

RESEARCH ARTICLE

Skeletal maturity and oxygen uptake in youth soccer controlling for concurrent size descriptors

Anderson S. Teixeira^{1,2}, Luiz G. A. Guglielmo¹, Juliano Fernandes-da-Silva², Jan M. Konarski³, Daniela Costa⁴, João P. Duarte^{4,5,6}, Jorge Conde⁷, João Valentim-dos-Santos^{4,8,9,10}, Manuel J. Coelho-e-Silva^{4,5*}, Robert M. Malina¹¹

1 Physical Effort Laboratory, Sports Center, Federal University of Santa Catarina, Florianópolis, Brazil, **2** Research Group for Development of Football and Futsal, Sports Center, Federal University of Santa Catarina, Florianópolis, Brazil, **3** Poznań University of Physical Education, Theory of the Sport Department, Poznań, Poland, **4** CIDAF (uid/dtp/04213/2016), University of Coimbra, Coimbra, Portugal, **5** Faculty of Sport Sciences and Physical Education, University of Coimbra, Coimbra, Portugal, **6** Portuguese Foundation for Science and Technology (SFRH/BD/101083/2014), Lisbon, Portugal, **7** School of Health and Technology, Polytechnic Institute of Coimbra, Coimbra, Portugal, **8** Portuguese Foundation for Science and Technology (SFRH/BPD/100470/2014), Lisbon, Portugal, **9** Institute for Biomedical Imaging and Life Sciences (IBILI), Faculty of Medicine, University of Coimbra, Coimbra, Portugal, **10** Faculty of Physical Education and Sport, Lusófona University of Humanities and Technologies, Lisbon, Portugal, **11** Department of Kinesiology and Health Education, University of Texas, Austin, Texas, United States of America

* mjcesilva@hotmail.com



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Abstract

Interrelationships among skeletal maturity status, body size, ventilator thresholds (VT) and peak oxygen uptake (VO_{2peak}) were considered in 47 adolescent male soccer players aged 12.5–15.4 years. Body mass, stature, and the triceps and subscapular skinfolds were measured. The latter were used to estimate fat mass and fat-free mass. Skeletal age was assessed with the Fels method. VO_{2peak} and VO_2 at the first (VT_1) and second (VT_2) ventilatory thresholds were determined during an incremental maximal exercise test on a motorized treadmill. Ratio standards and allometric models were used in the analysis. Scaling exponents suggested linearity for all combinations between size descriptors and physiological variables, except between log-transformed values of VT_1 and body mass ($mL \cdot kg^{-0.801} \cdot min$, 95%CI: 0.649 to 0.952). Early maturing players attained greater values than players classified as “on-time” in skeletal maturity for the three ventilatory parameters expressed in absolute terms (d ranged from 0.65 to 0.71). The differences were attenuated after normalizing for mass descriptors using ratio standards and scaled variables (d ranged from 0.00 to 0.31). The results suggested significant variability between maturity groups when moving from VT_1 to maximal metabolic conditions expressed by unit of stature (VT_1 : $t = -2.413$, $p = 0.02$, $d = 0.60$; VT_2 : $t = -2.488$, $p = 0.02$, $d = 0.65$; VO_{2peak} : $t = -2.475$, $p = 0.02$, $d = 0.65$). Skeletal maturity status and associated variation in overall body size affects VT_1 , VT_2 and VO_{2peak} . The observed scaling of ventilatory outputs for body size may be related to the better running economy and smaller body size of average maturing athletes.

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Introduction

Assessment of the fitness level of soccer players along with information about match performance are essential components of youth soccer particularly for the purpose of long-term athletic training [1]. Available evidence suggests that adult male players covered approximately 10–12 km during a soccer match [2]. Although less abundant, studies of youth soccer report shorter distances covered during competitions [3]: 6.5 km (Under-13), 7.4 km (Under-14), 8.1 km (Under-15). Seven international matches were analysed during the Under-20 South American Championship using global positioning technology and noted that in the final 15 minutes of a game, total distance and high-intensity running were 20–35% lower than during the initial 15 minutes [4]. More recently, the influence of predicted biological maturity status on match performance based on global positioning system in elite youth male soccer players 8–16 years was examined in 80 outfield players in a British academy [5]. Players of contrasting maturity status differed in total distance covered during a match; late maturing players covered greater distance per hour while early maturing spent a longer percentage of time in high speed running [5]. Though of interest, the latter results should be considered in the context of the limitations of the protocol used to predict maturity status [6].

Activities of young players during competitions were generally sustained at about 85% of individual peak heart rate and 75–80% of peak oxygen uptake (VO_{2peak}) [7, 8]. Corresponding match performance data of youth players are rather limited. Although significant correlations between time-motion variables and VO_{2peak} were not noted among adolescent soccer players [9], it is well recognized that a better aerobic fitness level is associated with high external training/match loads [3] and an improved tolerance to fatigue in high-intensity activities [10], suggesting a better ability to maintain physical performance for a prolonged period. Not surprisingly, ventilatory thresholds (VT) and running speed were suggested for monitoring aerobic training in soccer players, specifically during the early phase of the preseason [11]. Anthropometric characteristics, physical fitness and technical skills of adolescent soccer players were compared by competitive level and playing position (goalkeeper, central defender, fullback, midfield, forward) and it was found that stature and body mass discriminated elite from non-elite players among goalkeepers and central defenders [12]. By inference, coaches would benefit from better awareness of relationships among skeletal maturity status, body size and ventilatory parameters for the purpose of talent development and conditioning of youth soccer players.

Inter-individual variability in absolute VO_{2peak} was strongly correlated with body size, but was also influenced by function of the lungs, heart and skeletal muscle [13]. Body size indicated as body mass, stature, fat-free mass and thigh volume have been identified as significant predictors of aerobic fitness in adolescents [14–17]. Oxygen uptake is often expressed per unit of body mass (ratio standard: $ml \cdot min^{-1} \cdot kg^{-1}$), although this has theoretical and mathematical limitations [18]. Allometric models were suggested as a valid alternative approach to normalize physiological variables for inter-individual variability in body size [18]. Biological maturity status introduces further inter-individual variability in body size descriptors. Consequently, inadequate normalization for body size may contribute to misleading in the interpretation of ventilatory oxygen parameters during an incremental maximal test for soccer players contrasting in biological maturity and body size. Skeletal age (SA) of the hand-wrist is consensually considered the best of biological maturity status [19] and available literature combining maturation and VO_{2peak} in adolescent athletes has not systematically used this maturity indicator. Actually, the relationships between skeletal maturation and VO_{2peak} and VT in youth sports have not been extensively addressed, particularly in soccer [14–16, 20]. In this context, the purpose of the present study is to evaluate the interrelationships among skeletal maturity status,

body size as reflected in body mass, fat-free mass and stature, and VO_2 outputs in adolescent male soccer players 12 to 15 years of age. It was hypothesized that ventilatory oxygen parameters would be substantially explained by inter-individual variance in whole-body size descriptors that are influenced by skeletal maturation. By inference, it was also hypothesized that differences on VO_2 outputs between soccer players of contrasting skeletal maturity status would be attenuated after adequate normalization for body size descriptors.

