BoneKEy-Osteovision. 2005 September;2(9):6-13
http://www.bonekey-ibms.org/cgi/content/full/ibmske;2/9/6
DOI: 10.1138/20050173

PERSPECTIVES

11β-HSD: Guardian or Gate Crasher?

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Physicians have long been perplexed by the occasional patient who becomes Cushingoid, as characterized by a moon face, buffalo hump, violaceous striations, central adiposity, hypertension, and diabetes, when treated with relatively small amounts of glucocorticoids while other patients appear to be remarkably resistant. The explanation may be that the sensitivity to exogenous glucocorticoids is mediated by inherited gradations in the 11β-HSD shuttle (Fig. 1). This remarkable system is a natural pre-receptor controller of corticosteroid action as well as a unique tool that can be used to distinguish the direct effects of excess glucocorticoids on bone cells from the indirect effects that occur in almost every other tissue.

The 11β-HSD Shuttle

Two isoenzymes of 11β-hydroxysteroid dehydrogenase, 11β-HSD1 and 11β-HSD2, catalyze the interconversion of hormonally active glucocorticoids (such as corticosterol or cortisol, also known as compound F) and inactive glucocorticoids (such as corticosterone or cortisone, also known as compound E). The 11β-HSD1 track (the F train) is an activating route, and the 11β-HSD2 track (the E train) is an inactivating route (Fig. 1). The ability of any glucocorticoid to bind to glucocorticoid receptors (GR) as well as their biological activity depends on the presence of a glucocorticoid to bind to glucocorticoid

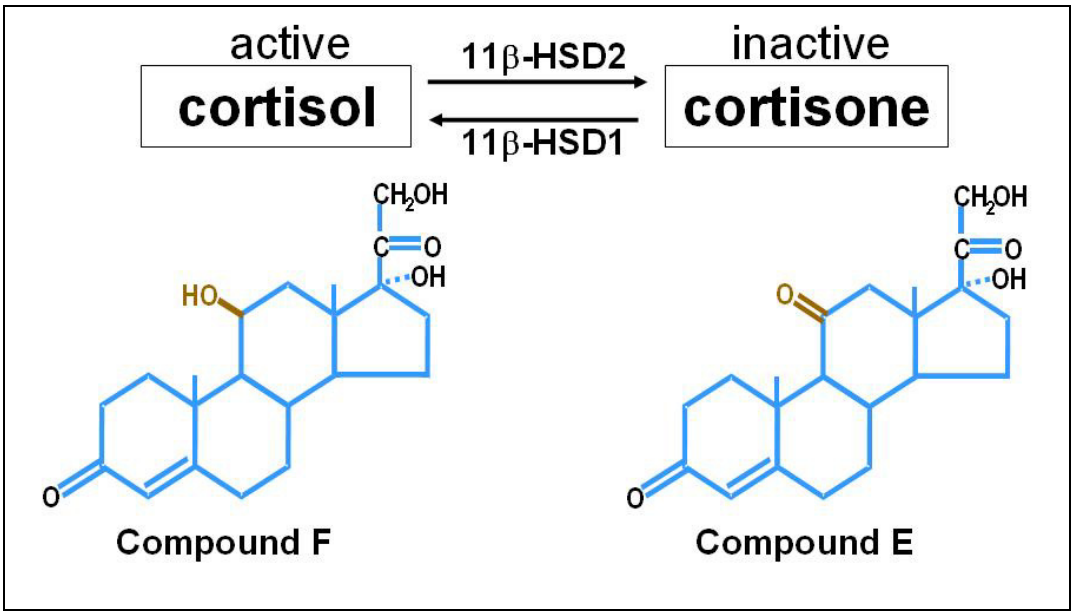

Figure 1. The 11β-HSD Shuttle

receptors (GR) as well as their biological activity depends on the presence of a hydroxyl group at C-11. Therefore, any tissue expressing 11β-HSDs can regulate the exposure of resident cells to active glucocorticoids (1).

The 11β-HSD shuttle also works on synthetic glucocorticoids (e.g., prednisolone or dexamethasone). Surprisingly, although total serum aldosterone levels are 100-400 pmol/L and those of cortisol are 200-700 nmol/L, both aldosterone and cortisol have similar affinity for the mineralocorticoid receptor (MR). How, then, is aldosterone able to bind to the MR and regulate mineralocorticoid responses in the presence of a 1000-fold higher circulating concentration of cortisol? The answer lies in the tissue-specific expression of the 11β-HSD shuttle components. To gain perspective on this shuttle, it is practical to review the role of 11β-HSD2 before that of 11β-HSD1.

