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13. ABSTRACT *(Maximum 200 Words)*

Peak bone mass is achieved during young adulthood, accompanying marked rises in gonadal and adrenal steroids [e.g., dehydroepiandrosterone-sulphate, (DHEAS)] and insulin-like growth factors (IGF-1). In these studies, we will test the hypothesis that DHEA increases bone mass through anabolic (e.g.,IGF-1), and antiosteolytic (e.g., interleukins) mechanisms as follows:

*Specific Aim I--to measure the effects of DHEA on bone and the regulation of androgens, IGF-1, and cytokines in anorexic women who have increased fracture risk. Data in 36 women enrolled in year 1 showed subnormal serum levels of DHEAS, IGF-I, and bone formation markers, and elevated urinary bone resorption markers. Low levels of DHEA-S were associated with elevated bone resorption markers.

* Specific Aim II--to measure the effects of DHEA on bone, cytokines, and growth factors in women with accelerated bone loss from treatment with gonadotropin-releasing hormone agonist (GnRH); assays are being pooled for measurement in subsequent years.

* Specific Aim III--to determine whether cytokine production and/or osteoclastogenesis is downregulated by DHEA and/or gonadal steroids. The studies from human marrow cultures revealed that testosterone and DHEA suppress production of IL-6. These clinical and basic studies will provide new information on the mechanisms whereby DHEA and sex steroids affect bone.

14. SUBJECT TERMS
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Meryl S. LeBoff, M.D. 9/25/97

PI - Signature Date

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(5) INTRODUCTION

Osteoporosis is a major public health problem. Some 200 million people worldwide, including approximately 28 million Americans, are at risk for or suffer from osteoporosis. Currently there are gaps in our knowledge of how to maximize peak bone mass. The dramatic rise in DHEA and insulin-like growth factor (IGF) levels during adolescence and bone accretion, the age-related decline in levels of dehydroepiandrosterone (DHEA) and insulin-like growth factors (IGF), and evidence that DHEA and IGF levels are positively correlated with bone density suggest that DHEA and IGF may have a key role in the development of peak bone mass. Adolescents and young adults with anorexia nervosa have reduced peak bone mass and an increased risk of osteoporotic fractures; subnormal DHEA and IGF levels may be linked to their reduced bone mass. In addition, according to cross-sectional studies, DHEA and its sulfated derivative are lower in osteoporotic than nonosteoporotic women. To gain new information about the mechanisms underlying skeletal accretion in young adults, to develop safe therapies to optimize skeletal health and prevent stress fractures in young military recruits, in this program of clinical and basic research studies we are testing the hypothesis that DHEA may increase bone mass through anabolic and antiosteolytic mechanisms.

(6) BODY

SPECIFIC AIM I: To measure the effects of DHEA on bone mass and circulating estrogens, androgens, IGF-I and cytokines in young patients with anorexia nervosa.

Adolescents and young adults with anorexia have reduced peak bone mass and an increased risk of osteoporotic fractures (1,2). For year 1 of this grant, we have completed enrollment of 36 patients with anorexia nervosa (AN) out of a planned total sample of 60 patients over 4 years. Our enrollment during this time interval exceeded our expectations. Only two participants have discontinued the study, for reasons unrelated to either the study medication (micronized DHEA) or the protocol. No adverse side effects have been noted in any subject. At baseline and 3-month intervals, a physical examination is performed, and heights and weights are obtained. Patients subsequently have vital signs, and a blood and urine sample obtained for hormonal parameters, cytokines, and growth factors. At baseline, 6, and 12 months, bone mineral density and body composition measurements are performed.

Baseline data obtained from this longitudinal protocol to date are summarized in an abstract that will be presented at the annual meeting of the American Society for Bone and Mineral Research on October 2, 1999 in St. Louis, MO (see attached). The mean age of the subjects was 18.4 ± 3.2 years. The median duration of anorexia nervosa (AN) was 22 months (range 4-96) and duration of amenorrhea was 15 months (range 3-90) (3).

As shown in Table I, we found subnormal baseline levels of DHEA, DHEA-S, IGF-I, and the bone formation markers, osteocalcin and bone specific alkaline phosphatase, and elevated urinary levels of N-telopeptides (NTx).

TABLE I:
Baseline levels of DHEA(S), IGF-I and Bone Turnover Markers

Variable	Median	Range	Normal range or mean for age
DHEA (ng/dl)	325	97-657	540
DHEAS (mcg/dl)	205	55-407	235
IGF-I (ng/ml)	173	52-372	460
Osteocalcin (ng/ml)	14	8.6-43	42-225
BSAP (ng/ml)	13	4.6-30	28-38
NTx (nmol/mmol Cr)	80	35-156	13-65

Correlation analyses using Spearman rank coefficients revealed significant relationships between the baseline variables shown in Table II below:

TABLE II:
Correlation Analyses on Baseline Variables (Spearman rank coefficients)

Variables	r	Significance level
DHEA and duration of amenorrhea	-0.42	p=0.015 *
DHEAS and duration of AN	-0.37	p=0.036 *
DHEAS and urinary NTx	-0.42	p=0.015 *
Weight and IGF-I	0.34	p=0.025 *
Age and urinary NTx	-0.42	p=0.018 *
Age and osteocalcin	-0.53	p=0.016 *
* p< 0.05		

These results confirm our earlier pilot data that patients with AN had low DHEA(S) and IGF-I levels, accompanied by both subnormal levels of bone formation markers and elevated levels of bone resorption markers. Importantly, low levels of DHEA-S were associated with high levels of bone resorption markers and duration of AN. NTx and osteocalcin levels decreased with increasing age in these young women, possibly as a result of a decrease in bone turnover after the attainment of peak bone mass.

As we are still blinded as to the treatment status of our study subjects, we are unable to comment on the effect of DHEA therapy on IGF-I and cytokine levels. Analysis of baseline cytokine levels from our first 36 patients revealed undetectable IL-6 levels. This was an unexpected finding that we are in the process of analyzing further. Patients with anorexia nervosa have very low sedimentation rates and low levels of this cytokine may reflect an overall decreased inflammatory state. We will also measure serum levels of interleukin (IL)-1β and IL-11, and tumor necrosis factor (TNF)-α, other cytokines that have been shown to stimulate bone resorption in in vivo and in vitro experiments.

SPECIFIC AIM II. To measure the effects of DHEA on bone, cytokines, and growth factors in young women with accelerated bone loss from treatment with gonadotropin-releasing hormone agonist (GnRH agonist).

Patients treated with GnRH agonist show acute bone loss (4,5). In year 1 of this grant, we enrolled 10 patients and we are actively screening an additional 3 patients with endometriosis treated with GnRH agonist. Advertisements were placed in newspapers, Brigham and Women's Hospital bulletins, and the Endometriosis Association (an association comprised of patients with endometriosis and Obstetrics/Gynecology practitioners), and the internet. Recruitment is also done through clinics and presentations. Questionnaires on emotional well-being, physical activity, calcium intake and endometriosis symptoms have been administered in accordance with the study design. An additional symptom score questionnaire has been modified for follow-up appointments. Physical examinations, vital signs, bone density of the spine, proximal femur and total body and body composition are measured on all enrolled subjects at baseline, 1, 3, 6, and/or 12 months. Laboratory tests for hormonal assays, growth factors, cytokines, and safety parameters are measured at baseline and subsequent follow-up visits at 1, 3, 6, and 12 months after baseline. Adverse events were closely monitored and promptly reported to Brigham and Women's Hospital Institutional Review Board. Events were also reported to the Department of Defense on a monthly basis. One serious adverse event of vertigo that was *not* related to possible use of DHEA was reported as requested within 48 hours to the Department of Defense; the patient underwent extensive evaluation of this symptom . These events were all reviewed by the medical monitor, Dr. Marc Laufer. Laboratory tests for hormonal assays, growth factors and cytokine levels continue to be pooled and will be assayed in years 2 and 4 as originally stated.

SPECIFIC AIM III. To determine whether cytokine production and/or osteoclastogenesis is downregulated by DHEA and/or gonadal steroids, using marrow cultured from women and men undergoing orthopedic surgery.

For Year 1, we collected marrow from 18 women for primary cell cultures and in addition used a human cell line of marrow stomal cells (KM 101) to assay the regulation of growth factor and cytokines by DHEA and gonadal steroids. Data from *in vitro* and *in vivo* studies indicate that part of the protective actions of adrenal and gonadal steroids may be mediated through inhibition of proresorptive cytokines such as IL-6. These experiments are designed to test the hypothesis that DHEA, estrogen, or testosterone treatment decreases IL-6 production by human marrow cells.

For each experiment, femoral bone marrow was obtained as discarded material from female subjects undergoing total hip replacement. Low-density mononuclear cells were isolated by centrifugation on Ficoll histopaque. Cells were cultured with IL-β (25 ng/ml) at 10^6 cells/ml/2cm^2 in phenol red-free α-MEM with 10% charcoal-stripped, heat-inactivated fetal bovine serum, with and without 10 nM dihydrotestosterone (T), DHEA or estradiol (E). Conditioned media were collected from human bone marrow cultures at days 2 and 5. An enzyme-linked immunosorbent assay (ELISA) for IL-6 was subsequently performed on the supernatant from these time points.

We currently have complete data analyzed from day 2 cultures of marrow from 18 women. As part of this study, we performed experiments on marrow from a 28-year-old womanwith congenital hip dysplasia and another postmenopausal subject on estrogen/progestin replacement therapy.

Table III summarizes these findings. Testosterone significantly suppressed IL-6 levels in 6 of 16 cultures ($p<0.05$) with a group mean of 86% (treated/control). The magnitude of significant inhibition was between 52 -88% of control. DHEA significantly suppressed IL-6 in 7 of 16 cultures with a group mean of 77%. The magnitude of suppression was between 11-87% of control. Estrogen significantly suppressed IL-6 in only 3 of 17 cultures with a group mean of 88%. The magnitude of significant suppression was between 29-53% of control. Dose-response curves for DHEA and testosterone (DHT) are shown in Figures 1 and 2. Significant inhibition of IL-6 (compared to control) is denoted by asterisks. In marrow from the estrogen-treated patient, there was no significant effect of any steroid on secretion of IL-6. In marrow from the 28-year-old, there was a significant increase in IL-6 secretion after both estrogen and testosterone treatment, but no significant effect after DHEA. Marrow from different subjects of different ages showed variable patterns of responses to these steroids that may be due to differences in receptors, genetic, or epigenetic factors. We will present preliminary results at the annual meeting of the American Society for Bone and Mineral Research in October 1999 (6). In summary, these data indicate that testosterone and DHEA suppress human marrow production of IL-6 in vitro. These steroids may lead to reduced bone resorption in vivo by direct inhibition of this proresorptive cytokine in human marrow.

TABLE III:
Effect of Gonadal and Adrenal Steroids on IL-6 Secretion by Human Marrow IL-6 (% Treated/Control)

Subject Age (yrs)	Control (pg/ml)			17βE2/C	DHT/C	DHEA/C
84	153	±	2	0.89	0.88	0.11*
84	70	±	7	1.27	1.02	1.05
80	104	±	18	0.69	0.68*	0.83
79	607	±	43	0.84	0.88	0.81
76	270	±	29	1.11	0.60*	0.57*
71	39	±	4	0.92	0.77	0.92
70	2891	±	380	0.53*	0.72	0.48*
69	2119	±	228	0.48	1.02	0.87*
67	46	±	16	0.52*	0.52*	0.32*
65	2554	±	21	no data	0.93	0.83
57	368	±	18	1.01	0.88*	0.86*
50	22024	±	2740	1.14	1.24	0.15*
49	1181	±	217	1.15	1.02	1.27
48	260	±	10	0.95	0.84*	0.87
42	67	±	21	0.95	0.78*	0.91
41	139	±	7	0.29*	0.89	1.2*
28	144	±	34	1.69*	2.2*	1.9
68PPV	398	±	73	1.2	1.12	0.97

*P < 0.05
PPV = Premarin/Provera
C = Control
17β E2 = 17β-estradiol
DHT = Dihydrotestosterone
DHEA = Dehydroepiandrosterone

Additional experiments for this project period focused on the use of a line of human marrow stromal cells, designated KM 101. These cells secreted 133.9 ± 7.4 pg/ml IL-6 after 2 days of treatment. Constitutive secretion increased to 391.2 ± 31.7 pg/ml after 5 days. As with normal human marrow cultures, treatment with IL-1 (25 ng/ml) stimulated IL-6 secretion 21-fold on day 3 and 23-fold on day 6. We tested the effects of steroids on IL-6 secretion by KM 101 cells. In cultures treated for 2 days, IL-6 secretion in the presence of estradiol was 9.8% of basal ($P<0.001$); in the presence of T, IL-6 secretion was 77.9% of basal ($p=0.006$); and in the presence of DHEA, it was 52.8% of basal ($p=0.013$). Inhibition was more dramatic after 5 days of treatment. Estradiol completely inhibited IL-6 secretion ($P<0.001$). Secretion of IL-6 was 2.6% of basal in cultures treated with T ($p<0.001$) and 3.8% in cultures treated with DHEA ($p<0.001$). We conclude that KM 101 cells may serve as a useful model system for the identification of mechanisms whereby DHEA and sex steroids inhibit cytokine production.

(7) KEY RESEARCH ACCOMPLISHMENTS:

For Specific Aim I

- Enrollment for our randomized controlled trial of oral DHEA vs. estrogen/progestin therapy has exceeded expectations. To date, DHEA appears to be a well-tolerated therapy for young women with anorexia nervosa, with no adverse effects noted.

- An analysis of baseline data (before treatment) from our initial 36 patients with anorexia nervosa revealed subnormal serum levels of DHEA, DHEAS, IGF-I, and bone formation markers, and elevated levels of urinary bone resorption markers. . As shown in Table I, the median serum IGF-I level was subnormal for age. This finding is significant, as IGF-I appears to be an anabolic factor for bone. Further DHEA replacement may increase free IGF-I levels.

- Before treatment, low levels of DHEA-S were associated with elevated levels on bone resorption markers and a longer duration of anorexia nervosa.

For Specific Aim II,

- We have established efficient protocols for care of patients including triage of patient visits, distribution of study drugs, and collection of study data. We are continuing patient enrollment. To reduce interassay variation, assays are pooled and will be measured in years 2 to 4.

For Specific Aim III,

- The in vitro studies for this project using primary cultures of human marrow stromal cells from 18 women revealed that testosterone and DHEA suppress human marrow production of IL-6 in some specimens. These steroids may lead to reduced bone resorption in vivo by inhibition of this proresorptive cytokine in human marrow.

- Experiments with a line of human marrow stromal cells, designated KM101, revealed significant inhibition of IL-6 secretion after treatment with estradiol, testosterone and DHEA after 2 and 5 days of treatment. We conclude that KM101 may serve as a useful model system for the identification of mechanisms whereby DHEA and sex steroids inhibit cytokine production.

(8) REPORTABLE OUTCOMES: Year 1 of grant period

Abstracts:

Gordon CM, Grace E, Emans SJ, Goodman E, Rosen CJ, LeBoff MS. "Relationship between DHEA, IGF-I and bone turnover markers in young women with anorexia nervosa," *J Bone Miner Res* **14**, S396 (September 1999) .

