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Review

DHEA and the Skeleton (Through the Ages)

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Dehydroepiandrosterone (DHEA) and its 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 10–20% of peak levels by age 70 yr. 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.

Key Words: DHEA; osteoporosis; aging; insulin-like growth factors; cytokines.

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 the prevention of the catabolic changes associated with aging and for certain disease states. This review summarizes 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.

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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 precursor for 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 yr, 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. Whereas serum DHEA levels rise with the advancement of chronological 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 and 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 *(10)* referred to this phenomenon that normally occurs during aging as the "adrenopause."

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 have shown 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)*. However, results from

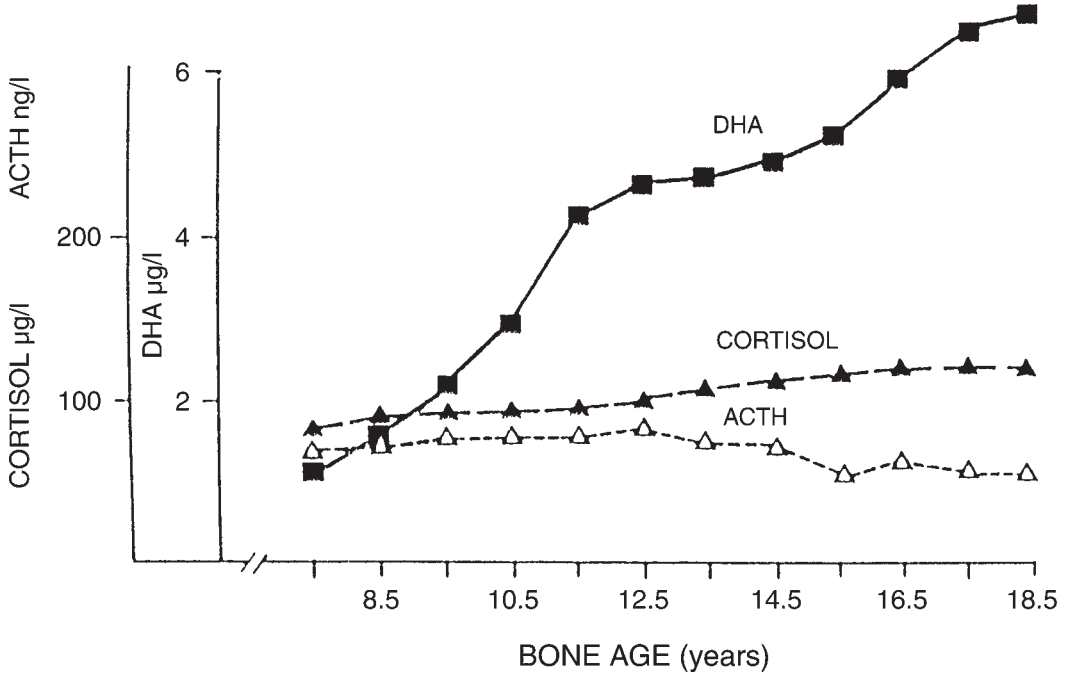

Fig. 1. Serum DHEA, ACTH, and cortisol in pubertal girls. (Reproduced with permission from ref. *5*.)

studies of DHEA's effects in rodent or other nonprimate 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. *(24)* studied the pharmacokinetics of 150 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. After oral administration, DHEA is largely absorbed and converted to DHEAS in the hepato-splanchnic system *(25)*. Using radioisotopic tracer techniques and calculations, the half-life of DHEA is estimated to be 15–30 min, whereas the half-life of DHEAS is much longer, at 7–10 h *(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 because 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 receptor (AR) and/or estrogen receptor (ER), 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, approx 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 (DHT). The enzymes responsible for these changes are 17β-hydroxysteroid dehydrogenase and 5α-reductase, respectively.

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)* that 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.

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 mo 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. *(49)* examined a small cohort of men between the age of 18 and 63 yr, and demonstrated that testosterone had a strong influence on pulsatile growth hormone (GH) secretion. Another study *(50)* examined associations between androgens and the GH-IGF axis. A significant correlation was found between components of the skeletal insulin-like growth factor (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)* that 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 AR 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 similar to that of unaffected female animals *(56)*. Another natural model is the case of a human with an ER mutation. The index case was a 28 yr-old man with tall stature, delayed closure of epiphyses, and reduced bone mineral density (BMD) *(57)*. A mutation in his ER resulted in resistance to even supraphysiological 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 owing 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).

IGFs 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 a recent report by Haden et al. *(64)*, there was a strong positive correlation between circulating DHEAS and IGF-I levels ($r = 0.43$, $p = 0.0001$). 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 *(64,66–68)*. 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 GH levels *(63)*, which suggests an acquired resistance to GH's action. A recent study showed that short-