Materials and methods

Research design and procedures

This cross-sectional study describes interrelationships among ventilatory oxygen uptake, body size descriptors and skeletal maturity status and includes a comparison between adolescent soccer players of contrasting skeletal maturity status. The *Ethics Committee* from the Federal University of Santa Catarina (protocol 2004-Federal University of Santa Catarina/2011) approved the research proposal. It was conducted according to the standards established by the declaration of Helsinki.

Adolescent soccer players were recruited in several soccer academies of professional clubs competing at the Brazilian national level after obtaining permission from the respective managers. Parents or legal guardians were informed about the nature of the study including objectives, protocols and related risks, and provided informed written consent. Participation was voluntary and players provided assent after being informed that they could withdraw from the study at any time.

All measurements were completed at the *Federal University of Santa Catarina* during the competitive season at the same time of day (afternoon, usually 16:00–19:00h). Participants were instructed to avoid heavy training for 48 h before the testing session. They were also instructed to avoid caffeinated drinks. The players followed a standardized diet provided at the soccer club academy including the day before testing (~50–60%, 30–25% and 20–15% of total energy intake composed of carbohydrates, fat, and protein, respectively). Anthropometry and the maximal running test were conducted in the laboratory on a single occasion. Air temperature and humidity were kept constant throughout the incremental treadmill test (20–22°C, 50–60% humidity). After the visit to the laboratory, players were then transported within the week to the Santa Catarina University Hospital for a radiograph of the left hand-wrist for the purpose of assessing SA.

Participants

The sample included 47 male soccer players aged 12.4–15.4 years ([S1 File](#)). Inclusion criteria were: (i) adolescent male soccer players; (ii) chronological age between 12 and 16 years; (iii) absence of injuries and clinical signs of cardiovascular or pulmonary conditions which would affect the achievement of maximum performances during an incremental test; (iv) a minimum of three years of experience in competitive soccer; (v) outfield players (goalkeepers were not included since they are typically selected for larger body size, specifically stature, and are not expected to cover long distances during a match). Players were classified as Under-14 (12.0 to 13.9 years) and Under-16 (14.0 to 15.9 years) according to the Soccer Federation of Santa Catarina (associated with the Brazilian Soccer Confederation). The players trained 3–5 regular sessions of 90–120 minutes per week and usually participated in one official game (usually on Saturdays). Of interest, three of the Under-16 players were selected for the Brazilian national team. Chronological age (CA) was calculated as the difference between date of birth and date of the hand-wrist radiograph. Goalkeepers were excluded.

Anthropometry

Measurements were taken by a single, experienced individual following standard procedures [21]. Body mass was measured to the nearest 0.1 kg (Scale Soehnle, Murrhardt, Germany). Stature was measured to the nearest 0.1 cm (Stadiometer Sanny, American Medical do Brazil, Brazil). The triceps and subscapular skinfolds were measured to 0.1 mm with a skinfold caliper (Adipometer, Cescorf, Porto Alegre, Brazil). Intra-observer technical errors of measurement were 0.2 cm for stature, 0.3 mm for the triceps and 0.3 mm for the subscapular skinfolds. Stage of pubic hair, using the criteria of Tanner [22], was assessed in a separate room by an experienced paediatrician who was a professor in the local medical school. The prediction equation for percentage fat mass which uses the triceps and subscapular skinfolds is specific for pubertal status [23]:

$$\text{Fat mass (\%)} = 1.21 \times (X) - 0.008 \times (X)^2 + \text{constant} \quad (1)$$

where X is the sum of the triceps and subscapular skinfolds (mm) and the constant (intercept) is specific for pubertal status: 1.7 for pre-pubescent, 3.4 for pubescent and 5.5 for post-pubescent males.

Skeletal maturation

The SA was estimated with the Fels method [24], which utilizes specific criteria for each bone of the hand-wrist and the ratios of linear measurements of epiphyseal and metaphyseal widths. Grades for each indicator and width measurements were entered into the Fels program (Felsw 1.0 Software) to estimate SA and the associated standard error of estimate. Details of the method and a critical overview of its applications were presented elsewhere [19]. All radiographs were assessed by a single, experienced observer. Independent assessments of 14 radiographs by the observer and an experienced assessor were used to examine quality control of the assessments. The two assessors performed 391 observations with an agreement rate of 93.1% and, as previously reported [25], intra-individual difference (-0.12 ± 0.34), inter-observer error of measurement (0.25 years) and coefficient of variance ($CV\% = 1.41$) were within normal range. Players were classified into contrasting maturity groups based on the difference of SA minus CA. An SA within ± 1.0 year of CA was classified as average or on time. An SA in advance of CA by >1.0 year was classified as early, while an SA delayed relative to CA by > -1.0 year was classified as late [19]. Among the 47 participants, 29 were classified as early maturing (62%) and 18 were average (38%); no players had an SA that classified them as late maturing and no players were skeletally mature.

Incremental maximal treadmill test

Oxygen uptake outputs (maximal and ventilatory thresholds) were obtained during an incremental running test on a motorized treadmill (Ibramed Millennium Super, Brazil). Pulmonary gas exchange was measured breath-by-breath using an automated open-circuit gas analysis system (Quark PFTergo, Cosmed, Rome, Italy). The gas analysers were calibrated immediately before each test using ambient air (assumed to contain 20.94% oxygen and 0.03% carbon dioxide), and certified alpha standard gases containing 16.0% oxygen and 5.0% carbon dioxide (White Martins Ltda, Osasco, Brazil). The turbine flowmeter used for the determination of minute ventilation was calibrated with a 3-L syringe (Quark PFT Ergo, Cosmed, Rome, Italy). Gas exchange responses were interpolated to 1-s intervals and averaged every 15s. After a 3-minute warm-up running at $6.6 \text{ km}\cdot\text{h}^{-1}$, the test started at $7.2 \text{ km}\cdot\text{h}^{-1}$ with subsequent increments of $0.6 \text{ km}\cdot\text{h}^{-1}$ every minute until voluntary exhaustion [26]. The slope was kept constant

at 1% across the entire test. All players were verbally encouraged to give a maximal effort during the test. All participants were familiar with progressive maximal running tests as part of their usual fitness assessment program. The first ventilatory VT (VT₁) was described as the point of transition from moderate to heavy intensity activity, and corresponds to the level of intensity at which the first disproportionate increment in ventilation (VE) to VO₂ consumption occurs. The second disproportionate increase in ventilation is labelled the second VT (VT₂), and represents the highest sustainable level of exercise intensity. As recommended [14], VT₁ and VT₂ were determined by two independent experienced technicians. VO_{2peak} was considered as the highest 15-s average achieved during the test [27] and was considered valid if two of the following criteria were attained [28]: (i) volitional exhaustion, (ii) heart rate within 10% of the age-specific estimated maximal heart rate [HRmax = 220 - age], (iii) a plateau in oxygen consumption despite increased exercise intensity (ΔVO_2 between 2 consecutive work rates < 2.1 mL·kg⁻¹·min⁻¹); (iv) a maximal respiratory exchange ratio ≥ 1.10 .

