CLINICAL STUDY

Impact of total cumulative glucocorticoid dose on bone mineral density in patients with 21-hydroxylase deficiency

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Abstract

Objective: It remains controversial whether long-term glucocorticoids are charged of bone demineralization in patients with congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency. The aim of this study was to know whether cumulative glucocorticoid dose from the diagnosis in childhood to adulthood in patients with CAH had a negative impact on bone mineral density (BMD).

Design: This was a retrospective study.

Methods: Thirty-eight adult patients with classical and non-classical CAH were included. BMD was measured in the lumbar spine and femoral neck. Total cumulative glucocorticoid (TCG) and total average glucocorticoid (TAG) doses were calculated from pediatric and adult files.

Results: We showed a difference between final and target heights (-0.82 ± 0.92 s.d. for women and -1.31 ± 0.84 s.d. for men; $P < 0.001$). Seventeen patients (44.7%) had bone demineralization (35.7% of women and 70% of men). The 28 women had higher BMD than the 10 men for lumbar (-0.26 ± 1.20 vs -1.25 ± 1.33 s.d.; $P = 0.02$) and femoral T-scores (0.21 ± 1.30 s.d. versus -1.08 ± 1.10 s.d.; $P = 0.007$). In the salt-wasting group, women were almost significantly endowed with a better BMD than men ($P = 0.053$). We found negative effects of TCG, TAG on lumbar ($P < 0.001$, $P = 0.002$) and femoral T-scores ($P = 0.006$, $P < 0.001$), predominantly during puberty. BMI was protective on BMD ($P = 0.006$).

Conclusion: The TCG is an important factor especially during puberty for a bone demineralization in patients with 21-hydroxylase deficiency. The glucocorticoid treatment should be adapted particularly at this life period and preventive measures should be discussed in order to limit this effect.

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Introduction

The 21-hydroxylase deficiency is an autosomal recessive disease that constitutes 95% of the congenital adrenal hyperplasia (CAH), the remaining 5% being due to other enzyme deficiencies. It is essentially responsible for impaired cortisol synthesis, which in turn leads to a positive feedback on CRH and adrenocorticotrophin (ACTH), and increased production of androgens and steroid precursors upstream from the enzymatic defect (1).

There is a fine balance between androgen levels and glucocorticoid treatment, the goal of which is to lower androgen levels without making them plummet and bringing on associated side effects. What is more, chronic glucocorticoid therapy is known to generate bone loss in many ways: a direct suppression of osteoblastic activity (2) and an inhibition of digestive calcium absorption with secondary hyperparathyroidism and increased bone

resorption by osteoclasts (3). Glucocorticoids also seem to regulate renal calcium excretion and parathormone secretion (4). Based on these findings, many studies have attempted to study bone mineralization status in patients with 21-hydroxylase deficiency, with the precise goal of identifying bone demineralization. Some studies reported no significant differences in bone mineral density (BMD) between patients and controls (5–9), whereas others found impaired BMD (10–15). These contradictory results may be explained by heterogeneous populations and methods. Indeed, a couple of studies included pre- and post-pubertal patients who had not yet reached their final height, while others sought to compare BMD with average glucocorticoid dose, at the time of the study.

To resolve this issue, we hypothesized that there could be a negative role of the total cumulative glucocorticoid (TCG) dose on BMD. To do so, we collected the daily treatment from the diagnosis in early infancy to inclusion in the

study. We considered adult patients more than 16 years old with classical and non-classical CAH that had been revealed by precocious puberty and those who had been continuously monitored and treated since childhood.