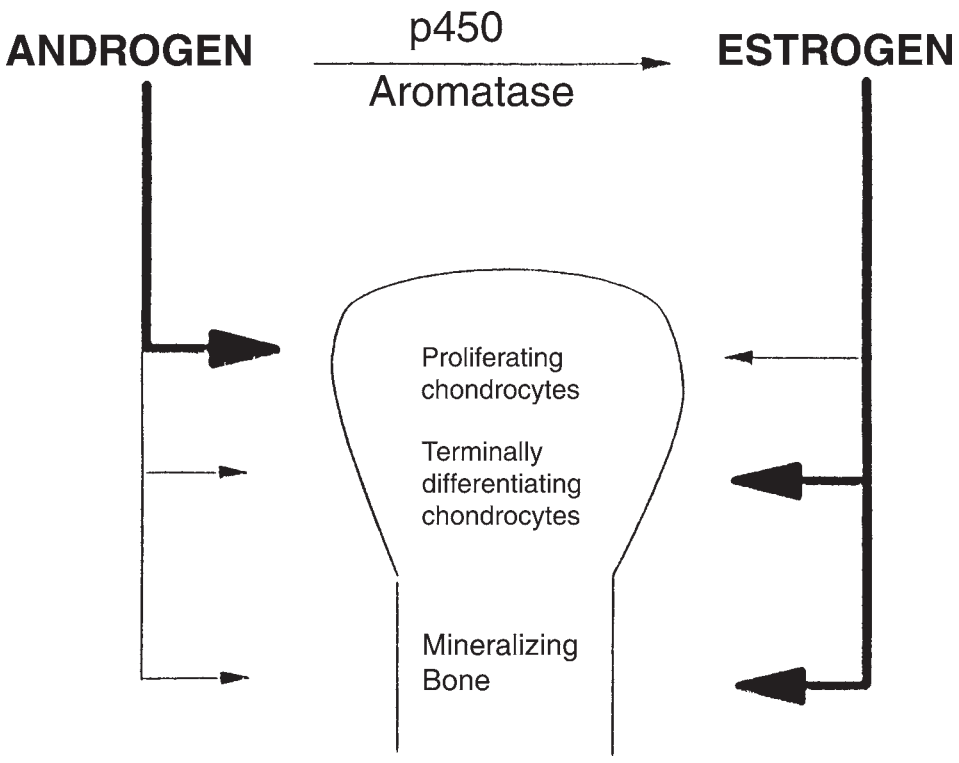

Fig. 2. Roles of estrogen and androgen on bone growth and development. (Reproduced with permission from ref. *61*.)

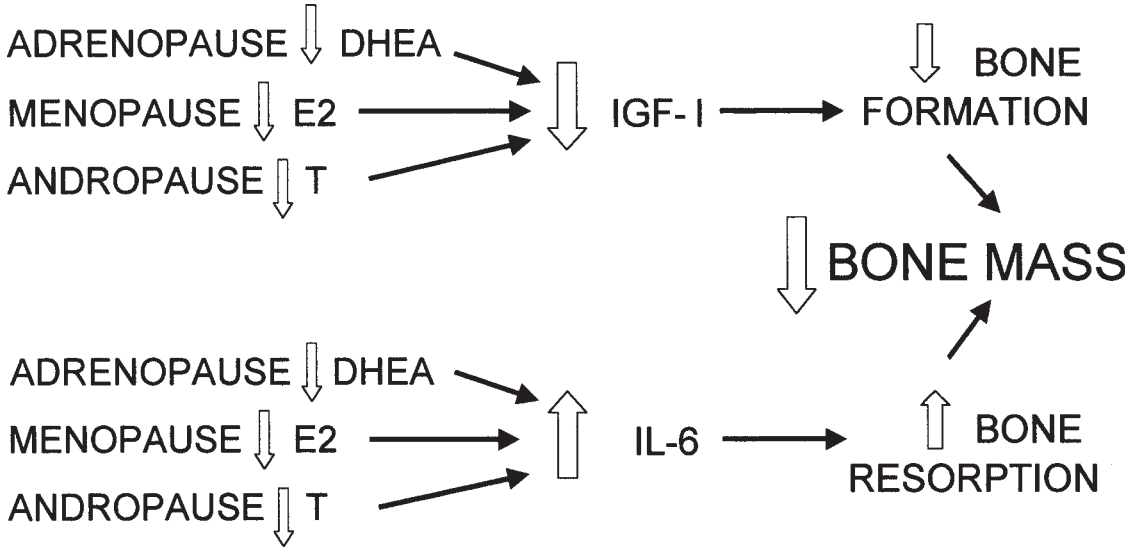

Fig. 3. Proposed mechanisms of bone loss associated with age-related declines in sex steroids. (Reproduced with permission from ref. *61a*.)

term iv replacement of recombinant human IGF-I to osteopenic patients with anorexia nervosa increased bone formation *(63)*. In a report by Gordon et al. *(65)*, after 3 mo of DHEA therapy, serum IGF-I was positively correlated with final serum osteocalcin levels in patients with anorexia nervosa. Those 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)*.

DHEA and Local Factors in Bone Turnover

IGF-I is both a systemic and local modulator of bone formation. GH is considered the prototypic systemic regulator of IGF-I synthesis in humans. An excess of GH is associated with increased levels of serum IGF-I, whereas a deficiency in GH is accompanied by low serum IGF-I and low BMD *(69)*. In vitro treatment of bone cells with IGF-I

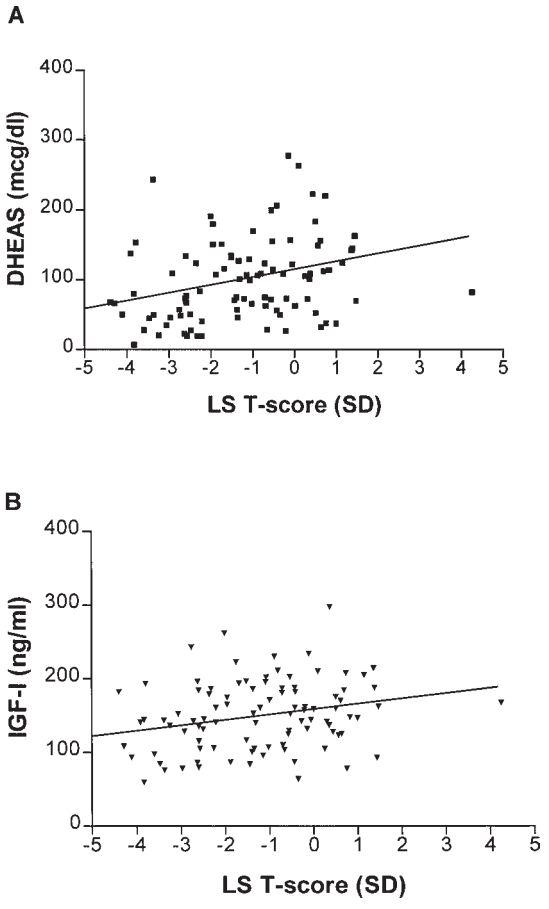

Fig. 4. Relationships between **(A)** serum DHEAS and **(B)** IGF-I levels, and T-scores at the lumbar spine. Serum DHEAS and IGF-I levels were positively correlated with T-scores of the lumbar spine ($r = 0.32$, $p = 0.001$ and $r = 0.27$, $p = 0.007$, respectively) (Adapted from ref. *64*).

