Association Between Dehydroepiandrosterone Sulphate Levels at 7 Years Old And Bone Mineral Density At 10 Years Old – a Prospective Cohort Study

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Abstract

We aimed to explore the effect of dehydroepiandrosterone sulphate (DHEAS) at age 7 on areal bone mineral density (aBMD) at age 10, and to distinguish the direct and indirect effects (explained by sexual maturity and by aBMD at age 7), for each sex, after adjustment for body mass index z-score (BMI).

In a subsample of 274 children (139 girls, 135 boys) from the Generation XXI cohort, aBMD was assessed with dual-energy x-ray absorptiometry (DXA) scan at age 7 and age 10. The increase in aBMD at age 10 for each 10 µg/dL increase in DHEAS levels at age 7 was estimated using path analysis. Both the direct and the indirect effects were calculated.

In girls, higher DHEAS levels at age 7 were associated with higher aBMD at age 10. No direct effect was observed. The indirect effect via higher aBMD at age 7 explained 61% of the total effect, and the indirect effect via higher Tanner stage explained 21%. After adjustment for BMI, the total effect remained statistically significant, explained in 33% by the indirect effect of DHEAS on Tanner stage and Tanner stage on aBMD. In boys, no effect of DHEAS on aBMD was observed.

Conclusion: An indirect effect of DHEAS at age 7 on aBMD at age 10 was found in girls, but not in boys, as higher DHEAS levels were associated with more advanced sexual maturity at age 10, and more advanced sexual maturity to higher aBMD. No direct effect of DHEAS on aBMD was observed.

What Is Known

- Conditions associated with elevated DHEAS, adrenarche's biomarker, are accompanied by advanced bone maturity.
- Whether adrenal androgens influence bone mineralization in childhood remains puzzling and longitudinal data is scarce.

What is new

- In girls, but not in boys, higher DHEAS at age 7 was associated with higher aBMD at age 10.
- This was partially explained by the indirect effect of DHEAS at age 7 on sexual maturity at age 10, as DHEAS at age 7 was positively associated with sexual maturity at age 10, which was further associated with aBMD.

Introduction

Adrenarche is the progressive peripubertal maturation of the adrenal *zona reticularis*, resulting in increasing amounts of androgen precursors, including dehydroepiandrosterone (DHEA) and its sulphate (DHEAS). Although new data suggest that 11-oxygenated C₁₉ adrenal-derived steroids are the main bioactive androgens during adrenarche [1], DHEAS has been widely used as the biomarker of adrenal androgen production [2–4]. DHEAS is generally detectable using standard laboratory techniques from 5 to 6 years of age onwards, while the clinical manifestations of adrenarche, including the appearance of pubic and axillary hair, apocrine body odor, acne, and seborrhea, are usually seen in girls after the age of 8 and in boys after the age of 9 [2–4].

DHEAS, weakly androgenic, is a precursor steroid in the pathway to more potent androgens and estrogens. Although it has been suggested that DHEAS has beneficial effects in aging, metabolism, and neurologic function [5; 6], it is unclear which functions it exerts on its own. Higher DHEAS levels in prepuberty have been associated with obesity and lower birth weight [7–10], and also with earlier puberty in girls, but not in boys [11; 12].

Bone growth accelerates during puberty, initially in length, followed by width, mineral content, and density [13]. Most of the bone mass is obtained during adolescence and young adulthood, and the rate of bone mineral accrual peaks around menarche in girls [13]. Bone modelling and growth in childhood and early puberty are influenced by genetic, intrauterine programming, nutrition, physical activity, and hormones, mainly gonadal sex steroids and insulin growth factor 1 (IGF1) [13–16]. Research has focused on the influence of estrogens on bone mass, while the role of the androgen hormones, especially adrenal-derived steroids, is less certain [17].

Androgens can affect the bone indirectly through aromatization to estrogens, which promote linear growth, increase calcium absorption, and decrease bone turnover [18; 19]. Androgens also directly stimulate osteoblast proliferation and differentiation, skeleton maturation, longitudinal bone growth and epiphyseal closure, via liaison with the bone's androgen receptors [17; 19-21].

