Sex Steroids and the Skeleton

Fuller Albright established in the 1940s that estrogen was a major regulator of bone metabolism in women. Using careful calcium balance studies, he demonstrated that estrogen deficiency was associated with negative calcium balance, which could be reversed with estrogen treatment. Subsequent studies confirmed his original findings, showing that oophorectomy or menopause in women leads to a marked increase in bone resorption, followed by a coupled increase in bone formation. However, because of estrogen deficiency, bone formation cannot keep pace with bone resorption, leading to rapid bone loss.

Sundeep Khosla, MD, of the Division of Endocrinology, Diabetes, Metabolism, and Nutrition at Mayo Clinic in Rochester, Minnesota, says: “While much less was known about sex steroid regulation of the male skeleton, studies in Czech prisoners who were orchiectomized for sexual crimes showed that male castration, which is associated with loss of testosterone, led to a similar pattern of bone loss. Thus, traditional scientific opinion held that, similar to estrogen in women, testosterone was the major regulator of bone metabolism in men.”

The pivotal role of testosterone in the male skeleton was challenged, however, by an important “experiment of nature.” Dr Khosla explains: “The description in 1994 of a male with mutations in both alleles of the estrogen receptor who had normal testosterone levels but marked osteopenia led to the hypothesis that even in men, estrogen may be the major regulator of the skeleton. This hypothesis was rigorously tested by our group using an experimental design in which sex steroid production was suppressed in adult men through a combination of a gonadotropin-releasing hormone agonist and an aromatase inhibitor, followed by the selective replacement of either estrogen or testosterone, both, or neither, by giving the men the respective drug patches. After baseline measurements of bone turnover markers, the men were randomly assigned to these 4 different groups, to define the relative contributions of estrogen and testosterone toward regulating bone turnover. The results demonstrated that estrogen accounted for 70% or more of the total effect of sex steroids on bone resorption in men, whereas testosterone could account for no more than 30% of the effect [Figure 1].”

Dr Khosla continues: “These findings, combined with observations from numerous other clinical investigative studies, have now established that estrogen is a key hormonal regulator of bone metabolism not only in women, but also in men. In a subsequent study, we tested whether aging men would have skeletal benefits from treatment with the selective estrogen receptor modulator (SERM) raloxifene, which has estro-
genlike activity on bone but is nonfeminizing. These studies showed that raloxifene reduced bone resorption in men, but only in those elderly men who had estradiol levels below a threshold for skeletal estrogen deficiency. On the basis of these studies, other investigators have demonstrated that men with prostate cancer who are given androgen deprivation therapy (which also leads to estrogen deficiency) do indeed benefit from treatment with a SERM. In addition, epidemiological studies have now found that fracture risk in older men is much more closely related to serum estradiol levels, rather than testosterone levels.

With aging, men have modest decreases in total testosterone levels with little or no change in total estradiol levels. Dr Khosla notes: “However, due to marked increases in sex hormone–binding globulin (SHBG) concentrations, bioavailable (or non-SHBG bound) testosterone and estradiol levels decrease markedly with aging in men [Figure 2]. It is these declining levels of bioavailable estradiol that appear to be most closely related to bone mineral density, bone loss, and fracture risk in elderly men.”

Recent studies using standard and high-resolution quantitative computed tomographic (QCT) imaging of bone are also revealing some important gaps in the understanding of bone loss with aging, independent of changes in sex steroid levels. Dr Khosla adds: “Although dual-energy x-ray absorptiometry (DXA) is a very useful clinical tool for assessment of bone mineral density, it cannot separately assess changes in cancellous bone (the ‘spongy’ bone in the vertebrae, pelvis, and metaphyses of long bones) vs cortical bone (the ‘compact’ bone in the diaphyses of long bones and surrounding the cancellous bone in the vertebrae and pelvis). The traditional thinking, based on studies using DXA, was that bone mass peaked in the 30s, remained stable until midlife, and declined thereafter, following the menopause in women and with aging in men. Using QCT, however, we have found that while cortical bone at multiple sites does follow this pattern, cancellous bone begins to decrease in young women and men, even those in their early 20s. These young individuals are sex-steroid sufficient, a fact that points to other, as-yet unknown mechanisms, leading to the initiation of cancellous bone loss soon after the completion of growth in humans. These findings in humans have now been confirmed in mouse models, where cancellous bone loss also begins shortly after skeletal consolidation (at the age of ~3 or 4 months). Collectively, these human and mouse studies are demonstrating that age-related loss of cortical bone is closely tied to estrogen deficiency (in women and in men), whereas age-related loss of cancellous bone, while accentuated by estrogen deficiency, occurs even in the presence of normal estrogen levels.”

