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The digit ratio (2D:4D) and testosterone co-predict vertical jump performance in athletic boys: Evidence of organizational and activational effects of testosterone on physical fitness

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ABSTRACT

The digit ratio (2D:4D) is a negative correlate of boy's physical fitness, and thought to arise from organizational effects of prenatal testosterone on different bodily systems. During human ontogeny, activational effects of testosterone on body size and strength offers another pathway to physical fitness. We tested these hypotheses by examining the organizational and activational effects of testosterone on vertical jump performance in athletic boys. Using a cross-sectional design, 173 boys (aged 9 to 18 years) were tested for standing height, body mass, body fat, fat-free mass, weekly training activity, training history, salivary testosterone and cortisol, R2D:4D, L2D:4D, and right-left 2D:4D (Dr-1), and vertical height in 3 different countermovement jump (CMJ) tests. A generalized additive model was employed to delineate age-related trajectories and predict CMJ performance. Our models yielded significant non-linear increases (or changes) in body size, current hormone concentration, training outcomes, and CMJ performance with chronological age. All 2D:4D measures were age invariant. The R2D:4D and testosterone were significant non-linear predictors of CMJ height with (R^2 = 66.2%) or without (R^2 = 54.3%) covariates, whereby a higher current testosterone concentration (up to a certain level) and a lower or higher R2D:4D were linked to better performance. The L2D:4D and Dr-1 had no predictive value. In conclusion, the R2D:4D and testosterone were co-predictors of CMJ height among athletic boys, with non-linear performance effects that differed in timing, tempo, and direction. Our findings confirm that testosterone can regulate a simple measure of boy's physical fitness through both an activational and organizational pathway.

1. Introduction

Intrauterine programming may play a significant role in early ontogenesis and development of adult traits related to physical fitness [1]. Whilst the mechanisms involved are still unclear, prenatal androgenization is thought to exert "organizational" effects on different bodily systems (e.g., reproductive, cardiovascular, musculoskeletal, central nervous) [1–3] that could, in theory, regulate physical fitness. The second-to-fourth digit ratio (2D:4D) provides a putative retrospective marker of 1st trimester prenatal testosterone exposure, relative to estrogens [2, 4]. Reviews affirm that the 2D:4D is a consistent negative correlate of sporting success, athletic ability, and physical performance [1, 5, 6]. In other words, individuals with a lower or "masculine" 2D:4D, indicating relatively high prenatal testosterone exposure relative to estrogens, tend to perform better than individuals with a higher or "feminine" 2D:4D, reflecting less exposure to testosterone relative to estrogens [4, 7].

The relationships described appear to be independent of age, at least among younger male cohorts, based on widespread reports of negative 2D:4D and physical fitness (e.g., grip strength, maximal oxygen uptake, explosive power, sprinting speed and jump performance) associations in boys and young men of varying age [8–14]. These relationships extend to the right-hand (R2D:4D) minus left-hand (L2D:4D) difference, often termed Dr-1 [15]. Since the 2D:4D itself is independent of age [16, 17], it could provide an early signature of athletic potential that persists throughout a life span. Even so, studies in this area are limited by

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customary use of statistical models that assume linearity (e.g., Pearson correlations, least squares linear regression). In other domains, 2D:4D connections to performance were better described by non-linear than linear relationships [18–20]. Further issues could arise from a failure to control for maturation factors relevant to young, developing boys.

Puberty is one of the main stages of human ontogeny, with boys experiencing accelerated growth and strength increases that are actualized, in part, by a surge in testosterone production [21–24]. Hence, 2D:4D linkages to physical fitness might also arise from "activational" effects of testosterone on body size and muscle strength at different concentrations. This is an important distinction to make from the organizational effects of testosterone, which occur in a narrow prenatal window and persist irrespective of postnatal testosterone secretion. Attempts to separate testosterone's activational role are limited to controlling, statistically, for age and body-size differences [8-10, 13, 15, 25]. To date, no 2D:4D study has evaluated physical fitness, body size, and current testosterone level in boys representing different stages of pubertal development. Addressing this gap would permit a more detailed analysis of testosterone-mediated effects on physical fitness, thereby providing a stronger basis for implementing the 2D:4D as a proxy marker in research and practice.

This study investigated the organizational and activational effects of testosterone on vertical jump performance in a cohort of athletic boys spanning a wide age range. The primary aims were to: (1) model age-related trajectories in the 2D:4D, testosterone, body size, and jumping ability, and (2) determine if the 2D:4D and testosterone measures can predict physical performance. Our first hypothesis was that testosterone would exert both organizational and activational effects on performance, operationally defined as significant 2D:4D and testosterone associations with jumping ability, when controlling for age and body-size differences. Our second hypothesis was that a lower 2D:4D and higher testosterone concentration would correlate with better performance. To advance research in this area, a generalized additive model (GAM) [26] was used to delineate the linear or non-linear nature of the aforementioned age-related trajectories and predictive relationships.

