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Declining Adrenal Androgens: An Association with Bone Loss in Aging Women (42625)

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Abstract. Bone loss in aging women is a major contributing factor to the onset of osteoporosis. To determine whether a decline in adrenal androgen output might be important in the loss of bone with age, we studied a highly selected group of 14 women, average age 70, and measured adrenal androgens in relationship to trabecular bone density. Dehydroepiandrosterone sulfate (DHEAS) levels were used as a marker of adrenal sex steroid output while quantitative, computerized tomography was used to determine trabecular bone density. Our results showed that both bone density ($r = -0.69$, $P < 0.01$) and DHEAS levels ($r = -0.68$, $P < 0.01$) declined with age, and that DHEAS was positively correlated with bone density ($r = 0.66$, $P = 0.01$). These data emphasize the association of declining adrenal sex steroid production with declining bone density during the process of aging.

Over 40 years ago Fuller Albright suggested that a decline in endocrine function may contribute to the phenomenon of skeletal fragility in elderly women (1). While the relationship of inadequate estrogen production in the menopause to declining bone mass is well established (2, 3), Albright's suggestion that a fall in the secretion of a "nitrogen retaining adrenal hormone" can also affect bone mass has until now received relatively little investigative attention.

We sought to examine if a relationship exists between age, a declining output of adrenal sex steroids, and bone density in a highly selected population of aged women who were at least 5 years beyond their menopause. These observations may lend credence to the suggestion that alterations in adrenal androgen output might be a contributing factor to changes in bone mass.

Since the sources of sex steroids may be gonadal, adrenal, or extraglandular in origin, and since in the menopausal female the predominant source of testosterone is the ovary (4) while androstenedione (A) comes from either the ovary or the adrenal (5), an assessment of adrenal function was determined through the measurement of cortisol and the major circulating adrenal androgen dehydroepiandrosterone sulfate (DHEAS). We reasoned that because cortisol concentrations do not diminish significantly with age (6), DHEAS would be an appropriate marker of declining adrenal androgen production.

Materials and Methods. *Subjects.* Fourteen Caucasian women of average age 70 years (range 55 to 85 years) were selected from the Bone Metabolism Clinic population of the Hershey Medical Center after meticulous screening to exclude any circumstance known to influence bone mass, calcium homeostasis, or sex steroid metabolism. Patients were referred for evaluation prior to intitiating hormonal or nonhormonal therapy for symptoms of the menopause. All patients had to be at least 5 years past menopause, living in independent homes, and ambulatory. Thirteen women had passed through a natural menopause, and one had been ovariectomized 40 years previously. Serum creatinine, calcium, phosphorus, alkaline phosphatase, thyroxine, and TSH were within the normal range. Exclusion criteria also included prior history of any smoking, excessive alcohol ingestion or abuse, ingestion of any sex steroids for any purpose, history of use of fluoride, calcitriol, pharmacologic doses of vitamin D, diuretics, anticonvulsants, or glucocorticoids. No evidence of any endocrine disorder, currently detectable neoplasia, or current use of other medications was allowed. One patient, on rereview of the data, was found to have diet-controlled diabetes mellitus, a bone density

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of 136 mg/ml, and no evidence of fracture as determined by plain films and was thus not deleted from analysis post facto. All procedures used in this study were approved by our Institutional Clinical Investigation Committee.

Measurements. Sex steroids and cortisol were measured in the Core Endocrine Laboratory of the Milton S. Hershey Medical Center. Blood was collected between 0800 and 1000 hr following an overnight fast. Serum obtained by centrifugation was drawn off and frozen at −70°C until assay. All samples were assayed in the same batch to eliminate interassay variation. Dehydroepiandrosterone sulfate (DHEAS) was measured by direct radioimmunoassay using material purchased from Diagnostic Products (Los Angeles, CA) (7). Interassay variability was 8% at an average concentration of 2380 mg/ml. The low-end sensitivity is 50 mg/ml. Androstenedione was measured by RIA following ether extraction and celite chromatography using material purchased from Radioassay Systems Laboratory (Carson, CA) (7, 8). Intraassay variability was 8% and interassay variability was 11% at an average concentration of 1.3 mg/ml. Low-end sensitivity is 0.1 mg/ml.