Gordon CM, Makhluf H, Blahut E, LeBoff MS, Glowacki J. "Gonadal and adrenal steroids inhibit IL-6 secretion by human marrow," *J Bone Miner Res* **14,** S268 (September 1999).

Manuscripts:

Gordon CM, Glowacki J, LeBoff MS. "DHEA and the Skeleton (Through the Ages)," *Endocrine* (1999 - in press).

Reportable outcomes will be available in years 2 to 4 of this award as prospective data become available.

(9) CONCLUSIONS

Reportable outcomes will be available in years 2 to 4 of this award as prospective data become available. These clinical and basic studies will provide new information on the mechanisms whereby DHEA and sex steroids affect bone.

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(2) Gordon CM, Glowacki J, LeBoff MS. "DHEA and the Skeleton (Through the Ages)," *Endocrine* (1999 - in press).

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(6) Gordon CM, Makhluf H, Blahut E, LeBoff MS, Glowacki J. "Gonadal and adrenal steroids inhibit IL-6 secretion by human marrow," *J Bone Miner Res* **14,** S268 (1999).

FIGURE 1

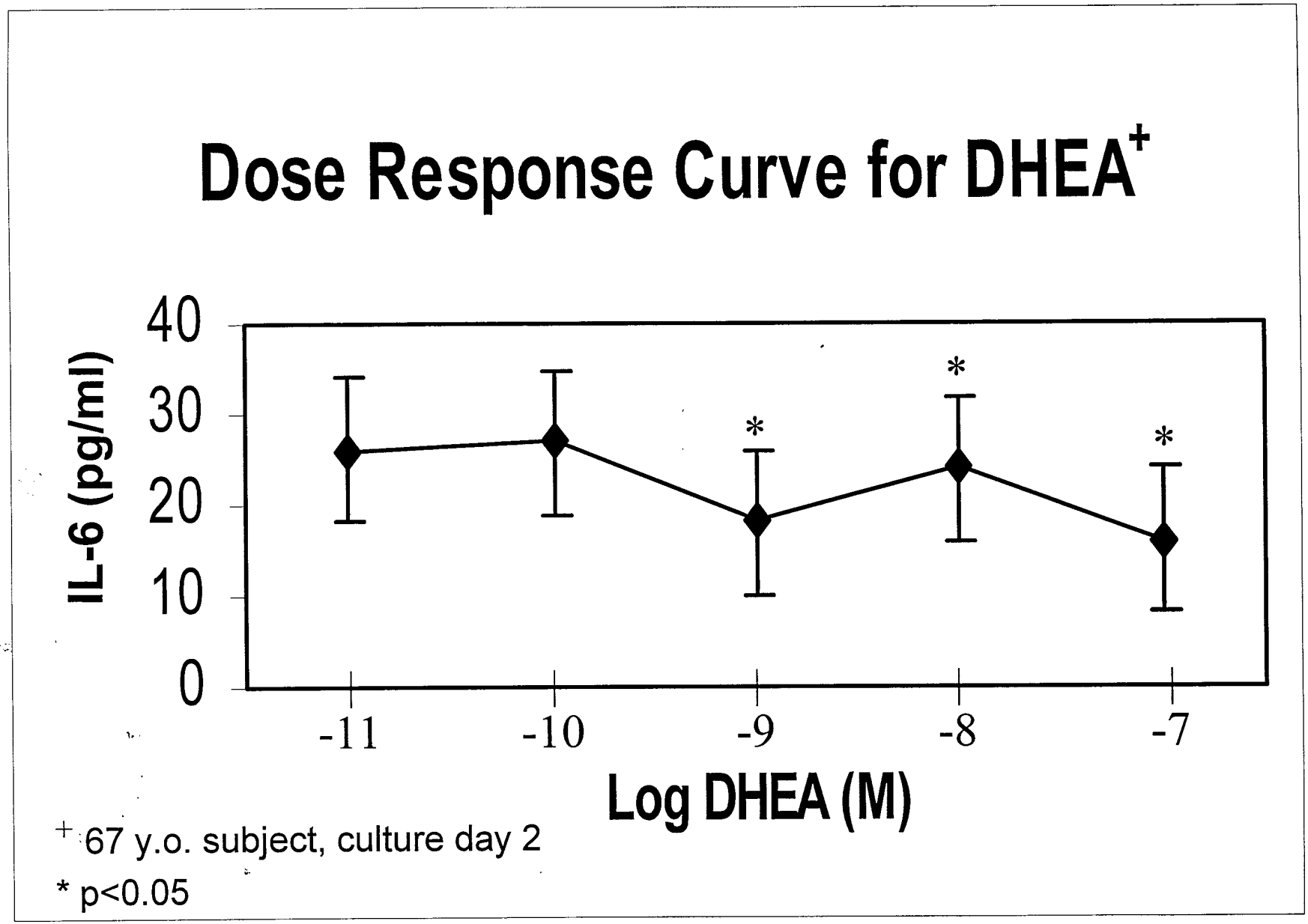
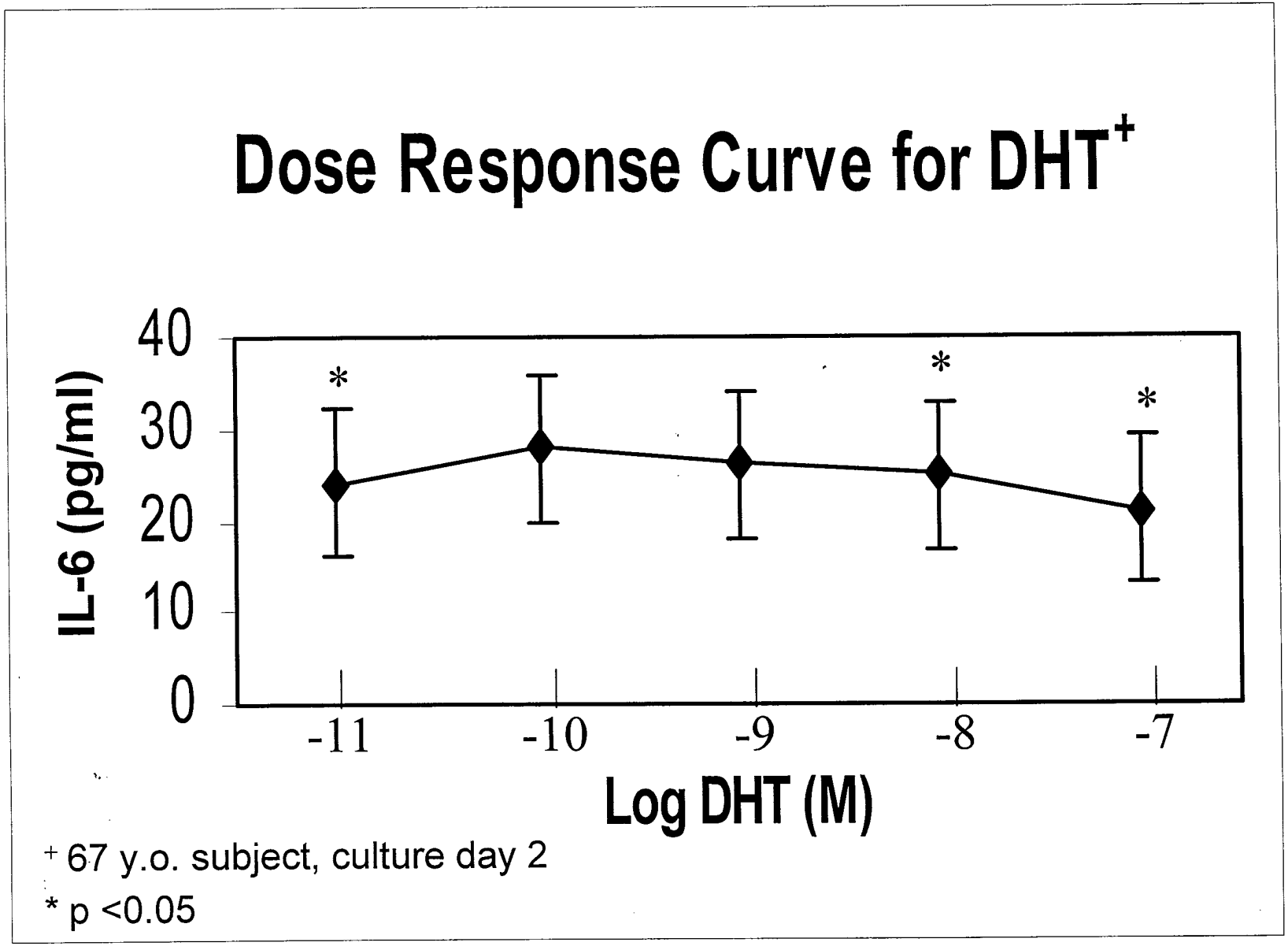

Dose Response Curve for DHEA+
IL-6 (pg/ml)
40
30
20
10
0
-11
-10
-9
-8
-7
Log DHEA (M)
*
*
*
+ 67 y.o. subject, culture day 2
* p<0.05

FIGURE 2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IL-6 (pg/ml)
40
30
20
10
0
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-11
-10
-9
-8
-7
Log DHT (M)
+ 67 y.o. subject, culture day 2
* p <0.05

1999
Program and Abstracts

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SA350

Relationship between DHEA, IGF-1, and Bone Turnover Markers in Young Women with Anorexia Nervosa. C. M. Gordon,[1,2] E. Grace,*[1] S. J. Emans,*[1] E. Goodman,*[1] C. J. Rosen,[3] M. S. LeBoff.[4] [1]Adolescent/Young Adult Medicine, [2]Endocrinology, Children's Hospital, [3]Maine Center for Osteoporosis Research and Education, St. Joseph Hospital, Bangor, ME, [4]Skeletal Health and Osteoporosis Program, Brigham & Women's Hospital, Boston, MA.

Young women with anorexia nervosa (AN) are at risk for early osteoporosis. In a pilot study, we recently showed that adolescents with AN have subnormal levels of dehydroepiandrosterone (DHEA), DHEA-S, and insulin-like growth factor (IGF-I) which are accompanied by subnormal serum bone formation markers and elevated urinary bone resorption markers (*JBMR* 1999;14:136-145). We examined the baseline characteristics of a larger cohort of young women with AN to identify relationships between these factors and bone turnover markers. We enrolled 36 Tanner stage V, postmenarchal young women, mean age 18.4 ± 3.2 years, who met psychiatric criteria for AN. No subjects were on estrogen replacement therapy. Fasting serum samples were obtained before 10:00 a.m. for DHEA(S), IGF-I, osteocalcin, and bone specific alkaline phosphatase (BSAP), and a second morning void for urinary N-telopeptide (NTx) levels. Median duration of AN was 22 months (range 4-96) and of amenorrhea, 15 months (range 3-90). The median DHEA level was 325 ng/dl, range 97-657 (normal mean 540); median DHEA-S level was 205 µg/dl , range 55-407 (normal mean 235); median IGF-I was 173 ng/ml, range 52-372 (normal mean 460); median osteocalcin was 14 ng/ml, range 8.6-43 (normal adolescent range 42-225); median BSAP level was 13, range 4.6-30 (normal adolescent range 28-38); and median NTx levels was 80 nmol/mmol Creatinine, range 35-156 (normal adult range 10-65). Correlation analyses using Spearman rank coefficients revealed the following significant relationships: DHEA was negatively correlated with duration of amenorrhea (r=-0.42, p=0.015); DHEA-S was negatively correlated with duration of AN (r=-0.37, p=0.036) and NTx levels (-0.42, p=0.015); weight was directly correlated with IGF-I (r=0.34, p=0.025); and age was negatively correlated with NTx levels (r=-0.41, p=0.018) and osteocalcin (r=-0.53, p=0.016). Results from this larger sample confirm our earlier pilot data that patients with AN have low DHEA(S) and IGF-I levels, accompanied by subnormal levels of bone formation markers and elevated levels of bone resorption markers. Low levels of DHEA-S were associated with high levels of bone resorption markers and duration of disease. Interestingly, NTx and osteocalcin levels decreased as a function of age in these young women, possibly reflecting decreased bone turnover after the attainment of peak bone mass.

F295

Gonadal and Adrenal Steroids Inhibit IL-6 Secretion by Human Marrow Cells.

C. M. Gordon,[1,3,4] H. Makhluf,*[1] E. Blahut,*[1] M. S. LeBoff,[2] J. Glowacki.[1] [1]Orthopedic Research, [2]Endocrine/Hypertension, Brigham & Women's Hospital, [3]Adolescent Medicine, [4]Endocrinology, Children's Hospital, Boston, MA.

Gonadal and adrenal hormones have protective effects on the skeleton. Data from in vitro and in vivo studies indicate that part of their action may be mediated through inhibition of bone resorptive cytokines such as interleukin-6 (IL-6). We tested the hypothesis that secretion of IL-6 by human marrow is inhibited by testosterone, dehydroepiandrosterone (DHEA) and estrogen. Femoral bone marrow was obtained as discarded material from 7 postmenopausal female subjects undergoing total hip arthroplasty, ranging in age from 48-84 years. In addition, there was one subject on systemic estrogen/progestin replacement. Low-density mononuclear cells were isolated by centrifugation on Ficoll histopaque. Cells were cultured in a 24-well plate at a seeding density of 1 million cells per well in phenol red-free αMEM supplemented with 10% charcoal-stripped, heat-inactivated fetal bovine serum, and IL-1β (25 ng/dl) with or without 10nM dihydrotestosterone (T), DHEA or estradiol (E). After 2 days in culture, conditioned media were collected and IL-6 levels were measured by high-sensitivity commercial ELISA kits. Testosterone significantly suppressed IL-6 levels in 5 of 7 cultures ($p<0.05$) with a group mean of 79% (treated/control). The magnitude of significant inhibition was between 60-88% of control. DHEA signficantly suppressed IL-6 in 3 of 7 cultures with a group mean of 78%. The magnitude of significant suppression was between 46-86% of control. Estrogen significantly suppressed IL-6 in only 1 of 7 cultures with a group mean of 91%. In marrow from the estrogen-treated patient, there was no significant effect of any steroid on secretion of IL-6. Marrow from different subjects showed different patterns of responses to the steroids, that may be due to differences in receptors, genetic or epigenetic factors. These data indicate that T and DHEA suppress human marrow production of IL-6 in vitro. These steroids may lead to reduced bone resorption in vivo by inhibition of this proresorptive cytokine in human marrow.

DHEA AND THE SKELETON (THROUGH THE AGES)

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ABSTRACT:

Dehydroepiandrosterone (DHEA) and it sulfate ester, DHEAS are the most abundant steroids in the human circulation, although their exact biological significance is not completely understood. DHEA(S) levels are high in fetal life, decrease after birth, and show a marked pubertal increase to a maximal level during young adulthood. In healthy adults, DHEAS levels decline to 10 to 20% of peak levels by age 70 years. This review summarizes information concerning the role of DHEA in skeletal physiology, including modulation of the skeletal insulin-like growth factor regulatory system, and its effects on secretion of proresorptive cytokines. The pattern of secretion of DHEA throughout the life cycle is discussed, as well as its potential usefulness in specific disease states as an agent with anabolic and antiosteolyic effects on bone.

