CLINICAL STUDY

Bone mass and body composition of adult women with congenital virilizing 21-hydroxylase deficiency after glucocorticoid treatment since infancy

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Abstract

Aim: To study bone mass, body composition and androgenic/anabolic activity in adult women with virilizing congenital adrenal hyperplasia (CAH) treated with glucocorticoids since infancy and to relate this to the postmenarcheal glucocorticoid impact.

Patients and methods: Thirteen adult women with virilizing CAH treated with gluco- and mineralocorticoids but otherwise medicine-free were investigated with respect to bone mineral content, body composition by dual energy X-ray absorptiometry and endocrine status. In addition an index of accumulated postmenarcheal exogenous glucocorticoid impact was calculated. Seven of the patients had regular menstrual periods, and six were oligomenorrheic but responded with withdrawal bleedings on cyclic progestagens. The data for the patients were compared with those of age-matched healthy reference subjects.

Results: In spite of their shorter stature, CAH patients were significantly heavier and had a significantly higher body mass index and fat/lean body mass ratio than the controls. Their bone mineral area density (BMD) was significantly lower than that of the controls. Serum concentrations of androgens were subnormal in all except two of the patients. Strong negative associations were found between BMD and the calculated index of accumulated postmenarcheal glucocorticoid dose but not between BMD and circulating androgen levels.

Conclusion: The results indicate that glucocorticoids were administered in excess in most of the patients, resulting in subnormal levels of adrenocortical androgens, increased body fat and bone demineralization. Increased catabolic activity due to hypercortisolism rather than decreased androgenic/anabolic steroids is probably the major cause of the subnormal BMD in the treated CAH patients.

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Introduction

In women with the classical form of congenital adrenal hyperplasia (CAH), timing of menarche and the menstrual cycle may be successfully normalized by glucocorticoid treatment. However, the rate of reproduction still remains low (1, 2). Direct effects on ovarian function (1, 3) or impairment of hypothalamic–pituitary function in adolescence by androgen excess (4) have been suggested to be causes of low fecundity. However, in a previous study of women with CAH under treatment with glucocorticoids we found a normal estrogen status as reflected by either regular menstruations or withdrawal bleedings on cyclic progesterone. Instead, most of the patients were hypoandrogenic rather than hyperandrogenic (2). This was

interpreted as a result of adrenocortical suppression by exogenous glucocorticoids and may indicate a state of overtreatment (2).

Besides the specific negative effects of glucocorticoid excess on the skeleton (5), the androgen deficiency noted during glucocorticoid overtreatment may cause additional bone loss. Androgen receptors have been demonstrated in osteoblasts and androgens promote osteoblast proliferation (6). In postmenopausal women positive associations between bone mineral area density (BMD) and endogenous androgens have been shown in several studies (for references see (7, 8)). Previous studies of BMD in CAH patients have yielded conflicting results, probably dependent on heterogeneous patient materials. Normal bone mineral density in treated CAH patients compared with

healthy controls has been reported in four studies (9–12). In the study of Mora and co-workers (11), serum androgens were measured and were reported to be at the upper limits of the normal range or slightly elevated. In another study no significant association was found between bone density and glucocorticoid impact as reflected by serum 17α -hydroxyprogesterone (17OHP) levels (13). However, the investigations of Cameron and co-workers (9), Girgis & Winter (13) and Gussinye and co-workers (12) included children with not fully mature bone status and the study of Guo and co-workers (10) dealt with a heterogeneous clinical material with classical as well as non-classical CAH including both men and women, half of the latter being postmenopausal. On the other hand, Jääskeläinen & Voutilainen (14) reported decreased bone mineral density in a mixed clinical material of adult glucocorticoid-treated men and women with CAH. The different results from the above mentioned investigations prompted us to investigate bone mineral density in a well-defined homogeneous group of treated adult women with classical CAH diagnosed at birth and having androgen deficiency secondary to glucocorticoid treatment, but seemingly normal estrogen status.

