Vitor Severino [approved: 18/20; in 27/09/2013].
5. Hector José Almeida Carvalho (2013). [Maximum oxygen consumption in male adolescent basketball players: contributions of size, maturity and training using allometry]. 7th MSc of Youth Sports Training. Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal. Jury: PhD António José Figueiredo, PhD Manuel João Coelho e Silva; PhD Amândio Cupido-dos-Santos, MSc João Valente-dos-Santos; [approved: 17/20; in 17/07/2013]
4. Paulo Paiva Soares (2012). [Body composition in futsal athletes]. 5th MSc of Youth Sports Training. Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal. Jury: PhD Manuel João Coelho e Silva; PhD António Figueiredo, MSc João Valente-dos-Santos [approved: 15/20; in 06/01/2012].

3. Ângelo Dario Ribeiro dos Santos (2011). [Analysis of the performance structure in Badminton - research applied to U-19 players]. 1st MSc of Youth Sports Training. Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal. Jury: PhD Manuel João Coelho e Silva, MSc Vasco Vaz, MSc João Valente-dos-Santos [approved: 16/20; in 22/01/2011].
2. Paulo Paiva Soares (2012). [Body composition in futsal athletes]. 5th MSc of Youth Sports Training. Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal. Jury: PhD Manuel João Coelho e Silva; PhD António Figueiredo, MSc João Valente-dos-Santos [approved: 15/20; in 06/01/2012].
1. Francisco Noronha (2011). Tactical Skills Inventory: Exploratory study in young tennis athletes]. 5th MSc of Youth Sports Training. Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal. Jury: PhD Manuel João Coelho e Silva; PhD Carlos Eduardo Gonçalves; MSc João Valente-dos-Santos [approved: 14/20; in 06/01/2012].

III.
TEACHING

3.1. Teaching service in higher education

3.1.1. Bachelor's degree

a) As an invited assistant

2011-2012: Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal

Year	Sem	Curricular Unit (year)	T	TP/ week	P	Classes
2011/12	**1**	Practical Studies II-Handball (2º)		8.00		4

2010-2011: Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal

Year	Sem	Curricular Unit (year)	T	TP/ week	P	Classes
2010/11	**1**	Practical Studies II-Handball (2º)		8.00		4

3.1.2. Master's Degree

a) As an invited assistant

[1] 2011-2012

7th MSc of Youth Sports Training. Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal: Readiness and Sports Talent (25 hours). October: 01, 02, 07, 08, 14, 15, 21, 22.

[2]2010-2011

6th MSc of Youth Sports Training. Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal: Readiness and Sports Talent (25 hours). October: 01, 02, 15, 22, 29.

3.2. Supervision of master's thesis

14. Alexandra Silva (awaiting admission to final exam). [Study of Female Youth Volleyball Athletes]. 9th MSc of Youth Sports Training. Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal. Supervision: Phd Manuel João Coelho e Silva; MSc João Valente-dos-Santos; Jury: [approved /20; in].
13. José Miguel Baptista de Almeida (awaiting admission to final exams). [Functional Evaluation of Young Rugby players]. 9th MSc of Youth Sports Training. Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal. Supervision: Phd Manuel João Coelho e Silva; MSc João Valente-dos-Santos; Jury: [approved /20; in].
12. Jorge Manuel de Oliveira Fernandes (2014). [Left ventricular variability in adolescent judo athletes]. 9th MSc of Youth Sports Training. Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal. Supervision: Phd Manuel João Coelho e Silva; MSc João Valente-dos-Santos; Jury: PhD António Figueiredo, MSc Joaquim Castanheira [approved 18/20; in 28/03/2014].
11. Tiago Emanuel da Costa e Sousa (2014). [Exploratory study and internal consistency of a system of observation and analysis of roller hochey teams and athletes]. 9th MSc of Youth Sports Training. Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal. Supervision: PhD Vasco Vaz; MSc João

Valente-dos-Santos; Jury: PhD Susana Ramos [approved 18/20; in 28/03/2014].

10. João Rafael Rodrigues Pereira (2013). [Youth Roller Hockey Players – characteristics by playing position]. 7th MSc of Youth Sports Training. Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal. Supervision: PhD Manuel João Coelho e Silva; PhD Vasco Vaz; MSc João Valente-dos-Santos; Jury: PhD António Figueiredo, PhD Amândio Cupido dos Santos; [approved 19/20; in 08/02/2013].
9. Nuno Gonçalo Ferreira Amado (2013). [A methodological proposal for sports training in youth volleyball]. 8th MSc of Youth Sports Training. Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal. Supervision: PhD Vasco Vaz; MSc João Valente-dos-Santos; Jury: PhD Elsa Silva [approved 15/20; in 27/09/2013].
8. William Roque Ferrari (2013). [Analysis of the offensive process in handball: identification of actions that differentiate winning teams]. 8th MSc of Youth Sports Training. Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal. Supervision: PhD Vasco Vaz; MSc João Valente-dos-Santos; Jury: PhD Manuel João Coelho e Silva; PhD António Figueiredo [approved 16/20; in 30/09/2013].
7. Luís Filipe costa Ferreira (2012). [Roller hockey-specific skills: individual technique and small-sided games]. 7th MSc of Youth Sports Training. Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal. Supervision: PhD Manuel João Coelho e Silva; PhD Vasco Vaz; MSc João Valente-dos-Santos; Jury: PhD Pedro Miguel Gaspar e MSc Miguel Ângelo Fachada [approved 17/20; in 24/10/2012].
6. Carlos Alberto Soares Ribeiro Marques (2012). [Association between isokinetic strength and the appendicular volume]. 7th MSc of Youth Sports Training. Faculty of Sport Sciences and Physical Education,

