

# Bone mineral density, bone turnover markers, lean mass, and fat mass in Egyptian children with congenital adrenal hyperplasia

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## Abstract

**Introduction:** Aim of this paper is to assess bone mineral density (BMD) and body composition, by dual energy X-ray absorptiometry (DXA), and various markers of bone growth, in a group of children with congenital adrenal hyperplasia (CAH) on long-term glucocorticoid therapy.

**Material and methods:** A case-control study included thirty patients with CAH with different states of metabolic control. Their mean age was  $7.5 \pm 4.2$  years. All patients are subjected to BMD using DXA at the neck of the femur and lumbar spine. A blood sample was taken for assessment of osteocalcin, osteoprotegerin, and procollagen type 1, as markers of bone formation, as well as RANKL and urinary deoxypyridinoline (DPD), as markers of bone resorption.

**Results:** We found no difference in BMD in patients and control subjects; however, patients showed significantly lower serum osteocalcin ( $p = 0.008$ ) and osteoprotegerin ( $p = 0.0001$ ) and significantly higher serum RANKL levels ( $p = 0.0001$ ). Our results show that patients had significantly lower lean body mass ( $p = 0.005$ ) and fat/lean ratio ( $p = 0.008$ ) compared to matched controls. The duration of treatment showed a significant negative correlation with procollagen type 1 ( $r = -0.49$ ,  $p = 0.02$ ) and lean mass % ( $r = -0.43$ ,  $p = 0.04$ ); however, it showed a significant positive correlation with total fat mass % ( $r = 0.6$ ,  $p = 0.0006$ ), and fat/lean ratio ( $r = 0.43$ ,  $p = 0.04$ ). Dose of steroid had a significant positive correlation with BMI SDS ( $r = 0.4$ ,  $p = 0.02$ ).

**Conclusions:** Bone mineral density is normal but bone turnover is low in patients with CAH. There is an increase in fat/lean mass in patients with CAH.

**Key words:** dual energy X-ray absorptiometry, bone mineral density, osteocalcin, osteoprotegerin, procollagen type 1.

## Introduction

Children with congenital adrenal hyperplasia (CAH) receive glucocorticoid therapy from the time of diagnosis, which may be at birth or even during fetal life. There is evidence that even modern glucocorticoid replacement therapy impacts on skeletal growth and maturation. Thus patients typically show some degree of short stature and delayed bone maturation during childhood [1].

In patients with CAH attributable to 21-hydroxylase deficiency, the synthesis of cortisol ( $\pm$  aldosterone) is impaired. Consequently, the secretion of ACTH is increased, resulting in excess androgen production. Treatment of CAH consists of substitution of cortisol and aldosterone,

thereby preventing an adrenal crisis and suppressing adrenal androgen overproduction [2]. Glucocorticoids must be dosed carefully to avoid over-suppression (leading to growth retardation) and under-suppression (leading to androgen excess and reduction of final height) [3]. Glucocorticoid administration, even in substitution doses, may cause decreased bone mineral density (BMD) [4] and obesity [5]. Previous reports on BMD in CAH patients showed increased [6, 7], decreased [8-11], or normal [12-14] BMD. These reports differed with respect to age selections and glucocorticoid regimens. Body mass index (BMI) is found to be elevated in most [8, 9, 11, 13, 15, 16], but not all [10, 14] reports on CAH patients, but it was not clear whether this resulted from increased fat mass (as a result of glucocorticoid treatment, despite the low dose regimen) or increased lean mass (as a possible result of androgen excess) [9, 10].

Bone mass measurement defines mineral content per area of bone. In the laboratory, bone density by DXA is a very strong predictor of bone strength [17]. Biochemical markers of bone turnover have been developed to look at bone metabolism. They provide potentially useful information but cannot be used alone to diagnose osteoporosis, to determine the severity of the disease, or to select a specific therapy. Urinary deoxypyridinoline (DPD) and serum RANKL are markers of bone resorption, while osteocalcin, osteoprotegerin, and serum procollagen I carboxyterminal propeptide are markers of bone formation [18].