Materials and methods

Subjects

Thirteen women aged 20–29 years with CAH due to 21α -hydroxylase deficiency and verified prenatal virilization participated in the study. They were all part of an earlier investigation (2), recruited from all over Sweden. Seven of the patients had regular menstrual periods, and six were oligomenorrheic but responded with withdrawal bleedings on cyclic progestagens. Twelve of the patients were salt-losing and one had simple virilizing disease. Mutation analysis of the CYP21 gene confirmed the diagnosis in all cases. Three of the patients had I 172 N/I₂ splice or I 172 N/Null, six had I₂ splice/I₂ splice or I₂ splice/Null and four had Null/Null genotype. All these genotypes are linked to classical, mostly salt-losing CAH. Five patients were treated with oral dexamethasone (0.5–0.75 mg daily), five with oral prednisolone (5.6–12.5 mg daily), one with oral cortisone acetate (37.5 mg per day), one with 8 mg oral triamcinolone daily and one patient with a combination of oral cortisone acetate (15 mg) and oral prednisolone (3.75 mg) daily. Twelve of the patients received oral fludrocortisone, 0.075–0.15 mg daily as additional mineralocorticoid therapy. At approximately the age of menarche most of the patients had been transferred from short- to long-acting glucocorticoids. With little variations this treatment had been maintained. In the statistical calculations the doses of the different glucocorticoids were transformed into cortisol equivalents as proposed by Migeon & Donohue (15). An index of accumulated postmenarcheal glucocorticoid medication was calculated by multiplying the daily glucocorticoid dose expressed as cortisol equivalents

with actual age minus age at menarche and dividing the result with the body surface area at the time of the study.

Except for the treatment specified above, none of the subjects were on medication or oral contraception. Two of the 13 women were light smokers (less than ten cigarettes/day); the others were non-smokers. Data about physical findings at birth were extracted from patient records. Clinical examination and gynecological history, obtained by a standardized interview, was performed by the senior gynecologist among the authors (K H).

Reference data on bone mineral density and body composition were obtained from a group of healthy sedentary age-matched women serving as controls in our previous study on bone mineral content (BMC) in female former gymnasts (16). They were recruited via announcements at Karolinska Institute and Stockholm University. They had menarche at normal age, 13.0 ± 0.7 (s.d.) years, which does not differ from that of the normal Swedish population, which is 13.2 ± 1.1 years (17). They were regularly menstruating and neither smoked nor used oral contraceptives or other medications. They took part in only moderate physical exercise in their leisure time. The study was approved by the ethics committee, Karolinska Hospital. Full and informed consent was obtained from all subjects participating in the investigation.

Measurement of BMD and body composition

BMD, BMC and lean/fat mass were determined from the whole body as well as from the lumbar spine (L2–L4) with the DEXA (dual energy X-ray absorptiometry) technique using a Lunar Model DPX equipment (Lunar Radiation, Madison, WI, USA). The results from the whole body, from the whole spine as well as from the lumbar spine (L2–L4) examination were used in the analyses. The whole spine as a part of the whole body measurement comprised the lower part of the cervical spine, the thoracic and most of the lumbar spine. Absolute BMD in g/cm^2 was used. The use of Z-scores was avoided as the algorithms matching for age, weight and sex were not available from the manufacturer. Thus the corrections introduced for individuals with high body mass index (BMI) (>30 , $n = 3$), could potentially affect the biological correlations (18). The accuracy of the whole body and L2–L4 determination was $0.01 \text{ g}/\text{cm}^2$ or $0.1 \times \text{s.d.}$ In addition the ancillary result of total bone calcium from the whole body DEXA determination was used.

Analytical methods

Venous blood samples were drawn between 0900 and 1200 h. In the patients with regular menstruations, the blood samples were collected in the early follicular phase. Serum was stored at -20°C until analyzed. Serum concentrations of dehydroepiandrosterone (DHEA), and its sulfate (DHEAS), 17OHP and 4-androstene-3,17-dione (A-4) were determined after extraction with diethyl ether

Table 1 Anthropometric and bone mineral data in treated patients with CAH ($n = 13$) and healthy controls of similar age ($n = 12$). With the exception of corticosteroid treatment in the CAH group, all subjects were free from medications. Means $\pm$ S.E.M.