University of Coimbra, Coimbra, Portugal. Supervision: PhD Manuel João Coelho e Silva; PhD Vasco Vaz; MSc João Valente-dos-Santos; Jury: MSc Mestre Filipe Simões [approved 15/20; in 09/10/2012].

5. Ricardo Milheiro Pimenta (2012). [Sports selection in basketball: comparative study of young basketball players aged 14-16 years]. 7th MSc of Youth Sports Training. Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal. Supervision: PhD Manuel João Coelho e Silva; MSc João Valente-dos-Santos; Jury: PhD António Figueiredo, PhD Susana Ramos, PhD Carlos Gonçalves [approved 15/20; in 09/10/2012].
4. Gabriel Gomes Gonçalves (2012). [Assessment of body composition by different methods in tennis players. 6th MSc of Youth Sports Training. Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal. Supervision: PhD Manuel João Coelho e Silva; PhD Vasco Vaz; MSc João Valente-dos-Santos; Jury: PhD Amândio Cupido dos Santos [approved 14/20; in 16/07/2012].
3. Marcelo Noef Brandão (2012). [Training of Young Tennis Players: Sports preparation and family support in players aged 13-14 years of the State of São Paulo]. 5th MSc of Youth Sports Training. Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal. Supervision: PhD Manuel João Coelho e Silva; MSc João Valente-dos-Santos; Jury: PhD Carlos E Gonçalves [approved 16/20; in 27/01/2012].
2. Francisco Noronha (2012). [Tactical Skills Inventory for Sports: exploratory study with young athletes. 5th MSc of Youth Sports Training. Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal. Supervision: PhD Manuel João Coelho e Silva; MSc João Valente-dos-Santos; Jury: PhD Carlos E Gonçalves [approved 14/20; in 06/01/2012].
1. Ricardo Rebelo Gonçalves (2010). [Morphological and functional profile of the young football goalkeeper]. Supervision: PhD Manuel

João Coelho e Silva; PhD António J Figueiredo; MSc João Valente-dos-Santos; Jury: PhD Luis Manuel Pinto Lopes Rama [approved 17/20; in 28/07/2010].

3.3. Scientific-pedagogical Internship Supervisor

[2013 – 2014] #13

Scientific-pedagogical internship Supervisor, under the MSc of Youth Sports Training. Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal:

1. André Bastos Coelho: Canoeing (Age group: U-15, U-17 and U-19), Clube Fluvial de Coimbra, Coimbra, Portugal.
2. Diogo A. G. Costa: Football (Age group: U-11), Escola Academia Sporting Coimbra, Coimbra, Portugal.
3. Diogo Vicente Martinho: Basketball (Age group: U-15), Academia de Basquetebol, Coimbra, Portugal.
4. Gustavo Henrique Leso: Football (Age group: U-15), Associação Académica de Coimbra - Organismo Autónomo de Futebol, Coimbra, Portugal.
5. João Carvalho Freitas: Futsal (Age group: U-15), Grupo Deportivo Ulmeirense, Granja do Ulmeiro, Portugal.
6. João Jacob Mateus: Rugby (Age group: U-21), Secção de Rugby – Associação Académica de Coimbra, Coimbra, Portugal.
7. João Oliveira Marques: Football (Age group: U-13), Escola Academia Sporting Coimbra, Coimbra, Portugal.
8. Miguel Batista Viegas: Canoeing (Age group: U-13), Sporting Clube de Aveiro – Secção de Canoagem, Aveiro, Portugal.
9. Paulo M. Silva: Kickboxing (Age group: U-19), Centro Cultural e Recreativo da Pena / Ginásio Polirithmus (Núcleos da Secção de Boxe da Associação Académica de Coimbra), Cantanhede, Portugal.
10. Pedro Neves Brás: Swimming (Age group: U-13, U-17), Secção de Natação – Associação Académica de Coimbra, Coimbra, Portugal.

11. Rafael D. Oliveira: Football (Age group: U-11), Escola Academia Sporting – Coimbra N10 II, Coimbra, Portugal.
12. Ricardo José Belli: Football (Age group: U-15), Associação Académica de Coimbra - Organismo Autónomo de Futebol, Coimbra, Portugal.
13. Shirley G. Souza: Volleyball (Age group: U-19), Secção de Voleibol – Associação Académica de Coimbra, Coimbra, Portugal.