Estradiol (E_2) was measured by RIA after ether extraction using specific antiserum (Diagnostic Products) (8). Interassay variation was 8% at 70 pg/ml. Low-end sensitivity is 10 pg/ml. Estrone was measured by RIA following ether extraction and celite chromatography (7, 8). Interassay variation was 4% at 50 pg/ml. The low-end sensitivity for the assay is 10 pg/ml.

Free and weakly bound testosterone was measured using the differential ammonium sulfate precipitation technique described by Stumpf *et al.* (9). Intraassay variability was 4% and interassay variability was 5% at an average concentration of 30%.

Cortisol determinations were performed by direct RIA using antibody-coated tubes purchased from Diagnostic Products. Intraassay variability was 4% and interassay variability was 6% at an average concentration 18 μg/ml. The low-end sensitivity is 1 μg/dl.

Calcium determinations were performed on a BECKMANASTRA chemical analyzer. Reference range is 8.4 – 10.6 mg/dl.

Trabecular bone density was measured by quantitative computerized tomography of the spine (10). The precision based on repeated measurements of a cadaver spine was 0.7% (11). The accuracy is reported to be 6% by ash weight and physical measurements (12, 13), and correlates well with dual-energy quantitative computerized tomography (14). It should be noted that negative values are occasionally detected as a function of the calibrations of standards at low bone density and the presence of bone marrow fat surrounding trabecular bone.

For purposes of this study, we decided to establish fracture threshold as a functional definition of osteopenia (11). Briefly, this involved evaluation of 96 women with careful documentation of bone density by quantitative computerized tomography of L_1, L_2, L_3, and fracture rate by anteroposterior and lateral radiographs of the thoracolumbar spine and age. Wider patient experience with 152 patients evaluated for bone density and 65 younger normal patients has confirmed the utility of the fracture threshold concept. Values less than 70 mg/ml have been considered to be below the fracture threshold; values between 70 and 100 mg/ml are considered to be at increased risk. Values between 100 and 120 mg/mL are considered to be at mild risk, values greater than 120 mg/ml are considered normal.

Percentage ideal body weight was calculated by dividing actual weight by ideal weight for height according to Metropolitan Life Table figures ×100.

Analysis. Statistical calculations were accomplished with the SPSS (15) and SAS (16) programs on an IBM 4381 computer. DHEAS values were subjected to a logarithmic transformation to achieve a normal distribution. The transformed DHEAS values were utilized in subsequent calculations. Relationships among variables were quantitated by Pearson product moment correlation and partial correlation.

Results. Descriptive statistics for the subjects are given in Table I.

Seven patients had bone densities below 70 mg/ml, which is considered to be the fracture threshold in our hands (11). Three patients had bone densities between 70 and 100 mg/ml and were considered to have a relative indication for prophylaxis. Three pa-

TABLE I. PHYSICAL CHARACTERISTICS AND MEAN STEROID LEVELS OF PATIENTS EVALUATED

	Age (years) (N = 14)	Years past menopause (N = 13)	% Ideal body wt (N = 14)	Bone density[a] (mg/ml) (N = 14)	Serum calcium (mg/ml) (N = 14)	E_1 (pg/ml) (N = 14)	DHEAS (μg/dl) (N = 14)	A (ng/dl) (N = 14)	FT (ng/dl) (N = 14)
Mean	70 ± 2.9	26 ± 3.5	104 ± 7.8	70 ± 9.9	9.35 ± .14	38.3 ± 4.3	49.1 ± 10.7	57.0 ± 13.3	25.0 ± 1
Range	55–85	6–41	77–170	−8.7–135	8.4–10.2	15–74	10.9–130.0	10.0–177.0	21.0–37.0

Note. All results are expressed as the means ± SEM.
[a] Trabecular bone density measured by CT scan.

tients had bone density values between 100 and 125 mg/ml and were considered for yearly bone density observations. One patient had a bone density of 136 mg/ml and was considered for serial observation at 2-year intervals. Five patients had one or more vertebral fractures as determined by plain films. Two of these patients had more than two fractures. All of these patients with demonstrable fractures had bone densities less than 70 mg/ml. The patient with diet-controlled diabetes had a bone density of 136 mg/ml, and no evidence of vertebral fracture.