I. INTRODUCTION

Dehydroepiandrosterone (DHEA) is an adrenal steroid that is currently being marketed as a "food supplement" in health food and grocery stores and does not require approval as a prescription drug by the U.S. Food and Drug Administration. Recent research studies suggest its usefulness in prevention of the catabolic changes associated with aging and for certain disease states. This review summaries the normal physiology of DHEA throughout the life cycle and highlights results of recent *in vivo* and *in vitro* studies with this hormone. Special emphasis is placed on its effect on skeletal physiology, and its potential anabolic and antiosteolytic effects on bone.

II. DHEA: NORMAL PHYSIOLOGY

DHEA and its sulfate ester, DHEAS are the most abundant steroids in the human circulation. Compared to other species, humans have high levels of circulating DHEA(S). In humans and other primates, DHEA circulates at concentrations 10-fold greater than cortisol (1,2). During pregnancy, DHEAS is synthesized in large amounts by the fetal adrenal glands where this hormone is a primary source of placental estrogens, either directly or after subsequent 16α-hydroxylation in the fetal liver (3). The fetal zone of the adrenal gland is transient, involuting during early infancy. After birth, DHEA(S) levels fall rapidly and remain at low levels until adrenarche. Between the ages of 6 and 8 years, serum levels of DHEA(S) and other androgens begin to rise (4). With the onset of puberty and activation of the hypothalamic-pituitary-gonadal axis, serum levels increase sharply to achieve peak levels during the early twenties. While serum DHEA levels rise with the advancement of chronologic and skeletal age, cortisol and ACTH levels remain relatively constant (5)(**Fig. 1**). Beginning with young adulthood, levels of DHEA decline and are between 10-20% of young adult levels by age 70. (2, 6-9). The fall in DHEA(S) levels with age is associated with a reduced synthesis of the 17,20-lyase enzyme. Albright referred to this phenomenon that normally occurs during aging as the "adrenopause" (10).

The diverse biological functions of DHEA in humans have become an area of intense interest and research, although the exact physiological roles of this hormone and its sulfate ester are not understood. Previous studies using animal models suggest that DHEA has protective effects on bodily functions and against diseases such as bone loss, atherosclerosis, systemic lupus, cancer, and diabetes mellitus (11-17). Some clinical studies of the elderly showed a significant positive correlation between serum DHEAS concentrations and both functional status (18-19) and psychometric parameters of well-being (20). DHEA may also act as a neurosteroid, influencing cognition, memory, and sleep patterns (21-22). Of note, results from studies of DHEA's effects in rodent or other non-primate models may not be applicable to humans because DHEA circulates in very low concentrations in lower species. Further research is needed to determine the significance and applicability of some of these animal studies to humans.

The daily young adult production rate of DHEA is 20-30 mg (23). Buster et al. studied the pharmacokinetics of 150 mg and 300 mg of DHEA in eight postmenopausal women and concluded that a dose of 50-75 mg is suitable to approximate peak adult levels of adrenal androgens (24). After oral administration, DHEA is largely absorbed and converted to DHEAS in the hepato-splanchnic system (25). Using radio-isotopic tracer techniques and calculations, the half-life of DHEA is estimated to be 15-30 minutes, while the half-life of DHEAS is much longer at 7-10 hours (21). The concentration of DHEA in the blood oscillates concurrently with cortisol, consistent with the response of adrenal DHEA secretion to ACTH, but there is no feedback control at the hypothalamus or pituitary.

Circulating plasma DHEAS levels result primarily from adrenal secretion of this hormone. In healthy children and adults, approximately half of the DHEA synthesized is converted to DHEAS by DHEA-sulfotransferase. This enzyme has been localized immunologically to the zona reticularis of the adrenal (26). Sulfation of DHEAS involves the transfer of a sulfonate group to

form a sulfate ester. The DHEAS that is formed accumulates at much higher levels in plasma than the unconjugated steroid because of its low clearance rate (27). Sulfation renders the steroid unavailable for enzymatic transformation within the circulation. Circulating DHEAS appears to serve as a transporter to local tissues where androgens or estrogens are synthesized (28-29). This is an important role in humans as active sex steroids are synthesized almost entirely in peripheral tissues, providing control to target cells for the necessary adjustment of sex steroid formation or metabolism (29-31).

The metabolism of DHEA(S) into active sex steroids typically occurs within specific cells that contain androgen and/or estrogen receptors, such as bone, adipose tissue, muscle, breast, prostate, skin, brain and liver (30-31). Plasma DHEAS levels in adult men and women are 100-500 times higher than those of testosterone and 1000-10,000 times higher than those of estradiol, providing a large reservoir of substrate for conversion into sex steroids (31). Approximately 50% of total androgens in adult men are derived from DHEA(S) (32-33). In women, approximately 75% of estrogens are formed from adrenal steroids within peripheral tissues and close to 100% after menopause (30). Within fat and other tissues, the adrenal androgens androstenedione and DHEA(S), and ovarian androgens are converted to the active estrogen, estradiol, through aromatase activity (aromatase cytochrome P450) (34-35). Adipose tissue is the primary source of aromatized estrogens in both women and men (36). Aromatases are also present in the liver, kidney and bone marrow, among other tissues (35-36). In both genders, more potent androgens are derived from conversion of DHEA(S) or androstenedione into testosterone and dihydrotestosterone. The enzymes responsible for these changes are 17β-hydroxysteroid dehydrogenase and 5α-reductase, respectively.

III. DEVELOPMENTAL CONSIDERATIONS AND NORMAL SKELETAL PHYSIOLOGY

Up to 50% of bone mass is achieved during adolescence (37-38). Bone density increases during adolescence in association with the rise in adrenal androgens and gonadal steroids, and declines with menopause and aging. Bone acquisition during puberty is closely linked to gonadal maturation (39). The importance of androgens to this process is suggested by the work of Mauras et al. (40) which demonstrated that administration of androgen to prepubertal boys increases calcium retention. In addition, young women with congenital forms of hyperandrogenism appear to have increased bone mass (41-42). After adulthood is reached, bone mass is normally maintained by the coupling of bone formation and resorption, in which osteoblasts deposit new bone at areas of bone resorption.

IV. EFFECTS OF SEX STEROIDS ON BONE GROWTH AND MAINTENANCE

Variations in circulating sex steroids exist throughout the life cycle and have important effects on bone. In pubertal girls and young women, estradiol is the major estrogen produced by ovarian granulosa cells. After menopause, ovarian production of estradiol decreases and adrenal androgens become increasingly important as the predominant precursors of estrogen. The significance of estrogen to bone is supported by the accelerated bone loss characteristically associated with the onset of menopause (43). Several clinical studies have documented that low estrogen levels contribute to both bone loss and fracture risk in elderly men and women (44-46). In men, serum testosterone and DHT are reliable markers of testicular secretion. In males, the adrenals are the primary source of testosterone during the first 6 months of life, (47) and by the seventh decade of life, contribute 40-50% of total androgens (48). Testosterone secretion rises during puberty coincident with peak growth velocity. Illustrating potential interactions of sex steroids with the hypothalamic-pituitary axis, Veldhuis et al. examined a small cohort of men between the age of 18 and 63 years, and demonstrated that testosterone had a strong influence on

pulsatile growth hormone secretion (49). Another study (50) examined associations between androgens and the growth hormone-IGF axis. A significant correlation was found between components of the skeletal IGF system (IGF-I, IGF-II and IGFBP-3) and levels of sex hormone binding globulin. Within this paradigm, free testosterone was positively associated with components of the IGF system. The importance of androgens to bone is suggested by the report of Finkelstein et al. (51) which documented osteopenia in men with a history of pubertal delay, a group of patients with subnormal androgen levels during a critical period for bone accretion.

Recently described clinical and laboratory models have differentiated the action of androgen from that of estrogen. First, it was reported that patients with androgen insensitivity had a mutation in the androgen receptor leading to resistance to even elevated androgen levels. These individuals had normal to elevated levels of estrogen, accompanied by markedly elevated androgens, but an impairment of androgen action (52-53). Two case reports documented decreased bone mineralization in these patients (54-55). An animal model of androgen insensitivity, the Tfm rat, also manifests a lower skeletal mass in affected genetic males that is similar to that of unaffected female animals (56). Another natural model is the case of a man with an estrogen receptor mutation. The index case was a 28 year-old man with tall stature, delayed closure of epiphyses, and reduced bone mineral density (BMD) (57). A mutation in his estrogen receptor resulted in resistance to even supraphysiologic levels of estrogen. The laboratory counterpart of this patient, a transgenic estrogen receptor knockout mouse, has a BMD 20-25% that of normal mice (58). Similarly, male and female patients with aromatase deficiency have a delayed bone age and osteopenia (59-60). These individuals have elevated androgens and markedly subnormal estrogens due to decreased serum aromatase levels resulting from a mutation in the P450 aromatase gene. **These natural models suggest that both androgen and estrogen exert a direct growth-stimulating action on the epiphysis, with acquisition of final BMD and final skeletal maturation being uniquely estrogen-dependent phenomena (61)(Fig. 2).**

V. INSULIN-LIKE GROWTH FACTORS AND THE SKELETON: Clinical aspects

The skeletal IGF system plays an important role in the maintenance of bone density (62). IGFs have anabolic effects on the skeleton and may be modulated by DHEA. IGFs, along with IGF-specific binding proteins (IGFBPs) and IGFBP-specific proteases, comprise the skeletal IGF regulatory system. IGF-I is the most abundant member of this family. Both postmenopausal women and patients with anorexia nervosa may have subnormal levels of IGF-I and DHEA(S), and each group has an increased incidence of osteoporosis (63-66). In the recent report of Haden et al. (64), there was a strong positive correlation between circulating DHEAS and IGF-I levels ($r= 0.43$, $p=0.0001$) (64). These data are consistent with the hypothesis that declining DHEAS production that occurs with aging contributes to lower IGF-I production. **(Fig. 3)**. Some reports have shown positive correlations between serum IGF-I levels and bone density in older individuals, although there are conflicting results (64, 67-68, 66). The report by Haden et al (64) also showed a positive correlation between serum IGF-I levels and BMD in women **(Fig. 4)**. In patients with anorexia nervosa, decreased IGF-I levels are accompanied by elevated growth hormone (GH) levels, (63) which suggests an acquired resistance to GH's action. A recent study showed that short-term intravenous replacement of recombinant human IGF-I to osteopenic patients with AN increased bone formation (63). In the report of Gordon et al. (65), after three months of DHEA therapy, serum IGF-1 was positively correlated with final serum osteocalcin levels. These studies emphasize the role of IGF-I as a local anabolic factor on bone, with IGF-I serving as an anabolic mediator of GH's actions (62).

VI. DHEA AND LOCAL FACTORS IN BONE TURNOVER

IGF-I is both a systemic and local modulator of bone formation. Growth hormone is considered the prototypic systemic regulator of IGF-I synthesis in humans. GH excess is associated with increased levels of serum IGF-I, while GH deficiency is accompanied by low serum IGF-I and

low BMD (69). *In vitro* treatment of bone cells with IGF-I stimulates proliferation, differentiation, or matrix synthesis, depending upon the stage of osteoblastic differentiation. Components of the IGF system are produced by bone cells are regulated by bone-active agents. Thus, bone is both a target of IGF action and a source of IGF, its binding proteins, and proteases. 17β-estradiol (70) and parathyroid hormone (71) stimulate IGF-I synthesis in cultured bone cells. This local pathway offers a potential mechanism by which agents have anabolic effects on the skeleton. Further details of regulation of cellular IGF are revealed by *in vitro* studies with other cell types. For example, testosterone has been shown to increase proliferation of IGF-I and IGFBP in cultured fibroblasts, and antibodies against either IGF-I or the IGF-I receptor blocked testosterone's mitogenic action (72). Rosen et al. reported that human marrow cells secrete IGF-I, several of the IGFBPs, and BP-proteases, and that there is an age-related increase in secretion of IGFBP-3 (73)(**Fig. 5**). Upregulation of IGFBP-3 with aging is consistent with other reports that senescent fibroblasts secrete elevated levels of IGFBP-3 (74).

There is some information about the anabolic effects of DHEA on bone formation *in vitro*. DHEAS influences mineralization of chick embryonic cells *in vitro* (75). DHEAS and, to a lesser extent, DHEA stimulated osteocalcin production by osteosarcoma and normal human osteoblast-like cells, but only in the presence of 1,25-dihydroxyvitamin D3 (76). DHEA stimulated cell proliferation and alkaline phosphatase activity in human osteoblasts, but these effects were blocked, respectively, by the androgen receptor antagonist hydroxyflutamide and by neutralizing antibodies against TGF-β. These data were interpreted as evidence that the mitogenic effect of DHEA was mediated by androgen receptor-mediated mechanisms and that its effect on alkaline phosphatase activity was mediated by increased TGF-β (77). Finally, it has been shown that DHEAS, alone or in combination with carnitine, has anabolic effects and promotes alkaline phosphatase activity and collagen I synthesis by porcine osteoblast-like cells (78). Anabolic

effects of DHEA are likely to be complex. DHEA was shown to stimulate IGF-I gene expression directly in rat granulosa cells, but only during the differentiated stage when these cells secrete estrogen (79). Both DHEA and IGF-I can stimulate aromatase P450 activity and estrogen synthesis in preimplantation porcine conceptuses *in vitro* (80). In addition, IGF-I stimulates aromatase activity in human granulosa cells (81). These results can be integrated into the likelihood that DHEAS has direct and indirect anabolic effects on various cell types. In one pathway, DHEAS stimulates IGF-I that, in turn, stimulates estrogen production, resulting in enhanced stimulation of bone formation.

A model has evolved that both bone cells and stromal cells of the bone marrow microenvironment contribute to skeletal homeostasis. Bone marrow is an important component of the skeletal system as a source of progenitors of osteoblasts and osteoclasts (82). Further, the differentiation and activities of osteoclasts are modulated by osteoblasts and bone marrow stromal cells. The bone marrow stromal cells produce cytokines that regulate specific aspects of bone turnover. In addition to IGF and other local factors in bone that mediate anabolic actions, there are several local factors that mediate osteolysis. A number of such cytokines act in a paracrine manner to regulate skeletal metabolism by promoting osteoclast differentiation and bone resorption. Interleukin –6 (IL-6) is a major cytokine mediator of bone resorption that stimulates osteoclast formation from marrow progenitors (82). In addition, tumor necrosis factor (83-85), interleukin-1 (86-88) and interleukin-11 (89) stimulate bone resorption and/or osteoclast formation. IL-6 is a multifunctional cytokine with complex activities. For example, this cytokine is also produced by giant cells and stromal cells from human giant cell tumors (90). Thus, it is possible that IL-6 has distinct effects depending on the state of cellular differentiation. In addition, it is likely that feedback mechanisms exist. IL-6 is expressed in human adrenal glands and stimulated *in vitro* release of the adrenal steroids aldosterone, cortisol, DHEA, and androstenedione (91).

