Long-term glucocorticoid effect on bone mineral density in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency

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Abstract

Introduction: Patients with 21-hydroxylase deficiency (21OHD) assume a lifelong glucocorticoid (GC) therapy. Excessive GC treatment increases the risk of osteoporosis and bone fractures, even though the role of substitutive therapy is not fully established: we analyzed the effect of GC dose on bone metabolism and bone mineral density (BMD) over time in patients with 21OHD.

Methods: We studied bone metabolism markers and BMD in 38 adult patients with 21OHD (19–47 years, 24 females and 14 males) and 38 matched healthy control. In 15 patients, BMD data were available at both baseline and after a long-term follow-up.

Results: BMD was lower in patients than in controls at lumbar spine ($0.961 \pm 0.1 \text{ g/cm}^2$ vs $1.02 \pm 0.113 \text{ g/cm}^2$, $P=0.014$) and femur neck ($0.736 \pm 0.128 \text{ g/cm}^2$ vs $0.828 \pm 0.103 \text{ g/cm}^2$, $P=0.02$); otherwise, after height correction, only femoral neck BMD was lower in patients ($0.458 \pm 0.081 \text{ g/cm}^2$ vs $0.498 \pm 0.063 \text{ g/cm}^2$, $P=0.028$). In those 21OHD subjects with at least 10 years follow-up, we observed an increase in lumbar BMD ($P=0.0429$) and a decrease in femur neck BMD values ($P=0.004$).

Cumulative GC dose was not related to bone metabolism or BMD. No patient experienced clinical fragility fractures.

Conclusions: BMD values are decreased in patients with 21OHD, which are in part explained by decreased height, but not by the dose of glucocorticoids. Nevertheless, bone status should be carefully monitored in patients with 21OHD.

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Introduction

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders characterized by impaired cortisol synthesis; its incidence ranges from 1:10 000 to 1:20 000 births. The most common form of CAH is caused by mutations in *CYP21A2*, the gene encoding 21-hydroxylase enzyme (P450c21) that converts 17-hydroxyprogesterone to 11-deoxycortisol and progesterone to deoxycorticosterone, respective

precursors for cortisol and aldosterone (1). Approximately, 95% of CAH are due to 21-hydroxylase deficiency (21OHD). In addition to the salt-wasting (SW, 75% of cases) and simple virilizing (SV) forms of 21OHD, there is also a mild nonclassic CAH form (NCCAH), which may show variable degrees of postnatal androgen excess and does not lead to severe cortisol insufficiency (1).

Clinical Study	F Ceccato and others	BMD in adult with 21OHD	175:2	102
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The goals of 21OHD therapy are to reduce excessive androgen secretion and to replace the deficient hormones with the lowest glucocorticoid (GC) dose. During childhood, the preferred GC is hydrocortisone, since its short half-life minimizes the adverse side effects of more potent long-acting GCs, especially growth suppression; otherwise, in adulthood, long-acting GCs are preferred. A more precise estimation of endogenous cortisol production rate of 9–11 mg/m²/day (2) has recently led to the reduction in the dosage of GC replacement therapy (3). Adults with 21OHD are treated to limit symptoms of adrenocortical deficiency; however, slight GCs overtreatment is not so rare, mimicking glucocorticoid's excess features that include osteoporosis through multiple mechanisms (4, 5). Previous reports on bone metabolism showed discrepant results in adult patients with 21OHD. Even though a lower bone mineral density (BMD) and an increased incidence of clinical fragility fractures have been observed in adult patients with 21OHD (6, 7), other authors reported that BMD of young adults was similar to normal controls (8, 9). These discrepancies in various studies may reflect differences in age, type and severity of 21OHD, various cumulative dose, and short- or long-acting GCs.

The aim of this study was to evaluate bone health of adult patients with 21OHD in a case-control study, considering BMD, markers of bone metabolism, and their relationship with the cumulative GC dose. Moreover, in a subset of patients, we analyzed BMD after a long-term follow-up.

Patients and methods

Patients and healthy controls

We studied 38 patients with CAH due to 21OHD referred to the Endocrinology Unit of Padova from 1 January 2000 to 31 December 2013. The mean age was 31±7 years (range 19–47 years), and all diagnoses were confirmed through biochemical analyses and genetic testing of *CYP21A2*. Diagnosis was established at birth (22 subjects), in the first 3 years (6 patients), between 4 and 6 years (6 patients), and after 8 years in the remaining 4 patients. All patients achieved full puberty and final height, and received a stable oral GC dose for at least 12 months: dexamethasone (*n*=34); prednisolone (*n*=1); hydrocortisone (*n*=3); 23 patients (61%) assumed also fludrocortisone. The doses of GCs were converted to hydrocortisone using anti-inflammatory equivalents (30 mg hydrocortisone=7.5 mg prednisolone=0.75 mg

dexamethasone) (6); each patient's total cumulative GC (TGC) treatment was calculated and divided by body surface (mg/m²). Clinical and biochemical follow-up of 21OHD patients was performed following standardized international criteria (1); dual-energy X-ray absorptiometry (DXA) data were available in all patients. DXA was performed at baseline and repeated after 8–10 years in 15 patients with the same device.