Conditions associated with elevated DHEAS levels, like premature adrenarche or congenital adrenal hyperplasia, are accompanied by advanced bone age, together with height velocity [14; 22-26], suggesting that DHEAS may have an effect in skeletal maturation. DHEAS also indirectly influences bone mineralization, as a substrate in the formation of more potent androgens or estrogens and as a stimulus to other bone remodeling hormones, such as IGF1 [19]. However, whether DHEAS plays a significant direct role in bone mineralization during childhood is not totally clear, and data on the effect of DHEAS on bone mass in prepubescent children and adolescents are scarce and inconsistent [15; 26-33].

Thus, we sought to explore the effect of DHEAS at age 7 on bone mineral density (BMD) at age 10, and to distinguish the direct and indirect effects (explained by sexual maturity and by aBMD at age 7), considering sex differences and after adjustment for body mass index (BMI) z-score.

Material And Methods

Study population and design

The study participants are a subsample of children included in a prospective birth cohort, Generation XXI, whose full details have been published elsewhere [34; 35].

The recruitment of participants was conducted in 2005/2006 in all five public maternities of the metropolitan area of Porto, Portugal. Of the invited mothers, 8495 agreed to participate (91%) and a total of 8647 newborns were enrolled. At ages 4, 7 and 10 years old, all Generation XXI participants were invited to a face-to-face follow-up evaluation. Of the 8647 initial cohort members, 7459 (86%), 6889 (80%), and 6392 (74%) were assessed at the 4-, 7-, and 10-year-old follow-up evaluations, respectively. The follow-up visits included a physical examination and fasting blood sample, according to standard procedures.

From those that attended the 7-year-old follow-up, 700 prepubescent children were randomly selected and their DHEAS levels were measured, as part of a study on adrenarche [10]. Among these 700 prepubescent children, 274 (139 girls and 135 boys) had complete information in all variables analyzed, including a full-body dual-energy X-ray absorptiometry (DXA) scan at 7 and 10 years old. A comparison between those of the 700 who were included in the complete information analysis and those who were not is depicted in Supplementary Table 1.

Data collection

At the baseline, data on maternal demographic and socioeconomic characteristics, lifestyle, obstetric history, pre-pregnancy anthropometrics, and personal history of diseases, were collected by trained interviewers, using structured questionnaires, during the hospital stay.

Data on delivery and newborn characteristics (including gestational age, birth weight, and length) were additionally extracted from clinical records [34; 35]. Birth weight and length were transformed in z-scores according to the Fenton growth charts [36].

Anthropometric measurements

At 4, 7, and 10 years of age, trained researchers performed anthropometric measurements, with the child in underwear and bare feet. Weight was measured to the nearest 0.1 kg using a digital scale (Tanita®, Arlington Heights, IL, USA), and standing height was measured to the nearest 0.1 cm using a wall stadiometer (Seca®, Hamburg, Germany). BMI was calculated by dividing weight (kg) by squared height (m²). BMI was transformed into age and sex-specific z-scores using World Health Organization (WHO) standards [37].

DXA-derived bone measures and body composition

At ages 7 and 10, whole-body DXA scans were performed using a Hologic Discovery QDR® 4500W device (software version 13.3.0.1; Hologic Inc., Bedford, MA, USA) according to standard manufacturer's protocol, while the child was in underwear and with the bladder emptied. Standard quality assurance tests were performed daily using the spine phantom according to the manufacturer's instructions. Scans were evaluated immediately after acquisition and later validated by a second technician. Total body less head (subtotal) bone mineral content (BMC) (g) and areal BMD (aBMD) (g/cm²) were obtained, according to the International Society of Clinical Densitometry recommendation [38]. Fat and lean mass (g), and body fat percentage (%) were also assessed by DXA scan.

Sexual maturity evaluation

Sexual development evaluation was conducted by trained observers according to the sexual maturity ratings including breast changes in females, genital changes in males, and pubic hair changes in both females and males (Tanner stages) [39]. In girls, breasts were evaluated by inspection and palpation, and in boys, testicular volume was assessed by palpation using the Prader orchidometer. Included participants were classified as Tanner stage I, II, III, IV or V. Prepuberty was defined as Tanner stage I.

Biochemical analysis

DHEAS levels were measured in blood (serum) collected at the 7-year-old follow-up visit, by electrochemiluminescence immunoassays on the Roche cobas e411 analyzer (Roche Diagnostics, Basel, Switzerland). An overnight fasting venous blood sample was obtained before 11:00 a.m., after applying topical analgesic with lidocaine/prilocaine (EMLA cream).

