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Effect of DHEA supplementation on serum IGF-1, osteocalcin, and bone mineral density in postmenopausal, glucocorticoid-treated women

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ABSTRACT

Purpose: DHEA therapy increases bone formation in postmenopausal women. We have found only a few reports of dehydroepiandrosterone replacement therapy in women receiving long-term glucocorticoid medication. The purpose of this study was to establish whether DHEA replacement therapy may be useful in the treatment of steroid-induced osteoporosis in postmenopausal women.

Materials and Methods: Nineteen women, aged 50-78 years, treated at least for three years with average daily doses of more than 7.5 mg prednisone, with T-score L2/L4<-1.5 and bisphosphonates intolerance, were enrolled to the study. For the first year of the study the patients were given calcium, vitamin D3 and thiazide diuretics. For another year the patients received orally micronized DHEA 25-50 mg daily. Before the study, after twelve months of Calcium/D3 therapy, then after six weeks and six months of DHEA therapy, serum concentrations of DHEAS, androstenedione, testosterone, estradiol, FSH, IGF-1 and osteocalcin were assessed. Bone mineral density (BMD) in lumbar spine and femoral neck was measured before the treatment, after a year on Calcium/D3 and after six and twelve months of DHEA replacement therapy.

Results: In all treated women, DHEA significantly increased serum DHEAS, androstenedione and testosterone concentrations. A significant elevation of serum IGF-1 and osteocalcin concentrations was found as early as after six weeks of DHEA treatment. A significant increase of bone mineral density in the lumbar spine and femoral neck was observed after six and twelve months of DHEA treatment.

Conclusion: Our results suggest a beneficial role of DHEA replacement therapy in the treatment of steroid-induced osteoporosis.

Key words: Glucocorticoid-induced osteoporosis, Dehydroepiandrosterone

INTRODUCTION

Glucocorticoid-induced osteoporosis is one of the most important adverse effects of long-term corticotherapy. Strong inhibition of bone formation develops during the treatment with anti-inflammatory doses of steroids, so antiresorptive treatment may be insufficient to prevent fractures. Thus, it would be important to develop a drug exerting anabolic effects and stimulating osteoblast activity. The hormones that could play such a role are as follows: synthetic anabolic steroids, parathormone (PTH), growth hormone (GH) and dehydroepiandrosterone (DHEA) [1-3].

Dehydroepiandrosterone (DHEA) is a weak adrenal androgen, produced in the adrenal cortex. Osteoblasts probably have a receptor for DHEA [4], while in the cytoplasm sulphatase was detected, that transforms DHEA sulphate (DHEAS) into DHEA [5]. DHEA increases production of Transforming growth factor-β_2 (TGF-β_2) in human osteoblasts, which is an evidence of stimulation of their activity [6]. In these cells dehydroepiandrosterone is converted into testosterone, estradiol and estrone [7]. Nawata *et al.* [8] found a positive correlation between serum DHEAS concentration and bone mineral density in the femoral neck. Szathmari *et al.* [9] demonstrated that patients with osteoporosis and osteopenia without other

risk factors had low serum DHEAS concentrations. In patients with SLE or rheumatoid arthritis a positive correlation between BMD and serum DHEAS concentration was found [10, 11].

Administration of DHEA to castrated female rats decreased the rate of bone mass loss [12]. In women with Addison's disease, with extremely low serum DHEAS, lower bone mineral density was found than in their healthy peers [13]. Two studies show, that bone mineral density increases after DHEA treatment in Addisonic patients, however they was performed on small groups of patients [14, 15]. In healthy postmenopausal women DHEA decreased bone resorption, enhanced osteoblasts activity and, finally, increased bone mass, [16]. Sun *et al.* [17] reported BMD increase, during administration of dehydroepiandrosterone to men with osteoporosis, however further studies failed to confirm this beneficial effect [18]. Jankowski and co-workers [19] demonstrated that DHEA replacement therapy for 1 year improved hip and spine BMD in older women but only hip BMD in older men .They assumed that these changes were due to increase of estrogens concentrations [20]. 100-200 mg of DHEA daily increased bone mass in women with SLE, treated with glucocorticoids [21], but such high doses of dehydroepiandrosterone may lead to excess of androgens and side effects of hyperandrogenism.

The above cited reports of DHEA effects on bone metabolism induced us to make an attempt at DHEA replacement therapy in women treated with anti-inflammatory doses of glucocorticoids. We aimed to establish the effect of dehydroepiandrosterone supplementation on bone mineral density as well as on rate of bone formation (concentrations of osteocalcin) and IGF-1 in postmenopausal women treated with anti-inflammatory doses of glucocorticoids.