Statistical analysis

Descriptive statistics (means and standard deviations) were calculated. Normality was checked with the Shapiro-Wilk test. Pearson correlations were calculated to examine the linearity of relationships among indicators of body size with VO_{2peak}, VT₂ and VT₁ expressed in absolute terms (L·min⁻¹). Allometric models were used to examine the relationship between body size descriptors and VO₂ parameters (peak, VT₂, and VT₁):

$$y = a \times x^k \times \epsilon \tag{2}$$

where “y” was the dependent variable of VO₂ (peak, VT₂ and VT₁) and values of “a” and “k” were derived from linear regressions of the logarithmic regression transformations in the form of:

$$\log y = \log a + k \times \log x + \log \epsilon \tag{3}$$

where “a” was the scaling constant, and “k” was the scaling exponent for the specific body size descriptor (body mass or fat-free mass). Relationships among residuals and scaled variables were examined for each allometric model using Pearson correlations. The magnitude of correlations was interpreted as 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 ($r > 0.9$) [29].

CA, anthropometric variables and VO₂ outputs expressed in absolute terms and as ratio standard and scaled values were compared between average and early maturing players using student t-tests for independent samples. The magnitude of the differences was assessed using standardized mean differences (Cohen’s *d* effect size) with thresholds of 0.20, 0.60, 1.20, 2.0 and 4.0 for small, moderate, large, very large and extremely large [30]. Statistical significance was set at 5%. Analyses were done with SPSS (SPSS version 17.0, Chicago, Illinois, USA) and GraphPad Prism (GraphPad Prism 5.0 Software Inc, San Diego, CA).

Results

Descriptive statistics are summarized in Table 1. CAs ranged from 12.36 to 15.41 years (range 3.05 years), while SAs ranged from 11.92 to 17.21 years (range 5.29 years). All anthropometric variables and physiological parameters derived from the incremental maximal treadmill test were normally distributed.

Bivariate correlations between log transformed VT₁ with log transformed stature, body mass, and estimated fat-free mass were moderately high: 0.76, 0.85, and 0.84, respectively (Table 2). Corresponding correlations for log transformed VT₂ and VO_{2peak} were slightly

Table 1. Descriptive statistics (n = 47) and normality of the distributions.

Variables		Descriptive statistics				Normality (Shapiro-Wilk)	
	unit	Mean	SEM	(95% CI)	Standard deviation	value	p
Chronological Age	years						
Skeletal age	years	15.33	0.18	(14.95 to 15.70)	1.27	0.962	0.13
Training experience	years	3.42	0.20	(3.02 to 3.82)	1.34	0.951	0.05
Stature	cm	167.4	1.5	(164.5 to 170.4)	10.0	0.975	0.41
Body mass	kg	56.4	1.5	(53.3 to 59.5)	10.5	0.964	0.16
Fat mass	%	12.9	0.4	(12.0 to 13.7)	2.8	0.977	0.48
Fat mass	kg	7.4	0.4	(6.7 to 8.1)	2.5	0.969	0.24
Fat-free mass	kg	49.0	1.5	(46.5 to 51.6)	8.6	0.979	0.54
VT ₁ : speed	km·h ⁻¹	10.35	0.19	(9.98 to 10.72)	1.27	0.974	0.37
VT ₁ : VO ₂	L·min ⁻¹	2.56	0.07	(2.43 to 2.70)	0.45	0.979	0.57
VT ₁ : VCO ₂	L·min ⁻¹	2.23	0.06	(2.10 to 2.35)	0.41	0.986	0.83
VT ₁ : RER		0.87	0.00	(0.85 to 0.88)	0.05	0.953	0.06
VT ₁ : % VO ₂ peak	%	75.6	1.06	(73.5 to 77.6)	6.9	0.981	0.63
VT ₁ : Heart rate	Bpm	160	1.9	(156 to 163)	13	0.975	0.40
VT ₂ : speed	km·h ⁻¹	13.62	0.16	(13.29 to 13.95)	1.12	0.980	0.57
VT ₂ : VO ₂	L·min ⁻¹	3.11	0.08	(2.94 to 3.28)	0.57	0.977	0.49
VT ₂ : VCO ₂	L·min ⁻¹	3.09	0.08	(2.91 to 3.26)	0.58	0.966	0.19
VT ₂ : RER		0.99	0.00	(0.98 to 1.00)	0.05	0.980	0.59
VT ₂ : % VO ₂ peak	%	91.2	0.6	(90.0 to 92.3)	3.8	0.954	0.06
VT ₂ : Heart rate	bpm	185	1.5	(182 to 188)	10	0.982	0.66
Peak VO ₂ : speed	km·h ⁻¹	15.95	0.16	(15.61 to 16.28)	1.13	0.968	0.22
Peak: RER		1.16	0.01	(1.14 to 1.18)	0.07	0.987	0.87
Peak: Heart rate	bpm	200	1.6	(197 to 204)	11	0.958	0.09
Peak VO ₂	L·min ⁻¹	3.42	0.10	(3.22 to 3.61)	0.66	0.978	0.50

Abbreviations: VT₁, first ventilatory threshold; VT₂, second ventilatory threshold; VO_{2peak}, peak oxygen uptake; VCO₂, carbon dioxide expired; RER, respiratory exchange ratio; bpm, beats per minute; SEM, standard error of the mean; 95% CI, 95% confidence interval.

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Table 2. Allometric modelling of log transformed VT₁, VT₂ and VO_{2peak} using log transformed indicators of body size.

Yi	Xi	allometric models [log Yi = log a + k × log Xi + log c]						
		A	Coefficients			r	model summary	
			value	standard error	(95% CI)		SEE	adjusted R ²
Log VT ₁	Log stature	-11.202	2.369	0.298	(1.768 to 2.970)	0.764	0.122	0.574
	Log body mass	-2.289	0.801	0.075	(0.649 to 0.952)	0.846	0.101	0.710
	Log fat-free mass	-2.341	0.843	0.083	(0.676 to 1.009)	0.835	0.103	0.691
Log VT ₂	Log stature	-12.589	2.678	0.269	(2.137 to 3.218)	0.830	0.110	0.682
	Log body mass	-2496	0.900	0.060	(0.780 to 1.020)	0.914	0.080	0.831
	Log fat-free mass	-2578	0.953	0.066	(0.821 to 1.085)	0.908	0.082	0.820
Log VO _{2peak}	Log stature	-13.294	2.834	0.272	(2.287 to 3.380)	0.841	0.111	0.701
	Log body mass	-2.600	0.949	0.059	(0.831 to 1.068)	0.923	0.079	0.849
	Log fat-free mass	-2.681	1.004	0.066	(0.872 to 1.136)	0.916	0.082	0.836

Abbreviations: VT₁, oxygen uptake at the first ventilatory threshold; VT₂, oxygen uptake at the second ventilatory threshold; VO_{2peak}, peak oxygen uptake.

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higher: 0.83, 0.91 and 0.91 between VT_2 with, respectively, stature, body mass, and fat-free mass; and 0.85, 0.92, and 0.92 between VO_{2peak} with, respectively, stature, body mass and fat-free mass.

The simple allometric models for VT_1 , VT_2 and VO_{2peak} are summarized in Table 2. The 95%CI for the scaling exponents suggested linearity for all combinations, except between log-transformed scores of VT_1 and body mass (beta exponent = 0.80). The coefficients were slightly higher than the respective scores using the original data (before logarithmic transformation). The absolute residuals of the allometric models (e.g., VT_1 and body mass) were not significantly correlated with the log-transformed scores of body size variables. Visual inspection of the absolute residuals did not suggest heteroscedasticity, indicating that scaled variables were independent of the size descriptors.