[2012 – 2013] #11

Scientific-pedagogical internship Supervisor, under the MSc of Youth Sports Training. Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal:

1. João Manuel Rodrigues: Swimming (Age group: U-19), Secção de Natação – Associação Académica de Coimbra, Coimbra, Portugal.
2. João Carlos Rodrigues: Tennis (Age group: U-16, U-18), Clube de Ténis de Pombal, Pombal, Portugal.
3. João Pacheco Amado: Athletics (Age group: Several), Secção de Atletismo – Associação Académica de Coimbra, Coimbra, Portugal.
4. Jorge Manuel Fernandes: Judo (Age group: U-13), Judo Clube de Coimbra, Coimbra, Portugal.
5. José Miguel Almeida: Rugby (Age group: U-21) Secção de Rugby – Associação Académica de Coimbra, Coimbra, Portugal.
6. Maria Alexandra Silva: Volleyball (Age group: U-17), Esmoriz Ginásio Clube, Esmoriz, Portugal.
7. Maria Lavinia Popa: Tennis (Age group: WTA), Secção de Ténis – Associação Académica de Coimbra, Coimbra, Portugal.
8. Miguel Cláudio Alfaiate: Rowing (Age group: U-17), Secção de Desportos Náuticos – Associação Académica de Coimbra, Coimbra, Portugal.
9. Pedro Gonçalves Mendes: Roller Hockey (Age group: U-17), Hóquei Clube da Mealhada, Mealhada, Portugal.
10. Teotónio Silva: Swimming (Age group: U-19), Clube Naútico Acadêmico de Coimbra, Coimbra, Portugal.

11. Tiago Sousa: Roller Hockey (Age group: U-19), Hóquei Clube da Mealhada, Mealhada, Portugal.

[2009 – 2010] # 1

1. Scientific-pedagogical internship supervisor under the Bachelor's degree in sport sciences and physical education. Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal:
   - Renato Fernandes (Roller Hockey; Age group: U-15), Lagonense Futebol Clube, Mira, Portugal.

[2008 – 2009] # 1

1. Scientific-pedagogical internship supervisor under the Bachelor's degree in sport sciences and physical education. Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal:
   - Filipe Simões (Roller Hockey; Age group: U-13), Lagonense Futebol Clube, Mira, Portugal.

3.4. Pedagogical publications

3.2.1. Publication of pedagogical tools

#1

Valente-dos-Santos J, Faria J, Pinho R (2013). Fair Play – Physical Education, Grades 10 to 12 – On-line Classes (1st Edition). Available in: **www.fairplay.te.pt**

3.2.2. Publication of a pedagogic book, with ISBN, that covers the program of a particular curricular unit

#2

Valente-dos-Santos J, Faria J, Pinho R (2013). Fair Play – Physical Education, Grades 10 to 12 – Teacher's Manual (1st Edition). Lisbon: Texto Editora. [ISBN: 978-111-11-3277-4]

Valente-dos-Santos J, Faria J, Pinho R (2013). Fair Play – The Physical Education Teacher Files, Grades 10 to 12 (1st Edition). Lisbon: Texto Editora. [ISBN: 978-111-11-3278-1]

IV.
TRANSFER AND USE OF KNOWLEDGE

4.1. Oral communications

4.1.1. National / International specialized training

36. Valente-dos-Santos J, Coelho-e-Silva MJ, Castanheira J, Cyrino ES, Esliger D, Sherar LB, Elferink-Gemser MT, Malina RM (2014). Developmental changes of left ventricular mass during pubertal years using static and ontogenetic allometric exponents in boys aged 11-14 years. 61st ACSM Annual Meeting, 5th World Congress on Exercise is Medicine and World Congress on the Role of Inflammation in Exercise, Health and Disease. May 27-31.
35. Castanheira J, Coelho-e-Silva MJ, Valente-dos-Santos J, Ferraz A, Ronque E, Sherar LB, Elferink-Gemser MT, Malina RM (2014). Normalizing left ventricular mass for different size descriptors using allometric exponents in adolescent boys 11-15 years. 61st ACSM Annual Meeting, 5th World Congress on Exercise is Medicine and World Congress on the Role of Inflammation in Exercise, Health and Disease. May 27-31.
34. Coelho-e-Silva MJ, Valente-dos-Santos J, Machado-Rodrigues A, Elferink-Gemser MT, Malina RM, Petrosk EL, Minderico CS, Silva AM, Baptista F, Sardinha LB (2014). Lower Limbs Lean Soft Tissue in Circumpubertal Boys: Agreement Between DXA Assessment and a New Model Derived From Anthropometry and Maturation. 10th Triennial Symposium on Body Composition Research. June 11-14.
33. Coelho-e-Silva MJ, Figueiredo AJ, Valente-dos-Santos J, Machado-Rodrigues A, Martins R, Santos R, Ronque EV, Cyrino ES, Ferrnandes RA, Malina RM (2014). Interrelationship between growth, maturation, BMI status, overfat and cardiorespiratory fitness in Azorean adolescents aged 11-15 years. 10th Triennial Symposium on Body Composition Research. June 11-14.

32. Castanheira J, Valente-dos-Santos J, Coelho-e-Silva M (2014). Left [Ventricular Morphology in Adolescents Athletes and Healthy Non-Athletes]. XIV International Forum of Sport. Coimbra 07 and 08 of March, Portugal.