The aim of the current study was to assess BMD, lean mass, and fat mass, by DXA, and various markers of bone growth in a group of children with CAH who have received long-term glucocorticoid therapy under careful monitoring at our clinic.

## Material and methods

It is a case-control study. Thirty patients with CAH were recruited from the Paediatric Department (Diabetes, Endocrine, and Metabolism Unit) at Cairo University Paediatric Hospital. We also studied 11 age- and sex-matched healthy controls who were included in our study. All subjects were healthy and appropriately physically active for their age; none was involved in competitive sport activities. Candidates were excluded if they had a history of chronic illness; they had one or more fractures; or they had taken any medication, hormone, vitamin preparation, or calcium supplements regularly.

Inclusion criteria were patients with CAH on treatment of more than 2 years' duration and exclusion criteria were newly diagnosed patients or treatment duration less than 2 years. The study included 27 patients with classic 21-hydroxylase deficiency, one patient with simple virilizing 21-hydroxylase deficiency and 2 patients with 3 $\beta$

hydroxysteroid dehydrogenase deficiency. Twenty-four patients were diagnosed in the first year of life. The remaining 6 were diagnosed at the age of 2-9 years; they were simple virilizing 21 OH deficiencies, late onset 3 $\beta$  hydroxysteroid dehydrogenase deficiency, and late diagnosis of the classic forms. All had been treated from the onset of diagnosis with glucocorticoids and mineralocorticoids in salt losing forms or glucocorticoid in simple virilizing forms.

All patients were followed up regularly with biochemical, hormonal, and anthropometric measurements. The following data were obtained for patients and controls: age at diagnosis and start of therapy, duration of treatment, dose of glucocorticoid and mineralocorticoid for patients only, height and weight measured with a stadiometer and a clinical scale at the time of examination, body mass index (BMI) calculated as kilograms per metre squared, height SDS and weight SDS calculated with a growth calculator as previously described [19], BMI SDS expressed with data published by Cole *et al.* [20], pubertal assessment, LHRH-analogue treatment for patients only, and bone age assessed by X-ray of the left hand using the Greulich and Pyle method [21].

In the 21-hydroxylase deficient subjects, tight control was defined as a mean serum 17OH-progesterone value of all clinic visits of less than 10 nmol/l, fair control was defined as a mean serum 17OH-progesterone value of 10-40 nmol/l, together with normal levels of plasma rennin, and poor control was defined as a mean serum 17OH-progesterone value over 40 nmol/l [22]; these were noncompliant patients who also showed elevated levels of serum testosterone and plasma rennin [1]. The 2 patients with 3 $\beta$ -hydroxysteroid dehydrogenase deficiency were tightly controlled since their DHEA was below 5 nmol/l and normal plasma renin activity. All enrolled patients and controls were subjected (after their verbal consent) to:

- 1) bone mineral content (BMC, g), areal BMD (BMD, g/cm<sup>2</sup>), fat mass, and lean mass were assessed by DXA (Norland – XR- 46, USA) at the lumbar spine (L1–L4) and the right femoral neck; absolute values were converted to Z-scores (standard deviations from the mean of a healthy age- and sex-matched reference population);
- 2) blood samples were collected by clean venipuncture into Vacutainer plane tubes under strict sterile conditions; 5 ml of venous blood was obtained and put into a water bath for 15 min and centrifuged at 4000 rpm; serum was separated and kept at –20°C for estimation of the different hormonal profiles as well as the following bone markers:
  - a) osteocalcin, using – ELISA kit (Biosource, Europe, S.A.) which is a quantitative sandwich enzyme-linked immunosorbent assay,