Comparisons of average and early maturing soccer players are summarized in Table 3 and illustrated in Fig 1. Compared to average maturing players, early maturing players were, respectively, moderately ($d = 0.69$) taller [170.3 ± 8.7 vs. 162.8 ± 10.5 cm, $t = -2.680$, $p = 0.01$, Fig 1A] and heavier [59.6 ± 9.4 vs. 51.3 ± 10.4 kg, $t = -2.828$, $p = 0.01$, Fig 1B], and had a higher estimated fat-free mass [51.7 ± 7.5 vs. 44.7 ± 8.6 kg, $t = -2.953$, $p = 0.01$, Fig 1C]. Comparisons of absolute outputs for VT_1 (2.71 ± 0.32 vs. 2.33 ± 0.55 L·min⁻¹, $t = -2.978$, $p = 0.01$), VT_2 (3.28 ± 0.50 vs. 2.84 ± 0.59 L·min⁻¹, $t = -2.724$, $p = 0.01$) and VO_{2peak} (3.61 ± 0.59 vs. 3.11 ± 0.67 L·min⁻¹, $t = -2.692$, $p = 0.01$) indicated moderately higher values ($p < 0.01$) in early than in average maturing players, respectively (Fig 1E). In contrast, average and early maturing players, respectively, did not differ in running speeds at VT_1 (10.09 ± 1.61 vs. 10.51 ± 0.10 km·h⁻¹, $t = -1.003$, $p = 0.33$), VT_2 (13.27 ± 1.21 vs. 13.84 ± 1.02 km·h⁻¹, $t = -1.753$, $p = 0.09$) and VO_{2peak} (15.53 ± 1.36 vs. 16.20 ± 0.89 km·h⁻¹, $t = -1.844$, $p = 0.08$); the effect sizes were small [Fig 1D].

Given the significant body size differences between early and average maturing players, ratio standards (per unit body mass, fat-free mass and stature) were also used to compare VT_1 , VT_2 and VO_{2peak} . The scaled value for body mass as the size descriptor was also used for VT_1 as this was the only scaling exponent that differed from linearity.

When tested using standard ratios, differences between maturity groups were non-significant and trivial for the three ventilatory variables (VT_1 , VT_2 , VO_{2peak}). For the dependent variable that required an allometric model (VT_1 using body mass as the size descriptor), the difference between maturity groups was small and not significant ($d = 0.31$, $t = -1.243$, $p = 0.22$). On the other hand, the results indicated significant differences between average and early maturing players for the three ventilatory outputs expressed per unit of stature (VT_1 : 14.23 ± 2.66 vs. 15.88 ± 1.51 mL·cm⁻¹·min⁻¹, $t = -2.413$, $p = 0.02$; VT_2 : 17.31 ± 2.74 vs. 19.18 ± 2.35 mL·cm⁻¹·min⁻¹, $t = -2.488$, $p = 0.02$; VO_{2peak} : 18.97 ± 3.15 vs. 21.12 ± 2.74 mL·cm⁻¹·min⁻¹, $t = -2.475$, $p = 0.02$; respectively), although the magnitude effect was small for VT_1 and moderate for VT_2 and VO_{2peak} (Fig 1F).

Fig 2 illustrates the scaling pattern of ventilatory variables for average and early maturing players with stature as the size descriptor. For average maturing players, the stature-scaling k -exponents were 2.90 for VT_1 (95%CI: 1.79 to 4.01; $R^2_{adj} = 0.66$), 2.86 for VT_2 (95%CI: 1.98 to 3.75; $R^2_{adj} = 0.73$) and 2.99 for VO_{2peak} (95%CI: 2.06 to 3.92; $R^2_{adj} = 0.73$). The corresponding stature-scaling k -exponents for early maturing players were 1.46 for VT_1 (95%CI: 0.74 to 2.18; $R^2_{adj} = 0.37$), 2.29 VT_2 (95%CI: 1.47 to 3.12; $R^2_{adj} = 0.55$) and 2.49 for VO_{2peak} (95%CI: 1.67 to 3.31; $R^2_{adj} = 0.57$).

Discussion

Interrelationships among skeletal maturity status, several indicators of body mass and stature on several oxygen ventilatory outputs were considered in adolescent male soccer players.

Table 3. Descriptive statistics (mean ± standard deviation) for adolescent soccer players of contrasting skeletal maturity status and comparison of the ventilatory outputs between maturity groups expressed in absolute values and using ratio standard and scaled values.

Yi: Variable	Xi: descriptor	unit	Descriptive statistics		Comparisons		Effect size	
			Average maturing (n = 18)	Early maturing (n = 29)	t	p	d-value	(qualitative)
Chronological Age		years	14.01 ± 0.9	14.08 ± 0.8	-0.255	0.80	0.07	(trivial)
Skeletal Age		years	14.22 ± 1.00	16.02 ± 0.87	-6.495	0.00	1.72	(large)
Stature		cm	162.8 ± 10.5	170.3 ± 8.7	-2.680	0.01	0.69	(moderate)
Body Mass		kg	51.3 ± 10.4	59.6 ± 9.4	-2.828	0.01	0.76	(moderate)
Fat-Free Mass		kg	44.7 ± 8.6	51.7 ± 7.5	-2.953	0.01	0.78	(moderate)
VT ₁ : Speed		km·h ⁻¹	10.09 ± 1.61	10.51 ± 0.10	-1.003	0.33	0.25	(small)
VT ₁ : Absolute output		L·min ⁻¹	2.33 ± 0.55	2.71 ± 0.32	-2.978	0.01	0.65	(moderate)
VT ₁ : Relative output	Body mass	mL·kg ⁻¹ ·min ⁻¹	45.58 ± 5.20	45.94 ± 5.00	-0.235	0.82	0.07	(trivial)
		mL·kg ^{-0.801} ·min ⁻¹	99.37 ± 11.98	103.21 ± 9.12	-1.243	0.22	0.31	(small)
	Fat-free mass	mL·kg ⁻¹ ·min ⁻¹	52.23 ± 6.44	52.80 ± 5.26	-0.329	0.74	0.08	(trivial)
	Stature	mL·cm ⁻¹ ·min ⁻¹	14.23 ± 2.66	15.88 ± 1.51	-2.413	0.02	0.60	(small)
VT ₂ : Speed	Speed	km·h ⁻¹	13.27 ± 1.21	13.84 ± 1.02	-1.753	0.09	0.46	(small)
VT ₂ : Absolute output		L·min ⁻¹	2.84 ± 0.59	3.28 ± 0.50	-2.724	0.01	0.71	(moderate)
VT ₂ : Relative output	Body mass	mL·kg ⁻¹ ·min ⁻¹	55.46 ± 4.15	55.22 ± 4.75	0.177	0.86	-0.06	(trivial)
	Fat-free mass	mL·kg ⁻¹ ·min ⁻¹	63.53 ± 4.83	63.50 ± 5.38	0.020	0.98	-0.01	(trivial)
	Stature	mL·cm ⁻¹ ·min ⁻¹	17.31 ± 2.74	19.18 ± 2.35	-2.488	0.02	0.65	(moderate)
Speed at peak VO ₂		km·h ⁻¹	15.53 ± 1.36	16.20 ± 0.89	-1.844	0.08	0.47	(small)
Absolute peak VO ₂		L·min ⁻¹	3.11 ± 0.67	3.61 ± 0.59	-2.692	0.01	0.71	(moderate)
Relative peak VO ₂	Body mass	mL·kg ⁻¹ ·min ⁻¹	60.72 ± 4.91	60.73 ± 4.87	-0.004	1.00	0.00	(trivial)
	Fat-free mass	mL·kg ⁻¹ ·min ⁻¹	69.52 ± 5.43	69.85 ± 5.86	-0.197	0.85	0.06	(trivial)
	Stature	mL·cm ⁻¹ ·min ⁻¹	18.97 ± 3.15	21.12 ± 2.74	-2.475	0.02	0.65	(moderate)

Abbreviations: VT₁, first ventilatory threshold; VT₂, second ventilatory threshold; VO_{2peak}, peak oxygen uptake.

