Significant differences in the microarchitecture and volumetric mineral density of bone assessed by HR-PQCT in patients with 21- and 17α-hydroxylase deficiency

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Research Article

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Abstract

Purpose

Due to disturbances in hormones and long-term glucocorticoid replacement therapy, congenital adrenocortical hyperplasia (CAH) patients are at risk of impaired bone structure and metabolism. The current study investigates bone microarchitecture in 21-hydroxylase deficiency (21OHD) and 17α-hydroxylase deficiency (17OHD) patients using high-resolution peripheral quantitative computed tomography (HR-pQCT).

Methods

A total of thirty-eight 21OHD and sixteen 17OHD patients were recruited with controls matched for age and sex at a ratio of 1:3. All underwent HR-pQCT scans of the nondominant radius and tibia. Comparisons of HR-pQCT indices between each cohort and the matched controls or between the two cohorts were conducted. Spearman analyses and multiple linear regression were performed to reveal the relations between clinical characteristics and HR-pQCT indices.

Results

Compared with the 17OHD group, the 21OHD group predominated in Tt.vBMD (P < 0.001), Ct.vBMD (P < 0.001), and Tb.vBMD (P < 0.001) at the radius. Comparisons of clinical characteristics between the two cohorts showed that 17OHD patients were taller, weighed more, and had higher levels of fasting blood glucose with shorter treatment course. Further correlation analyses revealed that some characteristics, such as height and FSH, contributed significantly to bone differences in HR-pQCT indices. However, treatment dosage and time were not correlated, indicating that the current glucocorticoid doses were within safety limits for bone impairment.

Conclusions

21OHD and 17OHD patients had different bone characteristics in BMD, the cortex and the trabecula, as assessed by HR-pQCT. Clinical manifestations, especially sex hormones and height, may contribute to these differences.

Introduction

Congenital adrenocortical hyperplasia (CAH) is a group of rare disorders with an autosomal recessive inheritance pattern and is caused by dysfunctions of catalyzing enzymes involved in various stages of corticosteroid synthesis in the adrenal gland. Insufficiency of adrenocortical steroids can further drive compensatory increases in adrenocorticotropic hormones and their precursors and even induce secondary hyperandrogenism in some patients [1]. 21-Hydroxylase deficiency (21OHD) has been reported to be the most common type, accounting for approximately 90%-95% of all CAH cases [2]. A rarer form of CAH, 17α-
hydroxylase deficiency (17OHD), is caused by *CYP17A1* mutations, is characterized by hypertension, hypokalemia and poor puberty or no puberty, and accounts for less than 1% of CAH cases [3].

Despite different mutated genes and dysfunctional enzymes, both 21OHD and 17OHD patients have the same features of insufficiency of adrenocortical hormones. Thus, the current predominant management for both disorders is glucocorticoid and mineralocorticoid replacement therapies, which involve simultaneous supplementation with exogenous glucocorticoids (GCs) and suppression of the accumulation of adrenal androgen precursors stimulated by elevated ACTH levels. Unfortunately, since CAH patients require long-term treatment, the risk of increased side effects of GCs should be considered, especially the adverse effects on bone metabolism and microarchitecture [4, 5]. It is estimated that up to half of patients with autoimmune and chronic inflammatory diseases requiring long-term use of intermittent high-dose GCs would suffer from at least one bone fracture [6, 7]. However, it is not clear whether the replacement GC dose would impair the bone structure. On the other hand, overproduction of androgens and their aromatic compounds, such as estrogens, may have protective effects against bone destruction [8–12]. One previous study on bone changes in CAH patients with 21OHD mainly enrolled juvenile patients. In this study, the discrepancies in BMD results were predominantly attributed to exogenous GCs [13–19]. However, a series of other factors should be considered, such as sex ratio, age, and growth factors. Although the baseline cortisol level was low in both types of CAH, it is noteworthy that there was excess androgen in 21OHD but a lack of sex hormones in 17OHD. It is still uncertain whether there are differences in the structure of bone in the two rare diseases.

Bone mineral density (BMD), usually measured by dual-energy X-ray absorptiometry (DEXA), has been widely used to evaluate bone quality and predict fractures, especially for the diagnosis of osteoporosis. However, as a two-dimensional method, DEXA may not present an accurate assessment of bone quality, and the measurement results are easily affected by disease conditions. Additionally, in certain diseases, fractures occur among patients with normal or above-normal BMD [20]. In such circumstances, high-resolution peripheral quantitative computed tomography (HR-pQCT) allows better quantitative assessment of peripheral bone microarchitecture and volumetric bone mineral density (vBMD) at distal parts of the radius and tibia with a three-dimensional view, which demonstrates its excellent performance in detecting changes in bone microarchitecture that might not be visible with conventional DEXA [21–23]. To date, no available data for bone microarchitecture and vBMD have been reported among patients with CAH. In this pilot study, we used a case–control design and correlation analyses to investigate alterations in bone geometry, microarchitecture and vBMD as assessed by HR-pQCT using cohorts of 21OHD and 17OHD patients.