11β-HSD2: The E Train

11β-HSD2 is a potent dehydrogenase that rapidly inactivates glucocorticoids at a pre-receptor level. It is primarily found in mineralocorticoid target tissues such as kidney, sweat glands, salivary glands, and colonic mucosa where it protects the indiscriminate MR from high cortisol concentrations (2). Thus, mineralocorticoid target tissue specificity is enzyme– not receptor– mediated (3). For this reason, 11β-HSD2 has been called the "guardian of the gate" (4). Other examples of pre-receptor-mediated ligand regulation include 5α-reductase for the androgen receptor and 5´monoiodinase for the thyroid hormone receptor. Overwhelming 11β-HSD2 with substrate (as occurs in the ectopic ACTH syndrome), inhibiting 11β-HSD2 activity by authentic, old-fashioned black licorice (glycyrrhetinic acid), or the presence of an inactivating mutation (as in the rare autosomal recessive syndrome of apparent mineralocorticoid excess or the "human 11β-HSD2 knockout") abolishes this selectivity and results in sodium retention, hypokalemia, and hypertension due to MR activation by both mineralocorticoids and glucocorticoids. Furthermore, increased expression of 11β-HSD2 in the anterior pituitary of patients with Cushing's disease may account for the relative resistance to corticosteroid feedback characteristic of the disease (8).

The Role of 11β-HSD2 as a Tool to Investigate Glucocorticoid-Induced Osteoporosis

Glucocorticoid-induced bone disease may be mediated by direct actions on bone cells, actions on extraskeletal tissues, or both. To distinguish the direct from the indirect effects of glucocorticoids on bone, O'Brien and coworkers over-expressed 11β-HSD2 in mice utilizing the murine osteocalcin gene 2 (OG2) promoter which is active only in mature osteoblasts and osteocytes (7). Under the control of this promoter, the transgene did not affect normal bone development or turnover as demonstrated by identical bone mineral density (BMD), strength, and histomorphometry in adult transgenic and wild type animals, suggesting that endogenous glucocorticoid action in osteocalcin-expressing cells is not required for normal skeletal development. Other workers over-expressed 11β-HSD2 in mice using the 2.3-kb Col1a1 promoter and did find effects on vertebral cancellous bone growth (12). These changes, however, were not found in males or in the long bones of either sex, suggesting that if endogenous glucocorticoids positively affect murine bone development, they do so in a site- and sex-specific manner. Strong evidence against a beneficial role for endogenous glucocorticoids on bone in adults is supplied by reports of patients with Addison's disease whose BMD values are normal until they are treated with greater than replacement amounts of glucocorticoids (13,14).

Mice harboring the OG2-11β-HSD2 transgene were protected from glucocorticoid-induced apoptosis of osteoblasts and osteocytes. Prevention of osteoblast/osteocyte apoptosis, in turn, resulted in the preservation of cancellous osteoblast numbers and osteoid production thereby preventing the expected decrease in bone formation caused by administration of

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excess glucocorticoids. More strikingly, bone strength was preserved in the transgenic animals in despite of a loss of BMD suggesting that the maintenance of osteocyte viability independently contributed to bone strength (Fig 2). Recently, the vital importance of the direct effect of glucocorticoids on osteoblasts that was shown enzymatically by O'Brien *et al*, was elegantly confirmed by GR deletion using osteoblast-specific $GR^{runx2cre}$ mice that were resistant to the decrease in bone formation typical of glucocorticoid excess (15).