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Differences between players of contrasting maturity status favoured early maturing players who were taller and heavier (see Fig 1A and 1B) and also attained higher absolute values in ventilatory thresholds and VO_{2peak} compared to average maturing players (see Fig 1E). After normalizing for inter-individual variability in body mass and fat-free mass using a ratio standard and the scaled variable, the advantages of the early maturing players were attenuated. On the other hand, relative values of VO₂ outputs normalized for stature indicated higher mean values for early compared to average players (see Fig 1F). The mean difference was more apparent for VT₂ and VO_{2peak}, which suggested a moderate difference in metabolic economy at the highest intensities associated with advanced skeletal maturity status. On the other hand, average maturing players were somewhat more efficient at identical running speeds.

Physical performances that require movement of body mass through space will benefit individuals who have greater stature-to-mass ratios [31]. Relative to U.S. age-specific reference data for males [32], mean stature and body mass of the sample of 47 Brazilian adolescent soccer players were higher than the respective reference medians (64th and 68th, for stature and body mass, respectively), consistent with observations for other samples of youth soccer players [16, 33, 34]. The advanced SA relative to CA of the present sample was also consistent with prior results for adolescent soccer players in Spain [35] and Portugal [33]. Absolute VO_{2peak} and VT₂ of the players of the current sample were also similar to those observed in soccer players of the same CA and competitive level [14, 16]. As expected, absolute VO_{2peak} of the soccer players in this study was, on average, higher than observed in previous studies of physically

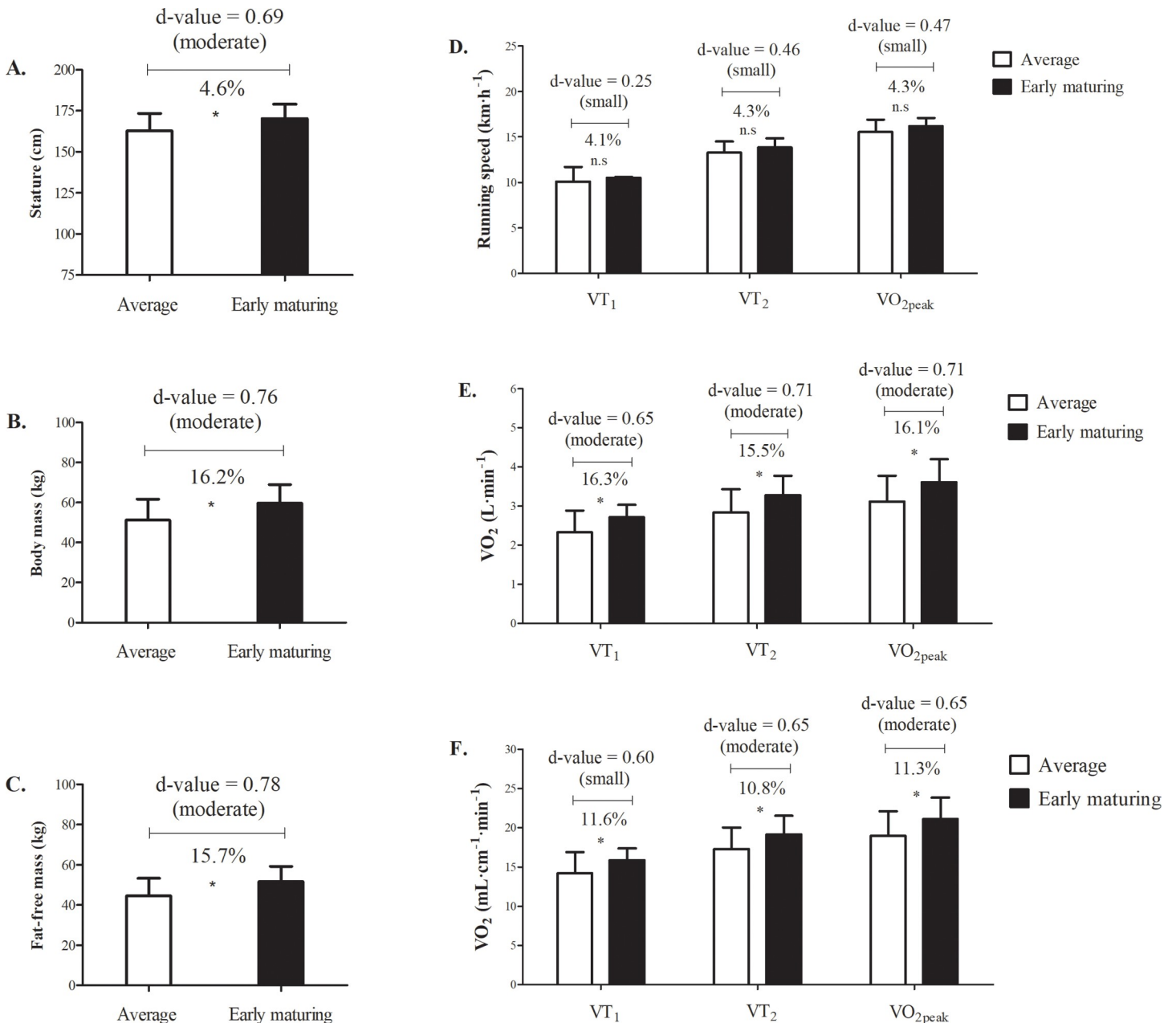


Fig 1. Stature (panel A), body mass (panel B) and fat-free mass (panel C) in average maturing (white bars) and early maturing players (black bars). Mean differences between maturity groups [average maturing (white bars) and early maturing players (black bars)] regarding running speed (panel D), absolute peak oxygen uptake (VO_{2peak}; panel E) and maximal metabolic conditions expressed by unit of stature (panel F) at each particular mark [first ventilatory threshold (VT₁), second ventilatory threshold (VT₂) and VO_{2peak}]. * indicates difference between the groups (p<0.05).