2. Material and methods

2.1. Participants

A cohort of 173 Caucasian boys (aged 9 to 18 years) were recruited from local schools and sporting clubs. Targeting a broad age range ensured a mixed sample of pre-pubertal, pubertal, and post-pubertal boys. A priori power analysis suggested a N of 136 subjects for a multiple linear regression (effect size $f^2 = 0.15$, probability = 0.05, power = 90%) with 8 predictors. We targeted 25% more subjects for the added complexity of a GAM. The following criteria were used for study exclusion: a learning disability and health problems, including a neurological disorder or injury, which could make it difficult to understand or complete the study protocols. The boys were active across 5 main sports (i.e., volleyball, swimming, biathlon, taekwondo, football) with an average training history of 4.5 years. On average, they reported close to 10 h of physical activity per week, dispersed across sport-specific activities and other training sessions. Informed consent was signed by each participant and their parents or legal guardians. Approval (KEBN-16–19-AP) was granted by the ethics committee of the Institute of Sport – National Research Institute, Warsaw.

2.2. Study protocols

Using a cross-sectional design, the boys completed a battery of tests during a single laboratory visit. The visit began with a procedural overview, collection of psychological data (not part of this study) and basic training information, before a saliva sample was taken for testosterone determination. Cortisol was also measured, because it can affect muscle function and human movement via broad actions on the neuromuscular system [27]. Next, selected body-size dimensions (i.e., height, body mass [BM], BM index [BMI], body fat [BF], and fat-free mass [FFM]) were evaluated, including an assessment of hand bone mineral content (not part of this study) using dual energy x-ray absorptiometry. Finally, both hands were scanned for the 2D:4D measurements and vertical jump performance was assessed. Participant testing was scheduled to begin at a similar time of day (0900 – 1100 h) to control for circadian variation in testosterone and cortisol secretion [28, 29]. Each test was implemented by the same investigator/s to eliminate any experimenter bias.

2.3. Saliva collection and assessment

Saliva sampling began at least 90 min after waking to eliminate the early morning surge in cortisol [28] and decline in testosterone [29]. To prevent sample contamination, instructions were given to avoid eating and drinking (except water) 60 min before sampling. Whole saliva (~1 mL) was self-collected by passive drool into 5-mL sterile containers and stored at −80 °C until assay. After thawing and centrifugation (3000 rpm × 15 min), the samples were tested for testosterone and cortisol using commercial immunoassay kits (IBL, Germany). Inter-assay coefficients of variation (CV) on low and high control samples were less than 10% (on average) for both hormones. Initial data inspection revealed 4 samples with an extremely low testosterone concentration (from 0.47 to 0.95 pg/mL). Subsequently, these values were truncated to the minimal detectable concentration of 1 pg/mL.

2.4. Body size evaluation

Body mass was measured to the nearest 0.1 kg using digital scales (Tanita, Japan). Standing height was assessed to the nearest 1 cm with a freestanding stadiometer (Siber-Hegner, Switzerland), after which a BMI was computed by dividing BM by height (expressed in m^2). Body fat percentage was estimated using a published formula for children [30], based on 3 skinfold measurements (i.e., triceps, subscapular, abdominal) taken with calipers (Siber-Hegner, Switzerland) by a trained technician. Relative repeatability error for the skinfold measurements ranged from 1.6% to 3.0%. Subsequently, FFM was estimated (FFM = BM – BF [% converted to kg]) to better index skeletal muscle mass, as it correlates more strongly with vertical jump height than BM [31]. The participants wore shorts and a shirt, without shoes and socks, during this evaluation.

2.5. 2D:4D assessment

The ventral surfaces of both hands were photocopied (1:1 ratio) simultaneously in a commercial scanner (Konica Minolta Bizhub, Poland). Each scan was printed out on A3 size paper and coded to ensure a blinded assessment by a single technician. The 2D and 4D lengths were measured from a mid-point in the proximal ventral flexion crease to the distal tip of that finger [7]. Finger length was measured to an accuracy of 0.01 mm using Vernier calipers (Mitutoyo, Japan). Each digit was assessed twice and averaged, before calculating the left-hand (L2D:4D) and right-hand (R2D:4D) ratios. Intra-class correlation coefficients on 76 printouts were 0.974, 0.978, and 0.950 for the respective L2D:4D, R2D:4D, and Dr-1 measures, indicating high accuracy and repeatability. The Dr-1 was also computed, as another metric of prenatal androgenization that correlates with boy's physical fitness [15]. Most boys (N = 170) reported being right-hand dominant.

2.6. Countermovement jump testing

Three variations of the countermovement jump (CMJ) test were performed on a Kistler force platform (amplifier Type 9281A, Switzerland). The first being a CMJ with hands held akimbo (ACMJ) on the hips, followed by a standard CMJ with arm swing permitted, and a CMJ with a 3–4 step run-up; commonly referred to as a spike jump (SPJ).

