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Relation of adrenal-derived steroids with bone maturation, mineral density and geometry in healthy prepubertal and early pubertal boys

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ABSTRACT

Background: Little is known about the effects of adrenal steroids on skeletal maturation and bone mass acquisition in healthy prepubertal boys.
Objective: To study whether adrenal-derived steroids within the physiological range are associated with skeletal maturation, areal and volumetric bone mineral density (aBMD and vBMD) and bone geometry in healthy prepubertal and early pubertal boys.
Methods: 98 healthy prepubertal and early pubertal boys (aged 6–14 y) were studied cross-sectionally. Androstenedione (A) and estrone (E1) were determined by liquid chromatography tandem mass spectrometry and DHEAS was determined by immunoassay. Whole body and lumbar spine aBMD and bone area were determined by dual-energy X-ray absorptiometry. Trabecular (distal site) and cortical (proximal site) vBMD and bone geometry were assessed at the non-dominant forearm and leg using peripheral QCT. Skeletal age was determined by X-ray of the left hand.
Results: Adrenal-derived steroids (DHEAS, A and E1) are positively associated with bone age in prepubertal and early pubertal children, independently of age. There are no associations between the adrenal-derived steroids and the studied parameters of bone size (lumbar spine and whole body bone area, trabecular or cortical area at the radius or tibia, periosteal circumference and cortical thickness at the radius or tibia) or BMD (aBMD or vBMD).
Conclusion: In healthy prepubertal and early pubertal boys, serum adrenal-derived steroid levels, are associated with skeletal maturation, independently of age, but not with bone size or (v)BMD. Our data suggest that adrenal derived steroids are not implicated in the accretion of bone mass before puberty in boys.

Introduction

In boys, estradiol (E2) and testosterone (T), produced in increasing amounts during puberty, play an important role in the regulation of bone growth, bone mass acquisition and bone maturation [1,2]. The contribution of the adrenal-derived steroids, which are secreted in increasing amounts from the age of 5–6 years (y), has not been well studied [3–5]. In animal studies adrenal androgens have been shown to accelerate bone maturation and bone growth [6] and several conditions with an elevated adrenal secretion such as premature adrenarche and congenital adrenal hyperplasia are associated with an advanced skeletal maturation [7–10] and increased areal bone mineral density (aBMD) [11,12]. There is, however, little data on the possible role of adrenal-derived steroids on bone maturation, areal and volumetric bone mineral density (aBMD and vBMD) or bone geometry in prepubertal and early pubertal boys. DHEAS and androstenedione (A) can be converted to the potent androgens T and dihydrotestosterone (DHT) in target tissues, whereas A is aromatized to estrone (E1). The effects of E1, a weaker estrogen compared to E2, on bone mass accretion have not been evaluated in prepubertal boys.

Therefore, this study aims to describe for different age groups of healthy prepubertal and early pubertal boys, serum levels of adrenal-derived steroids and bone-maturation, -mineral density (aBMD and vBMD) and -geometry, as well as their association. Our

Abbreviations: aBMD, areal bone mineral density; vBMD, volumetric bone mineral density; T, testosterone; E2, estradiol; E1, estrone; A, androstenedione; DHT, dihydrotestosterone; DHEAS, dehydroepiandrosterone sulfate; SDS, standard deviation score; BMI, body mass index; CV, coefficient of variation; LOQ, limit of quantification; DXA, dual energy X-ray absorptiometry; pQCT, peripheral quantitative computed tomography; LS, lumbar spine; WB, whole body; CSA, cross-sectional area; SSIp, strength–strain index.

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working hypothesis is that the rising production of adrenal-derived steroids from adrenarche might have an impact on bone maturation and accrual of bone mass and size in prepubertal and early pubertal healthy boys.

Subjects and methods

Subjects

Ninety-eight healthy male children and adolescents aged 6–14.5 y (mean age: 10.2 y) were included in this cross-sectional study. In total 65 were prepubertal Tanner genital stage 1 and 33 boys had Tanner stage 2. Eighty-one children were pre-pubarchal and 17 boys had pubic hair stage 2. Children were excluded if they were taking medication known to influence bone or mineral metabolism in the past year or if they had a metabolic bone disease, thyroid disorder or diabetes, if their height standard deviation score (SDS) was <−2.5 or >2.5 or if their body mass index (BMI) SDS was <−2 or >2. The study protocol was approved by the Ethics Committee of the Ghent University Hospital. Informed consent was obtained from the parents and all participants gave their assent. Participants were recruited by letters distributed in schools within the Ghent area.

Methods

Anthropometry and whole body composition and whole body and lumbar spine bone parameters by DXA

Information on medical history, lifestyle and socio-economic background was collected through a questionnaire. Standing height was measured to the nearest 0.1 cm using a Harpenden stadiometer (Holtain Ltd., Crymych, UK). Body weight was measured in light indoor clothing without shoes to the nearest 0.5 kg. The length of the forearm (from the olecranon to the processus ulnaris) and the tibia (from the medial knee joint line to the tip of the medial malleolus) were measured with a ruler to the nearest 0.1 cm. All anthropometric measurements were performed by the same trained physician (SV). Pubertal status was determined by the same trained physician (SV) according to the Tanner staging method (Tanner Genital Staging: stage 1: prepuberty; stage 2: early puberty). Standard bone parameters at the lumbar spine (LS) and whole body (WB) namely LS and WB bone area and LS and WB areal BMD, as well as WB fat and lean mass were measured using DXA (Hologic QDR 4500, software version 11.2.1; Hologic Inc., Bedford, MA). Areal BMD (aBMD) is obtained by dividing bone mineral content by bone area. Since the third dimension of the bone i.e. the depth is not taken into account, aBMD is strongly bone size dependent. The coefficient of variation (CV) for both LS and WB calibration phantoms was less than 1%, as calculated from daily and weekly measurements, respectively.