31. Valente-dos-Santos J, Coelho-e-Silva M (2013). [Overweight, growth, biological acceleration and physical activity]. 17th Portuguese Congress on Obesity: From Pathogenesis to Prevention of Obesity. Porto: 22 to 24 of November, Portugal.

30. Valente-dos-Santos J, Coelho-e-Silva M, Castanheira J, Elferink-Gemser M, Malina RM (2013). Modelling Developmental Changes In Left Ventricular Mass Using Multiplicative Allometric And Additive Polynomial Multilevel In Boys Aged 11-16 Years. 28th Pediatric Work Physiology Meeting. October 1-5

29. Duarte J, Severino V, Pereira J, Fernandes R, Simões F, Rebelo-Gonçalves R, Valente-dos-Santos J, Vaz V, Seabra A, Coelho-e-Silva MJ (2013). Reproducibility of repeated dribbling ability. 28th Pediatric Work Physiology Meeting. October 1-5

28. Pereira J, Vaz V, Valente-dos-Santos, Duarte J, Coelho-e-Silva MJ, Soles-Gonçalves R, Páscoa-Pinheiro J, Areces A, Atkinson G (2013). Advancement in the interpretation of isokinetic ratios derived from hamstrings and quadriceps. 28th Pediatric Work Physiology Meeting. October 1-5

27. Castanheira J, Valente-dos-Santos J, Duarte J, Pereira J, Rebelo-Gonçalves R, Severino V, Machado-Rodrigues A, Vaz V, Figueiredo AJ, Coelho-e-Silva MJ, Sherar L, Elferink-Gemser M, Malina RM (2013). Allometric scaling of left ventricular mass in relation to size, fat-free mass and maturation in 13-year-old-boys. 28th Pediatric Work Physiology Meeting. October 1-5

26. Severino V, Coelho-e-Silva MJ, Duarte J, Pereira J, Simões F, Rebelo-Gonçalves R, Valente-dos-Santos J, Castagna C, Figueiredo AJ (2013). Absolute and scaled peak power assessments in young

male soccer players: Variation by playing position. 28th Pediatric Work Physiology Meeting. October 1-5

25. Rebelo-Gonçalves R, Figueiredo AJ, Duarte J, Pereira J, Fernandes , Simões F, Severino V, Valente-dos-Santos J, Vaz V, Cupido-dos-Santos A, Coelho-e-Silva M, Tessitore A, Armstrong N (2013). Agreement between peak power outputs obtained from the application of common braking force and the estimated optimal load in soccer goalkeepers. 28th Pediatric Work Physiology Meeting. October 1-5
24. Batista R, Cupido-dos-Santos A, Duarte J, Pereira J, Rebelo-Gonçalves R, Severino V, Valente-dos-Santos J, Rêgo I, Coelho-e-Silva MJ, Fontes-Ribeiro CA, Capranica L, Armstrong N (2013). Allometric modelling of peak output obtained from a force-velocity protocol in prepubertal boys. 28th Pediatric Work Physiology Meeting. October 1-5
23. Wierike S, Yvonne-Tromp E, Elferink-Gemser M, Visscher C (2013). Coaches' judgment about current and future performance level of basketball players. 28th Pediatric Work Physiology Meeting. October 1-5.
22. Coelho-e-Silva M, Valente-dos-Santos J, Pereira J, Duarte J, Rebelo-Gonçalves R, Severino V, Vaz V, Figueiredo A, Malina RM (2013). [Growth, Maturation and Talent]. II International Congress of Sports Training - ISMAI. July: 04, Portugal.
21. João Valente-dos-Santos, Manuel J Coelho-e-Silva (2013). [Longitudinal modelling of short-term maximal performance]. XIII International Forum of Sport. Coimbra 02 of March, Portugal.
20. João Valente-dos-Santos, Manuel J Coelho-e-Silva (2013). Maximal Aerobic Power, Growth and Maturation In Adolescent Athletes. V Summer Course in Sports Science. University of Coimbra, Faculty of Sports Science and Physical Education. 04 of May.
19. Valente-dos-Santos, J. (2012). Developmental changes in functional capacities aligned by chronological age and skeletal age: A Mixed-Longitudinal Study. IV Summer Course in Sports Science. University

of Coimbra, Faculty of Sports Science and Physical Education. 04 of May.