Healthy subjects without previous fractures (*n*=38), matched for sex, age, and BMI were recruited among students, employees, and their family members of the Padova Endocrinology Unit, and were considered as controls. The study was performed in accordance with the guidelines in the Declaration of Helsinki, the Ethics Committee of the University-Hospital of Padova approved the protocol, and all subjects gave informed consent.

Biochemical analyses

Bone metabolism was evaluated in patients and controls with fasting serum calcium and phosphate levels, bone alkaline phosphatase, parathyroid hormone (PTH, Biochem Immunosystems, Italy), serum carboxyterminal telopeptide of type I collagen (CTX, Nordic Bioscience Diagnostics, Herlev, Denmark), and 25 hydroxyvitamin D (RIA, DIA SORIN, Italy).

Bone mineral density and clinical fragility fractures

BMD at lumbar spine (L1–L4) and femur (neck and total) was determined by DXA, using Hologic QDR 4500 C densitometer (Hologic Inc, Waltham, MA, USA). The instrument was calibrated on a daily basis according to the manufacturer's instructions. In our hands, coefficient of variation was 0.6% and 1.2% for instrument and *in vivo*, respectively. The data were analyzed by the same operator using the same software. We corrected BMD for height (dividing BMD by height in meters, BMD/m).

In all patients, a detailed documentation about previous fractures was obtained. In each patient with clinical symptoms of vertebral fractures (such as dorsal or lumbar back pain, or height decrease), spinal radiographs of the vertebrae T1–L5 were obtained. Vertebral fractures were diagnosed on visual inspection using the semiquantitative method described by Genant and coworkers (10). According to this technique, fractures assessed on lateral thoracolumbar spine radiographs were defined as reductions of more than 20% in anterior, middle, or posterior vertebral height.

Clinical Study	F Ceccato and others	BMD in adult with 21OHD	175:2	103
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Table 1 Antropometric data, bone metabolism markers, and DXA results among patients and controls.

	21OHD patients			Healthy controls		
	All (n=38)	Male (n=14)	Female (n=24)	All (n=38)	Male (n=14)	Female (n=24)
Age (years)	31±7	28±6	32±8	31±7	28±4	33±8
BMI (kg/m ²)	25.6±5.9	25.3±3.8	25.8±6.9	23±3.4	23.5±1.8	22.8±4.1
Weight (kg)	64.7±14.4	68.9±10	62.3±16.2	65.4±12.1	72.8±7.5	61±12.3
Height (cm)	159.1±8.7 ^a	165±7.8 ^b	155.7±7.4 ^c	166.7±8.9	175.5±6.5	161.6±5.4
Calcium (mmol/L, NV 2.10–2.55)	2.36±0.11	2.41±0.1	2.33±0.12	2.33±0.1	2.4±0.06	2.29±0.11
Phosphate (mmol/L, NV 0.87–1.45)	1.22±0.6	1.31±0.9 ^b	1.04±0.2	1.1±0.2	0.92±0.2	1.1±0.2
B-ALP (µg/L, NV 2.7–22.4)	11.1±5.4	12.9±6.4	10.3±4.8	11.2±3.8	13±3.9	10.2±3.45
CTX (pg/mL, NV <704)	380.2±166.6	418±177.7	368.9±171.4 ^c	331.7±208	476.5±202.5	237.7±152.7
25 OHD (nmol/L, NV 75–375)	56.3±36.1	78.4±44.1 ^b	44.7±25.2	39.2±15.1	31.4±10.8	43.9±15.6
PTH (ng/L, NV 17–73)	52.6±24.7	46.1±22.1	55.4±25.8	65.1±41.4	55.6±15.3	70.9±50.9
BMD L1–L4 g/cm ²	0.961±0.1 ^a	0.948±0.11	0.958±0.106 ^c	1.02±0.113	0.991±0.072	1.029±0.119
Z-score L1–L4	−0.1±1.0	−0.1±0.8	−0.6±0.8	−0.3±1.1	−0.5±0.8	−0.1±1.1
BMD femur neck g/cm ²	0.736±0.128 ^a	0.792±0.142 ^b	0.726±0.099 ^c	0.828±0.103	0.882±0.079	0.8±0.097
Z-score femur neck	−0.2±0.8 ^a	−0.2±0.9 ^b	−0.9±0.9 ^c	−0.2±0.9	−0.2±0.5	−0.3±0.9
BMD femur total g/cm ²	0.901±0.116 ^a	0.932±0.136 ^b	0.852±0.09	0.953±0.119	1.017±0.096	0.9153±0.116
Z-score femur total	−0.1±0.8 ^a	−0.1±0.7 ^b	−0.7±0.8	−0.2±0.9	−0.1±0.6	−0.1±1.1

^a*P*<0.05 vs controls; ^b*P*<0.05 vs healthy males; ^c*P*<0.05 vs healthy females.

