

Prepubertal Healthy Children's Urinary Androstenediol Predicts Diaphyseal Bone Strength in Late Puberty

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Context: During the physiological process of adrenarche, the adrenal glands of healthy children secrete increasing amounts of weak androgenic steroids partly metabolized to potent sex steroids.

Objective: The aim of the study was to examine whether adrenal androgen metabolite excretion rates before the onset of puberty may be prospectively associated with late-pubertal diaphyseal bone strength.

Setting: We conducted the study in an auxological and metabolic child nutrition research facility.

Study Population and Design: The sample included 45 healthy adolescents who underwent proximal forearm bone and muscle area measurements by peripheral quantitative computed tomography at the age of 16 yr (SD 1.5) and who had collected a 24-h urine sample 8 yr earlier, allowing to quantify the prepubertal urine metabolome. Prepubertal hormonal predictors quantified by gas chromatography-mass spectrometry were: dehydroepiandrosterone, its 16-hydroxylated downstream metabolites, 5-androstene-3 β ,17 β -diol (androstenediol), sums of total androgen and glucocorticoid metabolites, cortisol, and 6 β -hydroxycortisol.

Main Outcomes: Proximal forearm radius was measured.

Results: Of all prepubertal hormones analyzed, only sex- and age-specific androstenediol levels significantly predicted pubertal stage-, height-, and muscularity-adjusted diaphyseal bone modeling (periosteal circumference, $\beta = 0.67$, $P = 0.002$; cortical area, $\beta = 2.15$, $P = 0.02$), bone mineral content ($\beta = 2.2$; $P = 0.04$), and polar strength strain index ($\beta = 12.2$; $P = 0.002$). Androstenediol explained 5–10% of the late-pubertal diaphyseal radius variability.

Conclusions: Our prospective profiling of urinary steroid metabolites in 24-h urine samples collected before puberty suggests that androstenediol is an early predictor of the diaphyseal bone strength in late puberty. This predominantly peripheral conversion product of adrenarchal dehydroepiandrosterone by 17 β -hydroxysteroid dehydrogenase may hence be involved in a sustained improvement of radial bone accretion during growth. (*J Clin Endocrinol Metab* 94: 575–578, 2009)

Besides genetic, musculoskeletal, and metabolic factors, sex steroids along with other hormones substantially influence bone architecture and strength. Studies in pediatric patients with

elevated adrenal androgens due to premature adrenarche (1) or congenital adrenal hyperplasia (2) indicate that an excess of adrenal steroids may exert anabolic effects on bone even before

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Abbreviations: BMC, Bone mineral content; BMD, bone mineral density; Σ C19, sum of quantitatively most important urinary androgen metabolites; Σ C21, sum of quantitatively most important urinary glucocorticoid metabolites; CA, cortical area; DHEA, dehydroepiandrosterone; DHEA&M, sum of DHEA and its 16-hydroxylated downstream metabolites; GC-MS, gas chromatography-mass spectrometry; 17 β -HSD, 17 β -hydroxysteroid dehydrogenase; MA, muscle area; PC, periosteal circumference; pQCT, peripheral quantitative computed tomography; SSI, polar strength strain index.

puberty. Also, in healthy children, positive associations between adrenal androgens and radial diaphyseal bone parameters have been observed cross-sectionally (3). For androstenediol, a direct metabolite of the major adrenarchal secretion product dehydroepiandrosterone (DHEA), both relevant androgenic and estrogenic properties have been reported (4, 5). To examine whether prepubertal androgen levels may be associated with bone strength during late puberty, we analyzed—in a prospective study design—steroid hormone metabolites in 24-h urine samples of healthy children collected 8 yr before a peripheral quantitative computed tomography (pQCT) bone analysis at the proximal radius was performed. Hormone metabolites were profiled by gas chromatography-mass spectrometry (GC-MS).

Subjects and Methods

Subjects

The study sample consisted of a subgroup of 371 healthy children and adolescents participating in the Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) Study who underwent a one-time pQCT of the nondominant forearm (3, 6). Of those, 178 adolescents aged 12 to 18 yr were generally eligible for the present study. Further inclusion criteria for the adolescents were: 1) they had collected a 24-h urine sample 8 yr before their pQCT bone measurement; 2) they were in Tanner stage 1 for breast (girls) or genital (boys) development at the time of urine collection; and 3) they had reached Tanner stage 4 or 5, 8 yr later at pQCT analysis. A total of 45 children (21 boys) met these criteria. Tanner stages were assessed by one of three study pediatricians. In 10 children in whom the documented genital or breast staging was ambiguous, pubic hair stages (either less than Tanner 2 or more than Tanner 3) were also considered. The DONALD study was approved by the ethics committee of the University of Bonn, and the additional pQCT measurement was approved by the ethics committee of the medical faculty of the University of Cologne and the Federal Office for Radiation Protection (Salzgitter, Germany). Parental consent and (in older children) the child's assent were obtained both before entry into the DONALD study and before participation in pQCT measurement.