MATERIAL AND METHODS

Subjects

The study included 19 women receiving long-term glucocorticoid treatment, aged 50-78 years, at least two years after the menopause (2-30 years; 16.8 ± 8.1 years on the average). They were recruited from the endocrinology outpatient clinic of our Center.

The patients were treated with prednisone, 7.5-15 mg daily (9.1±2.4 mg) for at least three years before enrolment into the study. The duration of the steroid therapy was 3-21 years (7.5±4.6). Cumulative dose of steroids varied from 6000 to 80 000 mg prednisolone (28120 ±22600 mg) The indications to steroid treatment included chronic obstructive lung disease (10 patients), rheumatoid arthritis (8 cases) and systemic sclerosis (1 case). Patients with hyperthyroidism, hyperparathyroidism, osteomalacia and malignancies (at present or in the past) were not enrolled into the study. The body weight of the studied women was 50-80 kg (59.6±8.4 kg), and body mass index (BMI) was 18.8-30.4 kg/m^2 (23.7±3.7 kg/m^2). None of the studied subjects smoked tobacco, was on diet with reduced amount of calories consumed, drank more than one glass of wine per week.

During the steroid therapy, before enrolment into the study, nine women suffered compression fractures of vertebral bodies in the thoracic and lumbar segments, while in one patient hip fracture and in another forearm and vertebral body fractures occurred.

In all patients glucocorticoid-induced osteoporosis was diagnosed, according to criteria of UK 1998 Consensus Group [22]. The women had previous history of gastrointestinal disorders and then refused treatment with oral bisphosphonates.

All patients were informed about the purpose and method of the study and gave their written consent. The study protocol was approved by the Bioethical Committee at the Medical Centre for Postgraduate Education.

Method of therapy

1. In the first year of the study all studied patients were given:
   a. calcium carbonate in 1000-2000 mg daily dose (400-800 mg of elemental calcium, respectively), depending on the declared consumption of dairy products (diet questionnaire). The goal was to achieve 1500 mg elementary calcium intake.
   b. alfacalcidiol 0.5 µg daily;
   c. in cases of calciuria > 300 mg daily, hydrochlorothiazide 12.5-25 mg daily was administered (11 patients). Calciuria after this medication were reduced to value above 250 mg daily in all treated patients.
2. After a year, micronized DHEA (*Natural Nutrition, USA*) 50 mg was added to the drugs used as yet = two 25 mg capsules daily, directly after breakfast – between 8:00 and 9:00 a.m.
3. After six weeks of the treatment the dose administered was reduced by half (25 mg daily) if DHEAS serum concentration exceeded 4000 ng/ml. Following the same rule, the dose was reduced after six months.

Measurements

Before the study, after the twelve months of Calcium/D_3 treatment, than after six weeks and six months of DHEA therapy, serum concentrations of DHEAS, androstenedione, testosterone, IGF-1 and osteocalcin were assessed.

Bone mineral density (BMD) of lumbar spine (L2/L4) and femoral neck was assessed before the study, after twelve months of Calcium/ D_3 phase, and after the six and twelve months of DHEA therapy (18 and 24 months of the study).

Method of serum hormone concentrations measurement

Blood for the tests was withdrawn between 11:00 and 12:00 a.m.

DHEAS, Androstenedione, Testosterone concentration was determined by radioimmunoassay method using Spectria kits, *Orion Diagnostica.*. The ranges of normal values for

Table 1. **Serum hormones and BMD after Ca/D3 alone and after DHEA treatment.**

	Start of the study	12 months Ca/D3 (start of the DHEA)	6 weeks DHEA	6 months DHEA
DHEAS [ng/dl]	132 ±55.4	142.7 ±63,5	3943.9 ±2200.8 **	2845.7 ±1084.6**
Androstenedione [ng/dl]	38.2 ±23.6	36.7 ±27.4	270.3 ±119.4 **	177.5 ±128.9 **
Testosterone [ng/ml]	0.2 ±0.07	0.21 ±0.08	0.52 ±0.24 **	0.41 ±0.16 **
IGF-1 [mg/l]GF-1	100.6 ±29.1	102.0 ±28.9	173.5 ±68.3 *	213.5 ±69 *
OC [ng/ml]	11.3 ±6.0	9.6 ±4.9	17.4 ±4.8 ##	20.2 ± 7.1 ##
BMD L2/L4 [g/cm^2]	0,85 ±0.11	0.82 ±0.2 #	0.83 ±0.12 #	0.85 ± 0.12 ##
BMD f neck [g/cm^2]	0.73 ±0.11	0.7 ±0.12 #	0.71 ±1.85 #	0.73 ± 0.12 ##

# p<0.01 ## p<0.001 * p<0.0001 ** p<0.00001

women were 700-3900 ng/ml (DHEAS), 85-275 ng/dl (androstenedione) and 0.3-1.1 ng/ml (testosterone).