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active adolescents of similar age [27]. Note, however, the interpretation and comparison of VO₂ values obtained from different ergometers, gas analysers and protocols (speed at baseline, length of each stage, slope, among other factors such as criteria for checking data quality) requires caution. Running on a treadmill, for example, utilizes a larger muscle mass than does pedalling on a cycle ergometer. As a result, the highest VO₂ on a cycle ergometer is, on average, about 10% lower than on a treadmill [36]. The mean duration for the incremental ramp test

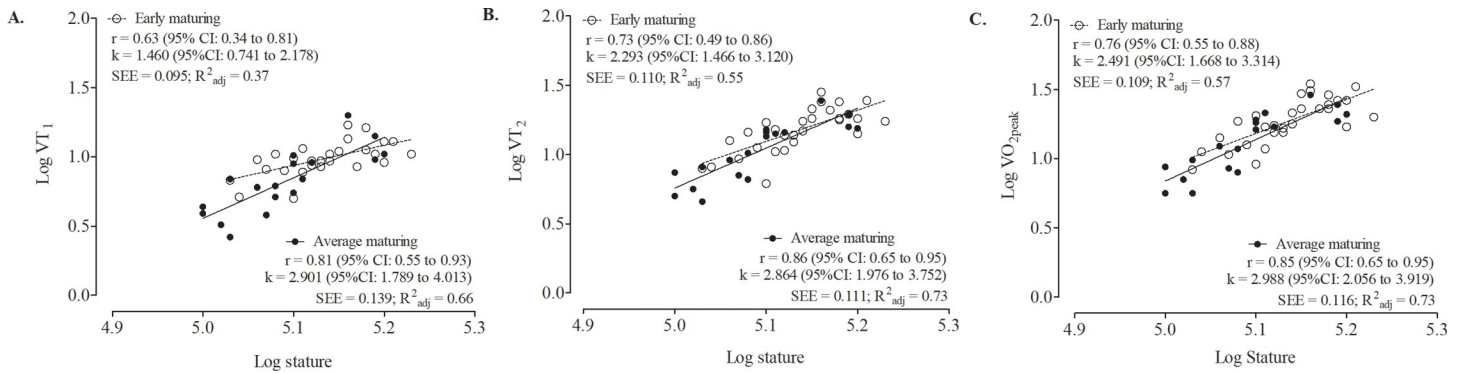


Fig 2. Allometric scaling pattern of log transformed VO₂ for different metabolic conditions: VT₁ (panel A), VT₂ (panel B) and VO_{2peak} (panel C) using log transformed stature as the size descriptor in early and average maturing players. Correlation coefficients (r), 95% confidence interval (95%CI), standard errors of estimate (SEE) and adjusted coefficients of determination (R²_{adj}) are also presented.

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was 14.6±1.9 min, resulting in a test duration close the recommended range of 8–12 min as previously suggested for healthy individuals [28, 37]. Furthermore, this protocol has successfully been used to elicit VO_{2peak} and to determine speed associated to VT and VO_{2peak} during treadmill running in young athletes [38].

Scaling coefficients using body mass as a size descriptor to normalize VO₂ outputs during submaximal intensities (70–85% VO_{2peak}) ranged 0.88–0.95 in both physically active and trained adolescents [14, 27]. In Portuguese adolescent soccer players, coefficients for log transformed VO_{2peak} and body mass decreased from 0.98 at 8–12 years to 0.86 at 13–15 years and to 0.51 at 16–18 years [16]. The relationship between log-transformed VO₂ parameters and body size in the current study tended to fluctuate above and below the line of unity, suggesting a linear relationship between the variables. Thus, the same scaling coefficient may not be appropriate for all ages [39]. Second, the exponents 0.67 or 0.75, which are commonly recommended for partitioning the influence of body mass on VO_{2peak} [14, 18, 27] may not be valid. Third, allometric coefficients should be developed for each particular sample [40]. Moreover, among highly trained adolescent soccer players, the postulates underlying the use power functions among tri-dimensional size descriptors and oxygen consumption may be potentially confounded by the effects of training including an increase in fat-free and muscle mass [16], a reduction in fat mass and changes in movement efficiency [41]. The selective retention of taller players at elite levels [25] may also influence the scaling exponents for stature.

Given the substantial variability in individual growth patterns in scaled VO_{2peak}, derivation of a single scaling factor is problematic. Estimated lean soft tissue of the lower limbs combined with pubertal status (pubic hair stages 3 and 4) explained a significant proportion of inter-individual variability in VO_{2peak} (41%) in a sub-sample of soccer players 13 to 15 years [16]. Note, however, stages of pubic hair are discrete (the individual is either in stage or not in stage) and as such may not be adequate for use with statistical techniques, which assume continuous variables with a normal distribution. The allometric models in the present study explained a large proportion of the variance in VO_{2peak}, 70% to 85%.

The current study did not include any players classified as late maturing. This was somewhat surprising as it contrasted the available SA literature for youth soccer players 12 to 15 years of age which commonly include late maturing players between 12 and 15 years of age, although numbers vary with method of SA assessment [19]. Nevertheless, numbers of late maturing players decline with increasing CA which suggests that the sport of soccer

systematically excludes late maturing players in favour of average and early maturing players as adolescence progresses.

The scaling of oxygen ventilatory outputs varied between the maturity groups. Progressively higher size exponents ($k = 1.46\text{--}2.49$) were noted in early maturing players ($r = 0.63\text{--}0.76$; $R^2_{\text{adj}} = 0.37\text{--}0.57$) as they progressed from sub-maximal exercise to maximal metabolic conditions but not in average maturing players ($k = 2.86\text{--}2.99$; $r = 0.81\text{--}0.86$; $R^2_{\text{adj}} = 0.66\text{--}0.73$). Overall, the results suggested that ventilatory outputs were dependent to a large extent on the player's physiological state [18] as expressed in his skeletal maturity status. Average maturing and smaller athletes, in contrast, exhibited greater capacity of the cardiovascular system and better running economy. Of potential relevance, the kinetic characteristics of running style in six early maturing and seven late maturing soccer players (based on SA) 12–16 years of age (mean 14.3 years) indicated longer strides relative to leg length at $8\text{ km}\cdot\text{h}^{-1}$, $9.5\text{ km}\cdot\text{h}^{-1}$ and $11\text{ km}\cdot\text{h}^{-1}$ among late maturing youth players [42]. In addition, development of aerobic performance as assessed with the Yo-Yo Intermittent Recovery Test (level 1) in 162 elite soccer players 11–17 years was related to muscularity and by inference to motor coordination [43].

The current investigation has several strengths, for example, objective measures of aerobic function, and also highlights the need to consider inter-individual variation in biological maturity status (using an established and valid indicator) and body size in the evaluation of aerobic fitness in competitive adolescent soccer players. Such variation can potentially confound the evaluation of players. There is also a need to experimentally determine and verify allometric exponents for partitioning the influence of body size and maturity status on VO_2 during both submaximal and maximal exercises. Given the trend for the selectivity of taller, earlier maturing adolescent players in soccer, their apparent disadvantage in running and metabolic economy should be noted. Coaches may perhaps implement motor-coordination drills into training programs at these ages.

Some methodological limitations should also be noted. Although SA is generally considered the best indicator of maturity status, obtaining radiographs is costly and its examination requires experienced assessors. Furthermore, the lack of late maturing players in the present sample did not permit comparisons among boys at the extremes of skeletal maturity status (late vs. early). The limitation of the relatively small sample size and the use of predicted body composition descriptors should also be noted.