Statistical analysis

Categorical and continuous variables were presented as counts (proportions), and mean and standard deviation (SD). The chi-square and the t-test for two independent samples were used to evaluate differences between sexes. The partial correlation test adjusted for age, sex and BMI z-score was applied to analyze the associations between aBMD and anthropometric and hormonal indicators. This analysis was performed using SPSS® (v.24; SPSS, IBM Corp., Armonk, NY, USA).

Path analysis was used to estimate crude and adjusted linear regression coefficients (β) and 95% confidence intervals (95% CI), which represent the increase in aBMD (g/cm²) at 10 years old for each 10 μ g/dL increase in DHEAS at 7 years old. Path analysis was conducted based on the theoretical model depicted in Figure 1. Since bone size and aBMD increase with body height and weight [15; 16; 40; 41], BMI, along with age, sex, and the stage of puberty, should be considered when assessing determinants of BMD in children and adolescents [16; 19]. Thus, our adjusted model included BMI z-score at 7 years old, aBMD at 7 years old, and Tanner stage at 10 years old as explanatory variables. Considering sex differences in pubescent development timing and bone mass increase, boys and girls were analyzed separately.

Path analysis was performed with the lavaan [42] package from R software version 4.0.3; 95% CI was calculated by bootstrapping. Full information maximum likelihood estimation was used to handle missing values, assuming missing at random [43]. The fit of the models was assessed using different indexes: the Comparative Fit Index (CFI) [44], the Tucker–Lewis Index (TLI) [45], and the Root Mean Square Error of Approximation (RMSEA) [46]. A good model fit is indicated by a CFI and TLI values ≥ 0.90 and values of RMSEA lower than 0.08. The final model had CFI 1.000, TLI 1.029, RMSEA 0.000 (girls) and CFI 1.000, TLI 1.003, RMSEA 0.000 (boys).

Results

Sample characteristics

Characteristics of the 274 participants (139 girls and 135 boys), and the comparison between sexes, are shown in Table 1. Evaluations were conducted at a mean age of 7.1 (SD: 0.2) years old and 10.1 (SD: 0.2) years old, with no sex differences. Neonatal and maternal characteristics were similar in boys and girls.

Table 1
Anthropometric, metabolic, and hormonal characteristics of the participants at birth, 7 and 10 years, and maternal characteristics, by sex