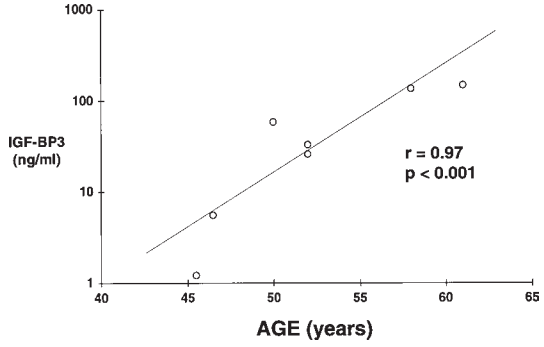

Fig. 5. Effect of age on secretion of IGFBP-3 by human marrow cultured for 7 d. (Reproduced with permission from ref. *73*.)

stimulates proliferation, differentiation, or matrix synthesis, depending on the stage of osteoblastic differentiation. Components of the IGF system 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 production 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. *(73)* 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 (Fig. 5). Upregulation of IGFBP-3 with aging is consistent with other reports that senescent fibroblasts secrete elevated levels of IGFBP-3 *(74)*.

Some information about the anabolic effects of DHEA on bone formation in vitro is available. 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 AR antagonist hydroxyflutamide and neutralizing antibodies against transforming growth factor-β (TGF-β). These data were interpreted as evidence that the mitogenic effect of DHEA was mediated by AR-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 which, in turn, stimulates estrogen production, resulting in enhanced stimulation of bone formation.

A model has evolved in which 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, several local factors 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)*, IL-1 *(86–88)*, and IL-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 stimulates 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. *(92)* delineated the importance of IL-6 in the mediation of bone resorption associated with estrogen deficiency. 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. ERs 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 *(92)* secretion of IL-6 and IL-11 was attenuated in marrow cultured from postmenopausal women on estrogen therapy *(97)*. Consistent with these conclusions, Ralston *(98)* 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 hormone replacement therapy, (HRT). 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 these differences, such as the state of ERs.

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. *(101)* found that these steroids did not affect IL-6 production, but that dexamethasone inhibited both basal and induced IL-6 expression. In a pilot study, Gordon et al. *(102)* reported that cultured marrow cells from seven postmenopausal women between age 48 and 84 yr of age showed different patterns of inhibition of IL-6 by gonadal and adrenal steroids. Testosterone significantly suppressed IL-6 levels in five of seven cultures with a range of 60 to 88% of control, and a group mean of 79%. DHEA significantly suppressed IL-6 in three of seven cultures with a range of 46–86% of control and a group mean of 78%. Estrogen significantly suppressed IL-6 in only one of seven cultures with a group mean of 91%. It has been shown previously that factors in medium and serum can mask in vitro regulation of IL-6 secretion by cultured marrow *(103)*. Variations in specimens 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. *(105)* reported that testosterone, DHT, and DHEAS inhibited activity of the IL-6 promoter, but only in cells with the AR. DHEA is an androgen, but can also be an estrogen precursor. Both female and male marrow contain 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.

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 androstenedione) 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 prolifera-

tion through AR-mediated mechanisms and alkaline phosphatase production through 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 *(110a)* 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 hormone replacement to prevent bone loss because it can be converted into both androgen and estrogen at levels sufficient to increase bone formation and decrease resorption, respectively *(1)*.

Several human studies have shown 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. *(64)* demonstrated a significant positive correlation between the serum DHEAS and BMD in women (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 yr *(115)*. However, more recent data from the same investigators included a larger number of men and women between age 50 and 89 yr 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/d for 28 d) on the production of endogenous androgens and estrogens in six menopausal women. Mortola and Yen *(28)* showed that DHEA treatment produced a marked rise in testosterone and a moderate rise in estradiol levels. Although no adverse clinical effects were noted in this study, high-density lipoprotein (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. *(23)* showed that 50 mg of nightly oral DHEA for 6 mo 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. DHEA also produced a rise in IGF-I and free IGF levels owing to a decline in IGFBP-1, 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 (QOL) assessments *(23)*. Oral administration of DHEA at a daily dose of 50 mg to elderly men for 20 wk resulted in a 20% increase in serum IGF-I and a 32% increase in the ratio of IGF-I/IGFBP-1 *(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 1 yr, 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.

Disease-Specific Considerations

Adrenal Insufficiency

Patients with adrenal insufficiency suffer from chronic DHEA(S) deficiency, because 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, because endogenous androgen deficiency is a frequently neglected issue *(118)*. It has been shown that despite otherwise adequate HRT in patients with this disease, QOL appears to be suboptimal *(119)*. In one protocol designed to reproduce the subnormal DHEA(S) levels found in Addison's disease, 4 d of suppressive doses of dexamethasone were 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.