There is a growing body of evidence that sex steroids decrease bone resorption by suppressing the secretion of proresorptive IL-6 (**Fig. 3**). In a landmark report, Jilka et al. delineated the importance of IL-6 in mediation of bone resorption associated with estrogen deficiency (92). In their *in vivo* studies, 17β-estradiol or antibodies against IL-6 prevented osteoclast development in ovariectomized mice. In their *in vitro* studies, 17β-estradiol suppressed IL-6 production by bone and marrow stromal cells. Estrogen receptors have been identified in bone cells (93-94) and estrogen treatment may thus protect against cytokine-mediated bone loss *in vivo* (95). Information on these pathways is now available from studies with human cells. There is a striking age-dependent increase in the *in vitro* generation of osteoclasts from marrow cells (96). This was attributed to the age-related increases in constitutive secretion of IL-6 and IL-11 by human marrow stromal cells (97). Furthermore, as was reported with murine studies (Jilka, 1992), secretion of IL-6 and IL-11 was attenutaed in marrow cultured from postmenopausal women on estrogen therapy (97). Consistent with these conclusions, Ralston showed expression of IL-6, IL-1α, and IL-1β more often in biopsies from postmenopausal women with fractures than with either women with normal BMD or women receiving hormonal replacement therapy (98). Another study with a line of human fetal osteoblasts showed that estrogen decreased IL-6 gene production and gene expression (99). Other studies testing for *in vitro* effects of estrogen (100) or of estrogen replacement therapy on cytokine release from human marrow did not find modulation of IL-6. However, there were major differences in patient selection criteria and culture conditions that could account for differences in the state of estrogen receptors, for example. *In vitro* studies provide important opportunities to test hypotheses and to unravel mechanisms, but it can be difficult to control for all of the variables introduced with clinical tissues. This point is further exemplified by recent studies on the effects of estrogen, testosterone, and DHEA on cytokine expression. In a study with bone-derived osteoblasts from healthy adults,

Hierl et al. found that these steroids did not affect IL-6 production, but dexamethasone inhibited both basal and induced IL-6 expression (101). In a pilot study, Gordon et al. reported that cultured marrow cells from 7 postmenopausal women between 48 and 84 years of age showed different patterns of inhibition of IL-6 by gonadal and adrenal steroids (102). Testosterone significantly suppressed IL-6 levels in 5 of 7 cultures with a range of 60 to 88% of control, with a group mean of 79%. DHEA significantly suppressed IL-6 in 3 of 7 cultures with a range of 46-86% of control and a group mean of 78%. Estrogen significantly suppressed IL-6 in only 1 of 7 cultures with a group mean of 91%. It was shown previously that factors in medium and serum can mask *in vitro* regulation of IL-6 secretion by cultured marrow (103). Variations in subjects cultured under exactly the same conditions suggest the possibility of major genetic or epigenetic factors influencing individual responses.

There are emerging data suggesting mechanisms by which steroids regulate cytokine production. Both estrogen and testosterone inhibited IL-6 production by murine bone marrow (104). In a more detailed study of the effect of androgen, Bellido et al. reported that testosterone, dihydrotestosterone, and DHEAS inhibited activity of the IL-6 promoter, but only in cells with the androgen receptor (105). DHEA is an androgen, but can also be an estrogen precursor. Both female and male marrow contains cytochrome P450 aromatase, the enzyme that can generate estrogen from androgen precursors such as DHEA (106). DHEA also stimulates the classic estrogen response element, but it is probably a direct effect because stimulation occurred even in the presence of the aromatase inhibitor, formestane (107). Not all data are as clear. Low doses of DHEA and DHEAS inhibited production of IL-6 in unstimulated human spleen cells, but enhanced its release by explant cultures (108).

In vitro studies are beginning to explain mechanisms that may be operating in bone at a very local or paracrine level. Emerging data support the hypothesis that with deficiencies of DHEAS,

estrogen or testosterone, low IGF-I and elevated IL-6 may contribute to bone loss (Fig. 3). Although this simplified view does not include the interactions of other local factors, it provides a basis for understanding clinical associations.

VII. DHEA REPLACEMENT THERAPY

Recent research has suggested that deficiencies of adrenal androgens jeopardize skeletal health. First, androgens exert independent and positive effects on peak bone mass (109). Second, DHEA (and androstendione) are steroid precursors of estrogens through aromatization in peripheral tissues (29, 35,110). As mentioned, *in vitro* data showed that DHEA stimulates human osteoblastic cell proliferation through androgen receptor-mediated mechanisms and alkaline phosphatase production through transforming growth factor-β (TGF-β) (77). Preliminary findings with oral DHEA treatment in young women with anorexia nervosa (65) and studies with topical DHEA used in older women (31) suggest that this androgen has both anabolic and antiosteolytic properties. In both studies, urinary bone resorption markers decreased, which may indicate suppression of osteoclast activity and bone resorption. Serum bone formation markers also increased, which may indicate a stimulation of osteoblast function and bone formation. The impact of long-term administration of this hormone on bone mass has not been studied. DHEA is an attractive form of hormonal replacement to prevent bone loss as it can be converted into both androgen and estrogen at levels sufficient to increase bone formation and decrease resorption, respectively (1).

Several human studies showed significant direct correlations between DHEA levels and bone density (64, 111-114), although the data are not consistent. The cross-sectional study of Haden et al. demonstrated a significant positive correlation between the serum DHEAS and BMD in women (64) (Fig. 4). A population-based study in an older retirement community showed no relationship between DHEA and BMD in men and women between age 50 and 74 years (115).

However, more recent data from the same investigators included a larger number of men and women between the ages of 50 and 89 years and showed that DHEA(S) levels in women were directly correlated with BMD in the forearm, spine, and hip, but that neither DHEA nor DHEAS was associated with bone density in men (46). These data suggest that gender differences exist in the relationship between DHEA(S) and bone.

To date, clinical research examining the effects of DHEA replacement has been carried out primarily as short-term studies in older patients. One study examined the effect of pharmacological doses of DHEA (1600 mg/day for 28 days) on the production of endogenous androgens and estrogens in 6 menopausal women. Mortola and Yen showed that DHEA treatment produced a marked rise in testosterone and a moderate rise in estradiol levels (28). While no adverse clinical effects were noted in this study, HDL levels decreased in both men and women. Other studies examined the effect of short-term DHEA replacement with lower doses to achieve young adult DHEA levels. Morales *et al.* showed that 50 mg of nightly oral DHEA for 6 months restored serum DHEA levels to those of young adulthood and produced a rise in serum androgens, no changes in levels of sex hormone binding-globulin, estrogen, or cholesterol, but a slight decrease in HDL (23). DHEA also produced a rise in IGF-I and free IGF levels due to a decline in IGFBP-I, supporting the potential anabolic effects of DHEA on bone. DHEA was without adverse effects and produced a sense of "well-being" in 70-80% of patients according to quality of life assessments (23). Oral administration of DHEA at a daily dose of 50 mg to elderly men for 20 weeks resulted in a 20% increase in serum IGF-I and a 32% increase in the ratio of IGF-I/IGFBP1 (116). In a preliminary report, topical DHEA at physiological replacement doses given to elderly patients increased bone density at the hip and spine by 2.3% in one year, with concomitant suppression of urinary bone resorption markers and increases in bone formation markers (31). These data suggest that DHEA may have beneficial effects on bone through conversion to estrogen and/or active estrogens or through androgenic effects on bone.

VIII. DISEASE-SPECIFIC CONSIDERATIONS

1. Adrenal Insufficiency

Patients with adrenal insufficiency suffer from chronic DHEA(S) deficiency, as conventional glucocorticoid and mineralocorticoid replacement therapy does not restore adrenal androgen concentrations (117). DHEA replacement therapy may be particularly relevant to female patients with adrenal insufficiency, as endogenous androgen deficiency is an issue that is frequently neglected (118). It has been shown that despite otherwise adequate hormonal replacement therapy in patients with this disease, quality of life appears to be suboptimal (119). In one protocol designed to reproduce the subnormal DHEA(S) levels found in Addison's disease, 4 days of dexamethasone suppression was given to healthy female volunteers. A 50 mg daily dose of DHEA resulted in normal levels of adrenal hormones and appeared to be an appropriate replacement dose for these women (117). One report documented that bone density was subnormal in women with adrenal insufficiency (120) which may in part reflect the low adrenal steroid levels seen. No prospective studies have examined the effect of DHEA replacement on bone density in patients with adrenal insufficiency. These studies will be needed to answer the questions of its benefit to bone density and other health parameters in this group.

2. Anorexia Nervosa

Patients with chronic anorexia nervosa often develop early osteoporosis and have a seven-fold increased incidence of fractures (121). Subnormal levels of the adrenal steroid DHEA may be causally linked to bone loss in this disease. Studies examining ACTH stimulation data reveal evidence of decreased adrenal 17-20 lyase activity in anorexia nervosa, with a predominance of glucocorticoid over androgenic pathways (122-123). This enzymatic block results in increased cortisol and decreased DHEA production. Combined with the low androgen and estrogen levels typically found in these patients, low DHEA levels appear to put adolescents with anorexia at

high risk for early osteoporosis (124-125). Although commonly prescribed for these patients, estrogen therapy alone has not been shown to prevent osteopenia in patients with anorexia (126). Because adolescence is a critical period for the acquisition of bone mineral (127-129), the identification of safe and effective strategies to preserve bone density in adolescents with eating disorders has become an important public health issue.

In a 3-month study, Gordon et al. (65) examined the effect of DHEA on bone metabolism in adolescents and young women with anorexia nervosa. Compared to literature normal values, these subjects had low levels of both DHEA and DHEAS. Patients treated with an oral dose of DHEA showed a significant decrease (25%) in urinary bone resorption markers, with NTx levels decreasing in 13 of 15 subjects between baseline and 3 months. After 3 months of therapy, there was also a significant increase within treatment groups in the serum bone formation marker, osteocalcin, and increased IGF-I levels in 9 of the 15 patients. A subgroup analysis revealed that increases in IGF-I in these 9 patients positively correlated with increases in the serum bone formation marker, bone specific alkaline phosphatase **(Fig. 6)**. Finally, a 50 mg dose was shown to restore physiologic levels of serum DHEA, estrogen, and testosterone in these patients. These data support the hypothesis that DHEA therapy has positive effects on bone mass, with anabolic effects mediated through IGF-I. Ongoing research in this area continues to provide new information on mechanisms leading to bone loss in anorexia and on the role of agents like DHEA in the prevention and treatment of osteoporosis in these young women.

The accelerated bone loss seen in patients with anorexia nervosa appears to reflect a state of increased bone turnover (63, 121, 126, 128, 130). IL-6 may mediate bone resorption in anorexia, as it does in bone loss of the postmenopausal period (97). A recent preliminary study examined the role of this cytokine in the bone loss of anorexia nervosa, documenting a level double that of

published normal controls in 14 young women with this disease (131). The role of DHEA in modulation of proresorptive cytokine secretion in these patients remains to be determined.

3. Osteoporosis in the Elderly

Estrogen deficiency is an important factor in postmenopausal bone loss. During the perimenopausal period, there is an acceleration of bone loss over 8 to 10 years and some women lose up to one-half of their bone density during menopause (43). In a longitudinal study of premenopausal women, there was an average loss of bone density at the hip of 0.3% per year (132). One longitudinal report showed that bone loss of the hip was weakly associated with circulating levels of androgens in premenopausal women (133) and was correlated with serum levels of both androgens and estrogens in postmenopausal women (133). Several studies concluded that low estrogen levels contribute to bone loss and fracture risk later in life (44-45, 134), although the data are contradictory. Earlier studies showed that total estrogen levels did not distinguish postmenopausal women with osteoporosis from those with normal bone density (135-137). However, in a subsequent cross-sectional study of postmenopausal women, Rozenberg et al. (138) found that estradiol levels were correlated with spinal trabecular bone mass. Additionally, DHEA(S) concentrations, characteristically reaching low levels by the age of 50 to 60 years, were related to changes in cortical bone mass in the forearm (138). In a longitudinal study of 84 women between the age of 42 and 58 years, Slemenda et al. showed that over 3 years, estrogen levels in perimenopausal and postmenopausal women predicted the rate of postmenopausal bone loss in the forearm (139). In that group, levels of testosterone were related to BMD changes in the distal forearm. In a larger prospective study of 231 women between the ages of 31 and 77 years, Slemenda et al (132) examined the role of sex steroids on bone mass of the spine, hip and forearm in peri-and postmenopausal women up to 25 years after the menopause. In postmenopausal women, estrogen and androgens were independent factors, both affecting bone loss. In women over 60 years, estrogen levels were lower in those losing more

bone in the spine and forearm, although testosterone levels were related to bone loss of the hip (132). These studies examining the effects of sex steroids on the bone of peri- and postmenopausal patients have relevance to DHEA physiology as this adrenal steroid is converted to active sex steroids in peripheral tissues.

There is some information about mechanisms of age-related bone loss. In human studies, (IL-6) levels increased with advanced age (15) and were less easily suppressed by estradiol (16-17, 23). Haden et al reported (64) an inverse correlation between DHEAS and IL-6 levels ($r = -0.32$, $p=0.02$) in older women. Treatment of animals with DHEA appeared to suppress the IL-6 production seen with aging (15, 31,140). These data suggest that the declining DHEAS production that occurs with aging may contribute to the increased IL-6 production that is characteristic of this period **(Fig. 3)**.

IX. CONCLUSION

DHEA and DHEAS are the most abundant steroids in the human circulation. Although incompletely understood at present, their biological effects are becoming better delineated. In both genders, the secretion of DHEA rises sharply during adolescence when bone mass is increasing, and reaches its peak during the third decade. DHEA levels subsequently decline with advancing age. Low DHEA levels appear to have a particularly detrimental effect on the skeleton. Androgens appears to have an anabolic effect on bone mass. Additionally, adrenal androgens are precursors of estrogens by aromatization in peripheral tissues. Some studies suggest that DHEAS is correlated with bone density and is lower in patients with osteoporosis. In elderly women, DHEA levels has been positively correlated with IGF-I levels and negatively correlated with the proresorptive cytokine IL-6. These data suggest that DHEA may be indicated for postmenopausal patients with osteoporosis. DHEA has also shown promise as a therapy to increase bone density in young women with anorexia nervosa, a group of patients who also

exhibit deficiencies in DHEA and IGF-I. The positive association between DHEA(S) levels and bone mass noted in some studies suggests that these adrenal steroids may play an important role in bone accretion (*e.g.*, during adolescence) and on the prevention of the bone loss associated with low DHEA states (*e.g.* anorexia nervosa and aging). More information on the mechanisms of DHEA's anabolic and antiosteolytic effects on bone may provide rationale for its potential role in the prevention of the catabolic effects of aging and disease.

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FIGURE LEGENDS:

Figure 1:
Serum DHEA, ACTH and cortisol in pubertal girls. (From Apter et al., (5), with permission)

Figure 2:
Roles of estrogen and androgen on bone growth and development. (From Bachrach et al., (61), with permission).