	All (n=274)	Girls (n=139)	Boys (n=135)	p-value
<i>At birth</i>				
Gestational age (weeks), mean (SD)	38.8 (1.5)	38.8 (1.6)	38.7 (1.4)	0.232*
Birth weight (grams), mean (SD)	3228 (463)	3153 (452)	3303 (463)	0.458*
Birth length (cm), mean (SD)	49.0 (2.2)	48.6 (2.1)	49.4 (2.1)	0.926*
<i>At 7 years old</i>				
Age (years), mean (SD)	7.1 (0.2)	7.1 (0.2)	7.1 (0.3)	0.210*
Weight (kg), mean (SD)	26.5 (5.1)	26.4 (5.5)	26.6 (4.8)	0.007*
Height (cm), mean (SD)	124.2 (5.2)	123.4 (5.2)	125.1 (5.1)	0.932*
BMI (kg/m ²), mean (SD)	17.1 (2.5)	17.2 (2.7)	16.9 (2.3)	<0.001*
BMI z-score, mean (SD)	0.7 (1.2)	0.8 (1.1)	0.7 (1.2)	0.712*
Fat mass (DXA) (g), mean (SD)	8026 (3628)	8908 (3814)	7121 (3194)	0.007*
Lean mass (DXA) (g), mean (SD)	15704 (2485)	15009 (2424)	16417 (2349)	0.647*
Body fat (%), mean (SD)	32.6 (7.8)	35.9 (7.3)	29.2 (6.7)	0.064*
DXA-derived aBMD (g/cm ²)	0.63 (0.06)	0.62 (0.06)	0.63 (0.05)	0.059*
DXA-derived BMC (g)	611.0 (89.1)	600.8 (91.4)	621.5 (85.8)	0.360*
DHEAS (µg/dL), mean (SD)	45.5 (32.5)	46.8 (31.6)	44.1 (33.4)	0.335*
<i>At 10 years old</i>				
Age (years), mean (SD)	10.1 (0.2)	7.1 (0.2)	7.1 (0.2)	0.705*
Weight (kg), mean (SD)	38.2 (8.6)	38.5 (9.2)	37.8 (7.9)	0.002*
Height (cm), mean (SD)	141.8 (6.3)	141.6 (6.5)	142.0 (6.0)	0.373*
BMI (kg/m ²), mean (SD)	18.9 (3.4)	19.1 (3.6)	18.6 (3.1)	0.004*
BMI z-score, mean (SD)	0.7 (1.2)	0.7 (1.2)	0.8 (1.2)	0.378*
Fat mass (DXA) (g), mean (SD)	14079 (6628)	14892 (6712)	13245 (6454)	0.578*
Lean mass (DXA) (g), mean (SD)	25252 (4894)	25084 (4782)	25424 (5013)	0.515*
Body fat (%), mean (SD)	34.5 (8.1)	35.9 (7.6)	32.9 (8.3)	0.299*
Tanner stage, n (%)	127 (46%)	31 (22%)	96 (71%)	<0.001*
- Tanner I	94 (34%)	58 (42%)	36 (27%)	
- Tanner II	46 (17%)	43 (31%)	3 (2%)	
- Tanner III	7 (3%)	7 (5%)	0 (0%)	
- Tanner IV	0 (0%)	0 (0%)	0 (0%)	
- Tanner V				
DXA-derived aBMD (g/cm ²)	0.81 (0.08)	0.81 (0.09)	0.80 (0.07)	<0.001*
DXA-derived BMC (g)	1041.2 (222.8)	1065.2 (238.4)	1016.6 (203.3)	0.005*
<i>Maternal characteristics</i>				
Maternal height (cm), mean (SD)	159.5 (5.6)	159.8 (5.7)	159.2 (5.5)	0.452*
Maternal pre-pregnancy BMI (kg/m ²), mean (SD)	23.9 (4.2)	24.0 (4.3)	23.9 (4.0)	0.313*
Weight gain during pregnancy (kg), mean (SD)	13.7 (5.9)	13.3 (5.9)	14.1 (5.9)	0.959*
Maternal age at menarche (years), mean (SD)	12.3 (1.5)	12.3 (1.5)	12.3 (1.5)	0.554*
Abbreviations: DHEAS – dehydroepiandrosterone sulphate; BMI – body mass index; DXA – dual-energy x-ray absorptiometry; aBMD – areal bone mineral density; BMC – bone mineral content				
*T-Test †Pearson's Chi-square Test				
BMI z-scores according to the WHO standards [37]				

At age 7, girls had higher mean fat mass than boys (8,908 [SD: 3,814] *versus* 7,121 [SD: 3,194] g, $p=0.007$). Mean DHEAS levels were similar in both sexes (girls: 46.8 [SD: 31.6] $\mu\text{g/dL}$; boys: 44.1 [SD: 33.4] $\mu\text{g/dL}$; $p=0.335$). No differences were found in DXA-derived bone parameters (Table 1).

At 10 years old, girls presented higher mean aBMD than boys (0.81 [SD: 0.09] *versus* 0.80 [SD: 0.07] g/cm^2 , $p<0.001$). Differences in Tanner stage at 10 years old were also observed, as most boys were Tanner stage I (71%), while only 22% of the girls were prepubescent ($p<0.001$). No other significant differences were found in anthropometry or body composition at age 10 (Table 1).

Partial correlations

Partial correlation coefficients between aBMD at 7 and 10 years old and independent variables, for the whole sample, are summarized in Table 2. Areal BMD at 7 years old correlated positively with birth length, height, fat and lean mass at 7 years old, and DHEAS levels at 7 years old, after adjustment for age, sex and BMI z-score. Areal BMD at 10 years old correlated positively with height, fat and lean mass at 7 years old, and with height and lean mass (but not fat mass) at 10 years old, after controlling for age, sex and BMI z-score. Areal BMD at 10 years old also correlated positively with DHEAS levels at 7 years old and aBMD at 7 years old, adjusted for age, sex and BMI z-score.