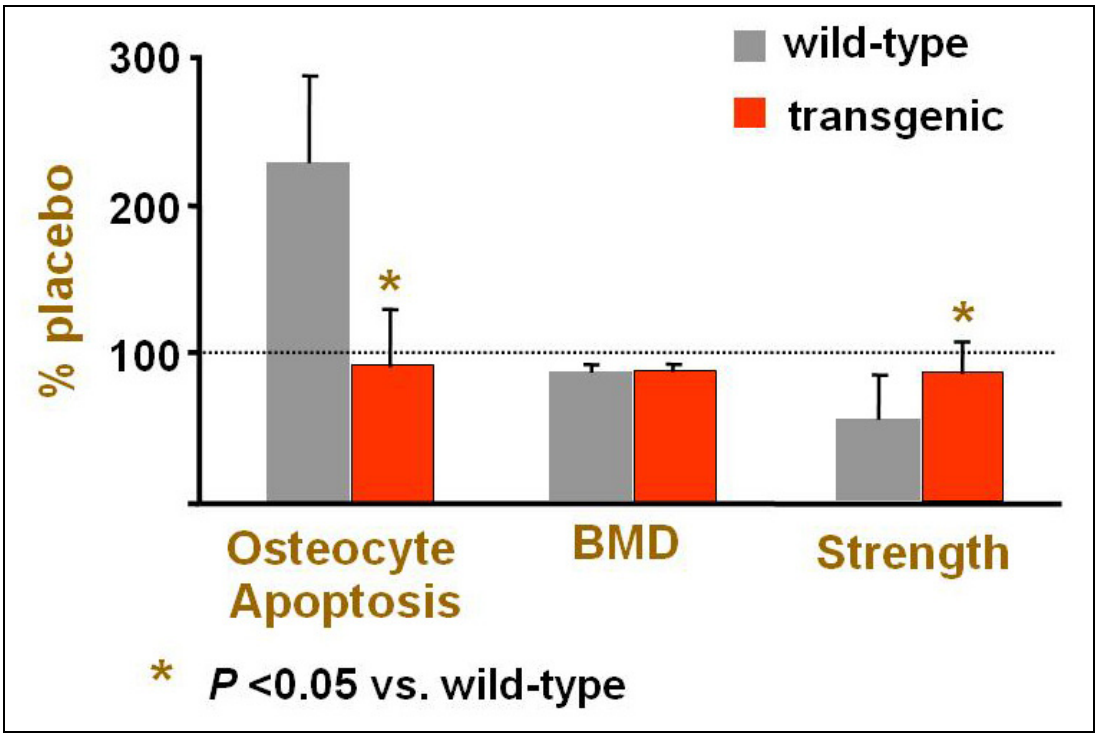

Figure 2: Bone strength and osteocyte viability are preserved in prednisolone-treated OG2-11β-HSD2 transgenic mice.

To further dissect the direct effects of glucocorticoids on bone, we over-expressed 11β-HSD2 in mice utilizing the murine (TRAP) promoter, which is only active in osteoclasts. Morphometric measurements of body size, vertebral and femoral dimensions, and vertebral histomorphometry revealed that the transgene had no impact on normal skeletal development. Therefore, we challenged the animals with glucocorticoids. In a 10-day experiment, prednisolone induced a greater than 4-fold loss of spinal BMD in wild-type mice when compared with their placebo-treated counterparts. This decrease was significantly greater than that seen in transgenic animals. Moreover, the prednisolone-induced bone loss in transgenics was indistinguishable from that seen in their placebo-treated counterparts (Fig. 3). These results indicate that the early rapid loss of bone characteristic of glucocorticoid-induced osteoporosis is due, at least in part, to the direct effects of glucocorticoids on osteoclasts (16).

11β-HSD1: The F Train

11β-HSD1 primarily functions as a low-affinity reductase, catalyzing the reactivation of inert 11-keto forms of glucocorticoids to their active 11-hydroxyl congeners (2). The enzyme is found in most glucocorticoid-sensitive tissues, and its synthesis and activity are modulated by glucocorticoids, stress, sex steroids, growth hormone, cytokines, and peroxisome proliferator-activated receptor-γ agonists (1). Congenital defects in 11β-HSD1 activity (apparent

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cortisone reductase deficiency or the "human 11β-HSD1 knockout") result in defective cortisone to cortisol conversion, enhanced peripheral clearance of cortisol, and decreased negative feedback suppression of corticotropin-dependent steroids (2). Affected female patients present with features of adrenal androgen excess such as oligomenorrhea, infertility, hirsutism, acne, plethora, and obesity while males present with precocious puberty (1).