Anorexia Nervosa

Patients with chronic anorexia nervosa often develop early osteoporosis and have a sevenfold 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 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

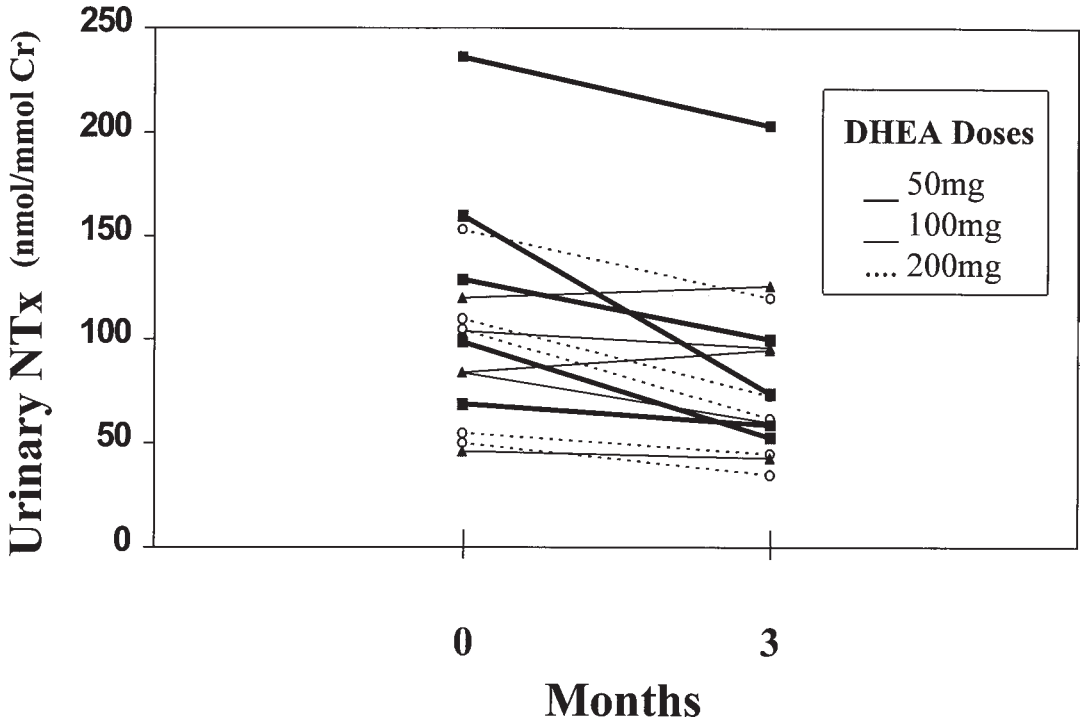

Fig. 6. Urinary NTx levels among treatment subgroups. Baseline and 3-mo urinary NTx levels are depicted for the three DHEA subgroups: 50(■), 100(▲), and 200(○)-mg dosage groups. A significant decrease was seen comparing baseline and 3-mo levels in the 50(■) ($p = 0.018$) and 200(○)-mg ($p = 0.016$) groups. (Reproduced with permission from ref. *65*.)

strategies to preserve bone density in adolescents with eating disorders has become an important public health issue.

In a 3-mo 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, those subjects had low levels of both DHEA and DHEAS. After treatment with oral DHEA, over half of those patients showed a significant decrease (25%) in the urinary bone resorption markers, NTx, between baseline and 3 mo (Fig. 6). After 3 mo of therapy, there was also a significant increase within treatment groups in the serum bone formation marker, osteocalcin. Finally, a 50-mg dose was shown to restore physiological 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 such as 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.

Osteoporosis in the Elderly

Estrogen deficiency is an important factor in postmenopausal bone loss. After menopause, there is an acceleration of bone loss over 8–10 yr, and some women lose up to one-half of their bone density *(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 *(13–137)*. 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 50–60 yr, were related to changes in cortical bone

mass in the forearm *(138)*. In a longitudinal study of 84 women between the age of 42–58 yr, Slemenda et al. *(139)* showed that over 3 yr, estrogen levels in peri- and postmenopausal women predicted the rate of postmenopausal bone loss in the forearm. 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 age of 31 and 77 yr, 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 yr after menopause. In postmenopausal women, estrogen and androgens were independent factors, both affecting bone loss. In women over 60 yr, 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). Those studies examining the effects of sex steroids on the bone of peri- and postmenopausal patients have relevance to DHEA physiology because this adrenal steroid is converted to active sex steroids in peripheral tissues.

Some information about mechanisms of age-related bone loss is available. In human studies, IL-6 levels increased with advanced age *(15)* and were less easily suppressed by estradiol *(16,17,23)*. Haden et al. *(64)* reported 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 characteristic of this period (Fig. 3).

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 have 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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References