**Materials And Methods**

**Study participants**

The study was designed as an observational cross-sectional study. Patients diagnosed with CAH caused by 21OHD or 17OHD at Peking Union Medical College Hospital (PUMCH) and aged 18–50 years were recruited from Jan 2019 to May 2020 for assessment of bone geometry, microarchitecture and vBMD by HR-pQCT. All patients were aged 18–50 (the women were all premenopausal) and had reached their full height, as confirmed by multiple follow-ups. The diagnosis of CAH relies on clinical manifestations and biochemical and hormonal testing. For those cases with CAH was difficult to identify, genetic testing was used. The patients were
followed up in the same endocrinologist outpatient clinic over a long period so that medical treatment could be standardized as much as possible. Healthy controls were recruited from medical staff and individuals presenting to our hospital for routine physical examination via flyers and posters, which described the inclusion criteria, a brief introduction of HR-pQCT and the potential benefits that they could obtain from the study. As in our previous study, diagnoses of 21OHD and 17OHD were confirmed by symptoms of disorders of sex development, abnormal electrolyte and adrenal hormone assays. Some of the patients underwent genetic detection of \textit{CYP21A2} and \textit{CYP17A1} mutations in blood samples using Sanger sequencing and multiplex ligation-dependent probe amplification, respectively [24]. After diagnosis, the patients received GC replacement treatment with an equivalence algorithm of 25 mg cortisone acetate = 5 mg prednisolone = 20 mg hydrocortisone = 0.75 mg dexamethasone each day. The exclusion criteria included the following: 1) incomplete clinical records or no confirmation of diagnosis by genetic testing; 2) other gene mutations that caused CAH; 3) a previous diagnosis of other diseases such as hypothyroidism that may have effects on bone physiology; 4) hepatic or renal insufficiency; 5) pregnancy; and 6) recent use of calcium agents, bisphosphonates, parathyroid hormone or other treatments that may have an effect on bone metabolism. This study was carried out in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of PUMCH. All patients and controls signed written informed consent forms.

**Laboratory tests**

The laboratory tests included measurements of serum morning adrenocorticotropic hormone (ACTH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, progesterone, and testosterone; serum potassium (K), calcium (Ca), sodium (Na), and chlorine (Cl); and serum phosphate fasting blood glucose (FBG) and uric acid (UA). All the abovementioned tests were performed when patients underwent HR-pQCT scans. Serum ACTH measurement was performed using a chemiluminescence immunoassay (Advia Centaur XP; Siemens). LH, FSH, PRL, estradiol, progesterone and testosterone were measured with chemiluminescence (ACS: 180; Automatic Chemiluminescence Systems, Siemens). For female patients with periods, sex hormones LH, FSH, estradiol, progesterone and testosterone were assessed during the early follicular phase.

**HR-pQCT**

All patients and controls underwent second-generation HR-pQCT scans (Xtreme CT II; ScancoMedical, Bruttisellen, Switzerland) with an isotropic voxel size of 61 µM. The nondominant distal radius and tibia were measured and filmed with three-dimensional bone images of 10.2 mm in the axial direction. Standard analyses were further performed to obtain parameters of morphology, vBMD and microarchitecture based on our previously reported protocol [25], including total volumetric bone mineral density (Tt.vBMD), cortical vBMD (Ct.vBMD), trabecular vBMD (Tb.vBMD), total bone area (Tt.Ar), cortical area (Ct.Ar), trabecular area (Tb.Ar), cortical pore diameter (Ct.Pm), intracortical porosity (Ct.Po), cortical thickness (Ct.Th), trabecular bone volume fraction (Tb.BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), and inhomogeneity of the network [St. Dev of 1/Tb.N (Tb.1/N.SD)].

**Statistical analysis**

Statistical analyses were performed using IBM SPSS 25.0 and R software 4.1.0 (https://www.r-project.org). A two-tailed $p$ value $<$ 0.05 was considered indicative of statistical significance. Distributions of categorical variables were compared using Pearson’s chi square test (or Fisher’s exact test if necessary). For continuous
variables, the Kolmogorov–Smirnov test was primarily conducted to identify distribution patterns. The normal variables are expressed as the mean value ± standard deviation (SD) and were compared using Student’s t test, while the nonnormal variables are presented as the median value with interquartile range (IQR) and were analyzed using the Mann–Whitney U test.

Two groups of controls matched with 21OHD and 17OHD patients by age and sex were recruited at a ratio of 1:3. Parameters of geometry, microarchitecture and volume densitometry at the radius and tibia were compared between the patients and controls. Spearman correlation analyses were performed between potential clinical characteristics and the bone parameters assessed by HR-pQCT. The significant variables in the correlation analyses were selected for the construction of multiple linear regression models for each HR-pQCT index. All variables were preprocessed by z score normalization before correlation analyses.