18. Duarte J, Pereira J, Rebelo-Gonçalves R, Severino V, Valente-dos-Santos J, Vaz V, Coelho-Silva MJ (2012). Repeated dribbling ability in youth soccer: test properties, interrelantionship with repeated sprint tests and variation by age group. II International Congress of Sports Training - ISMAI. July: 04, Portugal.
17. Valente-dos-Santos J, Coelho-e-Silva MJ, China NB, Pereira J, Duarte J, Simões F, Severino V, Rebelo-Gonçalves R, Carvalho H, Elferink-Gemser MT, Malina RM (2012). Modelling repeated sprint ability by skeletal maturity status in young soccer players. 3rd World Conference on Science and Soccer. Ghent. May 14-16 [poster]
16. Coelho-e-Silva MJ, Figueiredo AJ, Pereira J, Duarte J, Colares-Pinto JH, Simões F, Rebelo-Gonçalves R, Severino V, Valente-dos-Santos J, Carvalho H, Leite N, Malina RM (2012). Inter-individual variability in skeletal age determined by the FELS protocol: implications for age verification in youth soccer. Plenary session at 3rd World Conference on Science and Soccer. 16 de Maio.
15. Severino V, Coelho-e-Silva MJ, Duarte J, Simões F, Rebelo-Gonçalves R, Valente-dos-Santos J, Carvalho H, Vaz V, Santos AC, Figueiredo AJ, Castagna C (2012). Inter-relationship between skeletal maturation and peak VO2 before and after normalization for body size in youth soccer players. 3rd World Conference on Science and Soccer. Ghent. May 14-16 [poster]
14. Rebelo-Gonçalves R, Coelho-e-Silva M, Duarte J, Pereira J, Simões F, Severino V, Valente-dos-Santos J, Carvalho H, Santos AC, Figueiredo AJ, Luz L, Armstrong N (2012). Peak Power in Soccer Goalkeepers: relationship of the peak power assessed using the Wingate anaerobic test and a force-velocity test. 3rd World Conference on Science and Soccer. Ghent. May 14-16 [poster]
13. Simões F, Carvalho H, Rêgo I, Machado-Rodrigues A, Valente-dos-Santos J, Leite N, Ronque E, Cyrino ES, Rama L, Coelho-e-Silva MJ

(2012). Agreement between estimated fat mass by anthropometry and bioimpedance in girls aged 11-15 years. European Colege of Sport Science. Bruges. July 4-7 [poster]

12. Valente-dos-Santos, J. (2012). Effect of Bone Age, Body Size and Body Composition on Left Ventricular Mass of Young Athletes. XII Congress of the Midlands Delegation of the Portuguese Foundation of Cardiology. Fundação Portuguesa de Cardiologia, Delegação Centro. Coimbra, Quinta das Lágrimas, 11-12 May, Portugal.
11. Rêgo I, Ronque E, Cyrino E, Valente-dos-Santos, Machado-Rodrigues A, Rêgo MA, Coelho-e-Silva MJ (2011). Prevalence of normal weight, overweight and obesity and physical fitness in Mondego Valley. XV Congress of the Portuguese Society for the Study of Obesity. 11-12 November. Coimbra, Portugal [poster]
10. Valente-dos-Santos J, Figueiredo AJ, Franco S, Huijgen BCH, Elferink-Gemser MT, Malina RM, Coelho-e-Silva MJ (2011). Repeated sprint ability and skills in adolescent soccer players. 16th Annual Congress of the European College of Sport Science. Liverpool. 23-26 de Junho [poster].
9. Valente-dos-Santos J, Pimenta R, Gonçalves CE, Figueiredo AJ, Philippaerts RM, Malina RM, Coelho-e-Silva MJ (2011). Multivariate relationship between morphology, functional capacities and sport-specific skills in adolescent male basketball players. 16th Annual Congress of the European College of Sport Science. Liverpool. 23-26 June [poster].
8. Valente-dos-Santos J, Vaz V, Santos AC, Figueiredo AJ, Elferink-Gemser MT, Malina RM, Coelho-e-Silva MJ (2011). Short-and long-term maximal protocols and its contribution to differentiate under-17 hockey players by competitive level. XXVII European Group of Pediatric Work Physiology. University of Exeter, UK. September 19-23. [poster]
7. Coelho-e-Silva MJ, Carvalho HM, Vaz V, Valente-dos-Santos J, Figueiredo AJ, Elferink-Gemser MT, Malina RM (2011). Isokinetic

strength and risk of muscle imbalance in U-17 hockey players by competitive level. XXVII European Group of Pediatric Work Physiology. University of Exeter, UK. September 19-23. [poster]

6. Coelho e Silva MJ, Valente dos Santos J, Pinho R, Simões F, Figueiredo AJ, Malina RM (2010). Discriminating U-14 soccer players by level and position: a behavioural approach. 15th Annual Congress of the European College of Sport Science. Antalaya (Turkey). 23-26 June [poster].
5. Valente dos Santos J, Vaz V, Castanheira J, Figueiredo AJ, Santos A, Elferink-Gemser MT, Malina RM, Coelho-e-Silva MJ (2010). Maturity-associated variation of aerobic peak power and echocardiographic parameters in youth hockey players. 15th Annual Congress of the European College of Sport Science. Antalaya (Turkey). 23-26 June [poster].
4. Coelho e Silva MJ, Francisco P, Valente dos Santos J, Figueiredo AJ, Ronque E, Malina RM (2010). Application of the skinfold equations of Slaughter in Portuguese non-overweight adolescent males. ISAK World Conference. Estoril. 11-12 November [poster]
3. Coelho e Silva MJ, Moreira Carvalho H, Valente dos Santos J, Fonseca JN, Figueiredo AJ, MalinaRM (2010). Skeletal age and concurrent assessments of anaerobic fitness in adolescent basketball players. ISAK World Conference. Estoril. 11-12 November [poster]
2. Vaz V, Valente dos SantosJ, Moreira Carvalho H, Gonçalves RS, Páscoa Pinheiro J, Coelho e Silva M (2009). Isokinetic profile of knee extensors and flexors among Portuguese adolescent roller-skate [RS] hockey players by level of practice. 14th Annual Congress of the European College of Sport Science. Oslo. 24-27 June [poster].
1. Valente dos Santos J, Simões F, Vaz V, Santos A, Castanheira J, Figueiredo A, Coelho e Silva N, Fontes Ribeiro C, Elferink-Gemser M, Malina RM (2009). The effects of body size and maturation on aerobic power among Portuguese adolescent roller-skate [RS] hockey