Measurements

An XCT-2000 device (Stratec, Inc., Pforzheim, Germany) equipped with a low-energy (38 keV) x-ray tube was used to conduct the pQCT analysis of the nondominant forearm. The effective radiation was approximately 0.1 μ Sv from a radiation source of 45 kV at 15 μ A. The scanner was placed on the forearm, where the distance from the ulnar styloid process was 65% of the forearm length. A 2-mm-thick single tomographic slice was sampled at a voxel size of 0.4 mm along with the cross-sectional forearm muscle area (MA) at 65% of the ulnar length (7). Further specifications of these measurements have been published in detail (3, 6).

Body weight was measured to the nearest 0.1 kg using an electronic scale and standing height to the nearest 0.1 cm using a digital telescopic stadiometer. Height measurements taken 1 yr before pQCT were also included to calculate growth velocity (centimeters per year).

All 24-h urine collections (8 yr before pQCT) were performed at home under standardized conditions (8), and samples were stored at -20°C or below until analyzed. Urinary steroid profiles were determined using GC-MS analysis (8, 9).

To assess overall daily cortisol secretion of the adrenals, the seven quantitatively most important urinary glucocorticoid metabolites (C21-steroids) were profiled and summed (Σ C21): tetrahydrocortisone, tetrahydrocortisol, 5 α -tetrahydrocortisol, α -cortolone, β -cortolone, α -cortol, and β -cortol (9). Furthermore, we analyzed cortisol and its highly polar, unconjugated direct metabolite 6 β -hydroxycortisol (9). Total androgen secretion was determined as the sum (Σ C19) of andro-

sterone, etiocholanolone, 5-androstene-3 β ,17 α -diol, 5-androstene-3 β ,17 β -diol (androstenediol), DHEA, 16 α -hydroxy-DHEA, and 5-androstene-3 β ,16 α ,17 β -triol (8). The sum of the latter three steroids, *i.e.* DHEA and its 16-hydroxylated downstream metabolites (DHEA&M) represents the main metabolites of DHEA and DHEA-sulfate (8). Testosterone, estrone, estradiol, and estriol were also profiled but were below the detection limit (3 μ g/liter) in almost all samples.

11 β -Hydroxysteroid dehydrogenase type 1, which reactivates cortisone to cortisol, was assessed by the ratio of [α -THF + THF]/THE, and 5 α -reductase, which inactivates cortisol, was assessed by the ratio of 5 α -THF/THF.

Statistical analysis

All analyses were done using SAS version 8.2 (SAS Inc., Cary, NC). Values are reported as mean \pm SD or median and interquartile range when appropriate. Because diaphyseal bone growth normally differs between boys and girls at any particular pubertal stage, we performed tests (analysis of covariance) for sex-by-hormone interactions before the final multiple regression analyses. No significant sex-by-prepuberty hormone interactions ($P > 0.1$) were found, indicating comparable associations between prepubertal hormone levels and pubertal diaphyseal bone parameters for both sexes. Thus, analyses were not stratified by gender. Stepwise multiple regression analysis was used to examine the long-term predictive value of 24-h hormone excretion rates for the pQCT bone variables. Excretion rates were entered in the regression as age- and sex-specific SD scores (8, 9), apparent enzyme activities as metabolite ratios, and Tanner stages as Tanner 4 = 0 and Tanner 5 = 1. Additional covariates included in the model were forearm MA, height, age, growth velocity at pQCT, and sex (boys = 1, girls = 0). A P value < 0.05 was considered statistically significant.

Results

Anthropometric, hormonal, and diaphyseal characteristics of the study group are given in Table 1. Sex differences in height were not seen at baseline but were present at follow-up. Of the steroid metabolites determined, only androstenediol differed consistently between sexes (*i.e.* with and without body surface area correction) (Table 1). Testosterone was detected only in three, estradiol only in one, and estrone and estriol in none of the samples. The corresponding 24-h excretion rates were below 8 μ g/d and 4 μ g/d in boys and girls, respectively, and thus below the lower quartile of daily androstenediol excretion.