Results

Patient characteristics

A total of 54 CAH patients were enrolled in this study, consisting of 38 and 16 patients with 21OHD and 17OHD, respectively (Table 1). All patients were under 50 years old, with a median [M (Q1, Q3)] age of 29.5 (24.00, 34.25) years for 21OHD and 29.00 (21.50, 35.00) for 17OHD. For 21OHD patients (30 females and 8 males), most of them manifested the classic subtype (86.8%, 33/38; including 32 simple virilizing cases and only one salt-wasting case), while 5 manifested as the NC subtype. All 17OHD patients exhibited a female phenotype, whereas 7 (43.7%) of them had the 46XY karyotype and 9 (56.3%) had the 46XX karyotype. In addition to conventional GC treatment, some patients received estrogen replacement therapy (twelve 17OHD cases).
Table 1
Comparisons of clinical characteristics between patients of 21OHD and 17OHD.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>21OHD (N = 38)</th>
<th>17OHD (N = 16)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (female, %)</td>
<td>30 (78.9)</td>
<td>16 (100)</td>
<td>0.088</td>
</tr>
<tr>
<td>Current age (year)</td>
<td>29.5 (24.00, 34.25)</td>
<td>29 (21.50, 35.00)</td>
<td>0.791</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>154.30 ± 52.73</td>
<td>173.01 ± 7.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>62.30 ± 10.91</td>
<td>71.32 ± 21.46</td>
<td>0.045</td>
</tr>
<tr>
<td>BMI (kg/cm²)</td>
<td>26.25 ± 4.89</td>
<td>23.51 ± 5.53</td>
<td>0.077</td>
</tr>
<tr>
<td>Duration of treat (year)</td>
<td>8.44 (4.53, 20.89)</td>
<td>3.19 (1.80, 10.18)</td>
<td>0.047</td>
</tr>
<tr>
<td>Initial dosage of hydrocortisone</td>
<td>40 (30.00, 40.52)</td>
<td>20.00 (20.00, 30.00)</td>
<td>0.002</td>
</tr>
<tr>
<td>Current dosage of hydrocortisone</td>
<td>30.00 (20.00, 31.00)</td>
<td>20.00 (17.52, 20.00)</td>
<td>0.110</td>
</tr>
<tr>
<td>ACTH (pg/ml)</td>
<td>51.10 (8.60, 129.00)</td>
<td>47.25 (31.23, 182.00)</td>
<td>0.557</td>
</tr>
<tr>
<td>LH (IU/L)</td>
<td>3.84 (1.62, 5.11)</td>
<td>21.92 (9.93, 39.29)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FSH (IU/L)</td>
<td>5.74 (3.36, 7.39)</td>
<td>55.15 (27.92, 90.95)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>81.00 (46.50, 114.00)</td>
<td>50.0 (19.25, 71.50)</td>
<td>0.008</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>5.85 (2.74, 19.46)</td>
<td>7.67 (2.79, 11.63)</td>
<td>0.880</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>0.62 (0.17, 1.63)</td>
<td>0.10 (0.10, 0.14)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Testosterone² (ng/ml) *</td>
<td>0.32 (0.12, 0.95)</td>
<td>0.10 (0.10, 0.14)</td>
<td>0.005</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>4.30 (4.08, 4.43)</td>
<td>4.10 (3.53, 4.60)</td>
<td>0.336</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>138.00 (137.00, 139.00)</td>
<td>140.00 (139.00, 142.75)</td>
<td>0.002</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>103.50 (103.00, 105.00)</td>
<td>104.00 (103.00, 105.75)</td>
<td>0.427</td>
</tr>
<tr>
<td>Phosphorus (mmol/L)</td>
<td>1.24 (1.16, 1.31)</td>
<td>1.30 (1.11, 1.44)</td>
<td>0.142</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.38 ± 0.09</td>
<td>2.42 ± 1.11</td>
<td>0.160</td>
</tr>
<tr>
<td>FBG (umol/L)</td>
<td>4.80 (4.58, 5.10)</td>
<td>4.95 (4.80, 5.80)</td>
<td>0.007</td>
</tr>
<tr>
<td>Uric acid (umol/L)</td>
<td>336.0 (282.50, 369.00)</td>
<td>301.00 (255.25, 378.00)</td>
<td>0.500</td>
</tr>
</tbody>
</table>

BMI: body mass index; ACTH: adrenocorticotropic hormone; LH: luteinizing hormone; FSH: follicle-stimulating hormone; FBG: fasting blood glucose.*Remove male patients