The average age of evaluation was approximately 20 years past the median age of menopause in the United States. All FT, A, E_1, and E_2 levels were within reference range for postmenopausal women.

With increasing age, both DHEAS ($r = -0.68$, $P < 0.01$) and bone density ($r = -0.69$, $P < 0.01$) declined (Figs. 1 and 2). Likewise, years postmenopause correlated with bone density ($r = -0.64$, $P < 0.01$) and declining DHEAS ($r = -0.66$, $P < 0.01$). DHEAS was positively correlated with bone density ($r = 0.66$, $P = 0.01$) (Fig. 3). Androstenedione was also significantly associated with bone density ($r = 0.51$, $P < 0.05$) but not FT ($r = 0.21$, P = NS). Neither estradiol nor estrone correlated positively with bone density. The mean cortisol levels were reflective of the AM sampling times and were not found to correlate significantly with age.

When the effect of DHEAS was controlled by partial correlation, the correlation between age and bone density became nonsignificant ($r = -0.43$, $P > 0.05$). Consideration of squared correlation coefficients suggests that 48% of the variance in bone density could be explained by age; 18% of the variance remained after controlling for the effect of DHEAS. Controlling for the effect of A produced minimal change on the association between age and bone density ($r = -0.65$, $P = 0.01$). Likewise, when the effect of estrone was controlled by partial correlation, the correlation between age and bone density was not changed substantially ($r = -0.70$).

Discussion. Our results showed a significant correlation between adrenal androgen DHEAS and vertebral bone density in this select population of postmenopausal women. Our studies looked specifically at a highly selected population of women who were not influenced by the many homeosta-

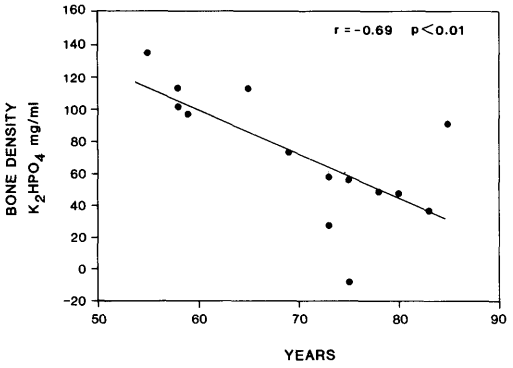

FIG. 1. Declining bone density with age.

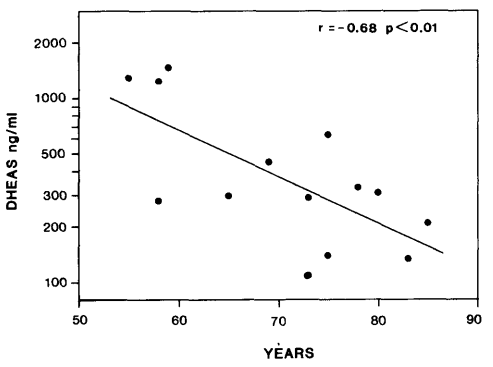

FIG. 2. Declining DHEAS with age.

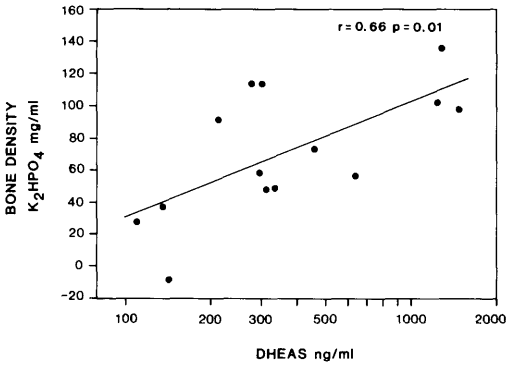

FIG. 3. The association of calculated bone density with plasma DHEAS.

tic factors which could affect bone density of adrenal steroid output. We selected patients at least 5 years after menopause to eliminate the perimenopausal fluctuations in ovarian sex steroid release. We eliminated patients with any abnormalities in calcium-regulating hormones, patients who had ever taken ovarian steroids, patients who had taken any medication known to influence bone or the adrenal, and patients who smoked. Although DHEAS was the most highly correlated adrenal androgen with bone density, androstenedione was also significantly correlated in this group of women. The association of bone density with DHEAS could not be accounted for by an indirect effect of estradiol or estrone as witnessed by the lack of an association of bone density wth estradiol and by a minimal effect of estrone as shown by partial correlation. Cortisol did not show a correlation with bone density nor was there a correlation of cortisol with age in these postmenopausal females. This suggests a greater association between the adrenal sex steroid pathway and the bone density than between the adrenal glucocorticoid pathway and the bone density.