	Patients	Controls
Age (years)	23.9 $\pm$ 0.8	22.3 $\pm$ 0.4
Menarcheal age (years)	13.2 $\pm$ 0.4	13.0 $\pm$ 0.2
Height (cm)	161 $\pm$ 2.0	166 $\pm$ 1.4
Body weight (kg)	67.6 $\pm$ 3.9**	58.5 $\pm$ 1.6
BMI (kg/m ²)	26.2 $\pm$ 1.7**	20.7 $\pm$ 0.3
Body fat (kg)	24.9 $\pm$ 3.0*	16.2 $\pm$ 1.1
Lean body mass (kg)	40.9 $\pm$ 1.5	37.6 $\pm$ 0.9
Lean/fat body mass ratio	1.88 $\pm$ 0.18	2.55 $\pm$ 0.30
BMC (kg)	2.34 $\pm$ 0.08	2.40 $\pm$ 0.04
BMD total (g/cm ²)	1.12 $\pm$ 0.02	1.13 $\pm$ 0.02
BMD spine (g/cm ²)	0.95 $\pm$ 0.03*	1.04 $\pm$ 0.02
BMD L2–L4 (g/cm ²)	1.14 $\pm$ 0.04	1.13 $\pm$ 0.03

* $P < 0.05$, ** $P < 0.01$.

by radioimmunological methods developed in our departments. In the assay of DHEAS, the conjugate was cleaved by thermal hydrolysis prior to extraction. Serum progesterone, testosterone and sex hormone-binding globulin (SHBG) were analyzed by RIA using commercial kits obtained from Diagnostic Products Corp., Los Angeles, CA, USA (Coat-a-Count Progesterone; Coat-a-Count Testosterone) and from Eurodiagnostics AB, Malmö, Sweden (SHBG).

Details of the methods as well as detection limits and within and between assay coefficients of variation have been given in previous reports (for references, see (2, 19)). Reference limits for healthy women in the appropriate age range were taken from the clinical reference materials of the Hormone Laboratory, Department of Obstetrics and Gynecology, Huddinge University Hospital and the Department of Clinical Chemistry, Karolinska Hospital.

Non-SHBG-bound testosterone (NST, sum of free + albumin-bound testosterone) was used as an index of biologically active testosterone as proposed by Pardridge (20). Apparent concentrations of NST were calculated from values for total testosterone, SHBG and a fixed albumin concentration of 40 g/l by successive

approximation using a computer program based upon an equation system derived from the law of mass action (21).

Statistical analysis

Correlations were tested using multiple regression test and by Spearman's rank correlation test. Differences between groups were tested with a t -test for unpaired observations or the Mann–Whitney U-test according to distribution. Normally distributed values are given as arithmetic mean and S.E.M., otherwise as median and range. DHEA and DHEAS values below the detection limit were found in all except three patients. In the calculations their values were set to 1 nmol/l for DHEA and 150 nmol/l for DHEAS.

Results

Anthropometric and bone mineral data for patients and controls are given in Table 1. Body weight, BMI and amount of body fat were significantly higher and spinal BMD significantly lower in the patients than in the controls. Serum concentrations of adrenal androgens in the patients were low compared with those of a reference population (Table 2). There were no significant associations between genotype on the one hand and anthropometric or bone mineral data on the other (data not shown). Multiple regression analysis failed to show any associations between bone on the one hand and steroids with direct (NST) or indirect (A-4) androgenic activity on the other. The calculated index of accumulated postmenarcheal glucocorticoid medication was by far the strongest determinant (negative) for all bone variables except BMD spine for which A-4 turned out to have this role (Table 3). When the associations were tested with Spearman's rank correlation test, the calculated index of accumulated postmenarcheal exogenous glucocorticoid medication showed strong and significant negative correlations to all four bone variables (Fig. 1). A significant association between spinal BMD and A-4 was also found in this test, although weaker than

Table 2 Serum concentrations of adrenocortical steroids, testosterone and SHBG (median and range) in treated patients with CAH ($n = 13$) and reference limits for healthy subjects of the same age range. With the exception of corticosteroid treatment in the CAH group, all subjects were free from medications.

	Patients	Reference limits
17OHP (nmol/l)	3.6 (0.4–118)	0.8–4.0*
Progesterone (nmol/l)	1.3 (0.1–17.8)	0.6–5.6*
A-4 (nmol/l)	3.3 (0.7–7.8)	2.5–8.4
DHA (nmol/l)	1.0 (1.0–2.6)	8.0–30.0
DHAS (nmol/l)	150 (150–352)	2000–8000
Testosterone (nmol/l)	0.5 (0.1–2.8)	0.3–2.5
SHBG (nmol/l)	33.0 (8.7–69.7)	21–81
NST (nmol/l)	0.26 (0.04–2.38)	0.18–1.20

* Early follicular phase.

Table 3 Multiple regression analysis of associations between bone variables on the one hand and A-4, NST and index of postmenarcheal glucocorticoid impact (GC index) on the other in treated patients with CAH.