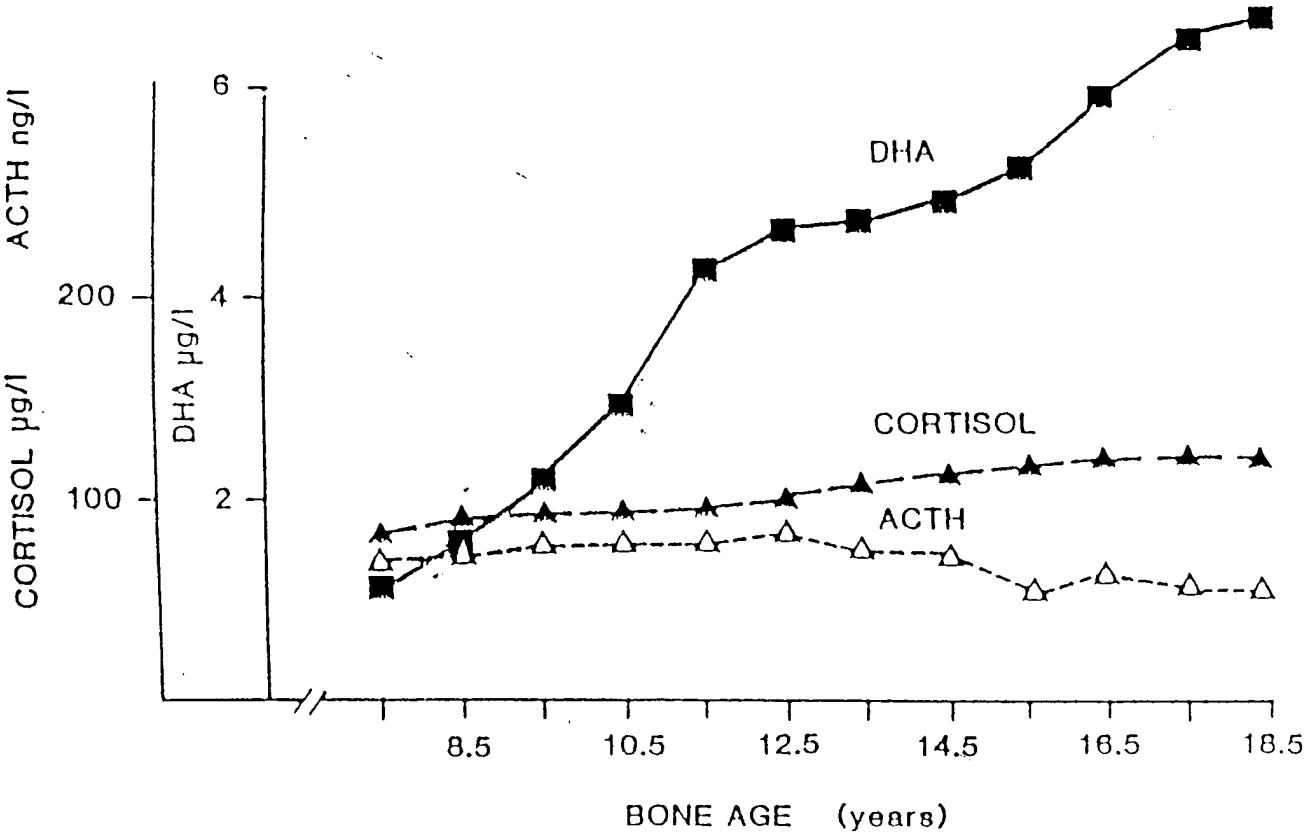
Figure 3:
Proposed mechanisms of bone loss associated with age-related declines in sex steroids. (From LeBoff, M.S. and Glowacki, J., Sex steroids, bone and aging (1999). In: *The Aging Skeleton*, Rosen, C., Glowacki, J.,and Bilezikian, J. (eds). Academic Press: San Diego, with permission).

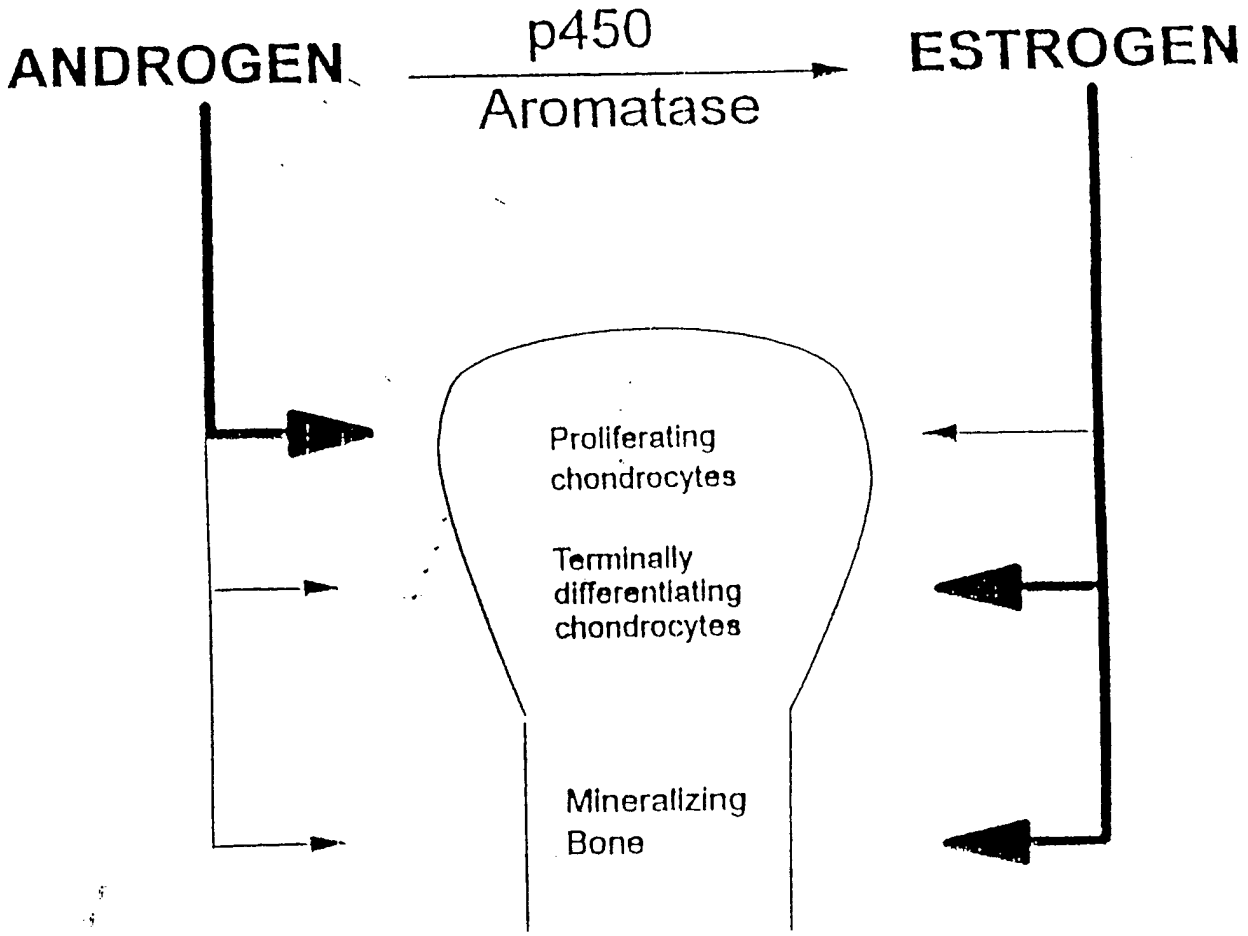
Figure 4:
Relationships between (A) serum DHEAS, (B) IGF-I levels and T-scores at the lumbar spine. Serum DHEAS and IGF-I levels were positively correlated with T-scores of the lumbar spine ($r = 0.32$, $p = 0.001$ and $r = 0.27$, $p=0.007$, respectively)[Adapted from Haden et al. (64)].

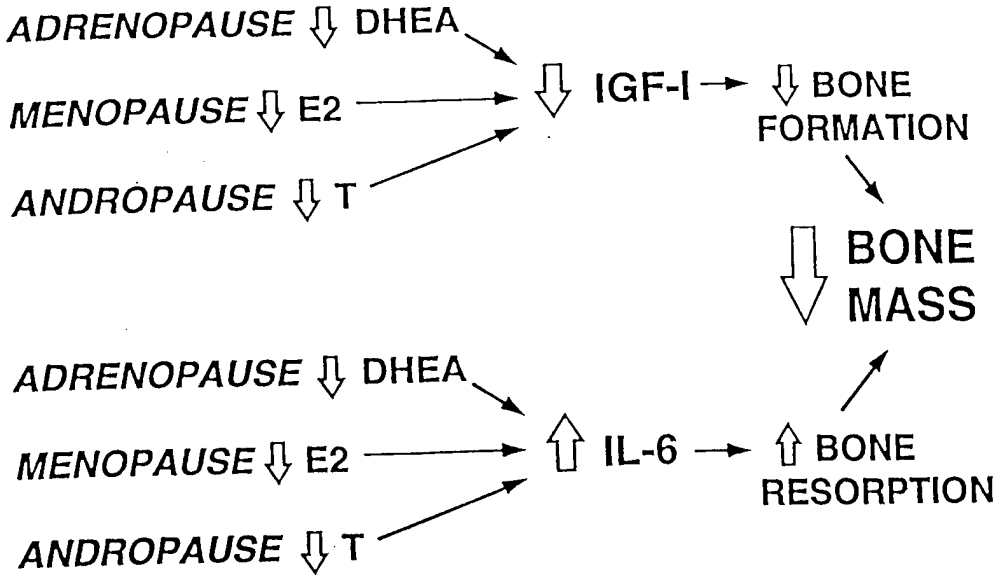
Figure 5:
Effect of subject age on secretion of IGFBP-3 by human marrow cultured for 7 days. (From Rosen, et al., (73), with permission).

Figure 6:

Urinary NTx levels among treatment subgroups. Baseline and 3-month urinary NTx levels are depicted for the three DHEA subgroups: 50, 100 and 200 mg dosage groups. A significant decrease was seen comparing baseline and 3-month levels in the 50 ($p=0.018$) and 200 mg ($p = 0.016$) groups. (From Gordon, et al. (65), with permission).


ACTH ng/l
CORTISOL µg/l
DHA µg/l
6
4
2
200
100
DHA
CORTISOL
ACTH
8.5
10.5
12.5
14.5
16.5
18.5
BONE AGE (years)


ANDROGEN
p450
Aromatase
ESTROGEN
Proliferating
chondrocytes
Terminally
differentiating
chondrocytes
Mineralizing
Bone


ADRENOPAUSE ⇩ DHEA
MENOPAUSE ⇩ E2
ANDROPAUSE ⇩ T
⇩ IGF-I
⇩ BONE FORMATION
⇩ BONE MASS
ADRENOPAUSE ⇩ DHEA
MENOPAUSE ⇩ E2
ANDROPAUSE ⇩ T
⇧ IL-6
⇧ BONE RESORPTION

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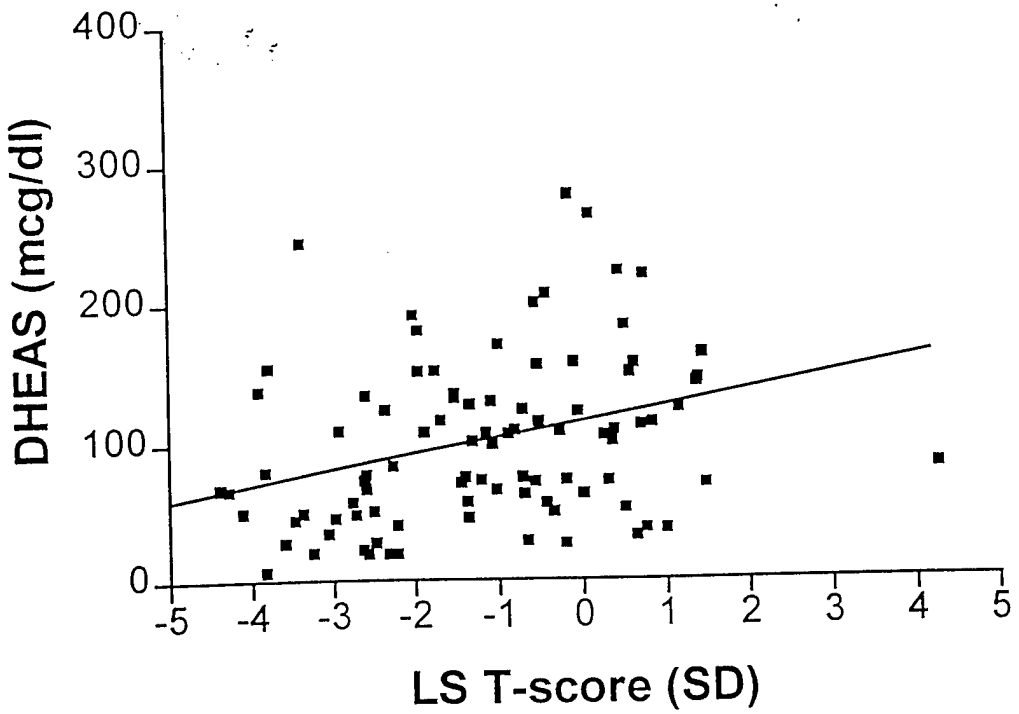
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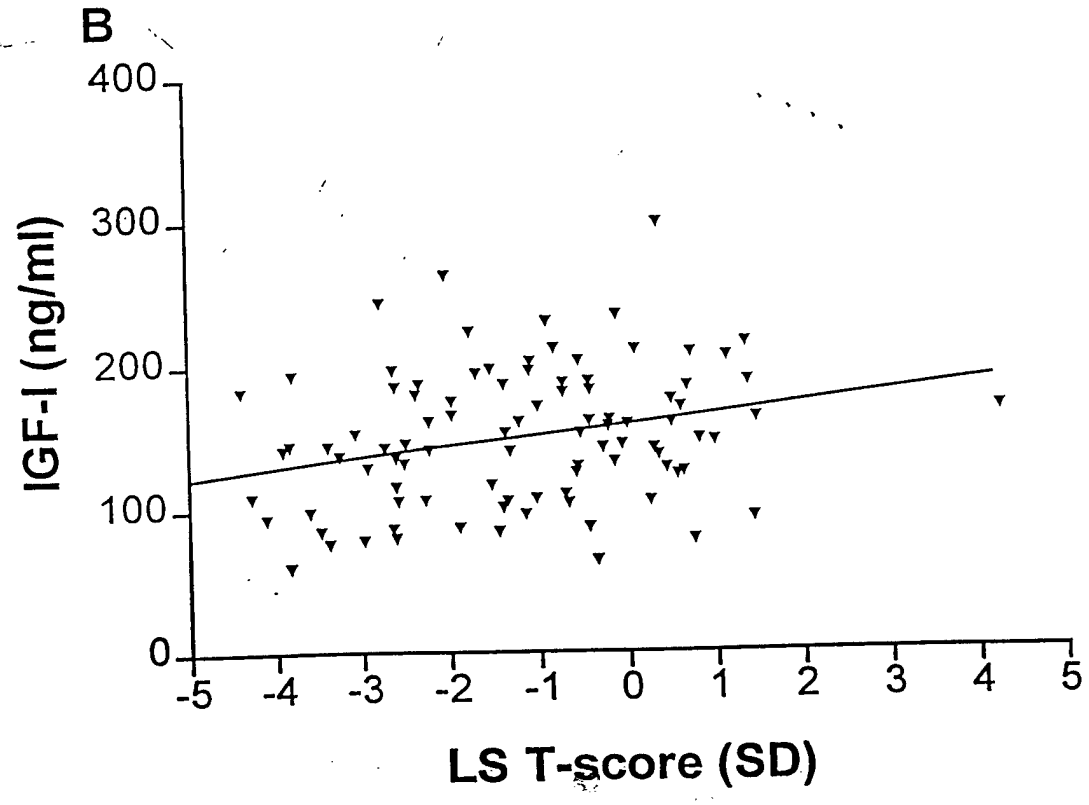
LS T-score (SD)