1. Frisch, R. E. (1990). *Prog. Reprod. Biol. Med.* **14,** 1–26.
2. Meikle, A. W., Daynes, R. A., and Araneo, B. A;L(1991). *Endocr. Metab. Clin. North Am.* **20,** 381–400.
3. Albrecht, E. D. and Pepe, G. J. (1990). *Endocr. Rev.* **11,** 124–150.
4. Sklar, C. A., Kaplan, S. A., and Grumback, M. M. (1980). *J. Clin. Endocrinol. Metab.* **51,** 548–556.
5. Apter, D., Pakarinen, A., Hammond, G. L., and Vihko, R. (1979). *Acta. Paediatr. Scand.* **68,** 599–604.
6. Orentreich, N., Brind, J. L., Rizer, R. L., and Vogelman, J. H. (1984). *J. Clin. Endocrinol. Metab.* **59,** 551–555.
7. Herbert J. (1995). *Lancet* **345,** 1193–1194.
8. Davis, S. R. and Burger, H. G. (1996). *J. Clin. Endocrinol. Metab.* **81,** 2759–2763.
9. Labrie, F., Diamond, R., Cusan, L., Gomez, J. L., Belanger, A., and Candas, B. (1997). *J. Clin. Endocrinol. Metab.* **82,** 2396–2402.
10. Albright, F. (1947). *Ann. Intern. Med.* **27,** 861.
11. Barrett-Connor, E., Shaw, K., and Yen, S. S. C. (1986). *N. Engl. J. Med.* **315,** 1519–1524.
12. Belanger, A., Candas, B., Dupont, A., Cusan, L., Diamond, P., Gomez, J. L., and Labrie, F. (1994). *J. Clin. Endocrinol. Metab.* **79,** 1086–90.
13. Suzuki, T., Suzuki, N., Engelman, E. G., Mizushima, Y., and Sakane, T. (1995). *Clin. Exp. Immunol.* **99,** 251–255.
14. Turner, R. T., Lifrak, E. T., Beckner, M., Wakley, G. K., Hannon, K. S., and Parker, L. N. (1990). *Am. J. Physiol.* **258,** E673–E677.
15. Daynes, R. A., Araneo, B. A., Ershler, W. B., Maoloney, C., Li, G. Z., and Ryu, S. Y. (1993). *J. Immunol.* **150,** 5219–5230.
16. Araneo, B. A., Shelby, J., Li, G. Z, Ku, W., and Daynes, R. A. (1993). *Arch. Surg.* **128,** 318–325.
17. Daynes, R. A. and Araneo, B. A. (1992). *J. Immunother.* **12,** 174–179.
18. Berr, C., Lafont, S., Debuire, B., Dartignes, J. F., and Baulieu, E. E. (1996). *Proc. Natl. Acad. Sci. USA* **93,** 13,410–13,415.
19. Ravaglia, G., Forti, P., Maioli, F., Boschi, F., Bernardi, M., Pratelli, L., Pizzoferrato, A., and Gasbarrini, G. (1996). *J. Clin. Endocrinol. Metab.* **81,** 1173–1178.
20. Cawood, E. H. and Bancroft, J. (1996). *Psychol. Med.* **26,** 925–936.
21. Baulieu E. (1996). *J. Clin. Endocrinol. Metab.* **81,** 3147–3151.

22. Friess, E., Trachsel, L., Gludner, J., Schier, T., Steiger, A., and Holsboer, F. (1995). *Am. J. Physiol.* **268,** E107–E113.
23. Morales, A. J., Nolan, J. J., Nelson, J. C., and Yen, S. S. C. (1994). *J. Clin. Endocrinol. Metab.* **78,** 1360–1367.
24. Buster, J. E., Casson, P. R., Straughn, A. B., Dale, D., Umstot, E. S., Chiamori, N., and Abraham, G. E. (1992). *Am. J. Obstet. Gynecol.* **166,** 1163–1170.
25. Longcope C. (1995). *Ann. N Y Acad. Sci.* **774,** 143–148.
26. Falany, C. N., Comer, K. A., Dooley, T. P., and Glatt, H. (1995). *Ann. N Y Acad. Sci.* **774,** 59–72.
27. Neville, A. M. and O'Hare, M. J. (1982). In: *The human adrenal cortex: pathology and biology—an integrated approach.* Springer-Verlag: Berlin.
28. Mortola, J. F. and Yen, S. S. C. (1990). *J. Clin. Endocrinol. Metab.* **71,** 696–704.
29. Labrie, F., Belanger, A., Simard, J., Luu-The, V., and Labrie, C. (1995). *Ann. N Y Acad. Sci.* **774,** 16–28.
30. Labrie, F. (1991). Mol. Cell. Endocrinol. **78,** C113–C118.
31. Labrie, F., Belanger, A., Cusan, L., and Candas, B. (1997). *J.Clin. Endocrinol. Metab.* **82,** 2403–2409.
32. Belanger, A., Brochu, M., and Cliché, J. (1986). *J. Clin. Endocrinol. Metab.* **62,** 812–815.
33. Moghissi, E., Ablam, F., and Horton, R. (1984). *J. Clin. Endocrinol. Metab.* **59,** 417–421.
34. Grodin, J. N., Siiteri, P. K., and MacDonald, P. C. (1973). *J.Clin. Endocrinol. Metab.* **36,** 207.
35. Frisch, R. E., Canick, J. A., and Tulchinsky, D. (1980). *J. Clin. Endocrinol. Metab.* **51,** 394–396.
36. Simpson, E. R. and Mendelson, C. R. (1990). In: *Adipose tissue and reproduction.* Frisch, R. E. (ed.). Karger, Switzerland: Basel.
37. Sandler, R. B., Slemenda, C. W., LaPorte, R. E., Cauley, J. A., Schramm, M. M., Barresi, M. L., and Kriska, A. M. (1985). *Am. J. Clin. Nutr.* **42,** 270–274.
38. Matkovic, V. (1992). *J. Intern. Med.* **231,** 151–160.
39. Bonjour, J. P., Theintz, G., Buchs, B., Slosman, D., and Rizzoli, R. (1991). *J. Clin. Endocrinol. Metab.* **73,** 555–563.
40. Mauras, N., Haymond, M. W., Darmaun, D., Vieira, N. E., Abrams, S. A., and Yergey, A. L. (1994). *J. Clin. Invest.* **93,** 1014–1019.
41. Dagogo-Jack, S., Al-Ali, N., and Qurttom, M. (1997). *J. Clin. Endocrinol. Metab.* **82,** 2821–2825.
42. Buchanan, J. R., Myers, C., Llyod, T., Leuenberger, P., and Demers, L. M. (1988). *J. Bone Miner. Res.* **3,** 673–680.
43. Mazess, R. B. (1982). *Clin. Orthop. Rel. Res.* **165,** 239–252.
44. Riggs, B. L., Khosla, S., and Melton, L. J. III. (1998). *J. Bone Miner. Res.* **13,** 763–773.
45. Cummings, S. R., Browner, W. S., Bauer, D. B., Stone, K., Ensrud, K., Jama, S., and Ettinger, B. (1998). *N. Engl. J. Med.* **339,** 733–738.
46. Greendale, G. A., Edelstein, S., and Barrett-Connor, E. (1997). *J. Bone Miner. Res.* **12,** 1833–1843.
47. Bidlingmaier, R., Dorr, H. G., Eisenmenger, W., Kuhnle, U., and Knorr, D. (1986). *J. Clin. Endocrinol. Metab.* **62,** 331–335.
48. Labrie, F., Belanger, A., Dupont, A., Luu-The, V., Simard, J., and Labrie, C. (1993). *Clin. Invest. Med.* **16,** 475–492.
49. Veldhuis, J. D., Liem, A. Y., South, S., Weltman, A., Weltman, J, Clemmons, D. A., Abbott, R., Mulligan, T., Johnson, M. L. Pincus, S., Straume, M., and Iranmanesh, A. (1995). *J. Clin. Endocrinol. Metab.* **80,** 3209–3222.
50. Pfeilschifter, J., Scheodt-Nave, C., Leidig-Bruckner, G., Woitge, H. W., Blum, W. F., Wuster, C., Haack, D., and Ziegler, R. (1996). *J. Clin. Endocrinol. Metab.* **81,** 2534–2540.
51. Finkelstein, J. S., Neer, R. M., Biller, B. M. K., Crawford, J. D., and Klibanski, A. (1992). *N. Engl. J. Med.* **326,** 600–604.
52. Griffin, J. E. (1992). *N. Engl. J. Med.* **326,** 611–618.
53. Quigley, C. A., De Bellis, A., Marschke, K. B., El-Awady, M. K., Wilson, E. M., and French, F. S. (1995). *Endocrine Rev.* **16,** 271–295.
54. Munoz-Torres, M., Jodar, E., Quesada, M., and Escobar-Jiminez, F. (1995). *Calcif. Tissue Int.* **57,** 94–96.
55. MacLean, H. E., Chu, S., Joske, F., Warne, G. L., and Zajac, J. D. (1995). *Biochem. Mol. Med.* **55,** 31–37.
56. Vanderschueren, D., Van Herck, E., Suiker, A. M. H., Visser, W. J., Schot, L. P. C., Chung K., Lucas, R. S., Einhorn, T. A., and Bouillon, R. (1993). *J. Bone Miner. Res.* **8,** 801–808.
57. Smith, E. P., Boyd, J., Frank, G. R., Takahashi, H., Cohen, R. M., Specker, B., Williams, T. C., LuBahn, D. B., and Korach, K. S. (1994). *N. Engl. J. Med.* **331,** 1056–1061.
58. Korach, K. S. (1994). *Science* **266,** 1524–1527.
59. Conte, F. A., Grumbach, M. M., Ito, Y., Fisher, C. R., and Simpson, E. R. (1994). *J. Clin. Endocrinol. Metab.* **78,** 1287–1292.
60. Morishima, A., Grumbach, M. M., Simpson, E. R., Fisher, C., and Qin, K. (1995). *J. Clin. Endocrinol. Metab.* **80,** 3689–3698.
61. Bachrach, B. E. and Smith, E. P. (1996). *Endocrinologist* **6,** 362–368.