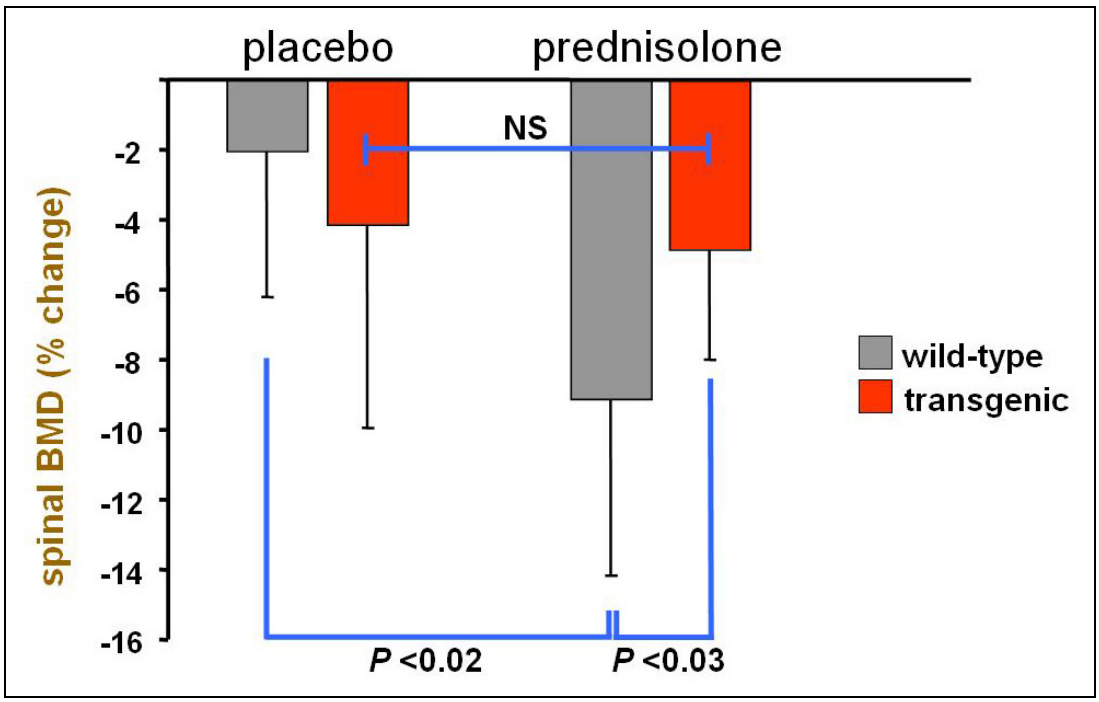

Figure 3: Overexpression of TRAP-11β-HSD2 prevents glucocorticoid-induced loss of BMD. The percent change in spinal BMD in wild type and TRAP-11β-HSD2 mice receiving placebo or prednisolone pellets for ten days. Wild-type mice are shown in the gray bars, and transgenic animals are shown in red. There are 8-10 animals per group. Analysis was by ANOVA.

Remarkably, a patient with Cushing's disease and defective 11β-HSD1 activity did not exhibit the characteristic features of the disease suggesting that the level of 11β-HSD1 expression may determine individual susceptibility to the effects of glucocorticoid excess (10). Resistance to hormone action is usually accompanied by normal or elevated levels of hormones due to regulatory feedback control, and it occurs through pre-receptor, receptor, or post-receptor mechanisms. In the case of glucocorticoids, however, it is unlikely that a reduction in GR number accounts for much resistance since only 5-10% binding to the GR is necessary for full hormone effects. Glucocorticoid resistance has been postulated to result from polymorphisms in the GR gene but current evidence suggests that such variations are not a major contributor. It is more probable that individual sensitivity to glucocorticoids is inherited in a tissue-specific manner by varying traffic through the 11β-HSD shuttle.

Local Amplification of Glucocorticoid Action

11β-HSD1 plays a major role in the local modulation of glucocorticoid levels and, hence, access of active steroid to the GR. Tissue-specific increases in 11β-HSD1 expression and the resultant amplification of glucocorticoid action have been reported in liver, abdominal fat, lung, pituitary, gonads, skeletal muscle, vascular smooth muscle, and brain (5). Increased activity of 11β-HSD1 in omental fat may explain the characteristic central adiposity of Cushing's

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syndrome as well as the visceral adiposity of the metabolic syndrome (2,9). Interestingly, 11β-HSD1 knockout mice are protected from the deterioration of cognitive function induced by age-related increases in endogenous glucocorticoids, suggesting that the enzyme plays an important role in the degenerative conditions of the elderly (11). Therefore, hereditary or acquired local changes in 11β-HSD1 activity could account for variable tissue sensitivity to endogenous or exogenous glucocorticoids. It is tempting to speculate that the predilection of glucocorticoid-induced osteonecrosis for the hip is due to increased 11β-HSD1 activity in the proximal femur.