Patients and methods

Patients

Sixty-one adult patients suffering from CAH due to 21-hydroxylase deficiency and treated since early infancy in pediatric centers were seen between 2003 and 2007 by the Department of Endocrinology and Reproduction Medicine ($n=53$, 87%), La Pitié-Salpêtrière Hospital and the Department of Endocrinology ($n=8$, 13%), St-Antoine Hospital, Paris, France. All patients had achieved full puberty and final height. The inclusion criteria were based on the availability of 1) dual-energy X-ray absorptiometry (DXA) and 2) complete patient files, thus enabling the calculation of the TCG treatment. Whereas 23 patients were not included because they simply underwent a BMD evaluation, 38 patients (62.3%) met all criteria. Out of the 38 patients, 24 (63.2%) have the salt-wasting form (SW; 14 women and 10 men), 5 the simple virilizing form (SV; 13.1%, women only in this group), and 9 the non-classical form revealed by precocious puberty (23.7%, women only in this group). Mutation analysis of the cytochrome P450 21-hydroxylase (CYP21A2) gene confirmed the diagnosis in each case. All patients underwent a medical examination to determine height, weight, and body mass index (BMI). They had all been on glucocorticoid treatment since diagnosis in infancy and 23 of them (60.5%) were also receiving mineralocorticoid (9 α -fludrocortisone) during the study. Out of the 28 women, 16 (57.1%) had received exogenous estrogens at some point in their life. At the time of the study, six women were under 17 β -estradiol and cyproterone acetate and nine under estroprogestative pills. None of the subjects presented other conditions known to affect bone metabolism. No fracture was recorded. Nobody was taking bisphosphate, calcium, or vitamin D supplementation.

Consulting files

Retrospective research was done by consulting the pediatric files of Necker-Enfants Malades, St-Vincent de Paul and Trousseau Hospitals, Paris, France. We were able to research the pubertal profiles of 32 subjects. The onset of puberty was noted (B2 in girls (16) and testes size greater than 20 $\times$ 30 mm in boys (17)) as well as height and bone age at the beginning of puberty. The age of menarche and the use of exogenous estrogens were recorded for women. The total pubertal growth was defined as growth from the onset of puberty until final height. Target height was calculated as (maternal height + paternal height $\pm$ 13 cm)/2 (18). All daily glucocorticoid treatment and yearly heights and weights

were noted for each patient from day one of the diagnosis to BMD testing, based on both pediatric and adult files. We decided not to record selective hormonal data from childhood because the frequency and the type of hormonal samplings were very variable for all patients.

Glucocorticoid treatment

Doses of the various glucocorticoids were converted to growth-retarding cortisol equivalents (1 mg dexamethasone = 16 mg prednisone = 80 mg hydrocortisone; 10, 12, 19). Each year, we summed every daily dose of treatment and divided the total by the corresponding body area and obtained annual cumulative doses of hydrocortisone (mg/m² per yr).

Each patient's TCG treatment (TCG in mg/m²) was found by compiling these annual cumulative hydrocortisone doses.

Average daily hydrocortisone treatments (mg/m² per d) were determined every year.

We also calculated the mean daily dose of hydrocortisone that the patient received over his/her entire therapy life period: total average glucocorticoid (TAG in mg/m² per d).

BMD assessment

The femoral neck and lumbar spine BMD were assessed by the same operator using a Hologic Densitometer Quantitative Digital Radiography (QDR) 1000. The daily quality control showed a 0.52% coefficient of variation during the realization of these tests. BMD results at the femoral neck and lumbar spine L2–L4 were evaluated and expressed as absolute values in g/cm² and T-scores ((BMD–Peak Bone Mass)/s.d.). OFELY Caucasian reference curves were used to calculate women T-scores (20) and Hologic's TK91 norms were applied for men. Adjusting BMD regarding subjects' heights was not necessary, because they were in the normal range even if some did not reach their target heights. We were able to use T-scores to interpret and compare the results since all patients were less than 40 years old, and the general population's peak bone mass does not decrease at this age. We used WHO criteria to identify different degrees of low bone mass. We acknowledged that the descriptors were set up for postmenopausal women and defined as osteoporosis (T-score < –2.5 s.d.), osteopenia (T-score between –2.5 and –1 s.d.), and normal bone densitometry (T-score > –1 s.d.) (21).

Statistical analysis

Descriptive statistics were performed for each variable; quantitative results were presented as mean $\pm$ s.d. and qualitative results as frequency. We accepted that all variables (except BMI) followed a normal distribution. The four groups (SW, SV, NC females, and SW males) were compared using ANOVA test (Kruskall–Wallis for BMI).