Table 2
Correlations between aBMD at 7 and 10 years old and anthropometric and hormonal indicators at baseline, 7 and 10 years old

	aBMD (g/cm2) 7y, r (p)	aBMD (g/cm2) 10y, r (p)
Birth weight z-score	0.108 (0.061)*	-0.033 (0.585)**
Birth length z-score	0.157 (0.006)*	0.054 (0.378)**
Height 7y z-score	0.631 (<0.001)*	0.549 (<0.001)**
Fat mass 7y (g)	0.136 (0.018)*	0.172 (0.005)**
Lean mass 7y (g)	0.365 (<0.001)*	0.552 (0.005)**
DHEAS 7y ($\mu\text{g/dL}$)	0.777 (<0.001)*	0.171 (0.005)**
aBMD (g/cm2) 7y, r (p)	-	0.717 (<0.001)**
Height 10y z-score	-	0.621 (<0.001)**
Fat mass 10y (g)	-	-0.005 (0.936)**
Lean mass 10y (g)	-	0.723 (<0.001)**
*Analyzed by partial correlation test adjusted for age, sex and BMI 7y z-score		
**Analyzed by partial correlation test adjusted for age, sex and BMI 10y z-score		
Abbreviations: DHEAS – dehydroepiandrosterone sulphate; BMI – body mass index; aBMD – areal bone mineral density; r – correlation coefficient		
Birth weight and length z-scores according to the Fenton growth charts [36] and BMI z-scores according to the WHO standards [37]		

Path analysis

A mediation analysis is depicted in Figure 1. It comprises the estimated total, direct, and indirect effects of 10 $\mu\text{g/dL}$ increase in DHEAS at age 7 in aBMD (g/cm^2) at age 10, stratified by sex.

In girls, crude analysis showed that higher DHEAS at age 7 was associated with higher aBMD at age 10 ($\beta = 0.007$ [95% CI: 0.004; 0.010], $p<0.001$) (Figure 1, ab + ed + c). This total association was mainly explained by indirect effects. Higher DHEAS at age 7 was associated with higher Tanner stage at age 10, and higher Tanner stage was associated with higher aBMD, and this indirect effect represented 21% of the total effect ($p=0.001$) (Figure 1, ed). Higher DHEAS at age 7 was also associated with higher aBMD at age 7, and higher aBMD at age 7 was associated with higher aBMD at age 10, and this indirect effect explained 61% of the total effect ($p<0.001$) (Figure 1, ab). No direct effect of DHEAS at age 7 in aBMD at age 10 was observed (Figure 1, c) (Table 3).

Table 3
Estimated total, indirect and direct effects of DHEAS at 7 years (per 10µg/dL increase) on aBMD at 10 years of age

Total effect (ab + ed + c)					Direct effect (c)				Indirect effect (mediated by aBM			
	Unstandardized coefficient	95% CI	Standardized coefficient	p	Unstandardized coefficient	95% CI	Standardized coefficient	p	Unstandardized coefficient	95% CI	Standardized coefficient	p
Girls												
Crude model	0.007	0.004; 0.001	0.348	<0.001	0.001	-0.001; 0.003	0.065	0.201	0.004	0.002; 0.007	0.201	0.001
Adjusted model* ^a	0.003	0.000; 0.006	0.148	0.033	0.001	-0.001; 0.003	0.062	0.201	0.001	-0.001; 0.003	0.001	0.033
Boys												
Crude model	0.003	-0.001; 0.009	0.148	0.218	-0.001	-0.003; 0.002	-0.037	0.480	0.004	0.000; 0.008	0.148	0.218
Adjusted model* ^b	0.001	-0.003; 0.006	0.062	0.591	-0.001	-0.003; 0.001	-0.037	0.480	0.002	-0.001; 0.005	0.062	0.591
Abbreviations: CI – confidence interval, aBMD – areal bone mineral density; BMI – body mass index												
*Adjusted model: adjusted for BMI at 7 years-old												
^a Comparative fit index: 1.000 Tucker-Lewis index: 1.029 Root Mean Square Error of Approximation: 0.000												
^b Comparative fit index: 1.000 Tucker-Lewis index: 1.003 Root Mean Square Error of Approximation: 0.000												

After adjustment for BMI z-score at 7 years old, the total effect remained statistically significant in girls ($\beta = 0.003$ [95% CI: 0.000; 0.006], $p=0.033$) (Figure 1, ab + ed + c), explained in 33% by the indirect effect of DHEAS on Tanner stage and Tanner stage on aBMD (Figure 1, ed). The indirect effect of DHEAS on aBMD at age 7 and aBMD at age 7 on aBMD at age 10 was attenuated and lost statistical significance (Figure 1, ab) (Table 3).