- b) serum osteoprotegerin using ELISA technique (Biovendor Laboratory Medicine, Inc., Czech Republic), Cat. No. RD 194003200; it is a biotin labelled antibody based sandwich enzyme immunoassay,
  - c) procollagen type 1 using Metra C1CP assay; it is a sandwich enzyme immunoassay in a microtitre plate format utilizing a monoclonal anti-C1CP antibody coated on the plate, a rabbit anti-C1CP antiserum, a goat anti-rabbit alkaline phosphatase conjugate, and substrate to quantify the C1CP in human serum,
  - d) serum RANKL levels by using ELISA kit, Biomedica group, Biomedica Medicine product, GmbH and Co; it is an enzyme immunoassay for the quantitative determination of sRANKL in serum Cat. No. BI – 20422 H,
  - e) serum testosterone was measured by chemiluminescence enzyme immunoassay (Immulite 2000 Diagnostic Products Los Angeles, CA, USA),
  - f) serum 17 OH was measured by ELISA kit DRG (Germany) cat. no. EIA 1292 and it is based on the competition principle and micro-plate separation,
  - g) Delta 4 androstenedione, dehydroepiandrosterone DHEA, and plasma renin were measured by Radioimmunoassay cat. no. IT 1322 Cis. Bioassay 30200 Bagnols/CIZA France; the procedure follows the basic principle of RIA, where there is competition between radioactive and non-radioactive antigen for a fixed number of antibody binding sites; the amount of 1.25 labelled antigen bound to the antibody is inversely proportional to their concentration; separation of the free and the bound antigen is achieved by decanting or aspirating the coated tubes;
- 3) urinary deoxypyridinoline (DPD) using Metra EIA DPD assay Cat. no. 8007; it was carried out by using preservative first morning void urine or secondary morning void urine; metra DPD assay is a competitive enzyme immunoassay in microtitre strip-well format utilizing a monoclonal anti-DPD antibody coated on the strip to capture DPD; DPD in the sample competes with conjugated DPD alkaline phosphatase for antibody and the reaction is detected with substrate; metra DPD results are corrected for urinary concentration by creatinine in urine.

### Statistical analysis

Statistical Package for the Social Sciences (SPSS) program version 9.0 was used for analysis of data. Data were summarized as mean and SD. Non-parametric tests were used: Mann-Whitney U test

was used for analysis of 2 quantitative data and Kruskal-Wallis U test for comparison of more than 2 quantitative variables. Friedman ANOVA was used to compare between the three groups of 21-hydroxylase deficiency patients (tight, fair, and poor control) and different parameters. Spearman correlation was done when appropriate.

## Results

### Descriptive data of the study group

Thirty patients with CAH were included in the study, mean age  $7.5 \pm 4.2$  (range 3-18 years). Twenty-two were females and 8 were males. Among patients with 21-OH deficiency, 16 patients were on tight control (mean 17-OHP  $2.4 \pm 2.3$  nmol/l), 9 patients showed fair control (mean 17-OHP  $15.4 \pm 2.4$  nmol/l, normal PRA), and 3 patients showed bad control due to non-compliance (mean 17-OHP  $47 \pm 4.3$  nmol/l, high PRA). Two patients with  $3\beta$  hydroxysteroid dehydrogenase deficiency were on tight control. Three of our patients received LHRH analogue due to central precocious puberty (1 with fair control and 2 with bad control). Descriptive data of the study group including their biochemical bone markers, body composition, and DXA results are shown in Table I.

### Comparison between patients and control subjects

Patients included in the study showed significant lower lean body mass and higher total fat mass, abdomen fat %, trunk fat %, soft tissue fat mass % and fat/lean ratio compared to matched controls (Table II). Also, our patients showed significantly lower serum osteocalcin and osteoprotegerin, and significantly higher serum RANKL levels.