players. 14th Annual Congress of the European College of Sport Science. Oslo. 24-27 June [poster].

4.2. Other services to the community

4.2.1. Courses for coaches

33. Valente-dos-Santos J (2014). [Sport Pedagogy: Parents Involvement in Sports Practice of Children and Youth] (26/03/2014). Aveiro, Portugal: Union of European Football Associations, Portuguese Soccer Federation and Aveiro Football Association. 2nd Course of the New Structure, UEFA "C" – Level 1.
32. Valente-dos-Santos J (2014). [Sport Pedagogy: The Pedagogical Intervention of the Level I Coach] (24/03/2014). Aveiro, Portugal: Union of European Football Associations, Portuguese Soccer Federation and Aveiro Football Association. 2nd Course of the New Structure, UEFA "C" – Level 1.
31. Valente-dos-Santos J (2014). [Sport Pedagogy: The Coach of Level I in the Context of the Pedagogy Applied to Sports] (21/03/2014). Aveiro, Portugal: Union of European Football Associations, Portuguese Soccer Federation and Aveiro Football Association. 2nd Course of the New Structure, UEFA "C" – Level 1.
30. Valente-dos-Santos J (2014). [Sport Pedagogy: Parents Involvement in Sports Practice of Children and Youth] (19/03/2014). Aveiro, Portugal: Union of European Football Associations, Portuguese Soccer Federation and Aveiro Football Association. 1st Course of the New Structure, UEFA "C" – Level 1.
29. Valente-dos-Santos J (2014). [Sport Pedagogy: The Pedagogical Intervention of the Level I Coach] (18/03/2014). Aveiro, Portugal: Union of European Football Associations, Portuguese Soccer

Federation and Aveiro Football Association. 1st Course of the New Structure, UEFA “C” – Level 1.

28. Valente-dos-Santos J (2014). [Sport Pedagogy: The Coach of Level I in the Context of the Pedagogy Applied to Sports] (15/03/2014). Aveiro, Portugal: Union of European Football Associations, Portuguese Soccer Federation and Aveiro Football Association. 1st Course of the New Structure, UEFA “C” – Level 1.
27. Valente-dos-Santos J (2014). [Sport Didactics: the Training Unit – Structuring Factor of Didactics Applied to Sports] (10/03/2014). Aveiro, Portugal: Union of European Football Associations, Portuguese Soccer Federation and Aveiro Football Association. 2nd Course of the New Structure, UEFA “C” – Level 1.
26. Valente-dos-Santos J (2014). [Sport Didactics: the Didactics Applied to Sports Context] (07/03/2014). Aveiro, Portugal: Union of European Football Associations, Portuguese Soccer Federation and Aveiro Football Association. 2nd Course of the New Structure, UEFA “C” – Level 1.
25. Valente-dos-Santos J (2014). [Sport Didactics: the Training Unit – Structuring Factor of Didactics Applied to Sports] (27/02/2014). Aveiro, Portugal: Union of European Football Associations, Portuguese Soccer Federation and Aveiro Football Association. 1st Course of the New Structure, UEFA “C” – Level 1.
24. Valente-dos-Santos J (2014). [Sport Didactics: the Didactics Applied to Sports Context] (25/02/2014). Aveiro, Portugal: Union of European Football Associations, Portuguese Soccer Federation and Aveiro Football Association. 1st Course of the New Structure, UEFA “C” – Level 1.
23. Pinho R, Valente-dos-Santos, J. (2013). [Motor development: Football 7] (14/11/2013). Aveiro, Portugal: Aveiro Football Association. 6th Course for "Graduate Monitors" on Basic Training Principles in Football and Futsal.