61a. LeBoff, M. S. and Glowacki, J. (1999). In: *The aging skeleton.* Rosen, C., Glowackli, J., and Bilezikian, J. (eds.) Academic Press: San Diego.

62. Rosen, C. J., Donahue, L. R., and Hunter, S. J. (1994). *Proc. Soc. Exp. Biol. Med.* **206,** 83–102.
63. Grinspoon, S., Baum, H., Lee, K., Anderson, E., Herzog, D., and Klibanski, A. (1996). *J. Clin. Endocrinol. Metab.* **81,** 3864–3870.
64. Haden, S. T., Hurwitz, S., Glowacki, J, Rosen, C. J., and Leboff, M. S. (1998). *Bone* **23,** S620.
65. Gordon, C. M., Grace, E., Emans, S. J., Goodman, E., Crawford, M. H., and LeBoff, M. S. (1999). *J. Bone Miner. Res.* **14,** 136–145.
66. Barrett-Connor, E. and Goodman-Gruen, D. (1998). *J. Bone Miner. Res.* **13,** 1343–1349.
67. Mohan, S. and Baylink, D. J. (1991). *Clin. Orthop. Rel. Res.* **263,** 30–48.
68. Boonen, S., Lesaffre, E., Dequeker, J., Aerssens, J., Nijs, J., Pelemans, W., and Bouillon, R. (1996). *J. Am. Geriatr. Soc.* **44,** 1301–1306.
69. Bing-You, R. G., Denis, M. C., and Rosen, C. J. (1993). *Calcif. Tissue Int.* **52,** 183–187.
70. Gray, T. K., Mohan, S., Linkhart, T. A., and Baylink, D. J. (1989). *Biochem. Biophys. Res. Commum.* **158,** 407–412.
71. McCarthy, T. L., Centrella, M., and Canalis, E. (1989). *Endocrinology* **124,** 1247–1253.
72. Ashton, W. S., Degnan, B. M., Daniel, A., and Francis, G. L. (1995). *Ann. Clin. Lab. Sci.* **25,** 381–388.
73. Rosen, C. J., Verault, D., Steffens, C., Cheleuitte, D., and Glowacki, J. (1997). *Endocrine* **7,** 77–80.
74. Goldstein, S., Moerman, E. J., Jose, R. A., and Baxter, R. C. (1991). *Proc. Natl. Acad. Sci. USA* **88,** 9680–9684.
75. Puche, R. C. and Romano, M. C. (1969). *Calcif. Tissue Res.* **11,** 39–47.
76. Scheven, B. A. and Milne, J. S. (1998). *Life Sci.* **62,** 59–68.
77. Kasperk, C. H., Wakley, G. K., Hierl, T., and Ziegler, R. (1997). *J. Bone Miner. Res.* **12,** 464–471.
78. Chiu, K. M., Keller, E. T., Crenshaw, T. D., and Gravenstein, S. (1999). *Calcif. Tissue Int.* **64,** 527–533.
79. Yan, Z., Lee, G. Y., and Anderson, E. (1997). *Biol. Reprod.* **57,** 1509–1516.
80. Hofig, A., Simmen, F. A., Bazer, F. W., and Simmen, R. C. (1991). *J. Endocrinol.* **130,** 245–250.
81. Christman, G. M., Randolph, J. F., Peegel, H., and Menon, K. M. (1991). *Fertil. Steril.* **55,** 1099–1105.
82. Manolagas, S. C. and Jilka, R. L. (1995). *N. Engl. J. Med.* **332,** 305–311.