In boys, no effect of DHEAS at age 7 on aBMD at age 10 was observed ($\beta = 0.003$ [95% CI: -0.001; 0.009], $p=0.218$) (Table 3).

BMI z-score at age 10 was also considered in the model, but it neither changed the results appreciably nor improved the fit of the model (data not shown), and therefore was excluded from the final model.

Discussion

The present study explores the effect of DHEAS at the age of 7 years on aBMD at the age of 10 years. Firstly, we found that aBMD at 10 years old correlated positively with DHEAS at 7 years old, after adjustment for age, sex, and BMI z-score. Secondly, using path analysis, we tried to distinguish a possible direct effect of DHEAS at age 7 on aBMD at age 10 from an indirect effect partially explained by sexual maturity or by aBMD at age 7. Although no direct effect of DHEAS at age 7 on aBMD at age 10 was observed, we found in girls, but not in boys, an indirect effect explained by sexual maturity, as higher DHEAS levels at 7 years old were associated with higher sexual maturity at 10 years old, which was further associated with higher aBMD, controlling for BMI.

To our best knowledge, this is the first study to address the longitudinal effect of DHEAS on aBMD in prepuberty and early puberty. So far, only a few cross-sectional studies have investigated the effect of circulating adrenal androgens on bone mass acquisition in mid-childhood, with mixed results, and a comparison with our findings is difficult due to different populations and methodological approaches. In accordance with our results, a positive effect of adrenal androgens on BMD was found in premenarcheal girls [47] and in two populations of children aged 5-8 years [15] and 6-18 years [32]. On the other hand, no association was found between DHEAS and bone mineral density in 255 children aged 7-8 years [31] and in a population of boys aged 6-14.5 years [26]. In a large cohort involving 472 Finnish children aged 6-8 years, the positive association of DHEAS with BMD disappeared after adjustment for fat and lean mass [33].

In our analyses, we have decided to study girls and boys separately, as we recognize the large sex differences in bone mass increase and the timing of puberty. At age 7, no statistically significant difference was found in aBMD between boys and girls, while, at the age of 10, girls presented higher aBMD than boys. Furthermore, at the age of 10, most of the girls had started puberty (78%), while 71% of the boys were still prepubescent. In girls, the effect of DHEAS at age 7 on aBMD at age 10 was partially explained by sexual maturity, as higher DHEAS at 7 years old was associated with higher Tanner stage at 10 years old, which was further associated with higher aBMD. In boys, no such effect was found, and some explanations for this sex discrepancy can be pointed out. Firstly, during puberty, girls accrue more bone mass than boys, and they do it in earlier Tanner stages [16]. Secondly, previous studies have shown that higher serum DHEAS at 7 years old is associated with earlier pubescent development in girls, but not in boys [11; 12]. Therefore, the indirect effect of DHEAS on sexual development and sexual development on aBMD is less relevant in boys than in girls, at this age.

It should be noted that the found effect of DHEAS on BMD at age 10, partially explained by sexual maturity, could also be the result of other unmeasured sexual hormones, such as estrogens. A direct effect of DHEAS on BMD, independent of estrogens, was not established.

Bone size and aBMD increase with height and weight [15; 16; 40; 41]. Hence, BMI, along with sex and the stage of puberty, was considered in our analyses. Obese and overweight prepubescent children present higher DHEAS levels [7; 9] and higher androgen levels are associated with changes in body composition,

such as increased central adiposity and lean mass [19], which can affect the bone. The association between BMI and BMD in children is mostly determined by lean mass [33; 41], but adiposity also appears to play a role, despite contradictory findings [33; 48; 49]. Adiposity may augment BMD through an increased mechanical load exerted on the skeleton by fat mass [18], or the aromatization of androgens in fat [16], or through unmeasured cytokines, growth factors or other hormones (leptin, insulin and estrogens) [33], which may exert direct stimulatory effects on osteoblasts [19]. Although we had other measures of adiposity, like waist circumference or body fat, they were not included in the model due to multicollinearity.