Dr Khosla summarizes: “Despite more than 70 years of multiple groups following a line of investigation first initiated by Fuller Albright, we continue to learn more about how sex steroids—and estrogen in particular—regulate bone metabolism. Given the key role of estrogen in the skeleton, unraveling the pathways by which estrogen acts on bone is likely to continue to provide new insights into both the mechanisms of bone loss with aging and the potential molecular targets for new therapies for osteoporosis and age-related bone loss.”

Figure 2. Bioavailable testosterone (T) and estradiol (E2) as a function of age in a population cohort of men from Rochester, Minnesota. Error bars indicate standard error of the mean. Data from Khosla S, Melton LJ 3rd, Atkinson EJ, et al. Relationship of serum sex steroid levels and bone turnover markers with bone mineral density in men and women: a key role for bioavailable estrogen. J Clin Endocrinol Metab. 1998;83(7):2266-74.
Men and women of normal weight have 15% and 30% body fat, respectively. Obese adults can have as much as 100 kg of body fat under extreme circumstances. The average size of a fat cell ranges from 0.2 to 1.4 mcg of lipid per cell, which means that someone with 20 kg of fat can have on the order of 33 billion fat cells. However, not all fat and not all fat cells are alike. Michael D. Jensen, MD, of the Division of Endocrinology, Diabetes, Metabolism, and Nutrition at Mayo Clinic in Rochester, Minnesota, says: “Our research program has focused largely on the attributes of human adipose tissue and body fat distribution as it relates to health and disease. For more than 20 years, we’ve been aware that persons with a preponderance of visceral (omental and mesenteric) fat and upper body fat are at greater risk for the metabolic complications of obesity. Persons with this body fat distribution also have greater concentrations of free fatty acid [FFA] in the blood.”

Dr Jensen continues: “It has been repeatedly shown that artificially elevating the FFA levels in healthy individuals can create insulin resistance and some of the other metabolic abnormalities seen in upper body obesity. One possible explanation is that those persons with excess visceral fat also take up and release too many fatty acids from the visceral fat. The adipocytes in the visceral fat, when studied in vitro, appear to have this potential. Excess FFA release from visceral fat could disproportionately affect hepatic metabolic function because the venous drainage of omental and mesenteric fat is into the portal vein—the so-called portal hypothesis. In contrast, adults with larger amounts of lower body (leg) fat often have relatively little visceral fat and have normal FFA.
Emerging Concepts in Ensemble Regulation of the Hypothalamo-pituitary-adrenal Axis

An emerging thesis in such multipathway biological systems as the corticotropic, gonadotropic, and somatotropic axes is that homeostasis is achieved by repeated incremental signaling among key components of the network (Figure 1). Johannes D. Veldhuis, MD, of the Division of Endocrinology, Diabetes, Metabolism, and Nutrition at Mayo Clinic in Rochester, Minnesota, says: “Direct sampling of hypothalamic-pituitary portal blood in the rat, sheep, and horse establishes that corticotropin-releasing hormone (CRH), arginine vasopressin (AVP), and corticotropin (ACTH) are secreted in discrete bursts of varying frequency, amplitude, and temporal concordance. Pulses of CRH and AVP drive bursts of ACTH secretion by activating cAMP–protein kinase A and protein kinase C, respectively. Pulses of ACTH, in turn, stimulate episodes of cortisol secretion levels. Our research has indicated that fat cells in visceral fat, leg fat, and upper body subcutaneous fat have unique characteristics that make them almost completely different types of fat. We also have discovered that much of what was believed about the visceral fat in the portal hypothesis was not entirely correct.”