25 OHD, 25 hydroxyvitamin D; B-ALP, bone alkaline phosphatase; BMD, bone mineral density; BMI, body mass index; CTX, carboxyterminal telopeptide of type I collagen; NV, normal value; PTH, parathyroid hormone.

Statistical analysis

Quantitative variables were presented as mean and standard deviation with PRISM 3.0 software (GraphPad). We compared the groups using the Mann–Whitney *U* test for nonparametrical data; the significance level was set at *P*<0.05 for all the tests. Linear regression was used to examine the relationship between quantitative parameters.

Results

Bone metabolism

As summarized in Table 1, patients and controls were matched for age, sex, and BMI; contrariwise, 21OHD subjects were shorter than controls; Table 2 described the duration of disease from the diagnosis to the last observation and TGC in patients. All considered

Table 2 Duration of disease and GC dose in CAH patients. Results are presented as mean±s.d.

21OHD form	Patients	F/M	Disease duration (years)	TGC 1 year (mg/m ²)	TGC 3 years (mg/m ²)
SW	21	10/11	32±6	12±6	12±5
SV	12	10/2	30±9	9±4	9±3
NCCAH	5	4/1	20±6	10±4	9±3
Total	38	24/14	30±8	10±5	11±5

NCCAH, nonclassical form of CAH; SV, simple virilizing; SW, salt-wasting; TGC, total cumulative GC treatment.

clinical features; TGC and DXA parameters were similar considering 21OHD phenotypes (SW, SV, and NCCAH).

Parameters of bone metabolism were similar among patients with 21OHD and controls. Considering gender-matched differences, females with 21OHD revealed higher serum CTX, while males with 21OHD exhibited higher serum phosphate and vitamin D levels.

Bone mineral density (BMD)

BMD values were lower in patients with 21OHD than in controls at L1–L4 and femur neck (Fig. 1). When BMD was adjusted for height (because patients were shorter than controls), only femur neck value resulted lower in patients than controls: L1–L4 0.598 ± 0.057 vs 0.618 ± 0.089 BMD/m, femur neck 0.458 ± 0.081 vs 0.498 ± 0.063 BMD/m ($P=0.028$ vs controls), also considering volumetric correction.

The linear regression among GC dose did not reveal any relationship between TGC and BMD or bone metabolism. Moreover, bone metabolism and BMD were similar among patients who received an early or a delayed diagnosis, also comparing those subjects who were diagnosed at birth ($n=22$) or in the first 3 years of age ($n=28$) vs others with 21OHD.

A subgroup of 15 patients with 21OHD (9 female, mean age 38 ± 4 years, range 23–47) performed a long-term DXA follow-up after at least 10 years from the first examination, with the same instrument every 2–4 years. Comparing the first and the last available DXA, we observed an increase in L1–L4 BMD ($P=0.0429$) and a

Figure 2

BMD values after long-term follow-up in 15 patients.

decrease in femur neck BMD ($P=0.0004$), as depicted in Fig. 2, without gender differences.

Clinical fragility fractures

During observation and analyzing the patient's history, we did not observe any vertebral or nonvertebral fragility fracture; otherwise, one male patient reported a traumatic wrist fracture.

Discussion

Treating adrenal insufficiency appropriately remains a challenge for endocrinologists, because there is no consensus on how to titrate the dosage of replacement therapy (3, 11). Other than androgen suppression with low GC dose in patients with 21OHD, several hormonal tools have been proposed to assess the GC dose in patients with adrenal insufficiency, using either urinary, single or multiple serum, or salivary cortisol samples (2, 3, 11, 12, 13) to avoid the risk of subtle chronic under- or overtreatment. Effects of GCs on bone metabolism consist in two phases: an early increase in bone remodeling and osteoclast activity, followed by a drastic drop in bone formation and an increase in osteocytes apoptosis (14, 15). Inconclusive data are reported in literature about bone metabolism in patients with 21OHD: a lower BMD and an increased incidence of clinical fragility fractures, as compared with healthy controls, have been observed only in adult women with 21OHD (6, 7); on contrary, other authors reported that the BMD levels of young adult were similar to normal controls (8, 9).

Figure 1

Panel (A): BMD in patients and controls; Panel (B): BMD/m values in patients and controls (male: empty circles; female: full circles).