Compared with 21OHD patients, 17OHD patients with taller height (173.01 ± 7.73 vs. 154.30 ± 5.73 cm; P < 0.001), higher weight (71.32 ± 21.46 vs. 62.30 ± 10.91 kg; P = 0.045), and higher levels of luteinizing hormone [21.92 (9.93, 39.29) vs. 3.84 (1.62, 5.11) IU/L; P < 0.001, Range 2.12–10.89 IU/L], follicle-stimulating hormone (55.15 (27.92, 90.95) vs. 5.74 (3.36, 7.39) IU/L; P < 0.001, Range < 10 IU/L), sodium (140.00 (139.00, 142.75))
vs. 138.00 (137.00, 139.00) mmol/L; P = 0.002, Range 135–145 mmol/L) and FBG [4.95 (4.80, 5.80) vs. 4.80 (4.58, 5.10) mmol/L; P = 0.007, Range 3.90–6.10 mmol/L], while they had lower levels of estradiol [50.0 (19.25, 71.50) vs. 81.00 (46.50, 114.00) pg/ml; P = 0.008, Range 27–122 pg/ml] and testosterone (female) [0.10 (0.10, 0.14) vs. 0.32 (0.12, 0.95) ng/ml; P = 0.005, Range 0.1–0.75 ng/ml]. Moreover, 21OHD patients also had a longer duration of GC treatment than 17OHD patients [8.44 (4.53, 20.89) vs. 3.19 (1.80, 10.18) years; P = 0.047].

**Comparison of HR-pQCT indices between 21OHD patients and controls**

As shown in Table 2, age, sex and weight were similar between the control and 21OHD groups. However, 21OHD patients had significantly shorter heights (P < 0.001) and higher BMIs (P < 0.001). Figure 1 shows representative images of the 21OHD and control bone microarchitecture. At the radius (Table 2 and Fig. 2), the 21OHD cohort was found to have decreased Tt.Ar (-12.4%, P < 0.001), Ct.Pm (-6.9%, P < 0.001), and Tb.Ar (-17.8%, P < 0.001) but showed significant increases in Tt.vBMD (+16.7%, P < 0.001), Ct.Ar (+6.9%, P = 0.041), Ct.vBMD (+4.7%, P < 0.001), Ct.Th (+18.8%, P < 0.001) and Tb.1/N.SD (+11.5%, P = 0.037). At the tibia, Ct.Ar (+7.2%, P = 0.028), Ct.vBMD (+2.2%, P < 0.001) and Ct.Th (+23.8%, P < 0.001) showed significant increases in 21OHD patients, while Tt.Ar (-18.8%, P < 0.001), Ct.Pm (-9.4%, P < 0.001) and Tb.vBMD (-9.6%, P = 0.045) significantly decreased.
Table 2
Comparisons of HR-pQCT indices between two cohorts of 21OHD and 17OHD and between each of them and matched controls.

<table>
<thead>
<tr>
<th>Demographics and parameters</th>
<th>21OHD</th>
<th>17OHD</th>
<th>21OHD vs. 17OHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (female, %)</td>
<td>90 (78.9)</td>
<td>30 (78.9)</td>
<td>1.000</td>
</tr>
<tr>
<td>Age (years)</td>
<td>29.60 (25.85, 35.15)</td>
<td>29.50 (24.00, 34.25)</td>
<td>0.372</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163.00 (159.00, 168.00)</td>
<td>154.50 (150.00, 158.13)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>60.00 (53.00, 66.00)</td>
<td>63.00 (52.88, 70.00)</td>
<td>0.363</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.69 ± 3.50</td>
<td>26.25 ± 4.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Radius</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tt.vBMD (g HA/cm³)</td>
<td>357.71 ± 61.65</td>
<td>417.37 ± 79.72</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ct.vBMD (g HA/cm³)</td>
<td>948.23 ± 42.64</td>
<td>993.09 ± 33.62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tb.vBMD (g HA/cm³)</td>
<td>149.18 ± 42.19</td>
<td>134.82 ± 47.64</td>
<td>0.072</td>
</tr>
<tr>
<td>Tt.Ar (mm²)</td>
<td>241.30 (218.50, 276.65)</td>
<td>211.30 (187.05, 239.93)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ct.Ar (mm²)</td>
<td>65.51 ± 11.84</td>
<td>70.02 ± 10.51</td>
<td>0.041</td>
</tr>
<tr>
<td>Tb.Ar (mm²)</td>
<td>180.80 (155.90, 211.75)</td>
<td>148.65 (120.28, 170.93)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ct.Pm (mm²)</td>
<td>65.10 (61.15, 69.45)</td>
<td>60.60 (56.08, 64.93)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ct.Po (%)</td>
<td>0.002 (0.001, 0.004)</td>
<td>0.003 (0.002, 0.004)</td>
<td>0.525</td>
</tr>
<tr>
<td>Demographics and parameters</td>
<td>21OHD</td>
<td>17OHD</td>
<td>21OHD vs. 17OHD</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-------</td>
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<td>------------------</td>
</tr>
<tr>
<td></td>
<td>Controls (N = 114)</td>
<td>Patients (N = 38)</td>
<td>P value</td>
</tr>
<tr>
<td>Ct.Th (mm)</td>
<td>1.17 ± 0.18</td>
<td>1.39 ± 0.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tb.BV/TV (%)</td>
<td>0.22 ± 0.06</td>
<td>0.20 ± 0.07</td>
<td>0.064</td>
</tr>
<tr>
<td>Tb.N (mm⁻¹)</td>
<td>1.36 ± 0.23</td>
<td>1.34 ± 0.33</td>
<td>0.480</td>
</tr>
<tr>
<td>Tb.Th (mm)</td>
<td>0.22 (0.21, 0.24)</td>
<td>0.22 (0.21, 0.24)</td>
<td>0.280</td>
</tr>
<tr>
<td>Tb.Sp (mm)</td>
<td>0.70 (0.60, 0.77)</td>
<td>0.73 (0.63, 0.90)</td>
<td>0.113</td>
</tr>
<tr>
<td>Tb.1/N.SD (mm)</td>
<td>0.26 (0.22, 0.30)</td>
<td>0.29 (0.23, 0.38)</td>
<td>0.037</td>
</tr>
</tbody>
</table>