When P value was <0.05 , Tukey's multiple comparison test was performed. A comparison between males and females was computed using Student's t -test and Mann–Whitney test for BMI. To assess the effect of treatment on BMD, with adjustment on covariates (gender, group, age, and BMI), a multivariate linear model was performed. Covariates were entered in the model using stepwise selection. Since BMD was characterized by two variables (lumbar and femoral T-scores), significance level was Bonferroni corrected: a $P<0.025$ was accepted as significant. Statistical analyses were performed using SAS 8.2 (SAS Institute, Cary, NC, USA) for Windows. Type I error was 0.05 for all analyses.

Results

Population general characteristics

Auxological data are shown in Table 1. All sub-groups of patients were comparable for age, the mean age being 24.6 ± 5.9 years (range: 16–39 years). We noted a lower treatment duration for non-classical CAH people (19.3 ± 9.3 years, $P=0.09$), but this population was probably not numerous enough to see a statistical difference with people suffering from classical CAH, 22.7 ± 6.9 years being the entire population mean. Mean height was 1.59 ± 0.06 m for women and 1.67 ± 0.04 m for men. Median BMI was 24.7 kg/m^2 (19.4 – 38.6) for women and 23.3 kg/m^2 (19.1 – 37.3) for men, without any significant difference. The mean growth-retarding current dose of hydrocortisone was $16.9 \pm 5.5 \text{ mg/m}^2$ per d. Annual hydrocortisone equivalent doses from birth to 18 years old, corresponding to the pediatric period, are represented in Fig. 1. Age at diagnostic was 19.9 ± 33.2 days for SW women, 45.9 ± 58.1 days for SW men, 3.9 ± 5.6 years for SV women, and 6.4 ± 1.5 years for NC women. Mean daily dose over a year could be distinguished in three periods: salt

wasters were treated with $23.8 \pm 8.8 \text{ mg/m}^2$ per d the first 2 years of life, patients with classical (SW and SV) and non-classical forms had respectively 18.2 ± 4.8 and $12.7 \pm 4.1 \text{ mg/m}^2$ per d during childhood ($P<0.001$) and 20.4 ± 6.4 and $13.8 \pm 3.0 \text{ mg/m}^2$ per d during puberty ($P=0.01$).

The 23 non-included patients for whom a TCG could not be calculated were significantly older (31.8 ± 8.2 years, $P=0.002$) and therefore had longer treatment duration (28.9 ± 9.8 years, $P=0.005$) than the included ones. They were comparable for height, BMI, mean current dose of hydrocortisone ($17.3 \pm 5.5 \text{ mg/m}^2$ per d), and BMD.

Puberty data

The onset of puberty was at 10.3 ± 1.8 years for girls and 11.6 ± 1.6 years for boys. The mean age of menarche was 13.6 ± 1.6 years, 3.1 ± 2.1 years after B2. The height at the start of puberty was 0.90 ± 1.82 s.d. Bone ages were available for half of the patients only and were not statistically different from chronological ages ($+0.6$ year for girls, $P=0.29$ and $+0.9$ year for boys, $P=0.26$). Total pubertal growth for girls and boys was 18.5 ± 8.5 cm and 18.5 ± 5.5 cm respectively which is significantly less than in the reference population of Prader *et al.* (22) with a mean pubertal growth of 20.3 ± 6.8 cm in females and 28.2 ± 8.2 cm in males. We noted a significant difference between the final and target heights (-0.82 ± 0.92 s.d. for women and -1.31 ± 0.84 s.d. for men; $P<0.001$).

Bone mineral density

The 28 women had significantly better values than the 10 men for lumbar T-score (-0.26 ± 1.20 vs -1.25 ± 1.33 s.d.; $P=0.04$) and femoral T-score (0.21 ± 1.30 vs -1.08 ± 1.10 s.d.; $P=0.009$). This difference almost

Table 1 Clinical characteristics of the 38 included patients.