B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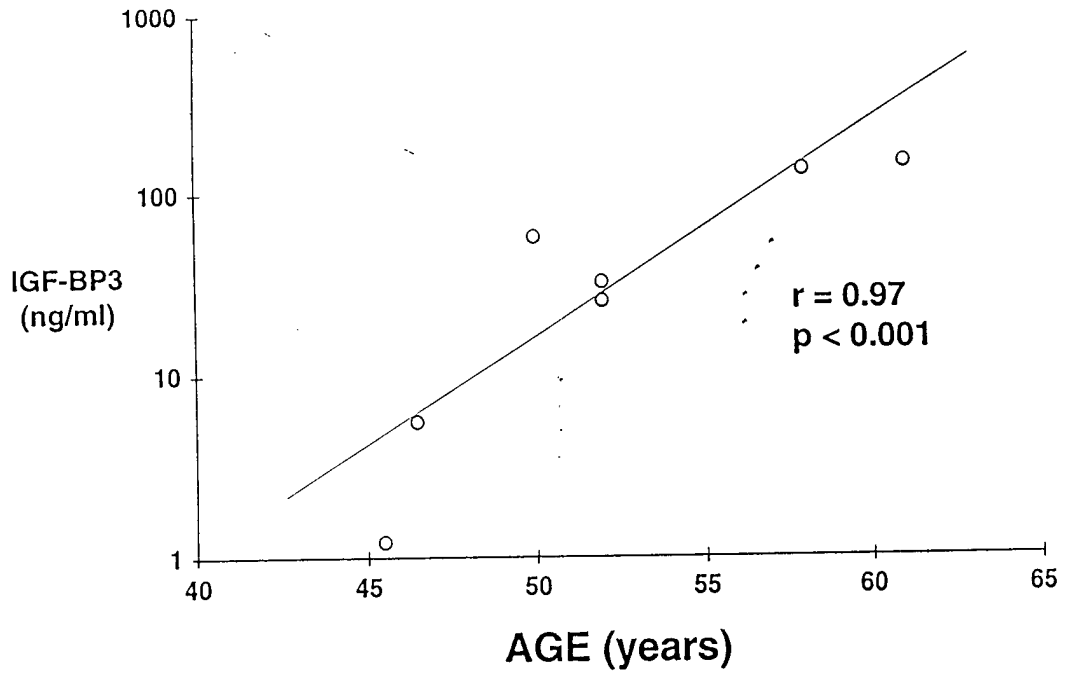
LS T-score (SD)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(ng/ml)
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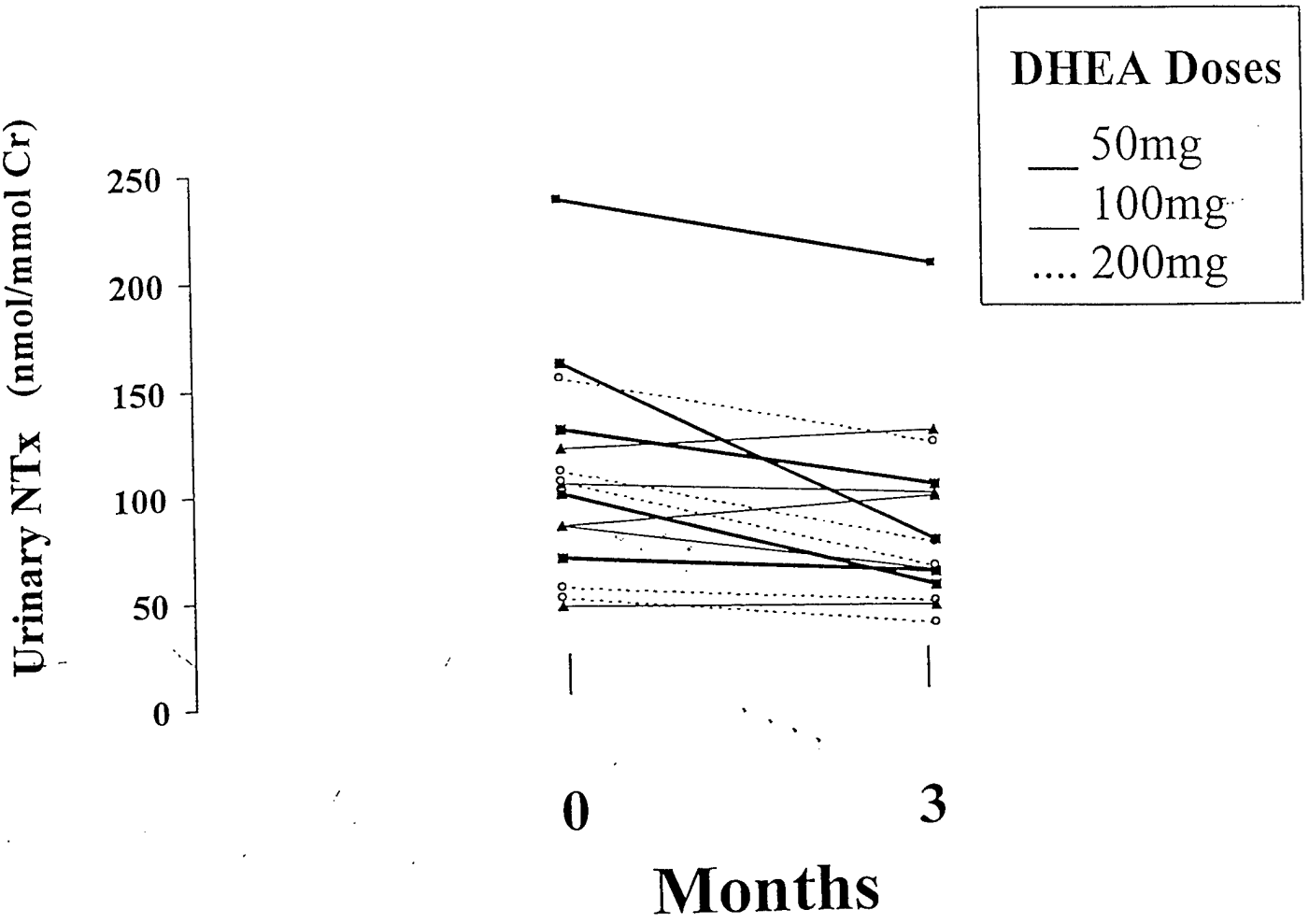
65
AGE (years)


DHEA Doses
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100mg
200mg
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200
150
100
50
0
0
3
Months

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Changes in Bone Turnover Markers and Menstrual Function After Short-term Oral DHEA in Young Women with Anorexia Nervosa

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ABSTRACT

Bone loss is a serious consequence of anorexia nervosa (AN). Subnormal levels of serum dehydroepiandrosterone (DHEA) are seen in patients with AN and may be causally linked to their low bone density. We hypothesized that oral DHEA would decrease markers of bone resorption (urinary N-telopeptides [NTx]), and increase markers of bone formation (serum bone-specific alkaline phosphatase and osteocalcin [OC]). Fifteen young women (age 15–22 years) with AN were enrolled in a 3-month, randomized, double-blinded trial of 50, 100, or 200 mg of daily micronized DHEA. Blood and urinary levels of adrenal and gonadal steroids and bone turnover markers were measured. No adverse clinical side effects of DHEA were noted, and a 50 mg daily dose restored physiologic hormonal levels. At 3 months, NTx levels had decreased significantly in both the 50 mg ($p = 0.018$) and the 200 mg ($p = 0.016$) subgroups. OC levels simultaneously increased within treatment groups over time ($p = 0.002$). Eight out of 15 (53%) subjects had at least one menstrual cycle while on therapy. Short-term DHEA was well-tolerated and appears to normalize bone turnover in young women with AN. Resumption of menses in over half of subjects suggests that DHEA therapy may also lead to estradiol levels sufficient to stimulate the endometrium in this group of patients. (J Bone Miner Res 1999;14:136–145)

INTRODUCTION

A SERIOUS CONSEQUENCE OF anorexia nervosa (AN) is the compromise of bone density. Patients with chronic AN have a 7-fold increased incidence of fractures and often develop early osteoporosis.[1] Poor nutrition deprives these young women of calcium and other macronutrients that strengthen bone.[2] While physical activity is important for maintaining bone mass, patients with AN often exercise excessively, leading to weight loss with subsequent hormonal alterations that predispose these patients to skeletal losses.[3–8] Because adolescence is a critical period for the acquisition of bone mineral,[9,10] the identification of safe and effective strategies to preserve bone density in these young women is an important public health issue.

Subnormal levels of the adrenal steroid dehydroepiandrosterone (DHEA) have been observed in patients with AN. Adrenocorticotropic hormone stimulation tests have suggested decreased adrenal 17–20 lyase activity in AN, with a predominance of glucocorticoid over androgenic pathways.[6,7] This enzymatic block results in increased cortisol and decreased DHEA production.[6,7] In adolescents with AN, DHEA and gonadal steroid levels revert back to the range of a prepubertal child. Subnormal androgen levels, paradoxically accompanied by normal to increased levels of cortisol, have been speculated to represent a regression of hormonal function, with levels normalizing after weight restoration.[6] Normally, the secretion of DHEA rises sharply during adolescence, when bone mass is increasing, and reaches its peak during the third decade. DHEA levels subsequently decline with advancing age.[11–14] In patients with AN, declines in both DHEA and

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insulin-like growth factor I (IGF-I) can occur.[6–8] In some adult studies, DHEA levels are positively correlated with bone mineral density (BMD), suggesting that DHEA may play an important role in bone accretion and the prevention of bone loss associated with low DHEA states (e.g., AN and aging).[15,16]

Although many clinicians use estrogen replacement therapy (ERT) for adolescents with AN, results of this treatment have been conflicting.[17,18] A recent short-term study of combined androgen and estrogen replacement in postmenopausal patients noted that although ERT inhibits bone resorption, it also decreases bone formation, while androgen replacement stimulates bone formation.[19] As DHEA is converted into both estrogens and androgens, inhibiting bone resorption and stimulating bone formation, respectively, restoration of DHEA levels may counteract several of the factors that contribute to bone loss in this population. There are no prior data on the effect of DHEA repletion in patients with AN.

In the present study, we examined the effects of short-term supplementation of DHEA on levels of bone turnover, estradiol (E_2), androgens, and IGF-I in young patients with AN. Because DHEA is metabolized into estrogens and androgens, we hypothesized that supplemental DHEA would increase serum levels to a physiologic range in these patients, simultaneously increasing markers of bone formation (serum osteocalcin [OC] and bone-specific alkaline phosphatase [BAP]) and decreasing markers of bone resorption [cross-linked N-telopeptide, NTx]).

MATERIALS AND METHODS

Subjects

Fifteen young Caucasian women aged 15–22 years (mean age 17.3 ± 2.7 years) with AN by Diagnostic and Statistical Manual of Mental Disorders, Revised Fourth Edition criteria participated in the study. The subjects were recruited from the Eating Disorders Program at Children's Hospital, Boston and a local suburban adolescent medicine practice. All patients were hemodynamically stable, free of any acute or other chronic disease, and were taking no anticonvulsants, glucocorticoids, or sex steroids, medications known to affect BMD. All patients gave informed consent according to the guidelines of the Committee for Clinical Investigation at Children's Hospital, Boston.

Study design, treatment, and measurements

Subjects were randomized to receive a total daily dose of micronized DHEA of either 50, 100, or 200 mg. DHEA was taken twice daily. Micronized DHEA was used because increased lymphatic absorption has been documented with this preparation, thereby decreasing hepatic first-pass effects.[13] The doses of DHEA tested were intended to approximate the high levels of this steroid that are found during adolescence, doses higher than would be generally recommended for replacement therapy in adults. At baseline and 1, 2, and 3 months, subjects had venous blood drawn from the antecubital vein and a second morning urine collected in the outpatient division of the General Clinical Research Center, Children's Hospital, Boston. Samples were obtained between 7:00 a.m. and 10:00 a.m. after an overnight fast. At baseline and at 3 months, BMD (total body, femoral neck, and lumbar spine) and body composition were measured by dual-energy X-ray absorptiometry (DXA). Nutritional and activity questionnaires, including a detailed assessment of calcium intake (both dietary and supplemental calcium), were completed at baseline and 3 months.[20] Psychological questionnaires, including the Beck Depression Inventory, Speilberger State Inventory (an anxiety assessment), and the Eating Attitudes Test (a tool for evaluating body image and anorexic behavior),[21–24] were completed at baseline and at 3 months. Compliance was assessed using monthly interviews, pill counts, and serum DHEA levels.

At monthly visits, subjects' weight in kilograms and height in centimeters were determined in a hospital gown after voiding. All weights were obtained on the same scale at each visit. Height was obtained using the same stadiometer (Perspective Enterprises, Kalamazoo, MI, U.S.A.). Body mass index was calculated from these measurements in kilograms per square meter. Percentage of ideal body weight (%IBW) was estimated using standard percentile tables from the National Center for Health Statistics.[25]

Each participant had a BMD measurement of the total body, lumbar spine, and femoral neck at baseline and at 3 months by DXA with a Hologic 2000 machine (Hologic, Inc., Waltham, MA, U.S.A.). Body composition by DXA was also obtained at these time points. With this instrument, the precision error (CV% ± SEM) for BMD of the spine was 0.53 ± 0.075% and 0.77 ± 0.14% at the femoral neck for premenopausal females.[26] Bone density of the spine was compared with age- and gender-matched controls. BMD of the hip was extrapolated from adult normal bone density data (age 20 years).

At monthly intervals, subjects had venous blood samples obtained for OC, DHEA, and dehydroepiandrosterone-sulfate (DHEAS) measurements by double-antibody radioimmunoassay (RIA), and BAP levels by immunoradiometric assay. At monthly intervals, urinary levels of NTx were also determined using enzyme-linked immunosorbent assay on a second morning void. At baseline, levels of follicle-stimulating hormone, luteinizing hormone, thyroxine, thyroid-stimulating hormone, and prolactin were also measured by RIA. At baseline, 1 month, and 3 months, calcium, phosphorus, liver function tests, total cholesterol, and high-density lipoprotein (HDL) levels were measured by Ektachem methodology (cholesterol oxidase). At baseline and 3 months, parathyroid hormone, total estrogen and testosterone, sex hormone binding globulin (SHBG), IGF-I, and a fasting glucose and insulin were analyzed by RIA, and levels of free and total testosterone were determined by dialysis at Endocrine Sciences (Calabasas Hills, CA, U.S.A.). Blood glucose was determined by glucose hexokinase methodology (Boehringer-Mannheim, Indianapolis, IN, U.S.A.). Serum insulin levels were determined by a microparticle enzyme immunoassay (Abbott Laboratories, Abbott Park, IL, U.S.A.).

