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 glucocorticoids 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 a normal estrogen status as reflected by either regular menstruations or withdrawal bleedings on cyclic progestagens. Increased body fat and bone mineral density in treated CAH patients compared with

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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 & Vuotila (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 21-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 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 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 g/cm^2 or 0.1X 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 ºC 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.
by radioimmunological methods developed in our depart-
ments. 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
Partridge (20). Apparent concentrations of NST were
calculated from values for total testosterone, SHBG and
Pardridge (20). Apparent concentrations of NST were
biologically active testosterone as proposed by

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 gluco-
corticoid 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 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 ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23.9 ± 0.8</td>
<td>22.3 ± 0.4</td>
</tr>
<tr>
<td>Menarcheal age (years)</td>
<td>13.2 ± 0.4</td>
<td>13.0 ± 0.2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>161 ± 2.0</td>
<td>166 ± 1.4</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>67.6 ± 3.9*</td>
<td>58.5 ± 1.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.2 ± 1.7**</td>
<td>20.7 ± 0.3</td>
</tr>
<tr>
<td>Body fat (kg)</td>
<td>24.9 ± 3.0*</td>
<td>16.2 ± 1.1</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>40.9 ± 1.5</td>
<td>37.6 ± 0.9</td>
</tr>
<tr>
<td>Lean/fat body mass ratio</td>
<td>1.88 ± 0.18</td>
<td>2.55 ± 0.30</td>
</tr>
<tr>
<td>BMC (kg)</td>
<td>2.34 ± 0.08</td>
<td>2.40 ± 0.04</td>
</tr>
<tr>
<td>BMD total (g/cm²)</td>
<td>1.12 ± 0.02</td>
<td>1.13 ± 0.02</td>
</tr>
<tr>
<td>BMD spine (g/cm²)</td>
<td>0.95 ± 0.03*</td>
<td>1.04 ± 0.02</td>
</tr>
<tr>
<td>BMD L2–L4 (g/cm²)</td>
<td>1.14 ± 0.04</td>
<td>1.13 ± 0.03</td>
</tr>
</tbody>
</table>

* P < 0.05, ** P < 0.01.

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.

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.

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Reference limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>17OHP (nmol/l)</td>
<td>3.6 (0.4–118)</td>
<td>0.8–4.0*</td>
</tr>
<tr>
<td>Progesterone (nmol/l)</td>
<td>1.3 (0.1–17.8)</td>
<td>0.6–5.6*</td>
</tr>
<tr>
<td>A-4 (nmol/l)</td>
<td>3.3 (0.7–7.8)</td>
<td>2.5–8.4</td>
</tr>
<tr>
<td>DHA (nmol/l)</td>
<td>1.0 (1.0–2.6)</td>
<td>6.0–30.0</td>
</tr>
<tr>
<td>DHAS (nmol/l)</td>
<td>150 (150–352)</td>
<td>2000–8000</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>0.5 (0.1–2.8)</td>
<td>0.3–2.5</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>33.0 (8.7–69.7)</td>
<td>21–81</td>
</tr>
<tr>
<td>NST (nmol/l)</td>
<td>0.26 (0.04–2.38)</td>
<td>0.18–1.20</td>
</tr>
</tbody>
</table>

* Early follicular phase.
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 Jaäskeläinen & 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 Jaäskeläinen & 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

<table>
<thead>
<tr>
<th>Partial F-values</th>
<th>r</th>
<th>A-4</th>
<th>NST</th>
<th>GC index</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMC</td>
<td>0.82**</td>
<td>1.56</td>
<td>0.13</td>
<td>10.67**</td>
</tr>
<tr>
<td>BMD total (kg/cm²)</td>
<td>0.90**</td>
<td>0.75</td>
<td>0.03</td>
<td>17.80**</td>
</tr>
<tr>
<td>BMD spine (kg/cm²)</td>
<td>0.81*</td>
<td>8.39*</td>
<td>3.76</td>
<td>4.95</td>
</tr>
<tr>
<td>BMD L2–L4 (kg/cm²)</td>
<td>0.90**</td>
<td>4.36</td>
<td>0.13</td>
<td>11.41**</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01.

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 $r_s = -0.76, P < 0.01$. 

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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 & Vuotilaïnen (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).

Acknowledgements

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