	Number	Age (years)	Treatment duration (years)	Current dose of hydrocortisone (mg/m^2 per d) ^a	Height (m/s.d.)	Weight (kg)	BMI (kg/m^2) ^b
All patients	38	24.6 ± 5.9	22.7 ± 6.9	16.9 ± 5.5	$1.61 \pm 0.06/$ <i>-0.94 ± 0.95</i>	66.5 ± 14.0	24.2 (19.1 – 38.6)
All females	28	24.7 ± 6.3	22.1 ± 7.6	15.5 ± 5.4	$1.59 \pm 0.06/$ <i>-0.80 ± 0.99</i>	65.7 ± 14.0	24.7 (19.4 – 38.6)
SW females	14	23.5 ± 5.5	23.5 ± 5.5	15.2 ± 4.2	$1.59 \pm 0.05/$ <i>-0.84 ± 0.82</i>	64.7 ± 12.3	24.7 (19.4 – 32.9)
SV females	5	27.0 ± 5.7	23.4 ± 9.7	20.1 ± 2.9	$1.56 \pm 0.07/$ <i>-1.38 ± 1.29</i>	63.3 ± 15.5	22.0 (21.2 – 37.9)
NC females	9	25.3 ± 8.0	19.3 ± 9.3	13.4 ± 6.7	$1.61 \pm 0.06/$ <i>-0.41 ± 1.01</i>	68.5 ± 16.9	25.4 (19.9 – 38.6)
All males	10	24.2 ± 4.5	24.2 ± 4.5	20.4 ± 4.3	$1.67 \pm 0.04^*/$ <i>-1.35 ± 0.70</i>	$69.0 \pm 14.4^*$	23.3 (19.1 – 37.3)

SW, salt wasting; SV, simple virilizing; NC, non-classical. *Significant difference between all females and all males ($P<0.05$). Italics indicates standard deviations of height (m).

^aIn growth-retarding equivalent.

^bIn median and range.

Figure 1 Annual average hydrocortisone growth-retarding equivalent doses from birth to 18 years old. Each year corresponds to a range of 26–38 patients, with NCF beginning the treatment later and some subjects stopping it periodically. SWF, salt-wasting CAH females; SVF, simple virilizing CAH females; NCF, non-classical CAH females; SWM, salt-wasting CAH males.

reached the significance between women and men with the SW form ($P=0.053$). All women sub-groups (SW, SV, and NC) were comparable for BMD.

Seventeen patients (44.7%) had bone demineralization according to WHO criteria, including the ten women (35.7% of women: 4 SW, 4 SV, and 2 NC) and seven men (70% of men: all SW). Among the women, five suffered from lumbar osteopenia alone, two from femoral neck osteopenia alone, and three from both. Five men were affected by lumbar osteopenia and one man by lumbar osteoporosis, and while all of the men had femoral neck osteopenia, only one man had femoral neck osteopenia without lumbar osteopenia (Table 2).

We noted a protective effect of BMI on lumbar ($P=0.006$) and femoral ($P<0.001$) BMD for both sexes in the multivariate modeling. There was no change in BMD in women taking exogenous estrogens compared with those who were not.

TCG treatment

TCG are shown in Table 2. The patients were probably not numerous enough to conclude that there was a difference between those with non-classical and classical forms ($P=0.08$). Multivariate linear regression demonstrated a negative effect of TCG on lumbar ($P<0.001$) and femoral T-scores ($P=0.001$; Fig. 2).

TAG dose

ANOVA showed that TAG differed between groups ($P=0.002$; Table 2): according to Tukey's comparisons, TAG was lower in patients with non-classical CAH than with classical CAH (13.2 ± 4.6 vs 20.2 ± 4.9 mg/m² per d); the other groups were comparable. We found a negative effect of TAG on lumbar ($P=0.002$) and femoral

T-scores ($P=0.002$; Fig. 3). Multivariate linear regression established that the average glucocorticoid dose during puberty had the most deleterious impact on both lumbar and femoral T-scores ($P=0.02$). Childhood or adulthood therapy, 0–2 years, seemed less damaging on BMD since they were not emphasized by the multivariate analysis.

Discussion

In this study, our main objective was to determine whether a bone demineralization in adult patients with 21-hydroxylase deficiency might be related to a high TCG treatment. To do so, we retrospectively calculated the TCG dose of 38 adult subjects with CAH who had achieved their definitive height and undergone DXA.