Some boys did not complete the SPJ test, due to a failure to execute the correct jumping technique. Three trials were completed per exercise, each separated by a 1-minute rest, and the best trial was chosen for analysis. Peak power (absolute, relative) and maximum vertical displacement of the body's center of mass were computed from ground reaction force data (collected at 1000 Hz) using customized software ("JBA" ZB. Staniak, Poland) [32]. Strong reliability coefficients have been reported for CMJ power (CV = 3.4%) and height (CV = 3.0%) among young male athletes [33]. Since relative peak power is a simple function of height when CMJ depth is constant [32], we limit our focus to ACMJ, CMJ, and SPJ height (all expressed in m).

2.7. Statistical analyses

The study data were analyzed in the R (version 4.1.1) programming environment [34] using several packages (i.e., mgcv, ggplot2, gratia, visreg, readxl, psych, corrplot, dplyr, easystats) and built-in functions. Preliminary testing began with calculation of summary statistics for chronological age, calculated from birth-date to the day of assessment, body size (i.e., height, BM, BMI, BF, FFM), training background (i.e., training hours per week, training years), 2D:4D measures (i.e., R2D:4D, L2D:4D, Dr-1), salivary hormones (i.e., testosterone, cortisol), and vertical jump performance (i.e., CMJ, ACMJ, SPJ). Next, zero-order Pearson correlations were used to explore linear associations between variables. Using standard conventions [35], correlations can be interpreted as being small (0.1 to <0.3), medium (0.3 to <0.5) or large effects (0.5 to 1.0). These results were also used to identify, and remove, highly collinear variables to prevent overfitting in subsequent GAMs.

The GAM procedures were implemented in the mgcv package (version 1.8–36) [36] and estimated by a restricted maximum likelihood (REML) method. To address the 1st study aim, all variable observations were plotted against chronological age and fitted with a cubic regression spline. The nature of this association is denoted by the effective degrees of freedom (EDF); a summary statistic reflecting the real degree of non-linearity of a smooth function [26]. An EDF value of 1 approximates a linear relationship, whilst EDF values exceeding 1 reflect a non-linear relationship and increasingly so. Statistical significance (p value) was approximated for each GAM. Additionally, periods of significant change were estimated by extracting the first derivatives and identifying where the 95% confidence interval falls entirely above, or below, a zero baseline. To establish more credible age-related trajectories, we included data from a subgroup of boys ($N = 57$) tested 6 months earlier on the same outcomes, excluding jumping ability. Repeated observations were ignored in these models, being only a small component of the entire dataset.

To address the 2nd aim, selected 2D:4D and body-size variables, along with testosterone, were entered into a GAM to predict performance. To eliminate redundancies arising from strongly related outputs, only a single response variable (CMJ height) was chosen. The model-building procedure began with covariate and 2D:4D predictor selection using the shrinkage version of the cubic spline. This smoother introduces a penalty that shrinks the fitted function towards zero, whereby a term with an EDF of zero has no predictive value [26]. A final GAM was specified using a cubic spline (no shrinkage) and pre-selected covariates and predictors. Summary results are provided with parametric (intercept only) and smoother (covariate / predictor) estimates, significance values, and goodness of fit statistics, including the adjusted coefficient of determination (R^2), Akaike information criteria (AIC), and REML. Statistical significance was set at an alpha level of $p < 0.05$.

3. Results

Marked variation was seen for all body-size, training, hormonal, and jumping variables (see Table 1), as evidence of different pubertal stages in our sample of athletic boys. Data were plotted (see Fig. 1) to highlight these differences in the context of chronological age. The GAMs yielded significant non-linear increases in height (Fig. 1A), BM (Fig. 1B), BMI (Fig. 1C), and FFM (Fig. 1E) from 9.6 – 10.9 years up to 14.7 – 18.3 years of age. Subject BF exhibited a slight linear decrease (Fig. 1D) across all ages ($p < 0.05$). Weekly training activity was more variable; rising, falling and rising again (Fig. 1F), whilst training history rose steadily after 14.0 years of age (Figs. 1E-1F, $p < 0.001$). Testosterone (Fig. 1C) and cortisol (Fig. 1D) concentrations both increased ($p < 0.001$) in a non-linear manner after 11.5 and 14.6 years of age, respectively. We found no significant effect of chronological age on the L2D:4D (Fig. 1J), R2D:4D (Fig. 1K), or Dr-1 (Fig. 1L). Height achieved in the ACMJ (Fig. 1M), CMJ (Fig. 1N), and SPJ (Fig. 1O) tests also improved, significantly and non-linearly, from 10.5 – 12.1 years up to 14.4 – 16.3 years of age.

Table 1
Summary statistics for age, body-size variables, training activity and history, salivary hormones, 2D:4D measures, and physical performance in athletic boys. Data includes a sub-group of boys assessed twice on all tests, except for jumping ability.