IGF-1 concentration was determined by radioimmunoassay method using SM-C-RIA-CT kits, *Biosource*. The range of normal values for postmenopausal women was 66-278 ng/ml. Osteocalcin concentration was determined by immunoradiometric method using Elsa-Osteo kits. The range of normal values for postmenopausal women was 8.0-55.9 ng/ml.

Method of BMD assessment

The determination of bone mineral density (BMD) was carried out by dual-energy X-ray absorptiometry (DXA) using Lunar DPX equipment (*Lunar Corporation, Madison, USA)*, in the lumbar spine (L2-L4) and the femoral neck. The coefficient of variance for the above mentioned method was 0.5-1.0%. The measuring error measured for our Densitometry Unit and technician was 0.7-1.0%. The lowest significant change (LSC) for the laboratory was: 1.9% for the measurement at L2/L4 site and 2.8% in the case of the femoral neck (at 95% confidence interval).

Results were expressed in absolute values – g/cm^2 and as T-score. Bone mineral density changes after a given therapeutic period were calculated as percent of the baseline value.

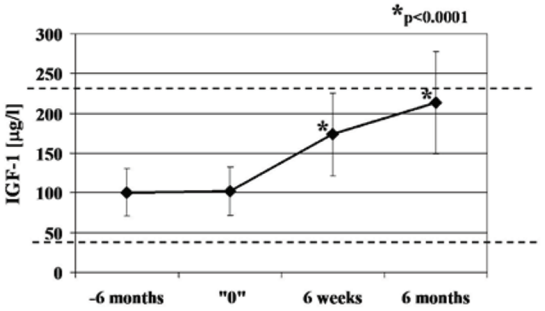
Statistical analysis

The results are presented as mean values ± standard error (SE). The normal distribution of variables in the groups was assessed by the Shapiro-Wilk test. The concentrations of the hormones before and after the treatment were assessed by the ANOVA test.

The changes of bone mineral density after a year of Calcium and 1OHD$_3$ administration, and after six months and one year of DHEA treatment were expressed as percent of the baseline value, calculating it following the formula: ΔBMD = (BMD" – BMD') : BMD'. Then, the values corresponding to BMD changes were compared using the ANOVA test.

The values p<0.05 were accepted as statistically significant.

Figure 1. **Serum IGF-1 after the 6 weeks and 6 months of the DHEA treatment.**



RESULTS

The baseline DHEAS concentrations were extremely low: 130.2 ±55.4 ng/ml before the treatment and 142.7 ±63.5 ng/ml after the Ca/D$_3$. DHEAS concentrations were negatively correlated with duration of steroid therapy (r= -0.84 p=0.02) and cumulative dose of steroids (r= - 0.76 p=0.03).

No significant changes were found after the Calcium/D$_3$ treatment in DHEAS, androstenedione, testosterone, IGF-1 and osteocalcin concentrations (*Tab. 1*).

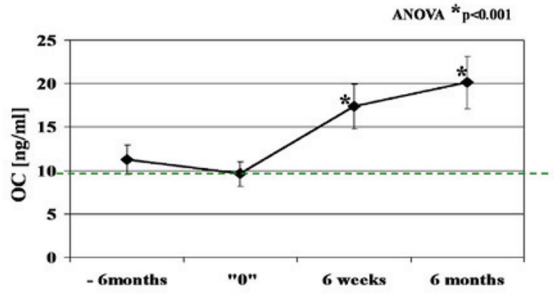
In all treated women, DHEA replacement therapy caused a significant increase of serum DHEAS, androstenedione and testosterone concentration after six weeks and six months of the treatment (*Tab. 1*).

Serum IGF-1 concentrations during the Ca/D3 treatment were stable (100.6±29.1 before and 102.0±28.9 µg/l after the 12 months). During DHEA therapy, IGF-1 concentration increased to 173.5±68.3 µg/l after six weeks and to 213.5±69 µg/l after six months of the treatment (p<0.0001) (*Fig. 1*). IGF-1 concentrations were positively correlated with DHEAS concentrations (r = 0.8 p = 0.03 after 6 weeks and r=0.88 p=0.009 after 6 months of the treatment).