Conclusions

Skeletal maturity status and associated variation in overall body size affected VT_1 , VT_2 and $\text{VO}_{2\text{peak}}$ responses varied with maturity status in elite adolescent soccer players. Inter-individual variability in mass descriptors (body mass and fat-free mass) using a ratio standard and the scaled variable attenuated the apparent higher values of oxygen uptake of the early maturing players noted for absolute values. In contrast, scaling exponents for stature increased with increasing metabolic demands in early maturing players, but were stable across increasing metabolic demands in average maturing players. The observed scaling pattern of ventilatory outputs for body mass and stature may be related to the smaller body size and better running economy of average maturing athletes.

Supporting information

S1 File. Full dataset.
(XLSX)

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Author Contributions

Conceptualization: Anderson S. Teixeira, Luiz G. A. Guglielmo, Juliano Fernandes-da-Silva, João Valente-dos-Santos, Manuel J. Coelho-e-Silva.

Data curation: Anderson S. Teixeira, Daniela Costa, João P. Duarte, Jorge Conde, Manuel J. Coelho-e-Silva.

Formal analysis: Jan M. Konarski, Daniela Costa, João P. Duarte, Jorge Conde, João Valente-dos-Santos, Manuel J. Coelho-e-Silva.

Funding acquisition: Anderson S. Teixeira, Luiz G. A. Guglielmo, Juliano Fernandes-da-Silva.

Investigation: Anderson S. Teixeira, Luiz G. A. Guglielmo, Juliano Fernandes-da-Silva.

Methodology: Anderson S. Teixeira, Luiz G. A. Guglielmo, Juliano Fernandes-da-Silva, Jorge Conde, João Valente-dos-Santos, Manuel J. Coelho-e-Silva.

Project administration: Luiz G. A. Guglielmo, Juliano Fernandes-da-Silva, Manuel J. Coelho-e-Silva, Robert M. Malina.

Resources: Anderson S. Teixeira, Luiz G. A. Guglielmo, Juliano Fernandes-da-Silva.

Software: Anderson S. Teixeira, Jan M. Konarski, Daniela Costa, João P. Duarte, João Valente-dos-Santos.

Supervision: Luiz G. A. Guglielmo, Juliano Fernandes-da-Silva, Jan M. Konarski, João Valente-dos-Santos, Manuel J. Coelho-e-Silva, Robert M. Malina.

Validation: Luiz G. A. Guglielmo, Juliano Fernandes-da-Silva, Jan M. Konarski, João P. Duarte, Jorge Conde, João Valente-dos-Santos, Manuel J. Coelho-e-Silva, Robert M. Malina.

Visualization: Anderson S. Teixeira, Daniela Costa, João P. Duarte, Jorge Conde, João Valente-dos-Santos, Robert M. Malina.

Writing – original draft: Anderson S. Teixeira, Jan M. Konarski, Daniela Costa, João P. Duarte, João Valente-dos-Santos, Manuel J. Coelho-e-Silva, Robert M. Malina.

Writing – review & editing: Luiz G. A. Guglielmo, Juliano Fernandes-da-Silva, Jan M. Konarski, Jorge Conde, João Valente-dos-Santos, Manuel J. Coelho-e-Silva, Robert M. Malina.

References

1. Carling C, Court M. Match and motion analysis. In: Williams AM, editor. *Science and soccer: developing elite performers*. 3rd ed. London: Routledge, Taylor & Francis Group; 2013: 173–198.
2. Stolen T, Chamari K, Castagna C, Wisloff U. Physiology of soccer: an update. *Sports Med* 2005, 35 (6):501–536. PMID: [15974635](https://pubmed.ncbi.nlm.nih.gov/15974635/)
3. Buchheit M, Mendez-Villanueva A, Simpson BM, Bourdon PC. Match running performance and fitness in youth soccer. *Int J Sports Med*. 2010; 31(11):818–825. <https://doi.org/10.1055/s-0030-1262838> PMID: [20703978](https://pubmed.ncbi.nlm.nih.gov/20703978/)
4. Ramos GP, Nakamura FY, Pereira LA, Junior WB, Mahseredjian F, Wilke CF, et al. Movement patterns of a U-20 national women's soccer team during competitive matches: Influence of playing position and

- performance in the first half. *Int J Sports Med.* 2017; 38(10):747–754. <https://doi.org/10.1055/s-0043-110767> PMID: 28783847
5. Goto H, Morris JG, Nevill ME. Influence of biological maturity on the match performance of 8 to 16 year old elite male youth soccer players. *J Strength Cond Res.* 2018; <https://doi.org/10.1519/JSC.0000000000002510> PMID: 29461418
 6. Malina RM. Assessment of biological maturation. In: Armstrong N, van Mechelen W, Editors. *Oxford textbook of children's exercise science and medicine.* Oxford: Oxford University Press; 2017: 3–11.
 7. Castagna C, Manzi V, Impellizzeri F, Weston M, Barbero Alvarez JC. Relationship between endurance field tests and match performance in young soccer players. *J Strength Cond Res.* 2010; 24(12):3227–3233. <https://doi.org/10.1519/JSC.0b013e3181e72709> PMID: 21068683
 8. Stroyer J, Hansen L, Klausen K. Physiological profile and activity pattern of young soccer players during match play. *Med Sci Sports Exerc.* 2004; 36(1):168–174. <https://doi.org/10.1249/01.MSS.0000106187.05259.96> PMID: 14707784
 9. Rebelo A, Brito J, Seabra A, Oliveira J, Krusturup P. Physical match performance of youth football players in relation to physical capacity. *Eur J Sport Sci.* 2014; 14(S1):S148–156.
 10. Ratel S, Duche P, Williams CA. Muscle fatigue during high-intensity exercise in children. *Sports Med.* 2006; 36(12):1031–1065. PMID: 17123327
 11. Helgerud J, Engen LC, Wisloff U, Hoff J. Aerobic endurance training improves soccer performance. *Med Sci Sports Exerc.* 2001; 33(11):1925–1931. PMID: 11689745
 12. Rebelo A, Brito J, Maia J, Coelho-e-Silva MJ, Figueiredo AJ, Bangsbo J, et al. Anthropometric characteristics, physical fitness and technical performance of under-19 soccer players by competitive level and field position. *Int J Sports Med.* 2013; 34(4):312–317. <https://doi.org/10.1055/s-0032-1323729> PMID: 23059558
 13. Rowland T. *Children's exercise physiology*, 2nd ed. Champaign, IL: Human Kinetics; 2005.
 14. Cunha G, Lorenzi T, Sapata K, Lopes AL, Gaya AC, Oliveira A. Effect of biological maturation on maximal oxygen uptake and ventilatory thresholds in soccer players: an allometric approach. *J Sports Sci.* 2011; 29(10):1029–1039. <https://doi.org/10.1080/02640414.2011.570775> PMID: 21590578
 15. Cunha GS, Vaz MA, Geremia JM, Leites GT, Baptista RR, Lopes AL, et al. Maturity status does not exert effects on aerobic fitness in soccer players after appropriate normalization for body size. *Pediatr Exerc Sci.* 2016; 28(3):456–465. <https://doi.org/10.1123/pes.2015-0133> PMID: 26694739
 16. Valente-Dos-Santos J, Coelho-e-Silva MJ, Tavares OM, Brito J, Seabra A, Rebelo A, et al. Allometric modelling of peak oxygen uptake in male soccer players of 8–18 years of age. *Ann Hum Biol.* 2015; 42(2):125–133. <https://doi.org/10.3109/03014460.2014.932007> PMID: 25296010
 17. Valente-dos-Santos J, Sherar L, Coelho-e-Silva MJ, Pereira JR, Vaz V, Cupido-Dos-Santos A, et al. Allometric scaling of peak oxygen uptake in male roller hockey players under 17 years old. *Appl Physiol Nutr Metab.* 2013; 38(4):390–395. <https://doi.org/10.1139/apnm-2012-0178> PMID: 23713531
 18. Nevill AM, Markovic G, Vucetic V, Holder R. Can greater muscularity in larger individuals resolve the 3/4 power-law controversy when modelling maximum oxygen uptake? *Ann Hum Biol.* 2004; 31(4):436–445. <https://doi.org/10.1080/03014460410001723996> PMID: 15513694
 19. Malina RM. Skeletal age and age verification in youth sport. *Sports Med.* 2011; 41(11):925–947. <https://doi.org/10.2165/11590300-000000000-00000> PMID: 21985214
 20. Bouchard C, Leblanc C, Malina RM, Hollmann W. Skeletal age and submaximal working capacity in boys. *Ann Hum Biol.* 1978; 5(1):75–78. PMID: 206183
 21. Lohmann TG, Roche AF, Martorell R. *Anthropometric standardization reference manual*, 3rd ed. Champaign, IL: Human Kinetics; 1988.
 22. Tanner JM. *Growth at adolescence.* Oxford: Blackwell Scientific Publications; 1962.
 23. Slaughter MH, Lohman TG, Boileau RA, Horswill CA, Stillman RJ, Van Loan MD, et al. Skinfold equations for estimation of body fatness in children and youth. *Hum Biol.* 1988; 60(5):709–723. PMID: 3224965
 24. Roche AF, Chumlea WC, Thissen D. *Assessing the skeletal maturity of the hand-wrist: FELS method.* Springfield, IL: Charles C. Thomas; 1988.
 25. Coelho-e-Silva MJ, Figueiredo AJ, Simoes F, Seabra A, Natal A, Vaeyens R, et al. Discrimination of U-14 soccer players by level and position. *Int J Sports Med.* 2010; 31(11):790–796. <https://doi.org/10.1055/s-0030-1263139> PMID: 20830654
 26. Teixeira AS, da Silva JF, Carminatti LJ, Dittrich N, Castagna C, Guglielmo LG. Reliability and validity of the Carminatti's test for aerobic fitness in youth soccer players. *J Strength Cond Res.* 2014; 28(11):3264–3273. <https://doi.org/10.1519/JSC.0000000000000534> PMID: 24845207