TABLE 1. DEMOGRAPHIC CHARACTERISTICS OF ALL SUBJECTS AT BASELINE (MEANS ± SD)

Variable	*Mean ± SD*	*Range*
Age (years)	17.3 ± 2.7	15.0–22.0
Weight (kg)	46.4 ± 6.0	37.0–57.3
% ideal body weight	77.3 ± 6.1	67–84
BMI (kg/m^2)	17.3 ± 1.5	14.0–20.2
Calcium intake (mg/day)	1159.6 ± 528.9	390–2373
Caloric intake (kcal/day)	1616.9 ± 746.9	618–3252
Exercise (hr/week)	15.7 ± 10.8	3.0–41.8
Duration of amenorrhea (months)	20.9 ± 14.1	4.0–48.0
Duration of anorexia nervosa (months)	29.1 ± 27.6	3.0–99.0

n = 15.

Statistical analysis

Data were evaluated statistically with paired or unpaired Student's t-tests in the case of a normal distribution. In the case of a skewed distribution, Wilcoxon signed-rank or rank-sum tests were used, as appropriate. A one-way analysis of variance (ANOVA) was used to compare baseline means (bone marker and hormonal levels, and demographic variables) among the three treatment groups. A two-way repeated measures ANOVA was used to evaluate changes in BAP, OC, and NTx levels within and among the three treatment groups over time. To examine relationships among continuous variables, a Spearman's correlation analysis was performed. Data are presented as mean ± SD with two-tailed significance levels reported. Bone marker and hormonal and demographic variables were not significantly different among groups at baseline, and a dose–response relationship was not seen in response to therapy. Therefore, data are presented as a pooled sample for baseline means and correlation analyses, and as individual dosage subgroups for selected variables. Statistical analyses were performed using SPSS software (SPSS, Inc., Chicago, IL, U.S.A.). A p-value < 0.05 was considered statistically significant. Given the small sample size, all p values ≤ 0.10 are reported.

RESULTS

Demographic characteristics

Baseline demographic variables, presented in Table 1, were not significantly different across treatment groups. The median duration of AN at study entry was 20 months, with a range of 3–99 months. None of the demographic variables changed significantly over the course of the study, including calcium intake and duration of weekly exercise. All subjects had Tanner stage 5 breast and pubic hair development. Six patients (40%) had a family history of osteoporosis and one patient had previously been on ERT (more than 1 year prior to the study).

Hormonal levels

Baseline and 3-month hormone levels for the 50, 100, and 200 mg subgroups are presented in Table 2. There were no mean baseline differences in hormonal levels among groups.

For the total sample (n = 15), the mean baseline DHEA level was 359.7 ± 204.5 ng/dl and the median 290 ng/dl (range of 92–849 ng/dl). Nine of the 15 (60%) subjects were below the normal physiologic range of 215–850 ng/dl. The mean baseline DHEAS level for the sample was 170.6 ± 70.5 μg/dl (range 77–289 μg/dl), with a normal range of 183–283 μg/dl. DHEA levels increased at each time point following therapy, with significant increases from baseline to 1 and 2 months in all dosage subgroups. Increases in DHEA and DHEAS from baseline to 3 months were directly correlated with increases in testosterone over the same interval (r = 0.62, p = 0.016 and r = 0.66, p = 0.011, respectively).

The baseline E_2 level was 25.1 ± 12.1 pg/ml for the entire sample (range 10–44 pg/ml), with a normal range of 30–300 pg/ml. Mean levels within treatment subgroups did not change significantly over the course of the study. Increases in E_2 over the study were moderately correlated with increases in body fat as measured by DXA (r = 0.55, p = 0.050).

The mean baseline total testosterone for the 15 subjects was 20.4 ± 10.0 ng/dl (range 5.2–41.0 ng/dl) with a normal range of 10–55 ng/dl. Although no adverse clinical side effects were noted, total testosterone levels in the 100 and 200 mg groups at 3 months approached or were in a supraphysiologic range. As is seen in Table 2, total testosterone levels for the 50 mg subgroup remained in a physiologic range, despite a significant increase over the 3 months.

The mean baseline free testosterone for the total sample was 1.7 ± 1.2 pg/ml (range 0.5–4.6 pg/ml), with a normal range of 1.1–6.3 pg/ml. As is seen in Table 2, final free testosterone levels in the 100 mg and 200 mg subgroups were elevated, while those in the 50 mg subgroup were normal.

The mean baseline SHBG level for the entire sample was 1.6 ± 0.6 ng/dl (range 0.8–3.8 ng/dl, normal range of 1.0–3.0 ng/dl) with levels significantly decreased after 3 months of DHEA (p = 0.008). However, subgroup analyses revealed no statistically significant changes after 3 months of therapy within any of the treatment groups.

The mean fasting baseline IGF-I level for the entire sample was 272.9 ± 101.6 ng/ml (range 166–566 ng/ml) with a normal range of 240–660 ng/ml. IGF-I levels did not change significantly over the 3-month study period within any of the treatment groups. However, IGF-I levels increased after 3 months of DHEA in 9 out of 15 (60%) subjects. Within this subgroup, increases in IGF-I correlated with increases in BAP (r = 0.67, p = 0.049). The mean 3-month IGF-I level for the total sample was also strongly correlated with the final mean E_2 level (r = 0.65, p = 0.011).

The baseline luteinizing hormone level for the entire sample was 2.1 ± 2.1 mIU/ml (range of 0.15–7.0 mIU/ml) with a normal range of 0.4–11.7 mIU/ml. The mean baseline follicle-stimulating hormone level was 4.28 ± 2.16 (range of 0.24–7.04 mIU/ml) with a normal range of 1.0–9.2 mIU/ml.

The mean baseline insulin level was 4.9 ± 2.1 μU/ml, accompanied by a mean glucose of 76.2 ± 8.7 mg/dl before

TABLE 2. HORMONAL PARAMETERS OF INDIVIDUAL SUBGROUPS OF DHEA TREATMENT: BASELINE AND 3 MONTHS

Hormone	*Baseline*	*3 Months*	*Significance (p)*	*Normal range*
50 mg group				
DHEA (ng/dl)	305.8 ± 205.1	510.4 ± 85.9	NS	215–850
E_2 (pg/ml)	36.0 ± 11.6	64.3 ± 55.3	NS	30–300
Testosterone				
total (ng/dl)	23.01 ± 15.1	44.4 ± 7.4	0.043	10–55
free (pg/ml)	1.96 ± 1.2	5.32 ± 2.0	0.043	1.1–6.3
SHBG (ng/dl)	1.6 ± 0.6	1.1 ± 0.4	0.068	1.0–3.0
IGF-I (ng/ml)	345.5 ± 156.4	486.5 ± 51.9	NS	240–660
NTx (nmol/mmol Cr)	141.6 ± 60.3	98.0 ± 61.4	0.018	10–65
OC (ng/ml)	2.4 ± 1.2	9.7 ± 7.3	0.079	2–24
BAP (ng/ml)	21.4 ± 12.54	25.2 ± 18.9	NS	2–22
100 mg group				
DHEA (ng/dl)	359.0 ± 117.5	852.4 ± 228.1	0.043	
E_2 (pg/ml)	18.0 ± 8.3	24.4 ± 17.4	NS	
Testosterone				
total (ng/dl)	16.8 ± 4.9	56.6 ± 36.3	0.068	
free (pg/ml)	0.9 ± 0.3	8.1 ± 5.9	0.043	
SHBG (ng/dl)	2.6 ± 0.8	0.8 ± 0.1	NS	
IGF-I (ng/ml)	173.5 ± 10.6	275.5 ± 163.3	NS	
NTx (nmol/mmol Cr)	85.2 ± 28.9	79.2 ± 30.7	NS	
OC (ng/ml)	1.6 ± 0.5	4.7 ± 5.4	NS	
BAP (ng/ml)	12.6 ± 4.2	13.6 ± 4.0	NS	
200 mg group				
DHEA (ng/dl)	414.4 ± 288.4	1223.0 ± 591.5	0.043	
E_2 (pg/ml)	23.6 ± 10.7	25.2 ± 10.7	NS	
Testosterone				
total (ng/dl)	21.8 ± 8.1	52.8 ± 15.1	0.068	
free (pg/ml)	2.6 ± 1.4	10.3 ± 4.9	0.068	
SHBG (ng/dl)	1.2 ± 0.6	0.80 ± 0.3	NS	
IGF-I (ng/ml)	254.5 ± 64.3	269.5 ± 72.8	NS	
NTx (nmol/mmol Cr)	96.0 ± 40.7	74.2 ± 34.7	0.016	
OC (ng/ml)	2.5 ± 1.9	6.40 ± 6.7	NS	
BAP (ng/ml)	17.4 ± 7.1	17.0 ± 2.4	NS	

Values are expressed as mean ± SD; $n = 5$ for each dosage subgroup. NS, not significant.

DHEA. After therapy, levels of both insulin and glucose did not change significantly within any of the treatment groups.

Bone resorption markers

Urinary NTx levels at baseline and 3 months for the 50, 100, and 200 mg subgroups are shown in Table 2. There were no differences in mean NTx levels at baseline across the three treatment groups.

The mean urinary NTx level was increased for the 15 subjects at baseline: 108.8 ± 47.9 nmol/mmol creatinine (normal 10–65). As is shown in Fig. 1, significant decrease in NTx levels was seen in the 50 ($p = 0.018$) and 200 mg ($p = 0.016$) dosage subgroups. At baseline, levels of NTx were inversely correlated with levels of DHEAS ($r = -0.74$, $p = 0.002$). There was also a strong inverse correlation between 1-month levels of both DHEA and DHEAS and the 1-month NTx levels ($r = -0.60$, $p = 0.023$ and $r = -0.92$,

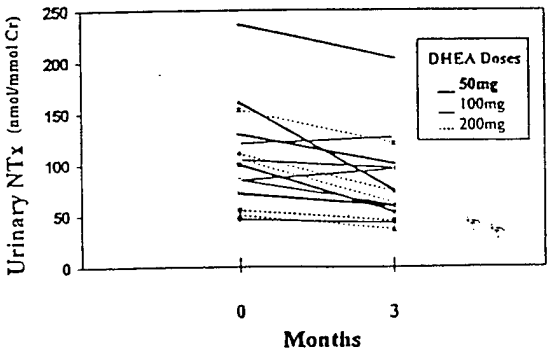

FIG. 1. Urinary NTx levels among treatment subgroups. Baseline and 3-month urinary NTx levels are depicted for the three DHEA subgroups: 50, 100, and 200 mg dosage groups. A significant decrease was seen comparing baseline to 3-month levels for the sample using Student's *t*-tests for paired data ($n = 15$, $p = 0.002$).

$p = 0.001$, respectively); a similarly strong inverse correlation was seen at 2 months for the same variables ($r = -0.56$, $p = 0.031$ and $r = -0.78$, $p = 0.001$, respectively). Increases in DHEAS from baseline to 3 months also weakly correlated with decreases in NTx over the same interval ($r = -0.45$, $p = 0.09$).

Bone formation markers

Baseline and 3-month BAP and OC levels for the three dosage subgroups are shown in Table 2. OC levels over the course of the study are shown in Fig. 2. There were no differences in levels of bone formation markers at baseline across treatment groups.

The mean baseline OC level was 2.2 ± 1.3 ng/ml for the entire sample, low normal compared with an adult normal range of 2–24 ng/ml, and subnormal compared with a normal range for adolescents of 42–225 ng/ml. As is shown in Fig. 2, OC levels increased significantly over time within all subgroups, particularly at the 1-month time point ($p = 0.002$). Increases in OC were strongly correlated with increases in BAP ($r = 0.70$, $p = 0.004$). The 3-month OC level was also strongly correlated with the final IGF-I level ($r = 0.63$, $p = 0.012$), and the final E_2 level ($r = 0.67$, $p = 0.009$).

The mean baseline BAP level for the sample was normal at 17.1 ± 8.8 ng/ml compared with a normal young adult range of 2–22 ng/ml, but was subnormal against expected levels of 28–38 ng/ml reported for healthy 15- to 18-year-old females.[27] There were no statistically significant changes in BAP over time or within subgroups. BAP increased in 9 out of 15 (60%) subjects from baseline to 3 months. Within this subgroup, the increase in BAP after DHEA was strongly correlated with both an increase in IGF-I ($r = 0.74$, $p = 0.034$) and an increase in E_2 levels ($r = 0.77$, $p = 0.010$). Four of the patients whose BAP levels increased were in the 50-mg treatment group.

Lipid panels

There was no significant difference between HDL levels at baseline and at 3 months; the mean HDL level was 48.6 ± 14.0 mg/dl before DHEA and did not change significantly in any subgroup after therapy. There was also no significant difference in total cholesterol with a level of 158.1 ± 41.9 mg/dl at baseline without significant changes at 3 months.

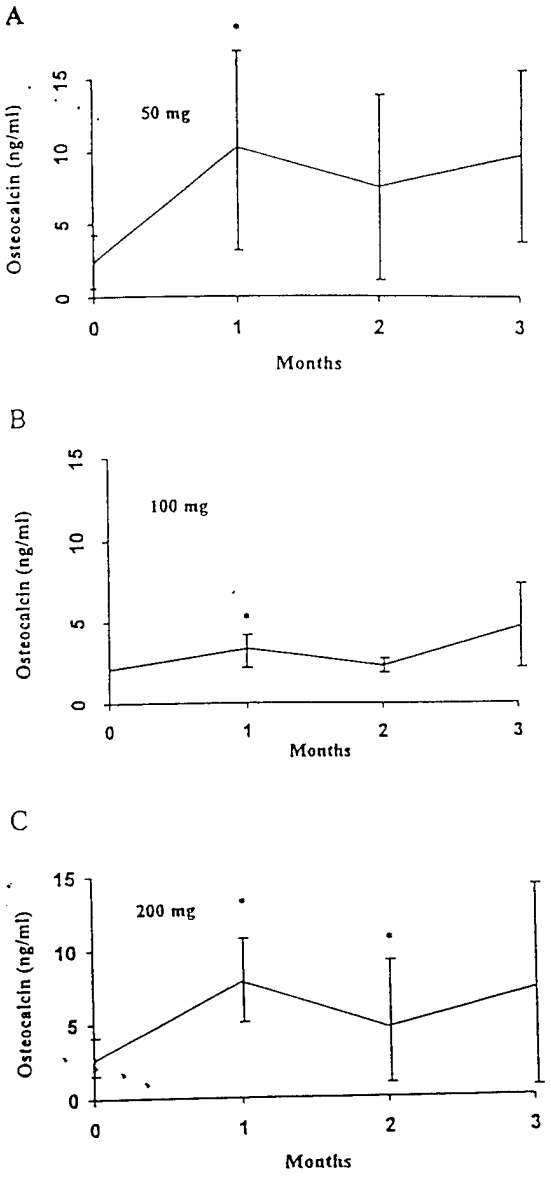

FIG. 2. Serum OC levels among treatment subgroups. Serum OC levels are shown at baseline, and after 1, 2, and 3 months of DHEA therapy for each of the three dosage groups: (A) 50 mg, (B) 100 mg, and (C) 200 mg. Over the 3-month study period, significant changes were noted within groups ($p = 0.002$) by repeated measures ANOVA. Significant changes from baseline are noted with an asterisk.