**Tibia**

<table>
<thead>
<tr>
<th></th>
<th>21OHD</th>
<th>17OHD</th>
<th>21OHD vs. 17OHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tt.vBMD (g HA/cm³)</td>
<td>313.84 ± 53.93</td>
<td>309.66 ± 71.52</td>
<td>0.689</td>
</tr>
<tr>
<td>Ct.vBMD (g HA/cm³)</td>
<td>962.80 (930.90, 986.90)</td>
<td>984.35 (971.40, 1010.05)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tb.vBMD (g HA/cm³)</td>
<td>157.29 ± 40.35</td>
<td>142.13 ± 38.98</td>
<td>0.045</td>
</tr>
<tr>
<td>Tt.Ar (mm²)</td>
<td>622.90 (573.85, 717.80)</td>
<td>505.55 (451.23, 572.68)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ct.Ar (mm²)</td>
<td>128.21 ± 22.45</td>
<td>137.41 ± 21.57</td>
<td>0.028</td>
</tr>
<tr>
<td>Tb.Ar (mm²)</td>
<td>507.6 (455.45, 586.40)</td>
<td>487.15 (443.05, 596.30)</td>
<td>0.416</td>
</tr>
<tr>
<td>Ct.Pm (mm²)</td>
<td>97.40 (93.00, 104.20)</td>
<td>88.20 (82.68, 92.98)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ct.Po (%)</td>
<td>0.012 (0.008, 0.019)</td>
<td>0.014 (0.009, 0.021)</td>
<td>0.410</td>
</tr>
</tbody>
</table>
Comparison of HR-pQCT indices between 17OHD patients and controls

Similar to the 17OHD patients, all 48 enrolled control subjects were female and showed a similar distribution in age and BMI between them (Table 2). Representative images of one 17OHD patient and the corresponding control depicting the bone microarchitecture are shown in Fig. 1. 17OHD patients had taller height (P < 0.001) and higher weight (P = 0.013). As shown in Table 2 and Fig. 2, compared with healthy controls, the HR-pQCT indices of the radius demonstrated that 17OHD had significantly higher values of Tt.Ar (+ 41.5%, P < 0.001), Ct.Pm (+ 20.3%, P < 0.001), Tb.Ar (+ 67.2%, P < 0.001), Tb.Sp (+ 25.7%, P = 0.003) and Tb.1/N.SD (+ 42.3%, P = 0.007) but had lower values of Ct.Ar (-31.6%, P < 0.001), Ct.vBMD (-15.1%, P < 0.001), Ct.Th (-43.9%, P < 0.001), Tb.vBMD (-38.9%, P < 0.001), Tb.BV/TV (-35.0%, P < 0.001), Tb.N (-17.0%, P = 0.013) and Tb.Th (-4.5%, P < 0.001). At the tibia, the 17OHD group presented higher Tt.Ar (+ 50.2%, P < 0.001), Ct.Pm (+ 22.2%, P < 0.001) and Tb.Ar (+ 17.5%, P = 0.005), with a lower Ct.Ar (-28.8%, P < 0.001), Ct.vBMD (-11.9%, P < 0.001) and Ct.Th (-39.0%, P < 0.001).

Comparison of HR-pQCT indices between 21OHD and 17OHD patients

There were many more significant differences in the HR-pQCT results related to changes in bone between the 21OHD and 17OHD cohorts (Table 2). Compared with the 17OHD group, the 21OHD group predominated in
Correlations between HR-pQCT indices and clinical characteristics of CAH patients

Spearman correlation analyses were first performed between HR-pQCT parameters and clinical manifestations (as listed in Fig. 3). The significant variables were further selected for multiple linear regression analyses to identify the relations between clinical characteristics and each HR-pQCT index (Table 3).