Multiple regression analyses yielded strong independent positive associations between muscularity and all bone parameters given in Table 2. The variation of diaphyseal bone's cortical area (CA), bone mineral content (BMC), and polar strength strain index (SSI) explained by muscularity alone ranged from 41–50%. Also, height and Tanner stages showed significant associations with some of the bone variables. Due to the adjustments for muscularity, height, and pubertal development, the variables age and growth velocity did not explain significant portions of variability of periosteal circumference (PC), CA, BMC, and SSI. Bone mineral density (BMD) (variable not shown in Table 2) was the only outcome that was significantly associated with growth velocity ($r^2 = 0.4$; $\beta = -11.6$; $P < 0.0001$) and sex ($r^2 = 0.13$; $\beta = -27.7$; $P < 0.005$), but not with any hormone metabolite and MA.

Of all prepubertal hormone parameters analyzed, only androstenediol significantly predicted pubertal bone geometry, mass, and strength (Table 2). Positive associations of androstenediol were seen

TABLE 1. Anthropometric and 24-h urinary hormonal (baseline) and diaphyseal (follow-up) characteristics of the study group

	Baseline (before puberty)		Follow-up (8 yr later; puberty)	
	Boys (n = 21)	Girls (n = 24)	Boys (n = 21)	Girls (n = 24)
Age (yr)	8.1 ± 1.5	7.8 ± 1.5	16.1 ± 1.5	15.8 ± 1.4
Years before and after attainment of Tanner 2	2.3 ± 1.6	2.1 ± 1.4	5.7 ± 1.6	5.9 ± 1.3
Height (cm)	130 ± 8.5	129 ± 10.5	177 ± 6.9	168 ± 9.0 ^a
Growth velocity (cm/yr)	6.0 ± 1.1	6.2 ± 0.9	4.1 ± 3.5	1.5 ± 2.1 ^b
Weight (kg)	27.7 ± 6.1	26.8 ± 5.7	65.8 ± 9.7	60.0 ± 12.3
BMI (kg/m ²)	16.2 ± 1.8	15.9 ± 1.4	21.0 ± 2.3	21.1 ± 2.8
DHEA&M (μg/d)	167 [76, 283]	128 [68, 185]		
Androstenediol (μg/d)	14 [9, 20]	8 [5, 17] ^b		
ΣC19 (μg/d)	453 [268, 623]	372 [226, 652]		
Cortisol (μg/d)	38 [30, 45]	29 [26, 35] ^{b,e}		
6β-Cortisol (μg/d)	69 [55, 97]	59 [43, 70]		
ΣC21 (μg/d)	3588 [2968, 4117]	3450 [2893, 4127]		
MA (mm ²)			3798 ± 583	2773 ± 333 ^c
PC (mm)			40 ± 3.0	37 ± 2.9 ^d
BMD (mg/cm ³)			1073 ± 36.4	1113 ± 21.7 ^c
CA (mm ²)			83.4 ± 13.2	72.0 ± 10.6 ^d
BMC (mg/mm)			89.6 ± 15.6	80.2 ± 11.9 ^b
SSI (mm ³)			307 ± 66.5	248 ± 53.0 ^d

Values are means ± sd or median [interquartile range].

^{a,b,c,d} Significantly different from boys according to unpaired *t* test or Wilcoxon-Mann-Whitney test (median values): ^a *P* < 0.001; ^b *P* < 0.05; ^c *P* < 0.0001; ^d *P* < 0.01.

^e No longer different from boys after correction for body surface area.

with PC and CA, which mostly reflect bone modeling, and BMC and SSI, which reflect a combination of modeling and remodeling. The urinary 24-h excretion of androstenediol around midchildhood explained an additional 5–10% of variability of the diaphyseal bone modeling, bone mass, and bone strength during late puberty after accounting for muscularity, height, and pubertal stage.

Discussion

Relating 24-h urinary hormone metabolites in healthy children to pQCT forearm bone measurements performed 8 yr later, we could show that the renal excretion levels of androstenediol approximately 2 yr before puberty onset were independently associated with the diaphyseal bone modeling, bone mass, and bone strength during late puberty. These associations were not mediated by the local bone-related muscularity, height, or pubertal stage at follow-up. Our prospective findings extend recent

cross-sectional results in children and adolescents showing positive correlations of DHEA metabolites and androstenediol with parallel diaphyseal bone measurements (3). The fact that of all steroids profiled, only androstenediol showed a long-term prediction of bone strength strongly suggests that adrenarchal DHEA and ΣC19 increases are not bone-anabolic *per se*. Rather, the formation of androstenediol from DHEA appears to play a crucial role. The enzymes 17β-hydroxysteroid dehydrogenase (17β-HSD) types 1, 3, and 5 catalyze not only the activation of sex hormone precursors (like androstenedione and estrone) to testosterone and estradiol (10), but also the conversion of DHEA to androstenediol (10, 11). This intracrine reduction at position 17β of the steroid nucleus converts DHEA to a sex steroid that can act as an estrogen or an androgen receptor agonist (5, 12, 13). Depending on the target tissue, androstenediol's transcriptional regulation of either the androgen or the estrogen receptor preponderates (4, 11–14). In accordance with its sex steroid effects,