### Effect of puberty, gender, control state, and treatment with LHRH analogue

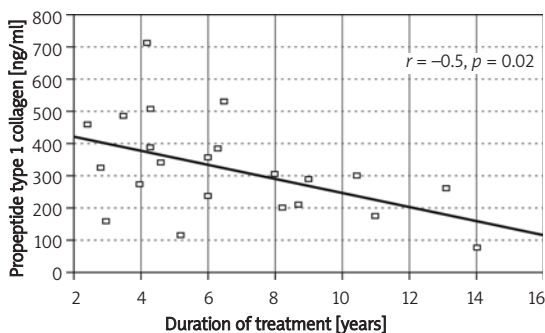
Our data showed no significant differences between pre-pubertal ( $n = 9$ ) and pubertal ( $n = 21$ ) patients regarding their biochemical bone markers and DXA parameters except for BMC, which is higher in the pubertal group than in the prepubertal group ( $1259.1 \pm 399.5$  g and  $840.3 \pm 458$  g, respectively,  $p = 0.05$ ). Moreover, no significant difference was found between patients who received LHRH analogue and the rest of the patients in any of the DXA parameters or bone turnover markers. Our results show no significant differences between the three groups with different control state as well as between male and female patients regarding their biochemical markers and DXA results.

Table I. Descriptive data of the study population

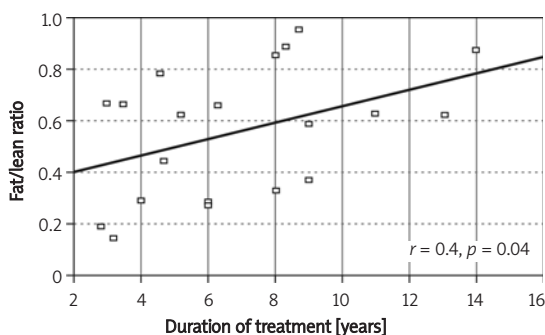
	Mean $\pm$ SD	Minimum	Maximum
Age at start of therapy [years]	1.86 $\pm$ 4.13	0.1	9
Duration of treatment [years]	6.47 $\pm$ 3.13	2.4	14
Average dose of glucocorticoids [mg/M <sup>2</sup> /day]	15.5 $\pm$ 3.68	5	21.5
Dose of mineralocorticoid [ $\mu$ g/day]	0.11 $\pm$ 4.1-02	0.05	0.2
Height SDS	-0.58 $\pm$ 1.58	-3.7	2.3
Weight SDS	7.14-03 $\pm$ 1.79	-3	3.2
BMI SDS	0.59 $\pm$ 1.6	-3.2	3
Procollagen type 1 [ng/ml]	325 $\pm$ 150.8	79	716
Osteocalcin [ng/ml]	21.1 $\pm$ 28.1	0.1	75
Osteoprotegerin [pg/l]	1.99 $\pm$ 0.99	0.3	3.6
Deoxyypyridinoline [nmol/mmol creatinine]	62.45 $\pm$ 67	1.13	285
RANKL [pmol/l]	116.98 $\pm$ 72.43	40	290
BMD total [gm/cm <sup>3</sup> ]	0.64 $\pm$ 9.9-2	0.44	0.83
BMC total [gm]	1144.86 $\pm$ 447.6	468	1954
Femur Z-score	-0.184 $\pm$ 0.66	-1.28	0.9
Vertebral Z-score	0.28 $\pm$ 0.76	-0.89	1.86
Total fat [%]	32.77 $\pm$ 10.1	12	47
Lean mass [%]	65.93 $\pm$ 10.44	51.25	87.28
Lean/fat ratio	0.55 $\pm$ 0.24	0.15	0.95
Fat/lean ratio	2.33 $\pm$ 1.46	1.1	6.86
Trunk fat [%]	28.2 $\pm$ 3.34	22.8	38.12
Abdomen fat [%]	16.5 $\pm$ 3.89	8.19	25.77
Soft tissue [%]	34.1 $\pm$ 10.44	12.72	48.75