At the radius, height and FSH were the factors most commonly related to bone changes, and both of them were negatively associated with Tt.vBMD, Ct.vBMD, Ct.Ar and Ct.Th but positively with Tt.Ar, Tb.Ar and Ct.Pm. LH was correlated with lower Tb.Ar and higher Ct.Th. FBG was found to be a positive predictor for Tt.Ar and Tb.Ar. Furthermore, a higher Tb.Th correlated with higher ACTH and lower serum chloride levels.

Likewise, at the tibia, patient height was negatively correlated with Tt.Ar and Ct.Pm but positively correlated with Ct.vBMD, Ct.Ar and Ct.Th. FSH was a negative predictor for Ct.vBMD, Ct.Ar, Ct.Th and Tb.N and was a positive predictor for Ct.Pm. LH was associated with higher Tb.N and lower Tb.Sp. Testosterone, sodium and chloride levels were positive predictors for Ct.Ar, Tb.Ar and Tb.Sp, respectively. Finally, at the tibia, uric acid was found to be positively correlated with Ct.Po and Ct.Th.

Figure 4 (Radius) and Fig. 5 (Tibia) depict the fitted curves for each HR-pQCT parameter using clinical variables by multiple regression analysis, and most performed very well in revealing the correlations. The linear fitting performance (R²) and p values are also shown. The results showed that clinical manifestations were closely related to changes in the radius and tibia, especially vBMD and bone areas. Specifically, at the radius (Fig. 4), Ct.vBMD demonstrated a strong linear dependence rather than Tb.vBMD. All area parameters (Tt.Ar, Ct.Ar and Tb.Ar), Ct.Pm and Ct.Th also had excellent linear relations with clinical characteristics. At the tibia (Fig. 5), the results were similar. Ct.vBMD had a strong linear dependence on clinical variables, while Tb.vBMD showed a nonsignificant relationship. The same strong linear dependencies were seen at the tibia for Ct.Pm and Ct.Th. However, unlike Ct.Ar, Tb.Ar at the tibia demonstrated only a weak correlation.

Discussion

Since CAH involves obvious sex hormone abnormalities and requires prolonged glucocorticoid therapy, which could be detrimental to bone health, clinicians have always paid close attention to the bone health of CAH patients. Previously, few studies reported two-dimensional features with regard to BMD and the trabecular bone score assessed by conventional DEXA in patients with CAH caused by 21OHD and indicated quite different results of bone mass density [26]. However, the two-dimensional characteristics may limit the accuracy and reliability of the evaluation of BMD, resulting in a lack of sensitivity and specificity of DEXA in the prediction of fracture risk [27]. HR-pQCT is an advanced technology that can reconstruct the three-
dimensional structure of human bone and measure quantitative parameters of volume bone density and microarchitecture by detecting cancellous bone and cortical bone independently [28]. Herein, using a case–control design, we describe differences in bone microarchitecture assessed by HR-pQCT in CAH patients with 21OHD and 17OHD. Correlations between HR-pQCT parameters and CAH patients’ clinical characteristics were also investigated.

In the current study, our results demonstrated a number of differences in bone geometry, architecture and density among the 21OHD, 17OHD and control groups (Table 2). Among 17OHD patients, compared to the controls, significantly lower bone density of total, cortical and trabecular areas at the radius was observed, while there was significantly lower Ct.vBMD only at the tibia. To our knowledge, none of the previous studies reported BMD in 17OHD patients except one published by Wu et al., in which 2 of 8 patients had partial or complete 17OHD that showed decreased BMD [29]. However, compared to controls, 21OHD patients had higher BMD of total and cortical areas at the radius than the controls, while at the tibia, they showed increased cortical BMD with mildly decreased trabecular BMD. Previous studies[26, 30–35] reported discordant results about BMD changes in 21OHD patients: 21OHD patients in different age groups have lower, higher or unchanged BMD in different bone parts compared to controls. Similar to this study, Filippo Ceccato et al.[36] showed an increase in lumbar BMD in 38 adult patients with 21OHD and 38 matched healthy controls. It was also found[15] that some 21OHD patients have normal BMD in a study of children with CAH. However, Jeremy A. King's study[16] concluded that 21OHD patients had an increased risk for bone loss due to the oversuppression of adrenal androgens by glucocorticoids, which is quite different from most studies. These controversial results indicated that the bone loss in 21OHD patients was relevant to the higher dose of glucocorticoids, not the disease itself. Furthermore, deviations between these studies may be attributed to the differences in the sex ratio, age and growth status. These studies all used DEXA to measure integral bone mass of the cortical and trabecular bone compartments divided by the two-dimensional projected area. Therefore, the areal BMD measurement provided by DEXA is highly influenced by bone size. However, HR-pQCT could evaluate biomechanical properties of bone by micro-finite element analysis. As a result, the increased and normal BMD were observed in 21OHD cases, while a significantly lower BMD was in 17OHD cases. The opposite changes in BMD between two cohorts of 21OHD and 17OHD may be attributed to the difference in hormone levels, such as androgen, which can be excessive in 21OHD patients but insufficient in 17OHD patients. Increased androgens could improve bone mineral density in 21OHD patients [11]. Furthermore, estrogen plays an important role in regulating bone metabolism, but no correlation was found accurately in measuring quantitative parameters of vBMD and bone microstructure by reconstructing the three-dimensional structure of bone, and it has never been used to evaluate CAH patients. Trabecular bone and cortical bone are detected independently, and biomechanical properties of bone can be evaluated by microfinite element analysis. Therefore, it is not necessary to correct the results by height. Our study enrolled adult 21OHD patients at their final adult height and used HR-pQCT to quantitatively assess bone changes. Increased or normal BMD was observed among 21OHD patients. On the other hand, BMD was significantly lower among 17OHD patients. The opposite changes in BMD between the 21OHD and 17OHD cohorts may be attributed to differences in hormone level between estrogen and such bone changes in this data analysis. This may be because only one estradiol test at 24 hours could not reflect the patient's actual ovarian function. Gonadotropin can better reflect the long-term ovarian function state and the state of estrogen reserve. In this study, adverse effects of increased FSH and LH on bone metabolism, which reflect the effect of a low estrogen
reserve, were found. Interestingly, this kind of low-dose GC replacement therapy was not related to impaired bone changes. Some studies have previously investigated fractures in patients with CAH, and the reported osteoporotic fracture rate varied from 0–20%[37]. In this study, most patients were young and had no higher fracture risk after FRAX score assessment. It is not currently possible to assess the real-time significance of these altered parameters with regard to fracture risk.