Clinical Study	F Ceccato and others	BMD in adult with 21OHD	175:2	105
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In our series of patients, we considered 38 patients with 21OHD, with a regular follow-up at the same tertiary Endocrinology office and with homogenous clinical characteristics: all adults, no menopausal women, stable oral GC dose. As described by Falhammar and coworkers (6, 7), we observed a lower BMD in patients, which could be related to their shorter height than controls. As a matter of fact, measurement of areal BMD by DXA is currently the accepted method for clinical diagnosis of osteoporosis and assessment of fracture risk; however, DXA measures integral bone mass of the cortical and trabecular bone compartments divided by the two-dimensional projected area. Therefore, the areal BMD measurement provided by DXA is highly influenced by bone size (16). To overcome this issue, we had corrected BMD for height, an easy clinical parameter that could be collected in every office, and finally only femur neck BMD resulted lower in patients. Our data confirmed those reported by Stikkelbroeck and coworkers that calculated volumetric BMD in 30 patients with 21OHD (8), and Christiansen and coworkers that proposed BMC adjusted for height in 18 patients (9). To conclude, there are no definitive data about volumetric BMD and fracture incidence, and our aim was to provide useful information for clinical practice, where there is widespread use of areal BMD. In our population, the only difference was height, a simple clinical parameter, so we adjusted BMD for height, although this correction is not widely used in adults. Other techniques could be used to directly assess bone size (i.e., peripheral quantitative computed tomography, pQCT), but DXA-derived BMD is able to predict fracture risk; moreover, pQCT is expensive, and present lower accessibility combined with large X-ray exposure (17). With our data, it seems that femoral neck size is less affected by height, but other studies in adults without 21OHD are needed to verify this hypothesis, since our patients with 21OHD were treated with substitutive low doses of GC, which were not able to suppress/retard skeletal growth.

Even though some authors reported a negative effect of TGC on lumbar and femoral BMD (18, 19), we found a relationship neither between median daily GC dose and TGC dose (calculated in the last 1 and 3 years) nor between patients with a diagnosis established at birth, early, or delayed, confirming that GC dose seems not to be related with BMD, as described previously (6, 8). In our routine clinical practice, we utilize low GC dose, similar to those previously reported (8, 20), in order to prevent from the risk of mild but chronic hypercortisolism. Moreover, although TGC in adults are likely different from those during childhood growth, with different effects for height vs bone density, in this study, we considered only adult patients who achieved final

height and puberty. In our patients, BMD values were lower in femur, suggesting that the major part of GC effect is on femoral bone rather than on lumbar spine. In conclusion, the effect of TGC on bone metabolism in patients with 21OHD remain a critical issue for endocrinologists: larger studies are needed to clarify this topic.

Bone turnover markers were similar among patients and controls, except higher CTX levels in women with 21OHD, as described by Zimmerman and coworkers (19). None of our patients were prescribed bisphosphonates or calcium supplementation, whereas patients with insufficient serum vitamin D levels assumed oral supplementation, explaining the higher vitamin D levels than controls. In our study, hypogonadism was not a key factor: all female patients had regular menses, and five took oral estro-progestinic pills as contraception. Taking into account all these considerations, lower BMD at femur neck might be more related to 21OHD *per se*, rather than a negative impact of GC on bone metabolism, or due to excessive androgens suppression (21, 22) or secondary to geometrical anatomic variability of femur neck, which is not considered with standard DXA analyses. Finally, none of the considered patients had a clinical fragility fractures.

Among our cohort, 15 patients performed a long-term DXA follow-up, with the same device. We observed a progressive increase in lumbar BMD and a decline in femur BMD. Considering the relative young age of patients, this phenomenon seem not related to spine osteoarthritis; moreover, our 21OHD patients did not display any clinical signs or symptoms related to lumbar arthrosis or arthritis. At our knowledge, this is the first report of long-term BMD follow-up in patients with 21OHD, reflecting general population: in a large study that comprised more than 500 of premenopausal woman, Adami and coworkers observed an increase in lumbar BMD from 26 to 30 up to 31–35 years, and decline in femur BMD in all ages brackets considered (23). Moreover, patients assumed low GC doses: probably, these low doses were not able to affect bone metabolism over years.

Our paper present some limitations: first, GC treatment is not yet standardized and patients took different dosage, so we tried to overcome this issue calculating equivalents and TGC. Moreover, this is a retrospective analysis, and we collected BMD data after a long-term follow-up in a subset of patients.

To conclude, we reported that in adult patients with CAH from 21OHD, the DXA estimates of BMD at lumbar spine were similar to those found in healthy age- and sex-matched controls. Moreover, it is fundamental to correct BMD value with height, in order to collect substantial data.

In addition, no correlation was found between the GC dose and the BMD, suggesting that CAH patients with standard GC regimens have an osteoporosis risk similar to controls; nevertheless, lower values of femur BMD in patients could be a consequence of the pathology itself, and bone health should be monitored carefully in patients with 21OHD.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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