Table II. Comparison between patients and control subjects

	Patients (n = 30)	Controls (n = 11)	P-value
BMD [gm/cm <sup>3</sup> ]	0.64 $\pm$ 1	0.6 $\pm$ 0.02	0.05
BMC [gm]	1144.0 $\pm$ 447.6	585.8 $\pm$ 149.9	0.06
Lean mass [%]	65.9 $\pm$ 10.4	76.1 $\pm$ 5.6	0.005
Fat mass [%]	32.8 $\pm$ 10.1	22.5 $\pm$ 5.6	0.006
Abdomen fat [%]	16.5 $\pm$ 3.9	13 $\pm$ 5.1	0.03
Trunk fat [%]	28.2 $\pm$ 3.3	21.6 $\pm$ 8.3	0.003
Soft tissue mass [%]	34.1 $\pm$ 10.4	23.9 $\pm$ 5.6	0.005
Fat/lean ratio	0.6 $\pm$ 0.2	0.3 $\pm$ 1	0.008
Lean/fat ratio	2.3 $\pm$ 1.5	3.4 $\pm$ 1.2	0.008
Osteocalcin [ng/ml]	21.1 $\pm$ 28.1	49.4 $\pm$ 34.5	0.008
Osteoprotegerin [pg/l]	2 $\pm$ 1	30.9 $\pm$ 33	0.0001
Procollagen type 1 [ng/ml]	325 $\pm$ 150.8	376 $\pm$ 218.5	0.4
Deoxyypyridinoline [nmol/mmol creatinine]	62.4 $\pm$ 67	60.8 $\pm$ 33.6	0.3
RANKL [pmol/l]	117 $\pm$ 72.4	19.5 $\pm$ 3.7	0.0001



**Figure 1.** Relation of the duration of treatment in patients with CAH and procollagen type 1



**Figure 2.** Relation of the duration of treatment in patients with CAH and fat/lean mass

#### Effect of duration and dose of steroid treatment

In our study, the duration of treatment showed a significant negative correlation with procollagen type 1 ( $r = -0.49, p = 0.02$ ) (Figure 1) and lean mass % ( $r = -0.43, p = 0.04$ ). However, it showed a significant positive correlation with abdomen fat % ( $r = 0.6, p = 0.006$ ), soft tissue % ( $r = 0.43, p = 0.04$ ), total fat mass % ( $r = 0.43, p = 0.04$ ), and fat/lean ratio ( $r = 0.43, p = 0.04$ ) (Figure 2). Dose of steroid showed a significant positive correlation with BMI SDS ( $r = 0.4, p = 0.02$ ).

#### Correlation between BMI, biochemical bone markers, and DXA parameters

BMI SDS showed a significant positive correlation with total fat % ( $r = 0.7, p = 0.001$ ), fat/lean ratio ( $r = 0.72, p = 0.0001$ ), and soft tissue % ( $r = 0.7, p = 0.0001$ ). Accordingly, BMI SDS showed a negative correlation with lean mass % ( $r = -0.7, p = 0.0001$ ) and lean/fat ratio ( $r = -0.6, p = 0.004$ ). Bone mineral density was not diminished in the distal femur (femur Z-score =  $-0.184 \pm 0.66$ ) and lumbar spine (vertebral Z-score =  $0.28 \pm 0.76$ ). No correlation was found between biochemical bone markers and any of the DXA parameters except for osteoprotegerin, a marker of bone formation, which showed a positive correlation with femur Z-score ( $r = 1, p = 0.00001$ ) and vertebral Z-score ( $r = 1, p = 0.00001$ ).