Furthermore, the 21OHD and 17OHD cohorts had significant differences in bone geometry, but the change pattern was exactly opposite in the two cohorts (Table 2). Compared to the controls, 17OHD patients had increased Tt.Ar and Tb.Ar with reduced Ct.Ar at both the radius and tibia. In contrast, among 21OHD cases, decreased Tt.Ar and Tb.Ar were observed, accompanied by increased Ct.Ar. Thus, the differences in bone geometry indices may reflect the opposite effects of 21OHD and 17OHD on bone metabolism, which was also verified by different changes in bone microarchitecture. For example, 21OHD patients had increased cortical thickness and decreased pore diameter at both the radius and tibia, while 17OHD patients had reduced cortical thickness and elevated pore diameter.

Clinical characteristics were assessed to analyze whether there was correlation with bone changes [26] (Fig. 3–5 and Table 3). Height and FSH had similar effects on bone differences and were negatively correlated with bone density, especially at the radius. In 17OHD patients, the insufficiency of sex hormones would lead to delayed closure of the epiphyseal line and a longer growth period, resulting in taller height and lower BMD [38]. Conversely, in 21OHD patients, the accumulation of sex hormone precursors causes hyperandrogenemia and thus may limit the period of growth [39]. This was consistent with our findings (Table 2) that 21OHD showed below-normal heights, while 17OHD showed the opposite trend. Additionally, 17OHD patients demonstrated much higher levels of FSH due to low levels of estradiol or testosterone than 21OHD patients (Table 1); FSH, thus, had similar correlations to height with HR-pQCT parameters. Moreover, height and FSH were also significantly positively associated with higher Tt.Ar, Tb.Ar and Ct.Pm but with lower Ct.Ar and Ct.Th, especially at the radius (Table 3). All the abovementioned differences may indicate that the two subtypes of CAH show opposite effects on bone microarchitecture, to which different hormone levels were the primary contributors. Furthermore, the significantly lower FSH level observed in 21OHD than in 17OHD can have positive effects on bone microarchitecture. A cross-sectional study of 699 healthy Chinese women found that serum FSH level were negatively associated with BMD changes in Chinese women [40]. Some vitro studies showed that FSH helped to increase the number of osteoclast and promoted osteoclast formation [41]. It may be possible to predict BMD changes based on serum FSH. Other hormone components, including estradiol, testosterone, progesterone and ACTH, presented fewer or limited contributions to the changes in bone microarchitecture in CAH patients (Table 3), although there were certain significant differences between 21OHD and 17OHD (Table 1). Interestingly, LH was strongly associated with higher Tb.N and lower Tb.Sp at the tibia, whereas it showed no such differences from those at the radius. Instead, LH mainly affected Ct.Th at the radius. Thus, there might be site-specific differences in changes in bone microarchitecture among CAH patients.

A lower level of fasting blood glucose (FBG) was observed among 21OHD patients than among 17OHD patients (Table 1). 17OHD patients usually had large Tt.Ar and Tb.Ar (Table 2), which might account in part for the positive correlation between FBG and areas of total bone or trabecula. In addition, due to reduced production of cortisol and aldosterone, 21OHD patients can demonstrate hyponatremia [42, 43], as described in Table 1, where 21OHD patients showed significantly lower serum sodium levels than 17OHD patients, which
similarly explained the correlation between sodium level and Tb.Ar at the tibia, since 21OHD usually had smaller areas of trabecula.