	<i>r</i>	Partial F-values		
		A-4	NST	GC index
BMC	0.82*	1.56	0.13	10.67*
BMD total (kg/cm ²)	0.90**	0.75	0.03	17.80**
BMD spine (kg/cm ²)	0.81*	8.39*	3.76	4.95
BMD L2–L4 (kg/cm ²)	0.90**	4.36	0.13	11.41**

* $P < 0.05$, ** $P < 0.01$.

corresponding association to the calculated index of accumulated postmenarcheal glucocorticoid medication ($r_s = 0.64$, $P < 0.05$ vs $r_s = -0.76$, $P < 0.01$).

Discussion

In contrast to most previous studies (9–13) but in accordance with Jääskeläinen & Vuotilainen (14) we found a distinctly decreased spinal bone density, significant correlations between bone variables and glucocorticoid impact and also low androgen levels in treated CAH patients. Our findings support the view of Jääskeläinen & Vuotilainen (14) and indicate that glucocorticoids have been given in

excess to our patients. The discrepancy between our results and most of the previous studies can be explained by different modes of glucocorticoid treatment and also by the fact that our study was restricted to a homogeneous group of adult women having their CAH diagnosed at birth, treated all their lives and studied in adulthood.

The aims of glucocorticoid treatment of CAH are to provide an adequate glucocorticoid substitution and to suppress excessive secretion of adrenal steroids, notably androgens. In untreated or poorly substituted women with 21-hydroxylase deficiency, the steroids measured in this study are all of predominantly adrenocortical origin and their suppression following glucocorticoid treatment reflects the glucocorticoid impact in treated CAH patients. The 3β -hydroxy-5-ene steroids DHEA and DHEAS are extremely sensitive to glucocorticoid treatment and are suppressed by far lower glucocorticoid doses than 3-oxo-4-ene steroids such as A-4, testosterone and 17OHP (22).

Theoretically, a subnormal BMD in glucocorticoid-treated menstruating adult CAH patients may be caused either by overtreatment with glucocorticoids or by subnormal androgen levels, the latter also being a consequence of excessive glucocorticoid treatment. However, bone loss is very common in women with Cushing's disease who frequently have elevated androgen levels (7). Furthermore, estrogen is far more important than androgen for bone mineralization in women and also in men (for references see (23)). Seven of the patients included in the present study

Figure 1 Associations between bone variables and index of accumulated postmenarcheal glucocorticoid medication (GC index) in treated patients with CAH. Spearman's rank correlation coefficients: BMC vs GC index $r_s = -0.86$, $P < 0.01$; BMD total vs GC index $r_s = -0.82$, $P < 0.01$; BMD spine vs GC index $r_s = -0.76$, $P < 0.01$; BMD L2–L4 vs GC index $r_s = -0.76$, $P < 0.01$.

were regularly menstruating and six responded with withdrawal bleedings on cyclic progestagens, indicating a sufficient estrogen status. This makes it unlikely that either androgen or estrogen deficiency is the cause of the noted lower BMD in our patients. Finally, when the two steroids that have a direct (NST) or indirect androgenic (A-4) activity were tested together with the index of accumulated postmenarcheal glucocorticoid medication, the accumulated glucocorticoid dose was by far the strongest determinant for bone variables. This strongly indicates that iatrogenic glucocorticoid excess is the major cause of the subnormal BMD in our patients. The subnormal spinal BMD in combination with normal peripheral BMD values also suggests an endocrine effect as the trabecular/compact bone ratio is higher in the vertebrae than in peripheral bone and trabecular bone is more strongly affected by hormonal factors than cortical bone (5).

In conclusion, our findings indicate that glucocorticoid excess may be present in most of the studied patients. As pointed out by Jääskeläinen & Vuotilainen (14), there is an urgent need for a better adjustment of the substitution therapy in this disease, since the effects of long-term hypercortisolism upon bone mineral status are deleterious. The common biochemical monitoring of the treatment, using serum 17OHP and urinary pregnanetriol, may not be sensitive enough in this respect. New drugs for therapy and new biochemical tools for therapy monitoring, which may serve as complements to or even replace these assays will, therefore, be needed. Meanwhile, adrenalectomy has been considered in patients with completely non-functioning 21-hydroxylase genes, in order to reduce the doses of glucocorticoids (24).

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