Variables	N	Mean	SD	Range (min – max)
Age (years)	235	12.8	1.9	9.6 – 18.3
Height (m)	235	1.63	0.17	1.35 – 2.01
BM (kg)	235	51.5	16.8	26.7 – 115.3
BMI (kg/m^2)	235	18.8	3.0	13.3 – 35.5
BF (%)	235	17.5	4.3	7 – 33.5
FFM (kg)	235	42.3	13.5	23.0 – 80.3
Training activity (hours)	235	9.8	2.8	5 – 19
Training history (years)	235	4.5	1.3	1 – 11
Testosterone (pg/mL)	235	29.0	32.3	1.0 – 191.8
Cortisol (ng/mL)	235	1.71	1.28	0.29 – 9.35
L2D:4D	232	0.97	0.03	0.90 – 1.08
R2D:4D	232	0.97	0.03	0.90 – 1.06
Dr-1	232	0.00	0.02	−0.06 – 0.07
ACMJ height (m)	171	0.33	0.07	0.18 – 0.55
CMJ height (m)	171	0.39	0.08	0.22 – 0.61
SPJ height (m)	149	0.47	0.10	0.29 – 0.75

Key: BM = body mass, BMI = body mass index, BF = body fat, FFM = fat-free mass, L2D:4D = left-hand 2nd to 4th digit ratio, R2D:4D = right-hand 2nd to 4th digit ratio, Dr-1 = R2D:4D minus L2D:4D, ACMJ = countermovement jump with hands held akimbo, CMJ = countermovement jump, SPJ = spike jump.

The correlational results are depicted as a matrix (Fig. 2) with hierarchical clustering to assist interpretation. Only significant results are shown and, since multiple comparisons increase the probability of false positives, we limit our reporting to large or strong effects. Several clusters of highly interrelated variables emerged. The first being a strong positive association between the L2D:4D and R2D:4D ($r = 0.68$); see a meta-analysis reporting an average L2D:4D and R2D:4D correlation of $r = 0.64$ [5]. Strong positive covariation was also seen amongst all tests of jumping ability ($r = 0.92 – 0.96$), which themselves were positively and strongly related ($r = 0.55 – 0.98$) to most body-size dimensions, chronological age, and current testosterone concentration. We found no strong inter-variable connections to the Dr-1, training history, cortisol, training activity, and BF measures.

To ensure model parsimony, the first GAM (Model 1) contained only 5 covariates; age, BF, cortisol, weekly training hours, and training years. The remaining body-size variables (i.e., height, BM, BMI, FFM) were excluded, due to strong linear associations with subject age and each other. The 2D:4D and Dr-1 measures were also entered to identify the most relevant predictor/s. Model 1 produced the following EDF values: age = 2.25, BF = 1.64, training activity = 0.94, training history = 1.98, cortisol = 0.00, L2D:4D = 0.00, R2D:4D = 2.73, Dr-1 = 0.00. Next, a second GAM (Model 2) was constructed, but excluding those variables with an EDF of zero. Model 2 summary revealed that all terms were significant contributors to CMJ height. A final GAM (Model 3) was specified with testosterone as an additional predictor. Model assumptions were assessed and verified by residual diagnostics, along with GAM-specific tests of K-basis functions and concurvity.