Serum osteocalcin (OC) concentrations during the Ca/D3 treatment were low and stable (11.3±6.0 ng/ml and 9.6±4.9 ng/ml). During the DHEA treatment, OC concentration increased to 17.4±4.8 ng/ml after six weeks and to 20.2±7.1 ng/ml after six months (p<0.001). (*Fig. 2*)

The initial bone mass in all patients was at least 2 SD below the mean peak bone mass in at least one site of axial

Figure 2. **Serum Osteocalcin after the 6 weeks and 6 months of the DHEA treatment.**



skeleton. After a year of Calcium/D_3, bone mineral density decreased 3.45±2.78% in L2/L4 ($p< 0.001$) and 3.86±3.85% in the femoral neck ($p< 0.01$). Individual changes varied from -6.8% to -0.3% of basal values in L2/L4 and from -9.2% to 0.3% of basal values in femoral neck. There was no difference between groups on hydrochlorothiazide (n=11) and without diuretic (n=8).

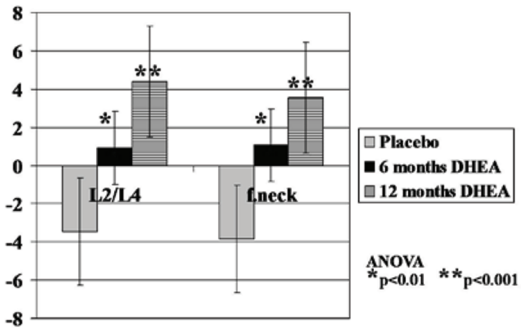
After DHEA replacement therapy the following was found:

a. After six months BMD increased: in lumbar spine 0.91±1.85% ($p<0.001$) and 1.07±1.85% in the femoral neck ($p< 0.01$), as compared to the Ca/D3 phase. Individual changes varied from -2% to 4.5% of basal values in L2/L4 and from -2.9% to 5.1% of basal values in femoral neck.

b. After twelve months BMD increased: in lumbar spine 4.39±3.05% and in the femoral neck 3.55±3.05% of the baseline value ($p<0.001$, as compared to the Ca/D3 phase). (BMD changes - see figure 3) Individual changes varied from 0.9% to 10.2 % of basal values for L2/L4 and from -0.9% to 10.5% of basal values in femoral neck. Seventeen women were treatment responders, achieving significant BMD gain in both sites (11 women) in L2/L4 (5 women) or in f. neck alone (1 women). In two remaining women we observed slight BMD increase after the one year of DHEA comparing to the year of D3/Ca treatment, however in was below the values of LSC. BMD gain was positively correlated with osteocalcin concentrations after the 6 weeks and 6 months of the treatment in femoral neck ($r=0.77$ $p=0.04$ and $r=0.94$ $p=0.002$), but not in lumbar spine. Response to DHEA was independent neither of age nor steroid cumulative and daily doses.

The tolerance of the treatment in most patients was good. In two patients a slight hirsutism developed, which regressed after dose reduction. Two studied subjects reported difficulties in falling asleep. In none of patients any significant exacerbation of the underlying disease was observed. Medical examinations and history taking, repeated during follow-up visits failed to reveal any significant abnormalities. No changes in standard blood examination results were found.

Figure 3. **Bone mineral density (BMD)- changes in % of initial values after the 6 and 12 months of the DHEA treatment.**



DISCUSSION

DHEA replacement therapy was effective and well tolerated. It restored normal DHEAS concentrations with only a moderate increase of serum testosterone. It increased also IGF-1 concentration what evidenced stimulation of anabolic processes, and osteocalcin concentration what reflected increased activity of the osteoblasts. The treatment caused a significant bone mineral density gain.

DHEA treatment is a potentially beneficial medication but the effects described in literature are frequently different in various groups of patients. Patients, qualified for some studies had DHEAS serum concentration exceeding even 1000 ng/ml, that is, hadn't deficit of this hormone at all. In our study, DHEAS concentrations were below 200 ng/ml. These women had profound DHEA deficit at the start point of the study, and in our opinion just therefore responded to the treatment.