We found a negative effect of TCG on lumbar and femoral T-scores. Moreover, we demonstrated the same negative effect with TAG, which represented the average daily dose of glucocorticoids that patients received over their entire therapy life period. In fact, 44.7% of the participants had bone demineralization according to WHO criteria, which is superior to a Gaussian repartition, in which 16% of the general population is under -1 s.d. These results are reinforced by the fact that patients do not always take their therapy as prescribed notably during childhood and adolescence; in other words, real glucocorticoid intakes may be sometimes lower than those written in files. Our results are reinforced by van Staa's *et al.* meta-analysis that underlined a strong correlation between cumulative dose and loss of BMD and between mean daily dose and risk of fracture independently of underlying disease requiring corticotherapy, age, and gender (23).

We found fairly normal pubertal development in boys (11.6 ± 1.6 years) and girls (10.3 ± 1.8 years) and a

Table 2 Results of bone mineral densities, total cumulative glucocorticoid, and total average glucocorticoid treatments.

No.	Form	T-score L (DS)	T-score F (DS)	BMD L (g/cm ²)	BMD F (g/cm ²)	Years of treatment	TCG (mg/m ²)	TAG (mg/m ² per d)
<i>Females</i>								
1	SW	1.35	0.57	1.175	0.881	21	119 574	15.6
2	SW	0.56	3.09	1.092	1.151	18	116 289	17.7
3	SW	0.28	0.62	1.062	0.886	20	131 400	18.0
4	SW	0.24	0.05	1.058	0.825	19	136 510	18.7
5	SW	0.01	1.06	1.034	0.933	18	113 661	17.3
6	SW	0.00	1.13	1.033	0.941	26	147 095	15.5
7	SW	-0.10	-0.16	1.023	0.803	29	237 104	22.4
8	SW	-0.38	-0.50	0.993	0.767	20	195 640	26.8
9	SW	-0.57	-0.02	0.973	0.818	31	268 166	23.7
10	SW	-0.77	-0.79	0.952	0.735	26	152 789	16.1
11	SW	-0.90	-1.02	0.939	0.711	35	286 160	22.4
12	SW	-1.12	1.21	0.915	0.949	20	118 990	16.3
13	SW	-1.75	-1.64	0.849	0.644	27	203 013	20.6
14	SW	-1.81	-0.78	0.843	0.737	19	91 542	13.2
	<i>Mean</i>	<i>-0.35 ± 0.88</i>	<i>0.20 ± 1.20</i>	<i>0.996 ± 0.092</i>	<i>0.842 ± 0.129</i>	<i>23.5 ± 5.5</i>	<i>165 567 ± 61 953</i>	<i>18.9 ± 3.8</i>
15	SV	1.26	2.20	1.165	1.055	22	118 041	14.7
16	SV	-1.11	-0.88	0.916	0.726	24	177 828	20.3
17	SV	-1.53	-1.54	0.872	0.655	9	82 454	25.1
18	SV	-1.85	-1.55	0.839	0.654	26	239 148	25.2
19	SV	-2.03	-0.67	0.820	0.748	36	391 572	29.8
	<i>Mean</i>	<i>-1.05 ± 1.34</i>	<i>-0.49 ± 1.55</i>	<i>0.922 ± 0.140</i>	<i>0.768 ± 0.166</i>	<i>23.4 ± 9.7</i>	<i>201 809 ± 121 699</i>	<i>23.0 ± 5.7</i>
20	NC	3.54	2.87	1.405	1.127	33	161 403	13.4
21	NC	0.69	0.84	1.105	0.910	26	187 902	19.8
22	NC	0.63	1.49	1.099	0.979	14	77 161	15.1
23	NC	0.18	1.51	1.052	0.982	12	27 156	6.2
24	NC	0.10	0.70	1.043	0.895	14	43 435	8.5
25	NC	0.01	0.22	1.034	0.844	9	32 850	10.0
26	NC	-0.17	-0.15	1.015	0.804	18	78 840	12.0
27	NC	-0.33	-1.58	0.998	0.651	34	239 513	19.3
28	NC	-1.65	-0.37	0.860	0.780	14	73 584	14.4
	<i>Mean</i>	<i>0.33 ± 1.38</i>	<i>0.61 ± 1.30</i>	<i>1.068 ± 0.145</i>	<i>0.886 ± 0.138</i>	<i>19.3 ± 9.3</i>	<i>102 427 ± 75 490</i>	<i>13.2 ± 4.6</i>
<i>Males</i>								
29	SW	1.60	1.20	1.239	1.044	16	94 024	16.1
30	SW	-0.01	0.07	1.090	1.080	29	209 510	20.5
31	SW	-0.40	-1.80	0.987	0.718	26	131 911	13.9
32	SW	-0.90	-0.10	0.928	0.871	26	133 809	14.1
33	SW	-1.56	-1.31	0.920	0.835	25	192 538	21.1
34	SW	-1.96	-1.84	0.875	0.777	18	111 690	17.0
35	SW	-1.99	-1.89	0.872	0.772	25	278 313	30.5
36	SW	-2.28	-1.16	0.840	0.922	23	205 678	24.5
37	SW	-2.41	-2.09	0.826	0.800	23	170 419	20.3
38	SW	-2.57	-1.83	0.808	0.834	31	313 426	27.7
	<i>Mean</i>	<i>-1.25 ± 1.33</i>	<i>-1.08 ± 1.10</i>	<i>0.939 ± 0.135</i>	<i>0.865 ± 0.118</i>	<i>24.2 ± 4.5</i>	<i>184 132 ± 71 165</i>	<i>20.6 ± 5.6</i>