As age increased, only trabecular separation (Tb.Sp) and heterogeneity (Tb.1/N.SD) became more severe at the radius (Table 3), indicating impaired microarchitectures of trabecular bones in CAH patients, although most of them were young (21OHD: 29.5 [24.00, 34.25] years; 17OHD: 29 [21.50, 35.00] years). Supplementation with exogenous GCs is the most common treatment for CAH patients. However, whether GCs cause bone changes and the maximal safe dose remain worrisome issues. Previous studies indicated that endogenous or exogenous hypercortisolism was associated with alterations in bone architecture and metabolism [21, 44–46]. Our study first reported the effects of replacement treatment time and doses of initial and current therapy on bone microarchitecture in CAH patients and found no correlation, which revealed that the replacement doses we used for CAH were within the safety limits for bone changes. Moreover, it also provided more evidence that serum ACTH had few correlations with HR-pQCT parameters.

Even so, limitations should be noted in our study. First, the cohort of CAH patients came from a single center. Not only is PUMCH the main referral center for endocrine diseases, but it is also the diagnostic and treatment platform for rare diseases in China where patients from all over the country come for consultation. Considering the small sample size (although impressive for such a rare disease) and cross-sectional nature of the study, longitudinal examination would be of interest in our future studies. Second, considering the complex and long-term medication history of CAH patients, accurately evaluating the dosage of GC exposure remains difficult. Thus, the use of initial and current doses in the analyses might not be representative. The safety doses of GCs for avoiding impairment of bone microarchitecture in CAH patients requires further study.

**Conclusion**

Overall, the present study reported bone differences in geometry, microarchitecture and vBMD among CAH patients as measured by HR-pQCT. Compared with the 17OHD group, the 21OHD group predominated in Tt.vBMD (P < 0.001), Ct.vBMD (P < 0.001), and Tb.vBMD (P < 0.001) at the radius. Significant differences in HR-pQCT indices between the two subtypes may indicate their distinctions in pathogenic mechanism and bone metabolism, including contrasting hormone levels. Furthermore, some clinical manifestations, such as height and sex hormones, had strong correlations with HR-pQCT indices. The replacement treatment doses of the GCs used were within the safety limits and might not cause changes in bone structure.

**Declarations**

**Disclosure:** Xu Sun, Yijun Wu, Lin Lu, Weibo Xia, Li Zhang, Shi Chen, Min Nie, Guanyao Zheng, Wan Su, Huijuan Zhu and Zhaolin Lu declare that they have no conflict of interest.

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References


Table 3

Table 3 is available in the Supplementary Files section.

Figures
Figure 1

Representative images of bone microarchitecture in patients with congenital adrenocortical hyperplasia (17OHD and 21OHD) and healthy controls. There were obvious differences in bone microarchitecture between each patient and his or her corresponding control. Compared with controls or 17OHD group, The 21OHD group had higher Ct.Th (P<0.001). Other specific differences are shown in the results. A: Control of 21OHD (20-year-old female); B: Patient with 21OHD (20-year-old female); C: Control of 17OHD (20-year-old female); D: Patient with 17OHD (20-year-old female). R: radius; T: tibia. A1-D1 and A4-D4 for cortical bone; A2-D2 and A5-D5 for trabecular bone; A3-D3 and A6-D6 for total bone
Comparisons of percentage differences in HR-pQCT indices between 17OHD and 21OHD patients and controls at the radius and the tibia. There were many significantly different parameters of bone microarchitecture and volumetric bone mineral density assessed by HR-pQCT between the two cohorts of patients and the corresponding control groups. *Significant difference (P<0.05) was observed between the patient group and controls. †Significant difference (P<0.05) was observed between 17OHD and 21OHD.
Correlations between clinical characteristics and HR-pQCT indices by Spearman analyses. Clinical characteristics were generally positively or negatively correlated with HR-pQCT indices. \*P<0.05; \**P<0.01; \***P<0.001. The blank represents a correlation coefficient less than 0.01. The correlation analysis performed after male patients were excluded showed the similar result, as shown in supplementary table 1.

Figure 4

The fitted curves between significant characteristics in Spearman analyses and each HR-pQCT index of the radius by multiple linear regression analysis. At the radius, Ct.vBMD demonstrated a strongly linear
dependence, unlike Tb.vBMD. All area parameters (Tt.Ar, Ct.Ar and Tb.Ar), Ct.Pm and Ct.Th also had excellent linear relations with clinical characteristics.

Figure 5

The fitted curves between significant characteristics in Spearman analyses and each HR-pQCT index of the tibia by multiple linear regression analysis. Clinical manifestations were closely related to some changes in bone microarchitecture and volumetric bone mineral density in the tibia with excellent linear performance ($R^2$ value) in some models. At the tibia, the results remained similar. Ct.vBMD had a strong linear dependence on clinical variables, while Tb.vBMD showed a nonsignificant relationship.

**Supplementary Files**

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- SupplementaryTable1.docx
- Table3.docx