#### Discussion

In children with congenital adrenal hyperplasia, concerns have been raised that glucocorticoid replacement therapy might reduce growth and final height and might predispose to later osteoporosis [2, 16]. In this study, we have shown that children and adolescents with CAH on long-term steroid therapy have normal bone mineral density; however, they have low serum osteocalcin, osteoprotegerin, and procollagen peptide of type 1 collagen and high serum RANKL levels, i.e. our patients have low bone formation markers and high bone resorption markers (Table II). We have found that vertebral Z-scores of our patients, which is a sensitive indicator to the effect of steroid therapy [23], are considered normal according to the World Health Organization [24]. Normal BMD in CAH patients has been reported earlier by other authors [13, 25, 26], who found that adolescents with CAH had BMD values comparable with controls, despite glucocorticoid treatment. These authors hypothesized that negative effects of glucocorticoids on bone could be balanced by positive effects of androgens. Evidently, this may be true as long as glucocorticoid doses in CAH patients are in the low currently suggested dose [27]. Although our patients have low bone turnover, they have normal BMD. The normal bone status may be explained by the differential effects of glucocorticoids on growing bone, beneficial androgen effects, and other disease-specific factors [25]. The normal BMD that we observed in our patients does not rule out that these patients may develop glucocorticoid-induced osteoporosis at an older age. Lower lumbar and femoral neck BMD scores than controls were found in patients who had completed puberty [9]. As regards the bone turnover markers and their relation to BMD, our data are in agreement with Guo *et al.* [12], who found that BMD was not significantly decreased and that bone turnover was decreased in congenital adrenal hyperplasia patients. However, they measured serum osteocalcin and bone alkaline phosphatase to assess bone formation, whereas serum tartrate-resistant acid phosphatase and urinary cross-linked N-telopeptides of type I collagen were used to assess bone resorption. Also Sciannambo *et al.* [28] found that bone resorption markers were higher in patients with CAH.

In our clinic a protocol of regular monitoring of steroid dose, growth, serum 17OH-progesterone, testosterone, and plasma renin activity were followed. The average dose of hydrocortisone and mineralocorticoid was  $15.5 \pm 3.68 \text{ mg/M}^2/\text{day}$  and  $0.11 \pm 4.1 \cdot 10^{-2} \text{ } \mu\text{g}/\text{day}$ , respectively. Although we had patients with different control states as previously mentioned, no significant difference between these groups regarding their BMD, BMC, body

composition, and bone turnover markers was found. Moreover, the dose of steroid replacement did not show any significant relation to BMD or BMC or any of the bone turnover markers. Girgis and Winter [1] found no significant difference between the three groups of metabolic control (tight, fair, and bad) regarding their vertebral BMD and serum osteocalcin, procollagen peptide, or collagen C-terminal telopeptide, or in urinary amino-terminal telopeptide. Mora *et al.* [13], Fleischman *et al.* [25], and Sciannamblo *et al.* [28] found that BMD measurements and bone turnover markers did not correlate with actual glucocorticoid dose or mean dose over the previous 7 years, which is consistent with our results. However, when glucocorticoids are given at higher doses, this will cause increased bone resorption and inhibition of bone formation, leading to reduced BMD. Previous reports showed that decreased BMD in adult CAH patients was associated with glucocorticoid overdosing [9, 10]. Bachelot *et al.* [29] found that hydrocortisone dose was negatively correlated with the BMD T-score at the femoral neck.

As in earlier studies, we found that BMI was significantly higher in CAH patients compared with controls [10, 21, 29]. As regards body composition, our patients showed higher fat content than controls, which is related to the dose of steroid and duration of treatment. The increased fat mass could be the result of glucocorticoid use; other possible explanations for the increased fat mass could be adrenomedullary dysfunction with decreased catecholamine secretion, as recently described in CAH patients. Additionally, children with CAH had increased leptin levels and decreased insulin sensitivity, which has been ascribed to their adrenomedullary dysfunction.

In conclusion, children and adolescents with congenital adrenal hyperplasia have low bone turnover, normal BMD, high body fat content, and low lean mass. We recommend follow-up of the bone mineral density of these patients as they might be predisposed to later osteoporosis. In addition, we recommend further studies to clarify the role of calcium supplementation in CAH patients and study the diet provided to these patients to avoid overweight and obesity.

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