TAG, total average glucocorticoid treatment; TCG, total cumulative glucocorticoid treatment; BMD L, lumbar bone mineral density; BMD F, femoral bone mineral density; T-score L, lumbar T-score; T-score F, femoral T-score. Italics indicates mean results.

mean age of menarche around 13.6 ± 1.6 years, which had also been reported by Hughes *et al.* (24) and Bonfig *et al.* (25). We noted low final height compared with target height, consistent with former publications (25–28), while the onset of pubertal height was normal. Height gain was also reported as poor during pubertal years in studies conducted by Stikkelbroeck *et al.* (29) and van der Kamp *et al.* (30). We observed a small and non-statistical difference between chronological and bone ages for both sexes but only half of the latter were available. This result might thus not be relevant; therefore, we could not exclude that potential bone age advancement might possibly be responsible for a reduced final height.

This underlines the impact of glucocorticoid/androgen balance as more important than altered puberty development in this population. Firstly, hyperandrogenism during infancy due to undertreatment leads to growth velocity acceleration and a premature closure of epiphyseal plates (31). Conversely, overtreated children suffer from growth retardation (32, 33). One could wonder whether peak bone mass acquired during puberty might be affected by this therapy as total pubertal growth diminishes. Indeed, we underlined that BMD was mostly affected by the glucocorticoid therapy during puberty. This corroborates the work of Gussinyé *et al.* in which BMD values of adolescent and young adult CAH patients were significantly lower than

Figure 2 T-score regression lines explained by total cumulative glucocorticoid dose. (a) TCG and lumbar T-score and (b) TCG and female T-score.

those of the age- and sex-matched controls, whereas it did not differ between prepubertal patients and controls (34). Multivariate linear regression did not underline another key period, maybe because of the lack of statistical power. Secondly, glucocorticoids may lead to osteoporosis (35), while androgens are known to increase bone formation markers (36, 37). Many studies have pointed out that normal endogenous cortisol production is around 7 mg/m² per d in healthy people (38, 39), whereas clinicians advocate 10–15 mg/m² per d of exogenous therapy (40), which can bring on corticosteroid side effects in bone and result in sub-normal androgen levels. In light of this, non-classical CAH had better BMD than classical CAH groups and their TAG was lower. We were nevertheless unable to determine longitudinal clinical and biological androgenic status, which prevented us from knowing whether low height gain was due to hyperandrogenism or overtreatment and whether or not BMD was influenced by this therapeutic balance.

However, conflicting results were observed in previous studies. This was mainly due to the small number of subjects, their young age, and the heterogeneity of CAH forms and treatments, and also to the fact that different bone parameters were measured and many factors were

used to express glucocorticoid overdose. For example, Jääskeläinen *et al.* (10) chose a current and mean long-term glucocorticoid dose, (Hagenfeldt *et al.* (11)), an index of accumulated post-menarchal glucocorticoid medication, (Sciannamblo *et al.* (13)), the median of the last 7 years of treatment, and a cumulative dose that was calculated exactly over 0.5, 2, and 5 years preceding the investigation (Stikkelbroeck *et al.* (8)). We opted for the TCG treatment, because it was a long-term parameter that covered medication over an entire lifetime. We lent from work by van Staa *et al.* on 81 patients in two randomized, double-masked, placebo-controlled trials (23), where they related the TCG to the bone loss.