In studies on DHEA administration in humans, described in literature, varying doses of this hormone were used. Doses higher than 50 mg daily caused, however, a non-physiological increase of serum testosterone in women and of estradiol in men [23-25]. In our study we used initially 50 mg dose. In 15 patients (in nine after six weeks, and in six after six months) the dose was reduced to 25 mg due to high (exceeding 4000 ng/ml) serum DHEAS concentrations. These non-physiological DHEAS concentrations in some women were connected with hirsutism and seborrhoea. Surprisingly, testosterone serum concentrations were in normal range in spite of "androgenic" adverse effects observed in such cases.

In our study, a significant increase of IGF-1 concentration was observed. The plausible mechanism of this changes may be increase of estrogens and androgens levels in tissues – the both hormones increases IGF-1 concentrations. The other explanation is direct agonistic action of DHEA on oestrogen receptor [26, 27].

IGF-1 increases the activity of osteoblasts [28]. An increase of IGF-1 concentration due to DHEA therapy was noted by Casson *et al.* [29] and Villareal *et al.* [30]. On the other hand, no further elevation of IGF-1 due to DHEA therapy was

observed in women with hypopituitarism [31]. Arlt *et al.* [32], administering DHEA to patients with primary and secondary adrenocortical failure, found that IGF-1 increased only in patients with maintained hypophyseal function [32]. Long-term steroid therapy decreases serum IGF-1 concentration [33]. In the studied group of women, the baseline IGF-1 level was very low. In all women, without exception, an increase of IGF-1 serum concentration was already seen after six weeks of the treatment. It was positively correlated with serum DHEAS level. Haden noted a similar relationship between concentrations of endogenous DHEAS and IGF-1 in women [34]. In our study, the concentration of the main IGF-1-binding protein, i.e. IGFBP-3 was not assessed. Casson demonstrated that during DHEA replacement therapy, IGF-1 concentration increase was accompanied by a decrease of serum IGFBP-3 level what additionally increased the free fraction of insulin-like growth factor [29].

Increasing level of IGF-1 during DHEA replacement therapy can exert a number of beneficial anabolic effects. It prevents catabolic action of high glucocorticoid doses, inhibits degradation of matrix proteins and stimulates bone formation [35]. The stimulation of bone formation is extremely important for treatment of steroid-induced osteoporosis, since too low activity of osteoblasts can be the cause of therapeutic failure during administration of drugs exerting antiresorptive effect [36].

Osteocalcin is a highly-specific marker of osteoblastic activity, because it is produced only by the active osteoblasts. The baseline concentration of osteocalcin in our patients was very low. It was in concordance with literature reports, in which slowing-down of bone formation was described as an element of bone changes during steroid treatment [37, 38]. Since serum level of osteocalcin demonstrates circadian fluctuations, 30% of the baseline value is accepted as the minimal significant change (MSC). In this study, the mean osteocalcin concentration increased; the increment exceeded two and three times the MSC value. The increase of osteocalcin level is the evidence of stimulation of osteoblasts activity. Rate of bone resorption in glucocorticoid-treatment patients can vary from normal to very high - depending on the time of corticotherapy, menopausal status, calcium/vitamin D substitution etc. Pre-treatment concentrations of resorption marker (NTx) in our group were normal or slightly elevated (data not included), we didn't repeat these measurements during the treatment, because our goal was to established the changes in bone formation, not resorption rate.

Bone mineral density loss, most dramatic in the first year of steroid therapy is usually maintained in the successive years of its duration. In this study, administration of vitamin D_3 active metabolites and calcium for a year failed to prevent bone loss. After one year of treatment with DHEA, in all patients BMD increase, that reached 4.39% of the baseline value in the lumbar spine and 3.55% in the femoral neck was seen.

According to evidence-based medicine (EBM), in order to accept a drug as an antiosteoporotic agent, its effectiveness has to be proven in preventing fractures in a prospective, placebo-controlled trial with adequately large group. The first limitation of our study is its cross-sectional design: each patient served as control for herself. The other is that the study group was too small for statistical analysis of reduction of the number of fractures. However, the treatment described above could restore the physiological equilibrium of bone metabolism owing to stimulation of activity of osteoblasts. Dehydroepiandrosterone can be administered orally, it is well tolerated and causes no irritation of the upper segment of the gastrointestinal tract. Other possible favorable effects of DHEA, such as antidepressant activity, prevention of catabolic effect of high steroid doses on the skin and muscles, and improvement of cell-mediated immunity also encourage administration of the described above therapy [39].

CONCLUSIONS

We hope that the results of this study, demonstrating IGF-1 concentration increase, stimulation of bone formation, and significant bone mineral density gain can be the foundation for further studies on the use of substitution DHEA doses in patient with steroid-induced osteoporosis.

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