Model 3 results are summarized in Table 2. Linear and non-linear

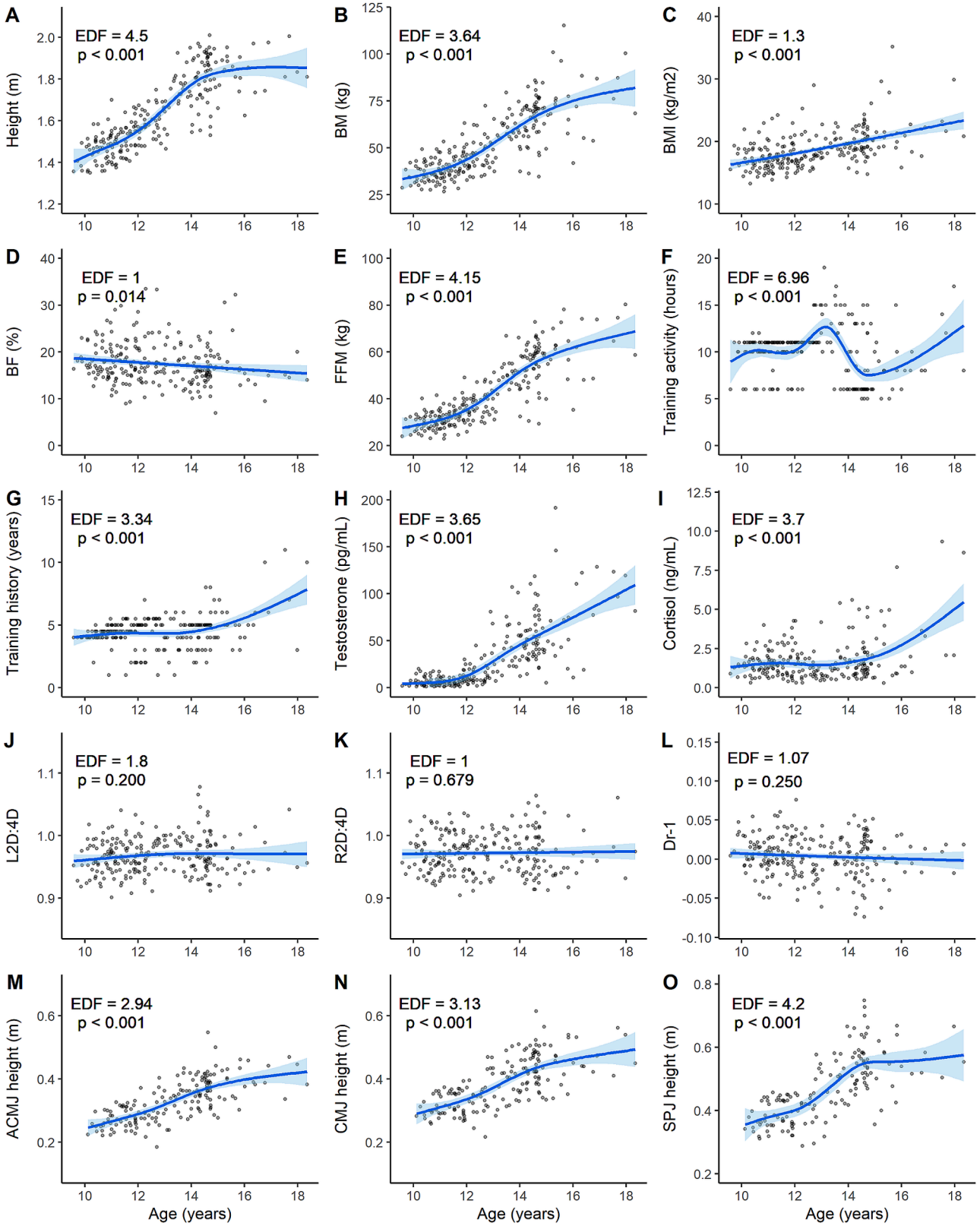

Fig. 1. Age-related trajectories in body size, training activity and history, salivary hormones, 2D:4D measures, and physical performance in athletic boys. Key: BM = body mass, BMI = body mass index, FFM = fat-free mass, L2D:4D = left-hand 2nd to 4th digit ratio, R2D:4D = right-hand 2nd to 4th digit ratio, Dr-1 = R2D:4D minus L2D:4D, ACMJ = countermovement jump with hands akimbo, CMJ = countermovement jump, SPJ = spike jump. The solid line represents the smoothed trend and the shaded region denotes the 95% CI.

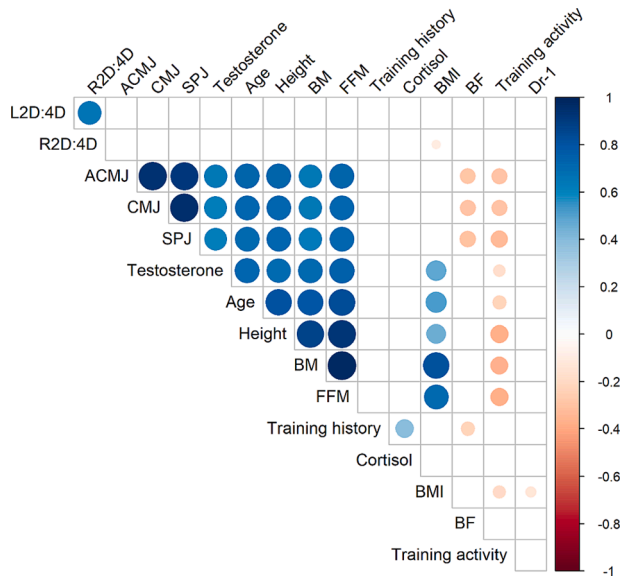

Fig. 2. Zero-order correlations presented as a correlational matrix with hierarchical clustering. Circle size and intensity of shading are proportional to the strength of each correlation. Key: BM = body mass, BMI = body mass index, BF = body fat, FFM = fat-free mass, L2D:4D = left-hand 2nd to 4th digit ratio, R2D:4D = right-hand 2nd to 4th digit ratio, Dr-1 = R2D:4D minus L2D:4D, ACMJ = countermovement jump with hands held akimbo, CMJ = countermovement jump, SPJ = spike jump. Only significant ($p < 0.05$) correlations are depicted.

Table 2
Summary statistics for the generalized additive model predicting countermovement jump height in athletic boys.