Bone age determination

Bone age reading of an X-ray of the left hand and wrist was done by two independent readers (a pediatric radiologist and a pediatrician), both blinded for the chronological age, using the Greulich and Pyle method [13]. The mean of both readings was taken as variable for analysis.

Regional body composition and vBMD and bone geometry parameters by pQCT

Standard bone parameters, estimates of bone strength [14] and regional body composition of the non-dominant forearm (radius) and the lower leg (tibia) were measured by pQCT (Stratec XCT-2000, Stratec Medizintechnik, Germany, version 6.0) which can provide three-dimensional information about bone mineral density (BMD), size and shape. A 2.0 mm slice (voxel size 0.5 mm) was performed at the 4 and 66% sites proximally from the distal end of the radius and at the 4 and 38% site proximally from the distal end of the tibia. Due to movement artifacts, radius 66% measurements of only 76 boys could be analyzed. The cross-sectional area (CSA) of the radius/tibia was determined after detecting the outer bone contour at a threshold of 280 mg/cm^3. For determining cortical vBMD, the threshold was set at 710 mg/cm^3, whereas for trabecular bone, it was set at 180 mg/cm^3. The cortical vBMD (mg/cm^3), cortical CSA (mm^2), muscle and fat CSA, endosteal and periosteal circumferences (mm), and cortical thickness (mm) were measured at the mid-radius (66% of bone length from the distal end) and mid-shaft tibia (38% of bone length from the distal end). The combined CSA of muscle and bone (fibula and tibia or radius and ulna) was determined at a threshold of 40 mg/cm^3 and the bone CSA was determined with the threshold set at 280 mg/cm^3. Muscle CSA was calculated by subtracting the bone CSA from the combined muscle and bone CSA. Fat CSA was calculated by subtracting the combined muscle and bone CSA from the total CSA. The strength–strain index (SSIp) of the radius 66% and the tibia 38% was calculated [14]. To assess the SSIp, a threshold of 480 mg/cm^3 was used. Trabecular vBMD (mg/cm^3) and area were measured using a scan through the distal metaphysis at the radius and the tibia (at 4% of bone length). The CSA of the radius/tibia was determined after detecting the outer margin; 55% of this cross-sectional bone area was peeled off to separate trabecular bone from the cortical shell. The CV for the calibration phantom was <1% as calculated form daily phantom measurements.

Hormonal measurements

Venous blood samples were collected between 0800 and 1000 h after a small breakfast. Serum samples were stored at −80 °C until batch analysis. Commercial immunoassays were used to measure serum DHEAS, SHBG, LH and FSH (Modular, Roche Diagnostics, Mannheim, Germany). The intra- and inter-assay CVs for these assays were less than 10%. The lower detection limit for DHEAS was 5 μg/dL and the inter-assay CV was 2.7% at 157.3 μg/dL. E1, E2, A, T and cortisol were determined by liquid chromatography tandem mass spectrometry (AB Sciex 5500 triple-quadrupole mass spectrometer; AB Sciex, Toronto, Canada). Serum limit of quantification (LOQ) was <0.5 pg/mL (1.9 pmol/L) for E2 and E1 and the inter-assay CV was 4.0% at 21 pg/mL (77 pmol/L) for E2 and 7.6% at 25 pg/mL (93 pmol/L) for E1 [15]. Serum LOQ was 1.2 ng/dL for T and the inter-assay CV was 8.3% at 36.7 ng/dL and 3.1% at 307.8 ng/dL. Serum LOQ was 4.25 ng/dL for A and the inter-assay CV was 2.9% at 59.8 ng/dL. Serum LOQ was 0.05 μg/dL for cortisol and the inter-assay CVs were 2.3% at 7.43 μg/dL and 3.1% at 24.7 μg/dL.

Statistics

Normality was checked using quantile–quantile plots. Data are presented as mean ± standard deviation or as medians (25th–75th percentile) in the case of a non-normal distribution. Differences between the age categories were evaluated using ANOVA, when criteria for normality were met. We used LSD test as post-hoc test. In the case of a non-normal distribution, Kruskal–Wallis tests were performed. The independent predictors of the various bone geometry, -density and -maturation parameters were tested using linear regression analysis including age, height for the analyses of the DXA parameters and bone length for the analyses of the pQCT parameters, body weight and serum E1, A or DHEAS levels. The difference was considered statistically significant at $p < 0.05$. Data were analyzed using SPSS software version 19.0.

Based on the available literature [16–18], sample size calculations were performed using G*power (version 3.1.5) (α: 0.05; β: 0.20). We calculated a necessary sample size of 10 to 14 children in each age-group to discern the published differences in bone size between the different age groups. To detect the effects of adrenal steroids on bone density and bone size, a sample size between 73 and 125 children was needed depending on the studied parameter.