Bone density and body composition measurements

The mean baseline lumbar BMD for all subjects was 0.92 ± 0.12 g/cm^2 (range 0.58–1.024 g/cm^2). Expressed as a Z score, the mean bone density was −0.73 ± 1.14 SD (range −4.02 to 0.64 SD) below that expected for age and gender. The mean whole body BMD was 1.1 ± 0.6 g/cm^2 (range 0.80–1.8 g/cm^2) with a Z score of −1.3 ± 0.8 SD (range −3.8 to −0.75 SD). The mean BMD of the femoral neck was 0.89 ± 0.10 g/cm^2 (range 0.54–0.99 g/cm^2) with a Z score of −0.71 ± 0.87 SD (range −3.5 to 0.19 SD). The mean baseline percentage body fat by DXA was 15.6 ± 6.9% (range 5.7–24.0%). There were no significant changes in either mean BMD at any site or body composition after 3 months of DHEA. Baseline BMD (g/cm^2) was inversely correlated with both duration of amenorrhea ($r = -0.52$, $p = 0.046$) and duration of AN ($r = -0.57$, $p = 0.028$). Percentage body fat was inversely correlated with the amount of weekly exercise at baseline ($r = -0.57$, $p = 0.027$).

TABLE 3. CLINICAL CHARACTERISTICS OF SUBJECTS WHO RESUMED MENSES

	Baseline	*3 months*	*Significance (p)*
Weight (kg)	44.9 ± 5.6	49.1 ± 8.9	NS
% body fat	12.7 ± 6.6	14.9 ± 6.8	NS
%IBW	0.8 ± 0.1	0.9 ± 0.1	NS
Duration of amenorrhea (months)	23.4 ± 16.5	N/A	N/A
E_2 (pg/ml)	29.7 ± 13.5	51.4 ± 43.6	NS
Testosterone (ng/dl)	23.6 ± 8.2	44.7 ± 12.8	0.018
DHEA (ng/dl)	444.0 ± 251.6	814.9 ± 438.4	0.028
NTx (nmol/mmol Cr)	110.6 ± 68.8	84.6 ± 60.0	0.036
OC (ng/ml)	1.6 ± 0.28	8.5 ± 7.1	0.012
BAP (ng/ml)	18.4 ± 11.9	24.3 ± 15.1	0.034

Values are expressed as mean ± SD. n = 8 for this subgroup. NS, not significant.

Psychological parameters

There were no significant changes in psychological measures over the study. The baseline mean Eating Attitudes Test score was elevated for the sample at baseline (48.3 ± 23.4 [normal < 15]) and did not change significantly at 3 months in any group. The baseline mean Beck Depression Inventory score was also increased at baseline at 18.5 ± 13.0 (normal < 9) and was unchanged at 3 months. Lastly, the Speilberger State Inventory score was 49.1 ± 11.8 (range of test, 20–80) at baseline and did not change significantly after DHEA.

Menstrual function

Eight of the 15 subjects (53%) reported having at least one menstrual period while on DHEA. Each episode of vaginal bleeding lasted a minimum of 3 days. This finding was unexpected because weight gain was significant in only 2 out of 8 (25%) subjects in this subgroup, with weight loss also seen in 2 out of 8 (25%). As is shown in Table 3, there was no significant change in weight, percentage body fat by DXA or %IBW within this subgroup after 3 months of DHEA. Compared with subjects who remained amenorrheic, there were no significant differences either at baseline or 3 months in these same variables, or duration of AN or amenorrhea. Significant increases in testosterone were seen in this subgroup comparing baseline to 3-month levels. Also shown in Table 3 are significant changes in bone turnover markers from a post hoc analysis of those subjects whose menses returned. This subgroup also had significant increases in BAP at 3 months (p = 0.027) compared with the amenorrheic group. Three-month E_2 levels were significantly increased in this subgroup, compared with those subjects who remained amenorrheic (mean 51.4 ± 43.6 vs. 17.7 ± 6.4 pg/ml, respectively; p = 0.010), although the baseline E_2 levels were not significantly different (29.7 ± 13.5 vs. 20.6 ± 9.2 pg/ml, p = NS). Within 3 months after discontinuing DHEA, seven of the above eight patients showed cessation of menses.

Adverse effects

One patient in the 200-mg subgroup had an elevation of transaminases after 1 month of DHEA, in association with mild upper respiratory symptoms. Without stopping therapy, laboratory values decreased within 48 h and were back within the normal range within 2 weeks. This episode was attributed to a viral illness rather than the DHEA, since parameters rapidly normalized despite continuation of therapy. No other abnormalities in other biochemical parameters and no signs of hirsutism or acne were noted.

DISCUSSION

The current study documents changes in several biochemical and clinical parameters after DHEA therapy in a cohort of adolescents and young adults with AN. To our knowledge, this is the first report to describe the effects of DHEA on bone turnover markers and menstrual function in young women with this disease. Our data suggest that DHEA may both decrease bone resorption and increase bone formation markers significantly in subjects with AN and may be associated with the resumption of menses unrelated to weight gain or changes in percentage of body fat. DHEA levels were subnormal in 60% of subjects studied, and a 50-mg dose of DHEA restored DHEA, E_2, testosterone, and IGF-I levels to a physiologic range. Although the 100-mg and 200-mg doses resulted in supraphysiologic-free testosterone levels, this therapy was well-tolerated without adverse clinical effects.

Recent research has affirmed that deficiencies of androgens jeopardize skeletal health.[29,30] For example, patients with complete androgen resistance have been documented to have a lower BMD, despite increased E_2 levels.[30] The androgen DHEA is a steroid precursor of estrogens through aromatization in peripheral tissues.[31–34] DHEA stimulates human osteoblastic cell proliferation through the androgen receptor, with alkaline phosphatase production through transforming growth factor-β.[35] A recent study[33] demonstrated that the stimulatory effect of DHEA on BMD and bone mineral content is primarily androgenic in nature. Our results corroborate with this report since the level of aromatization was not significantly increased at any of the DHEA doses studied. Our preliminary findings with oral DHEA; and those of Labrie et al. with topical DHEA,[36] suggest that this androgen has both anabolic and antiosteolytic properties. In both studies, uri-

nary bone resorption markers decreased, suggesting suppressed osteoclast production and bone breakdown. Serum bone formation markers also increased, implying a stimulation of osteoblast function and bone formation. Similar changes in bone turnover markers were also seen after 1 year of DHEA in the animal study of Martel et al.[33] However, larger prospective studies are necessary to determine the long-term effects on both bone turnover markers and bone mass.

Accompanying the increased bone resorption in AN, a decrease in bone formation has been previously described.[5,17] Our data support this observation because low BAP and OC levels and elevated NTx levels were observed at baseline in our study subjects. Our subjects also had a reduced mean body mass index, which has been identified as an independent risk factor for the development of osteoporosis.[37] The decreased bone formation in AN is particularly problematic in adolescent patients who normally acquire 45–60% of their bone mass during the teenage years and reach their peak bone mass during this period.[2,3] Bone loss associated with this disease has also been characterized as being of rapid-onset and often irreversible.[1,5] Furthermore, a compromise of peak bone mass increases these individuals' risk of developing subsequent osteoporosis and fractures.[37] To prevent bone loss, stimulation of bone formation with minimization of bone resorption is needed. These pilot data suggest that DHEA fulfills this need as it normalizes levels of endogenous hormones that should be present abundantly during adolescence, stimulating the critical bone formation of this developmental period.

The mechanisms behind the decrease in bone resorption observed in this study are unclear. NTx levels were inversely correlated with DHEAS levels at baseline, and significant increases in DHEA and DHEAS in all subgroups at 1 and 2 months were inversely correlated with decreases in NTx. DHEA may induce the release of certain mediators that inhibit bone resorption. For example, DHEA itself and/or DHEAS may suppress secretion of proresorptive cytokines. This suggestion is only speculative since cytokines were not measured in this study. Previous research has established that DHEA is converted into estrogen,[12] and supplemental estrogen has been well validated as a therapy for postmenopausal bone loss.[28] Additionally, 17β-E_2 in vitro inhibits the production of proresorptive cytokines.[38,39] Interestingly, NTx levels were not inversely correlated with E_2 levels in the current study. A report by Labrie et al. showed that changes in intracellular sex steroids, as may occur after DHEA administration, are not necessarily translated into parallel changes in circulating sex steroid levels.[40] Data from the study of Martel et al.[33] also corroborate our results, indicating that both the antiosteolytic and anabolic effects of DHEA on bone are due mainly to local formation of androgens in bone cells, rather than the estrogenic effects of DHEA. Other potential mechanisms behind the antiosteolytic actions of DHEA deserve further study.

IGFs play an important role in the maintenance of bone mass and may mediate the anabolic actions of DHEA on the skeleton.[41,42] Previous work has shown that IGF-I levels are low in patients with AN.[8,43] In vitro studies have shown that IGF-I has effects on osteoblast function and collagen formation.[44,45] One previous clinical study showed that DHEA produced a rise in IGF-I and free IGF-I as it decreased IGF binding protein-1 levels.[46] In the current study, it was hypothesized that IGF-I was an anabolic mediator of DHEA's actions, stimulating bone formation, although no significant increases were seen within the small sample studied. However, increases in the bone formation marker, BAP, were strongly correlated with increases in IGF-I, and the 3-month IGF-I levels were directly correlated with final levels of a second formation marker, OC. As only future protocols can explore, this therapy may increase secretion of anabolic mediators other than IGF-I, unidentified to date, that were not measured in the current study.

Direct correlations between levels of E_2 and bone formation markers were found in this study. We identified a strong correlation between increases in BAP and E_2, and the final OC and E_2 levels. DHEA is converted into E_2[12,34]; and previous studies have documented important effects of E_2 on bone formation and growth. Estrogen has been shown to stimulate transforming growth factor-β and IGF-I, each stimulating bone formation in vivo.[47] Although not examined in this study, estrogen also inhibits prostaglandin E_2 and interleukins 1 and 6,[48] thereby blocking bone resorption. Although an animal study has suggested that DHEA's effects on bone formation are primarily androgenic rather than estrogenic,[33] the beneficial effects of this steroid's conversion to estrogen in humans may be an important aspect of this hormonal agent and merits further study.

Androgenic hormones are receiving increased acceptance for use in female patients, but careful surveillance for androgenic side effects is warranted. Using pharmacological doses (1600 mg/day) for 28 days in six menopausal women, Mortola and Yen showed that DHEA produced increases in levels of testosterone, androstenedione, and E_2.[12] While no adverse clinical effects were noted on these doses for a short-term course, cholesterol, HDL, and SHBG levels decreased. Two other reports showed a trend of beneficial effects on serum lipid profiles after 12 months of percutaneous DHEA,[36,49] although in one of these studies[36] two women developed slightly increased facial hair and two others noted mild acne. Insulin resistance is another potential side-effect of DHEA therapy, although one report demonstrated evidence of decreased insulin resistance after 12 months of the topical form of this therapy.[49] In the current study, no significant changes in fasting insulin or lipid levels, acne, or hirsutism were seen after 3 months of replacement doses of DHEA. Given our data showing restoration of physiologic hormonal levels with the lower DHEA dose, and the potential for adverse side effects during longer treatment periods with higher doses, 50 mg appears to be an appropriate dose for long-term trials of DHEA in adolescent females with AN.

The resumption of menstrual bleeding in 53% of subjects was surprising and may contribute to our understanding of factors affecting return of menses in patients with AN. Studies have noted that anorexic patients resume menses at

varying body weights.[50–52] Data from a study by Golden et al. suggest that an E_2 level of 30 pg/ml may be a marker for the onset of menses in these patients.[51] We found that baseline E_2 levels were moderately correlated with percentage of body fat. We also documented significant increases in E_2 at 3 months in subjects who resumed menses with a mean level exceeding 50 pg/ml. Increases in E_2, combined with a significant increase in testosterone after DHEA, may explain why favorable, significant changes in bone turnover markers occurred within this subgroup. DHEA is a significant precursor of ovarian estrogen secretion.[34] Its conversion to estrogen in the ovary may explain the recurrence of menses seen in the current study. From the significant decrease in SHBG levels among the total sample, one can postulate that DHEA, and the testosterone to which it is converted, suppress SHBG, possibly providing increased bioavailable E_2 to stimulate the endometrium. However, Labrie et al.[36] found that 12 months of DHEA therapy did not induce estrogenic changes in the endometrium of postmenopausal patients. Whether DHEA administration stimulates the endometrium of young adolescent patients is currently unknown and deserves investigation. The fact that cyclic vaginal bleeding subsided within 3 months after discontinuing the medication further suggests that DHEA promoted the return of menses.

Our study has several limitations. First, given the small sample size of this pilot study, results must be considered preliminary. Second, the amount of activity and nutritional intake was determined by self-report, and patients with AN are known to under-report exercise and over-report dietary intake.[52] Furthermore, our study subjects were adolescents who may not have been compliant with therapy. Despite appropriate pill counts and histories of adhering to the protocol, varying DHEA and DHEAS levels throughout the study suggest intermittent compliance which may have impacted on our data. We also did not see a clear dose–response relationship among the 50, 100, and 200 mg dose groups, which could have been related to either noncompliance or other factors. Although not addressed specifically in this pilot study, factors relating to absorption and bioavailability may exist that are unique to patients with AN. There may be a threshold effect such that higher doses inhibit formation and/or increase resorption, or the maximal effect may be reached with the 50 mg dose. In addition, the small sample size (five subjects per group) gave us little power to determine definitive dose–response relationships and may have caused us to miss other significant associations. Last, only changes in markers of bone turnover were documented in the current study. Although increasingly sensitive,[53] they are not exact measures of changes in bone mass. As only a longitudinal protocol can address, the effect of DHEA on bone mass requires further study.

This preliminary pilot study was designed to evaluate the effect of a new therapy on bone turnover markers in order to begin to address its effects on reversing the skeletal loss associated with AN. This bone marker data, and the unexpected finding regarding menstrual function, indicate that DHEA may be an effective means of hormonal replacement for young women with this disease. Our data suggest that DHEA may be one of only a few agents which have shown promise in preventing the irreversible bone loss associated with AN. We have shown that short-term DHEA significantly decreased levels of bone resorption and increased markers of bone formation. DHEA was well tolerated and, as an oral therapy, was convenient for adolescent patients. These pilot data emphasize the therapeutic potential of DHEA. Longitudinal studies are needed to determine the long-term biological actions of this agent, including its effects on bone mass.

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