27. Armstrong N, Welsman JR. Assessment and interpretation of aerobic fitness in children and adolescents. *Exerc Sport Sci Rev.* 1994; 22:435–476. PMID: [7925551](#)
28. Howley ET, Bassett DR, Welch HG. Criteria for maximal oxygen uptake: review and commentary. *Med Sci Sports Exerc.* 1995; 27(9):1292–1301. PMID: [8531628](#)
29. Hopkins WG, Marshall SW, Batterham AM, Hanin J. Progressive statistics for studies in sports medicine and exercise science. *Med Sci Sports Exerc.* 2009; 41(1):3–13. <https://doi.org/10.1249/MSS.0b013e31818cb278> PMID: [19092709](#)
30. Batterham AM, Hopkins WG. Making meaningful inferences about magnitudes. *Int J Sports Physiol Perform.* 2006; 1(1):50–57. PMID: [19114737](#)
31. Nevill A, Tsiotra G, Tsimeas P, Koutedakis Y. Allometric associations between body size, shape, and physical performance of Greek children. *Pediatr Exerc Sci.* 2009; 21(2):220–232. PMID: [19556627](#)
32. Kuczumski RJ, Ogden CL, Guo SS, Grummer-Strawn LM, Flegal KM, Mei Z, et al. 2000 CDC Growth Charts for the United States: methods and development. *Vital Health Stat.* 2002; 11(246):1–190.
33. Malina RM, Pena Reyes ME, Eisenmann JC, Horta L, Rodrigues J, Miller R. Height, mass and skeletal maturity of elite Portuguese soccer players aged 11–16 years. *J Sports Sci.* 2000; 18(9):685–693. <https://doi.org/10.1080/02640410050120069> PMID: [11043894](#)
34. Figueiredo AJ, Goncalves CE, Coelho-e-Silva MJ, Malina RM. Youth soccer players, 11–14 years: maturity, size, function, skill and goal orientation. *Ann Hum Biol.* 2009; 36(1):60–73. <https://doi.org/10.1080/03014460802570584> PMID: [19085511](#)
35. Malina RM, Chamorro M, Serratos L, Morate F. TW3 and Fels skeletal ages in elite youth soccer players. *Ann Hum Biol.* 2007; 34(2):265–272. <https://doi.org/10.1080/03014460701207601> PMID: [17558596](#)
36. Turley KR, Rogers DM, Harper KM, Kujawa KI, Wilmore JH. Maximal treadmill versus cycle ergometry testing in children: Differences, reliability, and variability of responses. *Pediatr Exerc Sci.* 1995; 7(1):49–60.
37. Cunha FA, Midgley A, Montenegro R, Vasconcellos F, Farinatti P. Utility of a non-exercise VO₂max prediction model for designing ramp test protocols. *Int J Sports Med.* 2015; 36(10):796–802. <https://doi.org/10.1055/s-0034-1395590> PMID: [26038880](#)
38. Cottin F, Medigue C, Lopes P, Lepretre PM, Heubert R, Billat V. Ventilatory thresholds assessment from heart rate variability during an incremental exhaustive running test. *Int J Sports Med.* 2007; 28(4):287–294. <https://doi.org/10.1055/s-2006-924355> PMID: [17024637](#)
39. Thomis M, Rogers DM, Beunen GP, Woynarowska B, Malina RM. Allometric relationship between body size and peak VO₂ relative to age at menarche. *Ann Hum Biol.* 2000; 27(6):623–633. PMID: [11110226](#)
40. Balasekaran G, Robertson RJ, Goss FL, Suprasongsin C, Danadian K, Govindaswamy V, et al. Short-term pharmacologically induced growth study of ontogenetic allometry of oxygen uptake in children. *Ann Hum Biol.* 2005; 32(6):746–759. <https://doi.org/10.1080/03014460500329192> PMID: [16418048](#)
41. Malina RM, Bouchard C, Bar-Or O. Growth, maturation, and physical activity, 2nd ed. IL: Human Kinetics: Champaign; 2004.
42. Segers V, Clercq DD, Philippaerts R, Janssens M. Running economy in early and late mature youth soccer players. In: Aerts P, D'Août K, Herrel A, Van Damme R, editors. *Topics in functional and ecological vertebrate morphology.* Aachen: Shaker Publishing; 2002. pp. 125–138.
43. Deprez D, Valente-Dos-Santos J, Coelho-e-Silva MJ, Lenoir M, Philippaerts RM, Vaeyens R. Modeling developmental changes in yo-yo intermittent recovery test level 1 in elite pubertal soccer players. *Int J Sports Physiol Perform.* 2014; 9(6):1006–1012. <https://doi.org/10.1123/ijspp.2013-0368> PMID: [24664863](#)