22. Valente-dos-Santos, J. (2013). [Sports Pedagogy and Didactics: Soccer and Futsal] (08/11/2013). Aveiro, Portugal: Aveiro Football Association. 6th Course for "Graduate Monitors" on Basic Training Principles in Football and Futsal.
21. Pinho R, Valente-dos-Santos, J. (2013). [Motor development: Football 7] (22/10/2013). Aveiro, Portugal: Aveiro Football Association. 5th Course for "Graduate Monitors" on Basic Training Principles in Football and Futsal.
20. Valente-dos-Santos, J. (2013). [Sports Pedagogy and Didactics: Soccer and Futsal] (18/10/2013). Aveiro, Portugal: Aveiro Football Association. 5th Course for "Graduate Monitors" on Basic Training Principles in Football and Futsal.
19. Pinho R, Valente-dos-Santos, J. (2012). [Motor development: Football 7] (23/10/2012). Aveiro, Portugal: Aveiro Football Association. 4th Course for "Graduate Monitors" on Basic Training Principles in Football and Futsal.
18. Valente-dos-Santos, J. (2012). [Sports Pedagogy and Didactics: Soccer and Futsal] (18/10/2012). Aveiro, Portugal: Aveiro Football Association. 4th Course for "Graduate Monitors" on Basic Training Principles in Football and Futsal.
17. Pinho R, Valente-dos-Santos, J. (2012). [Motor development: Football 7] (02/10/2012). Aveiro, Portugal: Aveiro Football Association. 3rd Course for "Graduate Monitors" on Basic Training Principles in Football and Futsal.
16. Valente-dos-Santos, J. (2012). [Sports Pedagogy and Didactics: Soccer and Futsal] (27/09/2012). Aveiro, Portugal: Aveiro Football Association. 3rd Course for "Graduate Monitors" on Basic Training Principles in Football and Futsal.
15. Valente-dos-Santos, J. (2012). [Readiness and Talent in Judo: from Hand Morphology to Ventricular Volume] (07/09/2012). National Coaches Association and Portuguese Judo Association of Referees. Coimbra, Portugal: XVII CLINIC of Judo.

14. Valente-dos-Santos, J. (2012). [Sports Pedagogy and Didactics: Soccer and Futsal] (17/01/2012). Aveiro, Portugal: Aveiro Football Association. 2nd Course for "Graduate Monitors" on Basic Training Principles in Football and Futsal.
13. Pinho R, Valente-dos-Santos, J. (2012). [Motor development: Football 7] (23/01/2012). Aveiro, Portugal: Aveiro Football Association. 2nd Course for "Graduate Monitors" on Basic Training Principles in Football and Futsal.
12. Pinho R, Valente-dos-Santos, J. (2012). [Motor development: Football 7] (24/01/2012). Aveiro, Portugal: Aveiro Football Association. 2nd Course for "Graduate Monitors" on Basic Training Principles in Football and Futsal.
11. Valente-dos-Santos, J. (2011). [Sports Pedagogy and Didactics: Soccer and Futsal] (07/12/2011). Aveiro, Portugal: Aveiro Football Association. 1st Course for "Graduate Monitors" on Basic Training Principles in Football and Futsal.
10. Pinho R, Valente-dos-Santos, J. (2011). [Motor development: Football 7] (19/12/2011). Aveiro, Portugal: Aveiro Football Association. 1st Course for "Graduate Monitors" on Basic Training Principles in Football and Futsal.
9. Pinho R, Valente-dos-Santos, J. (2011). [Motor development: Football 7] (23/12/2011). Aveiro, Portugal: Aveiro Football Association. 1st Course for "Graduate Monitors" on Basic Training Principles in Football and Futsal.
8. Valente-dos-Santos, J. (2009). [Development of the young roller-hockey player] (04-05/10/2009). Tomar, Portugal: II Roller hockey International Symposium, Câmara Municipal de Tomar e a Universidade de Coimbra.
7. Valente-dos-Santos, J. (2008). [Project Skillitos 2 – Preliminary report] (03/10/2008). S. João da Madeira, Portugal: III Meeting International Meeting of Football Coaches, Câmara Municipal de S.João da Madeira e a Universidade de Coimbra.

6. Valente-dos-Santos, J. (2008). [Growth and maturation: Implications in sport organization] (03/10/2008). S. João da Madeira, Portugal: III International Meeting of Football Coaches, Câmara Municipal de S.João da Madeira e a Universidade de Coimbra.
5. Valente-dos-Santos, J. (2007). [New vision, another intervention in roller hockey] (05-06/10/2007). Mealhada, Portugal: Roller Hockey International Symposium, Câmara Municipal da Mealhada and the University of Coimbra.
4. Valente-dos-Santos, J. (2007). [Technical and tactical strategies in roller hockey] (27/05/2007). Coimbra, Portugal: Portuguese Roller-Skating Federation Coaching License: Level 3.
3. Valente-dos-Santos, J. (2007). [Training methodology: Roller Hockey] (15/04/2007). Coimbra, Portugal: Portuguese Roller-Skating Federation Coaching License: Level 3.
2. Valente-dos-Santos, J. (2007). [Training methods with children and young athletes] (28/03/2007). La Coruña, Spain: Spanish Association of Coaches of Roller Hockey and the Hockey Club Liceu.
1. Valente Valente-dos-Santos, J. (2006). [A multidimensional formation model of young roller hockey athletes] (26/12/2006). Barcelona, Spain: Spanish Association of Coaches of Roller Hockey and the Futbol Club Barcelona.

4.2.2. Municipalities

2. Valente-dos-Santos, J. (2011). [Motivational orientation of young athletes: Dilemmas and challenges] (17/11/2011). IV Training for sports agents. Lousã, Portugal: Câmara Municipal da Lousã.
1. Valente-dos-Santos, J. (2010). [The role of physical education in public health] (16/06/2010). 1st Formation of Healthy Life Styles. Fundão, Portugal: Câmara Municipal do Fundão.