83. Wallach, S., Avioli, L. V., Feinblatt, J. D., and Carstens, J. H. Jr. (1993). *Calcif. Tissue Int.* **53,** 293–296.
84. Pfeilschifter, J., Chenu, C., Bird, A., Mundy, G. R., and Roodman, G. D. (1989). *J. Bone Miner. Res.* **4,** 113–118.
85. Bertolini, D. R., Nedwin, G. E., Bringman, T. S., Smith, D. D., and Mundy, G. R. (1986). *Nature* **319,** 516–518.
86. Gowen, M., Wood, D. D., Ihrie, E. J., McGuire, M. K., and Russell, R. G. (1983). *Nature* **306,** 378–380.
87. Stashenko, P., Obernesser, M. S., and Dewhirst, F. E. (1989). *Immun. Invest.* **18,** 239–249.
88. Nguyen, L., Dewhirst, F. E., Hauschka, P. V., and Stashenko, P. (1991). *Lymphokine Cytokine Res.* **10,** 15–21.
89. Girasole, G., Passeri, G., Jilka, R. L., and Manolagas, S. C. (1994). *J. Clin. Invest.* **93,** 1516–1524.
90. Ohsaki, Y., Takahashi, S., Scarcez, T., Demulder, A., Nishihara, T., Williams, R., and Roodman, G. D. (1992). *Endocrinology* **131,** 2229–2234.
91. Path, G., Bornstein, S. R., Spath-Schwalbe, E., and Scherbaum, W. A. (1996). *Endocrin. Res.* **22,** 867–873.
92. Jilka, R. L., Hangoc, G., Girasole, G., Passeri, G., Williams, D. C., Abrams, J. S., Boyce, B., Broxmeyer, H., and Manolagas, S. C. (1992). *Science* **257,** 88–91.
93. Eriksen, E. F., Colvard, D. S., Berg, N. G., Graham, M. L., Mann, K. G., Spelsberg, T. C., and Riggs, B. L. (1988). *Science* **241,** 84–86.
94. Komm, B. S., Terpening, C. M., Benz, D. J., Graeme, K. A., Gallogas, A., Korc, M., Greene, G. L., O'Malley, B. W., and Hausler, M. R. (1988). *Science* **241,** 81–84.
95. Horowitz, M. C. (1993). *Science* **260,** 626–627.
96. Glowacki, J. (1995). *Calcif. Tissue Int.* **56S,** 50–51.
97. Cheleuitte, D, Mizuno, S., and Glowacki, J. (1998). *J. Clin. Endocrinol. Metab.* **83,** 2043–2051.
98. Ralston, S. H. (1994). *J. Bone Miner. Res.* **9,** 883–890.
99. Kassem, M., Harris, S. A., Spelsberg, T. C., and Riggs, B. L. (1996). *J. Bone Miner. Res.* **11,** 193–199.
100. Rifas, L., Kenney, J. S., Marcelli, M., Pacifici, R., Cheng, S. L., Dawson, L. L., and Avioli, L. V. (1995). *Endocrinology* **136,** 4056–4067.
101. Hierl, T., Borcsok, I., Sommer, U., Ziegler, R., and Kasperk, C. (1998). *Exp. Clin. Endocrinol. Diabetes* **106,** 324–333.
102. Gordon, C. M., Makhluf, H., Blahut, E., LeBoff, M. S., and Glowacki, J. (1999). 21st Annual Meeting, American Society of Bone and Mineral Research, St. Louis, MO.
103. Glowacki, J., Yates, K., Lesieur-Brooks, A., Bleiberg, I., and Cheleuitte, D. (1997). *J. Bone Miner. Res.* **12,** S337.
104. Girasole, G., Jilka, R. L., Passeri, G., Boswell, S., Boder, G., Williams, D. C., and Manolagas, S. C. (1992). *J. Clin. Invest.* **89,** 883–891.
105. Bellido, T., Jilka, R. L., Boyce, B. F., Girasole, G., Broxmeyer, H., Dalrymple, S. A., Murray, R., and Manolagas, S. C. (1995). *J. Clin. Invest.* **95,** 2886–2895.
106. Yeh, J., Kohlmeier, L., LeBoff, M. S., Connolly, M., and Glowacki, J. (1994). *Proc. Soc. Exp. Biol. Med.* **205,** 306–315.
107. Bruder, J. M., Sobek, L., and Oettel, M. (1997). *J. Steroid Biochem. Mol. Biol.* **62,** 461–466.
108. James, K., Premchand, N., Skibinska, A., Skibinski, G., Nicol, M., and Mason, J. I. (1997). *Mech. Aging Dev.* **93,** 15–24.
109. Buchanan, J. R., Hospodar, P., Myers, C., Leuenberger, P., and Demers, L. M. (1988). *J. Clin. Endocrinol. Metab.* **67,** 937–943.
110. Nimrod, A. and Ryan, K. J. (1975). *J. Clin. Endocrinol. Metab.* **40,** 367–372.
110a. Labrie, F., Diamond, P., Cusan, L., Gomez, J. L., Belanger, A., and Candas, B. (1997). *J. Clin. Endo. Metab.* **82,** 3498–3505.
111. Wild, R. A., Buchanan, J. R., Myers, C., and Demers, L. M. (1987). *Proc. Soc. Exp. Biol. Med.* **186,** 355–360.