Dr Jensen explains: “For example, men and women with small visceral fat adipocytes have a much greater tendency to store fat in their visceral depot, probably because the levels of proteins and enzymes related to fat storage are quite high in these fat cells. However, as omental and mesenteric fat cells (and thus visceral fat mass) become larger, most of the proteins and enzymes related to fat storage are dramatically suppressed and the cells become resistant to fat storage. Under conditions of stable fat mass, one would predict that reduced fat storage would be accompanied by reduced FFA release. We have found that, consistent with this hypothesis, visceral fat is not a major source of fatty acids in the circulation of upper body obese or viscerally obese men and women. In fact, the high FFA levels found in upper body obesity come primarily from upper body subcutaneous fat. We also found that the liver is exposed to some extra FFA’s coming from visceral fat in persons with large amounts of visceral fat. Leg fat and upper body subcutaneous fat also have their own unique characteristics that become even more apparent with weight gain.”

When normal-weight people gain modest amounts of fat, they do so by enlarging the fat cells in their upper body subcutaneous fat depot. In contrast, gain of lower body fat is largely the result of increasing the number of fat cells. Dr Jensen highlights: “We found that the gain of as little as 1.2 kg of leg fat resulted in an average gain of 2.6 billion new fat cells! This finding refuted the long-held belief that adult humans do not develop new fat cells but are instead ‘stuck’ with those that developed in their adolescence. We recently reported that adults do not lose these new leg fat cells when they lose weight.”

New fat cells in adults, like new fat cells in children, come from preadipocytes. Preadipocytes are one of the most common cell types found mixed in among fat cells within adipose tissue. They are typically thought of as capable of replicating and therefore of generating a continuous potential supply of preadipocytes and adipocytes. When a preadipocyte receives an appropriate signal from the body, it will cease replicating and develop into a mature adipocyte, which then stores and releases fatty acids under relatively strict hormonal and neural control. Adipocytes also release various hormones, known as adipokines.”

Dr Jensen adds: “One of the recent hypotheses regarding why some people become metabolically ill when they become obese and others do not relates to dysfunctional storage and release of fatty acids by adipose tissue. When adipose tissue releases excessive amounts of fatty acids or cannot store them effectively, the fatty acids accumulate in so-called ectopic sites, such as visceral fat, muscle, liver, heart, and islet cells. In these tissues, fatty acids can accumulate to excessive amounts in ceramides, diacylglycerols, and long-chain acyl coenzyme A’s. These fatty acid–containing molecules can serve a signaling function within cells and as such may cause insulin resistance and tissue dysfunction. Our laboratory is investigating why some tissues in some people convert fatty acids into abnormal amounts of signaling molecules and others are protected, either by not taking up fatty acids or by storing the fatty acids in the more benign triglyceride form. Understanding these pathways may provide an approach to preventing obesity–related metabolic disorders, such as insulin resistance diabetes, dyslipidemia, and hypertension.”
by the adrenal zona fasciculata via parallel and convergent signaling cascades. Systemic cortisol acts through delayed and rapid negative feedback to oppose CRH and AVP secretion from the hypothalamus and their actions on corticotropes [Figure 1]. According to this ensemble concept, regulatory interactions collectively—rather than any individual effector acting alone—maintain physiological glucocorticoid availability.”

**Tissue Actions of Glucocorticoids and Mineralocorticoids: Impact of Free and Protein-Bound Cortisol**

Cortisol acts on diverse target tissues, such as muscle, bone, brain, thymus, and pituitary gland, through type I (mineralocorticoid [MR]) and type II (glucocorticoid [GR]) receptors. Human MR and GR have nominal dissociation constants for cortisol of 0.043 and 0.36 mcg/dL, respectively. By comparison, diurnal peak and nadir free cortisol concentrations average 1.2 and 0.3 mcg/dL, respectively (Figure 2). Critical illness, ACTH injection, and major surgery elevate free cortisol levels to 2.2 to 8.7 mcg/dL. The importance of free cortisol as a negative-feedback signal is inferable in rare cases of patients with null mutations of the cortisol-binding globulin gene, who maintain normal ACTH concentrations despite a free cortisol concentration of 0.35 mcg/dL. This observation opens the possibility of using free cortisol measurements clinically.