4.2.3. Physical education professionals and school community

3. Valente-dos-Santos, J. (2012). [Growth, maturation and oxygen consumption in adolescent athletes] (18/12/2012). 2nd Scientific Update days in sport and Education. Coimbra, Portugal: Politécnico de Coimbra, Escola Superior de Educação.
2. Valente-dos-Santos, J. (2011). [Handball on Wheelchairs] (02/11/2011). Coimbra, Portugal: Faculdade de Ciências do Desporto e Educação Física.
1. Valente-dos-Santos, J. (2011). [Lifestyles, healthy nutrition and physical activity] (14/06/2011). Anadia, Portugal: Colégio Nossa Senhora da Assunção.

V.
SPORTS EXPERIENCE

5.1. Career as an athlete

2005-2007: Arsenal de Canelas first team, Goalkeeper, Portuguese 2nd League, Portugal.

2004-2007: Arsenal de Canelas first team, Goalkeeper, Portuguese National League of Beach Handball (3rd place, 2006), Portugal.

2003-2005: Estarreja Andebol Clube first team, Goalkeeper, Portuguese 2nd League, Portugal.

1997-2005: Associação Cultural de Salreu youth teams (U-13 to U-19), Goalkeeper (Captain), Portuguese Regional, 2nd and 1st Leagues, Portugal.

5.2. Coaching habilitations

2006: Roller Hockey Coaching License Level 2, Skating Portuguese Federation, Portugal.

2005: Handball Coaching License Level 1, Handball Portuguese Federation, Portugal.

5.3. Roller hockey coaching positions

2013-2014: Hóquei Clube da Mealhada, Portugal.

- Men's first team, assistant coach and strength and conditioning coach, Portuguese 1st League
- Men's second team, assistant coach and strength and conditioning coach, Portuguese 3rd League
- Woman's first team, assistant coach and strength and conditioning coach, Portuguese National League

- U-19 and U-17 teams, strength and conditioning coach, Portuguese National League

2013-2014: Associação Desportiva de Mira, Portugal.

- Coaches' supervisor
- U-11 and U-9 teams, coach, Portuguese Regional Leagues

2012-2013: Hóquei Clube da Mealhada, Portugal.

- Men's first team, strength and conditioning coach, Portuguese 2nd League
- Woman's first team, strength and conditioning coach, Portuguese National League
- U-19 and U-17 teams, strength and conditioning coach, Portuguese National League (4th place)

2011-2012: Lagonense Futebol Clube, Portugal.

- Coaches' supervisor
- U-15 team, coach, Portuguese National League

2010-2011: Hóquei Clube da Mealhada, Portugal.

- Men's first team, strength and conditioning coach, Portuguese 3rd League
- Woman's first team, strength and conditioning coach, Portuguese National League
- U-19 and U-17 teams, strength and conditioning coach, Portuguese National League

2010-2011: Lagonense Futebol Clube, Portugal.

- Coaches' supervisor
- U-15 team, coach, Portuguese Regional Leagues
- U-13 team, coach, Portuguese Regional Leagues

2009-2010: Hóquei Clube da Mealhada, Portugal.

- Men's first team, strength and conditioning coach, Portuguese 3rd League
- Woman's first team, strength and conditioning coach, Portuguese National League
- U-19 and U-17 teams, strength and conditioning coach, Portuguese National League

2009-2010: Lagonense Futebol Clube, Portugal.

- Coaches' supervisor
- U-13 team, coach, Portuguese Regional Leagues
- U-11 team, coach, Portuguese Regional Leagues

2008-2009: Hóquei Clube da Mealhada, Portugal.

- Men's first team, strength and conditioning coach, Portuguese 3rd League
- Woman's first team, strength and conditioning coach, Portuguese National League
- U-19 team, strength and conditioning coach, Portuguese National League (4th place)
- U-17 team, coach, Portuguese National League

2008-2009: Lagonense Futebol Clube, Portugal.

- Coaches' supervisor
- U-13 team, coach, Portuguese Regional Leagues
- U-11 team, coach, Portuguese Regional Leagues

2007-2008: Hóquei Clube da Mealhada, Portugal.

- Men's first team, strength and conditioning coach, Portuguese 3rd League

- Woman's first team, strength and conditioning coach, Portuguese National League
- U-19 team, strength and conditioning coach, Portuguese National League
- U-17 team, coach, Portuguese National League (regional champion)

2007-2008: Lagonense Futebol Clube, Portugal.

- Coaches' supervisor
- U-11 team, coach, Portuguese Regional Leagues

5.4. Handball coaching positions

2005-2006: Arsenal de Canelas, Portugal.

- Men's first team, strength and conditioning coach, Portuguese 2nd League
- U-17 team, coach, Portuguese Regional League
- U-15 girls team, coach, Portuguese Regional League (Champion)

5.5. Visiting coaching positions

2006-2007: Football Club Barcelona, Spain (Supervision: Joaquim Paüls i Bosch)

Men's professional team, assistant coach under the Leonardo da Vinci program, Spanish 1st League