Parametric coefficient	Estimate (SE)	t value	*p* value
Intercept	0.387 (0.003)	112.5	< 0.001
Smooth terms	**EDF**	**F**	***p* value**
Age (years)	1.00	37.67	< 0.001
BF (kg)	1.59	12.66	< 0.001
Training activity (hours)	2.21	2.25	0.078
Training history (years)	2.46	3.20	0.024
R2D:4D	3.06	3.85	0.005
Testosterone (pg/mL)	1.90	3.25	0.035
Model fit: Adjusted $R^2 = 0.662$, AIC = −548.95, REML = −257.73			

Key: BF = body fat, R2D:4D = right-hand 2nd to 4th digit ratio.

smoother effects on CMJ height were identified, and all terms were significant, except for weekly training activity. This model explained 66.2% of the variance in CMJ height. To aid interpretation, the smoother coefficients are plotted in Fig. 3. Subject age had a positive, linear effect on jumping performance (Fig. 3A), whilst other variables displayed non-linear associations. Physical performance declined in a curvilinear (concave) manner with a higher BF (Fig. 3B), likewise for weekly training activity (Fig. 3C), although this relationship was convex in shape before performance stabilized at ~10 h of activity per week. For training history (Fig. 3D), CMJ performance followed a classical inverted U-shape with inflection at around 4.5 years. The R2D:4D (Fig. 3E) effect on performance was convex in appearance, characterized by a flat negative slope and a steep positive slope with inflection at ~1.00 unit. The effect of testosterone (Fig. 3F) on CMJ height was concave in nature; being positive with increasing concentrations before plateauing at around 125 pg/mL.

Additional GAMs were conducted to evaluate the sensitivity of our results. First, all covariates were removed (Model 4) leaving only the R2D:4D and testosterone as predictors. In this model, the R2D:4D and testosterone remained highly significant ($p \leq 0.001$) non-linear contributors to CMJ height (Model 4 fit: adjusted $R^2 = 0.543$, AIC = −506.17, REML = −241.7). A deviance (Chi-square) test between Models 3 and 4 revealed a decrease in model fit following covariate exclusion (df = −8.46, deviance = −0.124, $p < 0.001$). Next, Model 3 was repeated, but all smoothers were omitted to test the linear effects of each covariate and predictor (Model 5). This analysis yielded significant effects ($p < 0.05$) of age, BF, weekly training activity, and testosterone on CMJ height, whereas the R2D:4D was no longer a significant predictor ($p = 0.504$). Model fit appeared to be compromised by the linear approach (adjusted $R^2 = 0.590$, AIC = −526.74, REML = −235.63), which we verified by a deviance test between Models 3 and 5 (df = −10.69, deviance = −0.081, $p < 0.001$). All model results and comparisons are provided as a supplementary file.

4. Discussion

This study tested the hypotheses that testosterone works through an organizational pathway, with the 2D:4D and Dr-1 as proxies, and an activational pathway, indexed by current testosterone concentration, to regulate a simple measure of physical fitness among athletic boys. Delineation of age-related trajectories revealed non-linear increases in overall body size, hormone concentrations, and jumping ability with chronological age, whilst the 2D:4D measures were age invariant. As hypothesized, significant non-linear effects of testosterone and R2D:4D on CMJ height emerged that also differed in timing, tempo, and direction.

Generally speaking, pubertal maturation is characterized by accelerated growth in boy's stature, weight, muscle size, and improvements in physical fitness (e.g., power, strength) that are mediated, in part, by a dramatic surge in testosterone production [21-24, 37]. Similarly, we observed non-linear growth in boy's height, BM, BMI, FFM, and jumping abilities with chronological age, and a rise in testosterone concentration from 11.5 years of age. The non-linear increase in cortisol concentration with age is also congruent with cross-sectional studies [24, 38], although this change occurred at a later age (14.6 years) than testosterone. Hand growth is another hallmark of human ontogeny, with 2D and 4D lengths increasing throughout infancy, childhood, adolescence, and even into adulthood [16, 17]. The R2D:4D and L2D:4D did not, however, change across the studied age range; a discrepancy that is likely attributable to similar proportional increases in 2D and 4D lengths [16]. These results add support to the concept of 2D:4D stability, especially during a major developmental stage, with little influence of allometric effects that could lead to biased 2D:4D measurements [16, 17]. This temporal constancy could also explain the age-related stability of Dr-1, as a composite measure of both 2D:4D outcomes [39].