**Target Cell–Specific Regulation of Cortisol Action**

The potency of glucocorticoids is specific to the target cell. Dr Velhuis explains: “For example, in the rat, the one-half maximally inhibitory (ID50) serum corticosterone concentration is approximately 1 mcg/dL for the CRH gene, 2 mcg/dL for the thymus, and 5 mcg/dL for the AVP gene. Differences in ID50 values putatively reflect the in vivo density of GR and MR, availability of coactivator and corepressor molecules, sequence characteristics of the targeted promoter, systemic and in situ CBG concentrations, and activities of 11β-hydroxysteroid dehydrogenase type I (activating cortisol to cortisol) and type II (inactivating cortisol).”

**Mechanisms of Negative Feedback**

Dr Velhuis continues: “Autoinhibition is a physiological hallmark of neuroendocrine systems. GR and MR mediate negative feedback by cortisol in the human because their respective antagonists, canrenone/spironolactone and mifepristone (RU486), increase mean cortisol concentrations. In the rat, corticosteroid feedback targets include the lateral septum, hippocampus, and bed

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**Figure 1.** Schema of interlinked stress-adaptive corticotropin (ACTH) axis. Bursts of hypothalamic corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) individually and jointly stimulate (+) pulsatile release of pituitary ACTH. Blood-borne ACTH drives adrenal secretion of cortisol, which feeds back (−) centrally on both CRH/AVP and ACTH outputs.

**Figure 2.** Pathophysiology of free cortisol in humans. Calculated free cortisol concentrations and kₙ's of cortisol’s binding to cortisol-binding globulin (CBG), mineralocorticoid (MR), and glucocorticoid (GR) in the human. ACTH indicates corticotropin; ICU, intensive care unit.
Sex Steroid Control of HPA Outflow

<table>
<thead>
<tr>
<th>Neuroanatomic site</th>
<th>Hormone</th>
<th>Receptor</th>
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<tbody>
<tr>
<td>Hypothalamus</td>
<td>CRH, AVP</td>
<td>GR</td>
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<td>BNST</td>
<td>AVP</td>
<td>MR</td>
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<td>Pituitary gland</td>
<td>ACTH</td>
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<td>Adrenal</td>
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Estradiol stimulates (+) or inhibits (-).

Testosterone potentiates AVP’s restraint.

Figure 3. Illustrative sites of sex steroid regulation of hypothalamic-pituitary-adrenal (HPA) axis in animals. The exact counterparts in humans have not yet been worked out. ACTH indicates corticotropin; AVP, arginine vasopressin; BNST, bed nucleus of stria terminalis; CBG, cortisol-binding globulin; CRH, corticotropin-releasing hormone; GR, glucocorticoid; MR, mineralocorticoid; PVN, paraventricular nucleus.

Day-Night Rhythmicity of ACTH and Cortisol Secretion

Circadian, hypothalamic-pituitary-adrenal (HPA) rhythms in animals arise from a combination of day-night variations in CRH and AVP gene expression, ACTH secretion, adrenal responsiveness to ACTH, and glucocorticoid feedback and clearance. In clinical studies, regulation of the mass (size) of ACTH and cortisol secretory bursts explain more than 95% and more than 85% of nyctohemeral rhythmicity, respectively. Thus, to obviate confounding, clinical comparisons need to be conducted at a uniform time of day.

Human CRH/AVP-ACTH Pulsing Mechanism

The time delays between successive ACTH secretory bursts are random about a mean probability with hormone-specific interpulse variability. This new model applies to other human neuroendocrine pulse generators also (eg, for gonadotropin-releasing hormone).

Sex and Age as Conjoint Determinants of HPA Responsivity

In animals, the HPA axis mediates adaptations to diverse physical (eg, exercise), psychosocial (anxiety), and metabolic (fasting) stressors in a manner that depends on age, sex, and sex steroids and the severity, persistence, novelty, and type of stimulus. Dr Velhuis adds: “Aging in the human does not limit maximal ACTH or cortisol secretion induced by AVP, CRH, or ACTH or by hypoglycemia, cortisol depletion, a cold-pressor test, or a 3.5-day fast. In 45 parallel-cohort studies involving 670 young and 625 older adults (mean [SD] age, 28 [5] and 69 [6] years, respectively), age accentuated stress-induced ACTH and cortisol secretion by a 2.4-fold increase and female sex did so by a 2.7-fold increase. Illustratively, young men often exhibit greater responses than premenopausal women to paradigms of competition stress [Table].