TABLE 2. Prepubertal hormonal predictors and pubertal nonhormonal covariates of late-pubertal diaphyseal bone

	PC (mm)			CA (mm ²)			BMC (mg/mm)			SSI (mm ³)		
	r ²	β	P	r ²	β	P	r ²	β	P	r ²	β	P
MA (mm ²)	0.10	0.002	<0.005	0.48	0.014	<0.0001	0.41	0.014	<0.0001	0.50	0.06	<0.0001
Height (cm)	0.48	0.15	<0.0001			>0.1			>0.1	0.06	2.00	<0.05
Tanner stage (4, 5)			>0.1	0.04	6.66	0.058	0.10	10.63	<0.01	0.03	28.43	0.07
Androstenediol (μg/d)	0.09	0.67	<0.005	0.07	2.15	<0.05	0.05	2.20	<0.05	0.10	12.21	<0.005
Total explained variation ^a	0.67		<0.0001	0.55		<0.0001	0.56		<0.0001	0.66		<0.0001

Forearm MA and height were measured at the time of bone analysis; r², partial R²; β, regression coefficient.

Urinary 24-h hormone excretion rates were entered as age- and sex-specific sd scores (8) and Tanner stages as a variable with stages 4 = 0 and 5 = 1. Sex, age, and logarithmized growth velocity (lg10) were not significant for any bone variable shown (*P* > 0.1 in all cases). Separate models were run for each, the sum of C19 steroid metabolites, DHEA&M, cortisol, 6β-hydroxycortisol or global enzyme activities (11β-HSD1, 5α-reductase); however, none of them significantly predicted any of the bone outcomes (*P* > 0.1).

^a Total r² includes only significant (*P* < 0.05) explanatory variables.

androstenediol has been shown to reduce circulating SHBG concentrations (15) and to inhibit the testosterone- and estradiol-inactivating 17 β -HSD type 2 (16) (expressed in human osteoblasts) (17) in favor of activating 17 β -HSD isozymes. The above mechanisms may operate from midchildhood onward, especially in children who persist with their androstenediol levels in the upper measurement range over years. However, it is currently not known whether tracking of androstenediol from childhood into adolescence is in fact high.

The fact that lower daily excretion rates of testosterone, estrone, estradiol, and estriol, compared with androstenediol, were seen in our prepubertal urine samples does not rule out the possibility that these sex steroids may additionally predict bone accretion from childhood onward. However, measurement of these hormones in prepubertal children requires a more sensitive analytical methodology than the GC-MS method used in the present study.

Both androgen and estrogen receptor activation is required for an adequate periosteal bone expansion (bone modeling) (18, 19). In our study, androstenediol levels significantly predicted modeling (*i.e.* PC and CA) and the modeling-related bone parameters BMC and SSI, but not the remodeling parameter BMD, known to be particularly dependent on estrogen action (20). It thus appears probable that the androgenic effect of androstenediol on diaphyseal bone (via androgen receptor binding) may prevail over its estrogenic effects.

Bone strength is closely adapted to the forces caused by the related muscles. It is therefore a strength of our study that we could specifically adjust for this major physiological influence on diaphyseal bone. This may allow a more specific identification of additional physiologically relevant bone determinants that, like androstenediol, explain only small proportions of the variability of bone parameters. Undoubtedly, other predictors like sex steroids in puberty and genetics contribute more to this variability than androstenediol.

Despite its prospective observational design, our study cannot prove causality. For this, measurements of the rate of change in bone parameters and of concurrent changes in 24-h androstenediol excretions would be required. Although repeated pQCT bone analyses in healthy children bear ethical problems, such carefully planned, longitudinal observational studies in children are essential as an integrative part of basic physiological research on human growth and development, thereby complementing specific molecular and cellular findings, *e.g.* on steroidogenic *in vitro* effects of estrogens, testosterone, and androstenediol.

Our profiling of urinary steroid metabolites in 24-h urine samples collected before puberty suggests that androstenediol, a mostly peripheral conversion product of adrenarchal DHEA by 17 β -HSD, is an early predictor of late-pubertal diaphyseal bone strength and may hence be involved in a sustained improvement of radial bone accretion during growth.

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