Current testosterone had a non-linear effect on CMJ height, such that physical performance improved in a curvilinear manner with a higher testosterone concentration, before stabilizing in the upper testosterone range. This pattern coincides with the non-linear age effect on explosive leg strength, seen here and in other research on athletic boys [31, 40] that, presumably, is due to a corresponding rise in skeletal muscle mass and contractile capabilities with the amount of testosterone in circulation as a driving force. It is noteworthy that the onset of a significant change in body size and CMJ performance (starting around 9.6 – 10.9 years of age) preceded the observed testosterone surge by several months, which we attribute to individual factors and the cross-sectional design. Whilst testosterone is undeniably important for promoting primary and secondary sexual characteristics in human males, more subtle day-to-day (or within-day) shifts in testosterone secretion could affect other neuromuscular processes (e.g., mood, motivation, cognition) that underlie human performance [27, 41]. Since these actions are expressed on a rapid timescale, they might complement the larger pubertal shifts in testosterone production to regulate physical fitness, but depending more on situational and environmental cues in daily life.

The R2D:4D effect on CMJ height was also non-linear in nature,

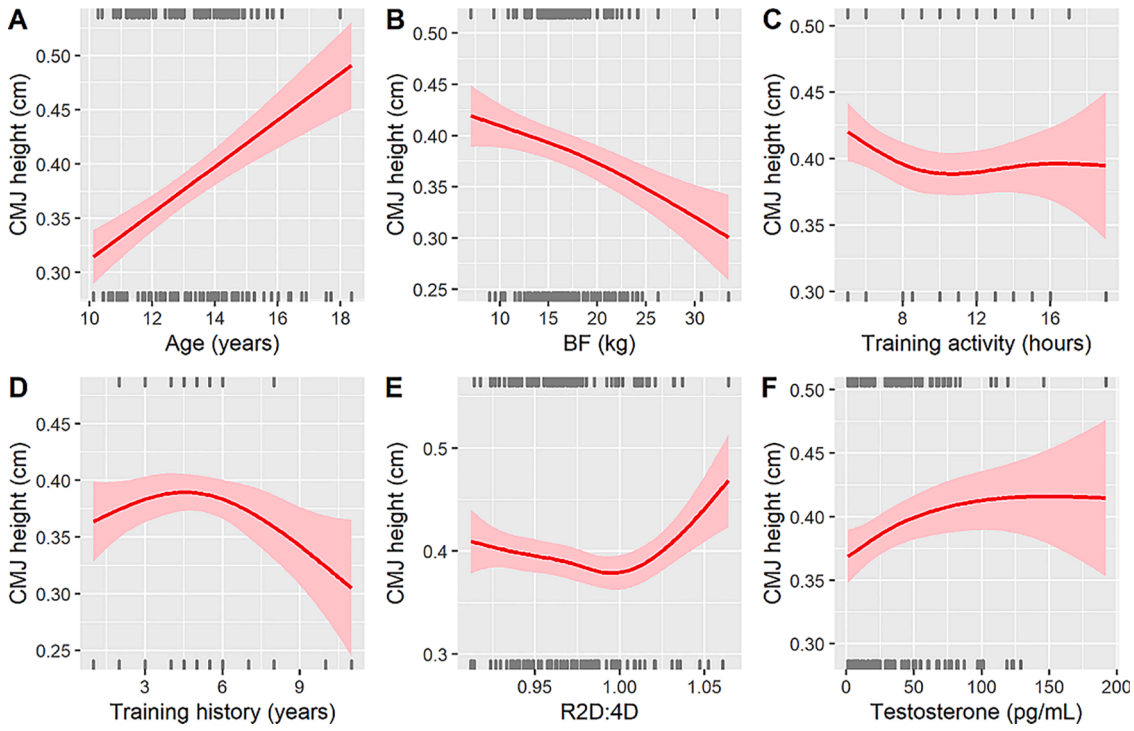

Fig. 3. Partial effects of age (3A), body fat (3B), weekly training activity (3C), training history (3D), R2D:4D (3E), and testosterone (3F) on countermovement jump (CMJ) height. The solid line represents the smoothed trend and the shaded region indicates the 95% CI.

characterized by a U-shaped curve. Other evidence of non-linearities in 2D:4D relationships emanates from academic [18], cognitive [19], and social preference domains [20]. This finding is novel in the context of pubertal development and physical fitness. Specifically, better CMJ performance was associated with a very low R2D:4D, at least for boys with a R2D:4D less than 1.0, and a very high R2D:4D, for boys with a R2D:4D exceeding 1.0. The former result is not surprising, as most studies on boys and young adults demonstrate that the R2D:4D is negatively related to different speed, power, and strength measures [8-10, 12-14]. Whilst the positive R2D:4D and CMJ association has less empirical support, this finding was largely driven by a smaller group of older boys. The curvilinear R2D:4D and CMJ relationship might be explained by a maturational effect of prenatal testosterone. Girls, for example, mature earlier than boys. One reason for this is the effect of prenatal testosterone (boys experience higher levels than girls) which delays maturation, as suggested by experimental studies on rats [42]. Whilst this is not surprising, the same effect has been identified in male rats [43]. Similarly, in humans, the prenatal effects of testosterone could delay sperm production [44] and a low R2D:4D relative to L2D:4D (or low Dr-l) was related to delayed maturation [39]. Consequently, the positive relationship in the small sub-set of boys could return to a negative one when low R2D:4D athletes catch up in pubertal maturation.