Dr Veldhuis summarizes: “Recent concepts in regulation of the corticotropic axis highlight the need for time of day, sex, and sex steroid plus age-controlled clinical studies of stress-adaptive endocrine systems.”

<table>
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<tr>
<th>Sex- and Age-Related Distinctions in Hypothalamic-pituitary-adrenal Responsivity</th>
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<tr>
<td>↑ CRH stimulation of ACTH and cortisol secretion*</td>
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<tr>
<td>↑ AVP/CRH (combined) stimulation*</td>
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<td>↓ Dexamethasone suppression of CRH effect</td>
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<td>↑ Paradoxical ACTH/cortisol response to somatostatin</td>
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<td>↑ Social stress effect*</td>
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<td>↑ Response to cognitive stress*</td>
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<td>↑ Stimulation by 5-HT&lt;sub&gt;1A&lt;/sub&gt; receptor agonist (ipsapirone)*</td>
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<td>↑ Response to hypothermic stress</td>
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<td>↑ Effect of physostigmine*</td>
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Abbreviations: ACTH, corticotropin; AVP, arginine vasopressin; CRH, corticotropin-releasing hormone; 5-HT<sub>1A</sub>, serotonin<sub>1A</sub>.

* Asterisk denotes a more prominent age contrast in women than in men.
2012 Graduating Clinical Endocrinology Fellows

Left to right (and upcoming appointment): Matheni Sathananthan, MD (Cadence Health System, Winfield, Illinois); Ekta Singh, MBBS (Department of General Internal Medicine, Mayo Clinic, Rochester, Minnesota); Neena Natt, MD, Program Director, Clinical Fellowship in Endocrinology, Diabetes, Metabolism, and Nutrition, Rochester, Minnesota; Irina Bancos, MD (Mayo Clinic Foundation Scholar program, Division of Endocrinology, Diabetes, Metabolism, and Nutrition, Mayo Clinic in Minnesota); Jaime P. Almandoz, MB, BCh (Division of Nutrition and Metabolic Diseases, University of Texas Southwestern Medical Center, Dallas, Texas).

2012 Graduating Endocrine Surgery Fellow

Louis C. Lee, MD, and his program director, Clive S. Grant, MD. Dr Lee’s new appointment is with Surgical Associates of Monterey Bay, Santa Cruz, California.

2012 Kroc Lecturer—Robert R. Henry, MD, Chief, VA Endocrinology and Metabolism and Professor of Medicine, University of California, San Diego, and clinical endocrinology fellows. Seated, left to right: Marcio L. Griebeler, MD, Robert R. Henry, MD, Jaime P. Almandoz, MB, BCh, and Caroline Davidge Pitts, MB, BCh. Standing, left to right: Jennie H. Law, MD, Adina F. Turcu, MD, Irina Bancos, MD, Neena Natt, MD (Program Director), Ekta Singh, MBBS, and Meera Shah, MB, ChB.
16th Mayo Clinic Endocrine Course
January 29–February 2, 2013, Marco Island, Florida
Designed for endocrinologists and interested internists and surgeons, the 16th Mayo Clinic Endocrine Course will address gaps in medical knowledge and barriers in clinical practice, in order to improve the outcomes of patients with endocrine and metabolic disorders. This 3 and 1/2–day course will span the full spectrum of endocrinology through lectures, debates, panel discussions, clinicopathologic sessions, “clinical pearls” sessions, informal breakfast roundtable discussions, and small-group discussions with experts. Attendees will have plenty of opportunity for interaction with the course faculty, who are selected from Mayo Clinic for their expertise and clinical acumen. An optional thyroid ultrasonography course will also be offered. For more information about this course, please call 800-323-2688 or visit www.mayo.edu/cme/endocrinology.

13th Annual Mayo Clinic Nutrition and Wellness in Health and Disease
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