Furthermore, we observed a large discrepancy between lumbar and femoral T-scores between women and men in this study. Authors of published papers explained that estrogens have more protective action for bone mineralization than androgen (36, 41), which could contribute to this result, all the more since the majority of women took exogenous estrogens. In addition, it appears that adult males with CAH face a dual problem: 1) adrenal steroid overproduction, especially androgen and progesterone that are less protective on BMD than testosterone, might interfere with follicle-stimulating hormone and luteinizing hormone production, resulting in gonadotropin

Figure 3 T-score regression lines explained by total average glucocorticoid treatment. (a) TAG and lumbar T-score and (b) TAG and femoral T-score.

deficiency and consequent small testicular size and lower testosterone levels (42, 43), and 2) adrenal rests can interfere directly with the endocrine function of normal testicular tissue in a mechanical way or by local steroid production. Furthermore, supraphysiological doses of glucocorticoids can inhibit gonadotropic axis and also deprive bone of the protector role of testosterone. We showed that BMD in women taking exogenous estrogens compared with those who were not were similar. Nevertheless, we did not distinguish between different hormonal treatments, their duration, or the periods over which they were administered.

BMI was positively correlated with lumbar and femoral bone mineralization in both sexes. This is consistent with the work of Bachelot *et al.* (14), which suggests that the higher BMI powerfully protects against bone density loss in adult patients with CAH. One could hypothesize two mechanisms: 1) estradiol production by aromatization in fat mass, 2) adipocyte secretion of leptin that regulates bone mass through a hypothalamic relay acting on osteoblasts (44).

Finally, our population was fairly young at 24.6 ± 5.9 years, the oldest patient being 39. It is recognized that men and premenopausal women may suffer from glucocorticoid-induced osteoporosis (45, 46), and

postmenopausal women also have a high risk of fracture during corticosteroid treatment (47). We may suppose that aging patients with CAH are at a risk of fracture as suggested by Falhammar *et al.* (15); therefore, preventive methods might be introduced, including physical activity and diet recommendations and vitamin D supplementation if necessary (48). Introducing or increasing 9 α -fludrocortisone in classical forms could be considered in order to diminish hydrocortisone needs, while some clinicians even tempted it in non-classical forms. Indeed, it reduces basal ACTH and 17-OHP by inhibiting hypothalamic–adrenal–pituitary axis (49).

We came up against several difficulties when collecting data from pediatric and adult files: sometimes old files were missing and we were not able to fill all the gaps in patients' medical history. This explains the fact that only 62.3% of our patients were included and that the non-included people were slightly older. Nevertheless, these two groups were comparable for height, BMI, mean current dose of hydrocortisone, and BMD. Moreover, since BMD was shown not to be related to age, the 38 patients included in this research were representative of our population with CAH. We endeavored to constitute a homogeneous group with

patients who went through the same key period of therapy in infancy and puberty, which is why we took the people with non-classical CAH with precocious puberty into consideration.

In conclusion, this trial contributes to the determination of bone mineralization in patients with 21-hydroxylase deficiency, based on the TCG dose measurement. We originally established that there was a negative relationship between 1) TCG and TAG and 2) lumbar and femoral BMD. This was effectively illustrated by the non-classical group whose TAG was inferior and bone mineralization was higher. Women might benefit from the preserving effect of estrogens compared with men. BMI also appeared to protect patients from bone loss. In light of this, physicians should bear in mind the potential consequences of glucocorticoids on bone by adjusting the treatment especially during puberty and improving clinical and biological surveillance from infancy. One could suppose that nowadays the youngest patients already benefit from lower doses when comparing with the oldest ones, consequently better results can be expected in the future. Furthermore, preventative measures against corticosteroid-induced osteoporosis should be discussed right from the beginning of glucocorticoid therapy. DXA might be done at least in adult patients on a regular interval. A prospective study taking into account all biases, such as a retrospective look at therapy based on old patient files, and the ensuing irregularities, may confirm our results.

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