It is important to emphasize that the GAM approach uncovered novel associations that might otherwise be obscured. In fact, a standard multiple linear regression failed to identify a significant R2D:4D effect on CMJ performance and it produced a relatively poor fitting model versus the GAM. Therefore, we encourage the use of flexible modeling approaches to highlight more nuanced 2D:4D and testosterone relationships with indices of physical fitness during key developmental stages. Other non-linear approaches in 2D:4D research include quadratic and polynomial regression models [18–20].

Regarding the 2D:4D role, there is speculation that prenatal androgenization can organize multiple physiological systems (e.g., central nervous, cardiovascular, musculoskeletal) [1–3] involved in the expression and development of physical fitness. The prenatal influences of testosterone could also be self-reinforcing, shaping individual differences in testosterone secretion and activational effects in later life. Partial support comes from negative relationships between testosterone and 2D:4D in a few adult populations [7, 19, 45]. Research involving boys are still inconsistent; one study finding no R2D:4D or L2D:4D relationship with testosterone [46], whilst another reported a weak (r = 0.11) R2D:4D and testosterone association that approached significance [47]. We explored this possibility (data not shown), but found no evidence that the 2D:4D or Dr-1 measures were able to predict current testosterone. Experimental work on adults [48–50] further support the possibility of an organizational effect (of prenatal androgenization) on the acute testosterone response to challenge situations [3], as another pathway to explain 2D:4D associations to sporting performance, but see Kowal et al., [51]. To our knowledge, no such research has been performed on athletic or non-athletic boys.

The L2D:4D and Dr-1 were both poor predictors of jumping ability. This finding contravenes an early review of 2D:4D literature, where neither hand out-predicted the other regarding general athletic prowess [5], but little data (2 out of 25 cohorts) originated from boys. Research on boys often favors the R2D:4D when assessing explosive power and other components of physical fitness [8, 9, 11-13, 25]. One explanation is that the R2D:4D is a more sensitive measure of prenatal sex-steroid exposure than the L2D:4D [2, 7], thereby magnifying any relationship with a target trait. Further examples exist of elite athletes or athletes possessing a lower R2D:4D than non-elites and controls [52–54], including differences between sporting groups [54], which implies that the R2D:4D could be a phenotypic indicator of athleticism. Resolving this laterality effect remains a challenge, primarily due to inconsistent methodological approaches. That is, many of the aforementioned studies did not assess the L2D:4D for comparative purposes [8, 9, 12, 13]. Fewer still have investigated the R2D:4D, L2D:4D, and Dr-1 together, and when doing so have produced somewhat mixed results [11, 15, 55].

There are limitations to this work that merit consideration. First, the prediction models are based on cross-sectional data and our population distribution was lower in the upper age range (16 – 18 years). Second, the recruitment of athletic boys prevents broader inferences being made,

as does the assessment of a single component of physical fitness. Future studies could benefit from comparisons between athletic and non-athletic boys to establish normative values and differences, whilst assessing a broader range of fitness qualities (e.g., power, endurance, agility, maximal strength) that reflect athletic prowess and health status. Third, we did not attempt to quantify biological (e.g., skeletal, reproductive, somatic) maturity, which can proceed independently of chronological age [56]. A longitudinal study is needed to obtain more precise population and individual estimates of growth and maturation (i.e., Tanner stage, testicular volume, skeletal age), how these changes are organized and/or activated by testosterone, and their collective role in mediating physical fitness. Fourth, hormone profiling from a single sample is problematic, although efforts were made to control for confounding factors. Maturational shifts in other steroid precursors (e.g., dehydroepiandrosterone, 17-hydroxyprogesterone) and growth factors (e.g., growth hormone) add to these complexities [37, 56]. Notwithstanding these limitations, controversy remains regarding the best method of 2D:4D assessment [57, 58] and whether it offers a retrospective marker of prenatal hormone exposure; see Swift-Gallant et al., [59] for evidence of an association between 2D:4D and prenatal sex steroids, and McCormick & Carré [60] for a counter view.

5. Conclusions

The cross-sectional assessment of athletic boys confirmed that testosterone exerts both activational and organizational effects on a simple measure of physical fitness. Specifically, a higher current testosterone concentration, up to a certain level, and a lower or higher R2D:4D were linked to better CMJ performance. The use of a flexible (GAM) modeling approach allowed us to characterize this complex interplay and it detected a significant R2D:4D effect on performance that a standard linear approach failed to identify.

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Conflicts of interest

The authors have no conflicts of interest regarding the material presented in this paper.

Data Availability

The data that has been used is confidential.

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Supplementary materials

Supplementary data associated with this article can be found, in the online version, at 10.1016/j.physbeh.2022.113816.

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