In our patient population, it is unlikely that a gonadal source of steroid was contributing to the observed correlation between adrenal sex steroids and bone density. It has been reported that the postmenopausal ovary continues to secrete testosterone (4). In addition, the adrenal contribution to circulating androstenedione appears to be greater than the gonadal contribution in the postmenopausal female (5). Our analysis of free and weakly bound testosterone showed no correlation with bone density, and was not significantly correlated with age. It has been well established that DHEAS is almost exclusively of adrenal origin (17). Thus it is unlikely that the observed correlation of DHEAS and A was influenced by gonadal secretion.

We selected DHEAS as a marker of adrenal sex steroid activity for several reasons. DHEAS, the most abundant adrenal androgen secreted by the adrenal cortex, has a long half-life, and its clearance is not affected by age (18–20). It provides an accurate assessment of the long-term secretory function of the sex steroid component of the adrenal cortex (21, 22). DHEAS does not appear to be influenced by the same circadian fluctuations which affect cortisol (17, 18). Circulating DHEAS levels are age related in that they increase with adrenarche, reach a maximum in the second and third decade of life, and diminish during the fourth decade (23–25). Our results substantiate that DHEAS, unlike cortisol, continues to decline with advancing years. Likewise, bone density as measured by CT scan peaks at approximately age 20–30 and starts to diminish during the fourth decade of life (26). This cross-sectional and longitudinal parallelism raises the possibility that these phenomena are more than merely associated events.

The association between adrenal sex steroids as reflected by DHEAS levels and bone mass should be explored in much greater depth. Premature ovarian failure and ovariectomy in both younger or older women seems to precipitate a decline in circulating DHEAS levels (27). In that the adrenal cortex contributes more than 90% of peripheral DHEAS (28), this suggests an effect of oophorectomy or adrenal androgen production. While diminished estrogen production has been the traditional explanation for the associated osteopenia (29–31), the decline in adrenal sex steroids needs to be evaluated in light of its potential effect on bone density. An influence of adrenal sex steroids upon bone physiology has been inferred from observations during times of bone maturation. Virilizing adrenal adenomas and carcinomas presenting in childhood have been associated with striking degrees of skeletal advancement (32–35). Children with congenital adrenal hyperplasia have bone ages advanced for

chronological age (36, 37). When evaluating the effects of an analog of gonadotropin-releasing hormone (GNRHa) on patients with central precocious puberty, while gonadal function was inhibited, DHEAS levels were found to correlate with skeletal maturation (38). Clinical, pharmacological, and physiological data then suggest that adrenal sex steroids may affect the skeleton at the time of the onset as well as at the time of decline of adrenal sex steroid output during an individual's lifetime.

If it is affecting bone density, the exact physiologic role that DHEAS would play in bone metabolism is unclear. Although DHEAS in pharmacologic doses induces both androgenic (sebum production) and estrogenic (vaginal smears) activity in humans through enhanced substrate availability (39), under normal conditions the utilization of DHEAS for extraglandular formation of estrone is considered quantitatively insignificant (40). On the other hand, androgen metabolism occurs in bone (41). DHEAS, or another adrenal sex steroid, could be providing additional substrate availability for extra adrenal formation of more potent androgens (i.e., dihydrotestosterone). Either DHEAS or other adrenal sex steroids might also act directly or indirectly by altering the normal hormonal control mechanisms that affect bone metabolism.

Our findings are consistent with other observations that adrenal androgens are associated with bone density in menopausal women (42, 43). The significance of this association awaits further study. It is emphasized that association of DHEAS with bone density does not prove that adrenal androgens are significant in maintaining bone density for the reason that age and DHEAS are correlated. A study of matched patients according to age where adrenal androgens and bone density are evaluated needs to be performed. Further investigation into the etiological role that androgens might play in maintaining bone density is warranted.

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