Bone modelling and growth in childhood and early pubescent years are influenced by endogenous and exogenous factors. Exogenous factors include nutrition (mainly calcium and vitamin D) and weightbearing physical activity, while endogenous factors include hormones (growth hormone, sex steroids, and various growth factors), cytokines, and growth plate aging [50]. BMD is also affected by genetic and early growth. In a previous study involving 1853 participants from the same birth cohort, Generation XXI, weight and height velocities up to the age of 6 were associated with increased aBMD at 7 years with the strongest associations observed for growth in early childhood [51]. Moreover, in the same population, children that between zero and 4 years followed a trajectory of persistent weight gain, had clearly increased bone mass at 7 years old, and weight gain seemed slightly more beneficial when it occurred later than on a normal trajectory during the first years of life [52].

Strengths and limitations

The strengths of our study include the novelty, as previous longitudinal data on the study subject is minimal. Furthermore, we have used a population-based cohort, with detailed information regarding birth and early childhood, physical examination, anthropometry, biochemical data, and DXA evaluation, according to standardized procedures, at ages 7 and 10 years, as well as DHEAS levels in prepuberty. Consequently, our results cannot be generalized to other age groups, as they would differ because of the effect of increased growth hormone and sex steroid levels on BMD during puberty.

Nevertheless, some limitations must be acknowledged. DXA is a two-dimensional estimate of volumetric bone density, so differences in bone size may confound the androgen-BMD association assessed by this technique. Nevertheless, adjustment for BMI partially attenuates this effect. Bone age evaluation was not part of the research protocol due to radiation exposure, and therefore no conclusions regarding skeleton maturation can be drawn. It is possible that the effect of DHEAS on BMD is not fully evident at the age we have assessed, especially among boys, who start puberty later than girls. As we continue to follow this cohort, we may carry on further investigation in different age ranges.

We have used path analysis to answer our main objective, but it is worth noting that path analysis is not intended to prove causation but rather to test if observed results are consistent with a priori hypothesis. Our statistical model is necessarily oversimplified, given the complex relationships between the variables analyzed. These variables may be influenced by several genetic and environmental factors that were not measured in this study. Furthermore, it assumes that the observed relations follow a particular direction that may not be totally realistic. Thus, the observed statistical associations demand careful interpretation regarding causality.

Conclusion

In girls, DHEAS at 7 years old affected aBMD at 10 years old. This effect is indirect, as higher DHEAS levels were associated with more advanced sexual maturity at the age of 10, and more advanced sexual maturity was associated with higher aBMD. No direct effect of DHEAS on aBMD was observed. No effect of DHEAS at 7 years old on aBMD at 10 years old was seen in boys.

Abbreviations

aBMD - areal bone mineral density

BMI - body mass index

BMC - bone mineral content

BMD - bone mineral density

CFI - Comparative Fit Index

CI - confidence intervals

DHEAS - dehydroepiandrosterone sulphate

DXA - dual-energy x-ray absorptiometry

IGF1 - insulin growth factor 1

RMSEA - Root Mean Square Error of Approximation

SD - standard deviation

TLI - Tucker–Lewis Index

WHO - World Health Organization

Declarations

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Rita Santos-Silva: contributed to the design of the study and the acquisition of data, conducted the analysis and interpretation of data and drafted the article.

Manuel Fontoura: contributed to the conception of the study, the interpretation of data and revised the article critically for relevant knowledgeable content.

Milton Severo: conducted the statistical analysis and contributed to the interpretation of data.

Raquel Lucas: contributed to the conception of the study and revised the article critically for relevant knowledgeable content.

Ana Cristina Santos: contributed to the conception of the study and the acquisition of data, participated in the analysis and interpretation of data, and revised the article critically for relevant knowledgeable content.

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Figures

Figure 1

Diagram of hypothesized causal relations Figure 1 presents the pathway tested using path analysis to assess the plausibility of a direct effect of DHEAS in aBMD, adjusted for BMI. Total effect: $ab + ed + c$ Direct effect: c Indirect effect (mediated by aBMD at age 7): ab Indirect effect (mediated by Tanner stage at age 10): ed Abbreviations: BMI – body mass index; DHEAS – dehydroepiandrosterone sulphate; aBMD – areal bone mineral density

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