112. Nordin, B. E., Robertson, A., Seamark, R. F., Bridges, A., Philcox, J. C., Need, A. G., Horowitz, M., Morris, H. A., and Deam, S. (1985). *J. Clin. Endocrinol. Metab.* **60,** 651–657.
113. Taelman, P., Kaufman, J. M., Janssens, X., and Vermeulen, A. (1989). *Maturitas* **11,** 65–73.
114. Steinberg, K. K., Freni-Titulaer, L. W., DePuey, E. G., Miller, D. T., Sgoutas, D. S., Coralli, C. H., Phillips, D. L., Rogers, T. N., and Clark, R. V. (1989). *J. Clin. Endocrinol. Metab.* **69,** 533–539.
115. Barrett-Connor, E., Kritz-Silvertein, D., and Edelstein, S. L. (1993). *Am. J. Epidemiol.* **137,** 201–206.
116. Khorram, O., Vu, L., and Yen, S. S. (1997). *J. Gerontol. A. Biol. Sci. Med. Sci.* **52,** 1–7.
117. Arlt, W., Justl, H. G., Callies, F., Reincke, M., Hubler, D., Oettel, M., Ernst, M., Schulte, H. M., and Allolio, B. (1998). *J. Clin. Endocrinol. Metab.* **83,** 1928–1934.
118. Oelkers, W. (1996). *N. Engl. J. Med.* **335,** 1206–1212.
119. Riedel, M., Wiese A., Schurmeyer, T. H., and Brabant, G. (1993). *Exp. Clin. Endocrinol.* **101,** 106–111.
120. Devogelaer, J. P., Crabbe, J., and Nagant De Deuxchaisnes, C. (1987). *BMJ* **294,** 798–800.
121. Rigotti, N. A., Neer, R. M., Skates, S. J., Herzog, D. B., and Nussbaum, S. R. (1991). *JAMA* **265,** 1133–1138.
122. Zumoff, B., Walsh, B. T., Katz, J. L., Levin, J., Rosenfeld, R. S., Kream, J., and Weiner, H. (1983). *J. Clin. Endocrinol. Metab.* **56,** 668–672.
123. Devesa, J., Perez-Fernandez, R., Bokser, L., Gaudeiero, G. J., Lima, L., and Casanueva, F. F. (1988). *Horm. Metab. Res.* **20,** 57–60.
124. Boyar, R. M., Hellman, L. D., Roffwarg, H., Katz, J., Zumoff, B., O'Connor, J., Bradlow, H. L., and Fukushima, D. K. (1977). *N. Engl. J. Med.* **296,** 190–193.
125. Walsh, B. T., Roose, S. P., Katz, J. L., Dyrenfurth, I., Wright, L., Vande Wiele, R., and Glassman, A. H. (1987). *Psychoneuroendocrinology* **12,** 131–140.
126. Klibanski, A., Biller, B. M., Schoenfeld, D. A., Herzog, D. B., and Saxe, V. C. (1995). *J. Clin. Endocrinol. Metab.* **80,** 898–904.
127. Ott, S. M. (1991). *N. Engl. J. Med.* **325,** 1646–1647.
128. Bachrach, L. K., Guido, D., Katzman, D., Litt, I. F., and Marcus, R. (1990). *Pediatrics* **86,** 440–447.
129. Dhuper, S., Warren, M., Brooks-Gunn, J., and Fox, R. (1991). *J. Clin. Endocrinol. Metab.* **71,** 1083–1088.
130. Bachrach, L. K., Katzman, D. K., Litt, I. F., Guido, D., and Marcus, R. (1991). *J. Clin. Endocrinol. Metab.* **72,** 602–606.
131. Grinspoon, S., Wolf, L., Pinzone, J., Herzog, D., and Klibanski, A. (1997). Abstract, Annual Meeting of the Endocrine Society, Minneapolis, MN.
132. Slemenda, C., Longcope, C., Peacock, M., Hui, S., and Johnston, C. C. (1996). *J. Clin. Invest.* **97,** 14–21.
133. Slemenda , C. W., Longcope, C., Zhou, L., Hui, S., Peacock, M., and Johnston, C. C. (1997) *J. Clin. Invest.* **100,** 1755–1759.
134. Riis, B. J., Christansen, C., Deftos, L. J., and Catherwood, B. D. (1984) In: *Osteoporosis.* Christiansen, C., Arnaud, C. D., Nordin, B. E. C., Parfitt, A. M., Peck, W. A., and Riggs, B. L. (eds.). Glostrup, Denmark: Aalborg Stiftsbogtrykkeri.
135. Davidson, B. J., Riggs, B. L., Wahner, H. W., and Judd, H. L. (1983). *Obstet. Gynecol.* **61,** 275–278.
136. Riggs, B. L., Ryan, R. J., Wahner, H. W., Jiang, N. S., and Mattox, V. R. (1982). *J. Clin. Endocrinol. Metab.* **36,** 1097–1099.
137. Longcope, C., Baker, R. S., Hui, S. L., and Johnston, C. C. J. (1984). *Maturitas* **6,** 309–318.
138. Rozenberg, S., Ham, H., Bosson, D., Peretz, A. and Robyn, C. (1990). *Maturitas* **12,** 137–140.
139. Slemenda, C., Hui, S. L., Longcope, C., and Johnston, C. C. (1987). *J. Clin Invest.* **97,** 1261–1269.
140. Spencer, N. F., Norton, S. D., Harrison, L. L., Li, G. Z., and Daynes, R. A. (1996). *Exp. Gerontol.* **31,** 393–408.