Glucocorticoids, 11β-HSD1, Osteoporosis and Aging

Basal morning glucocorticoid levels increase with age in mice and humans (11,17). In healthy elderly men, aged 61 to 72 years, cortisol levels and BMD measurements taken at baseline and four years later reveled that the trough cortisol concentration and rate of bone loss at the lumbar spine and femoral neck were related ($r = 0.38$; $p < 0.05$ and $r = 0.47$; $p < 0.001$) (17). Importantly, these relationships remained significant after adjusting for adiposity, cigarette smoking, alcohol consumption, dietary calcium intake, physical activity, and serum testosterone and estradiol levels. Furthermore, using primary cultures of human osteoblasts, 11β-HSD1 activity has been shown to increase with donor age and glucocorticoid exposure (6). The increase in bone 11β-HSD1 expression would be expected to augment the impact of the ambient corticosterone levels on bone cells and may therefore contribute to involutional osteoporosis.

In preliminary work done at the University of Arkansas for Medical Sciences, 11β-HSD1 mRNA was also found to increase with age in C57Bl/6 mice (Fig. 4). In addition, there was an inverse correlation between 11β-HSD1 expression levels and vertebral compression strength ($r = -0.67$, $p < 0.05$). These results suggest that the local amplification of endogenous glucocorticoid levels affects bone strength, contributing to the disparity between bone quantity and quality typical of aging—perhaps by increasing the prevalence of osteocyte apoptosis (7).

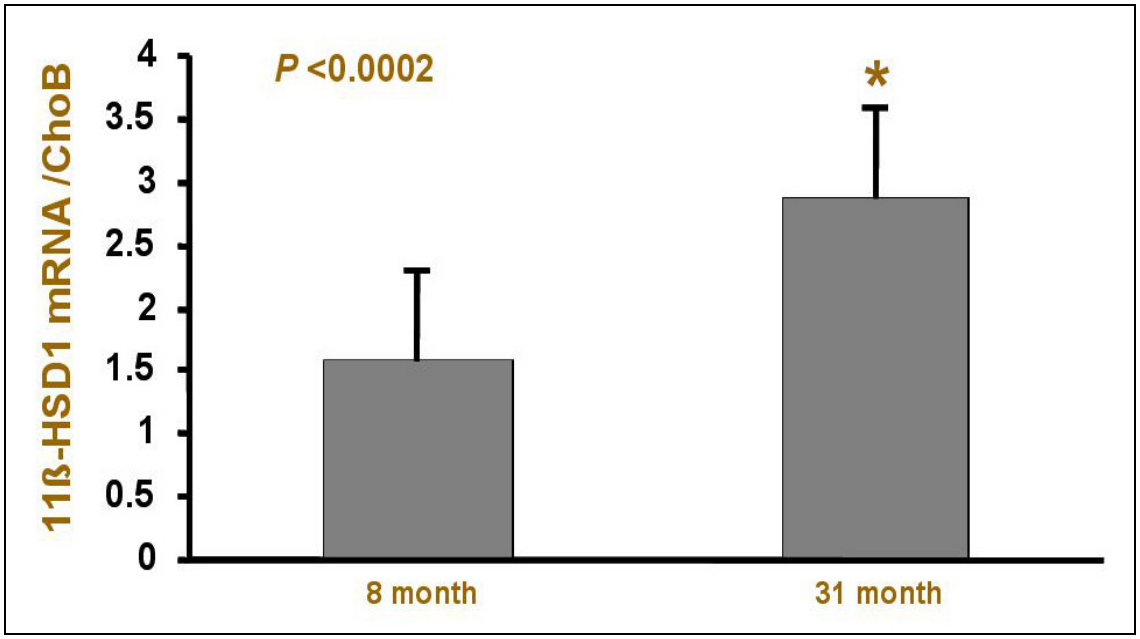

Figure 4: RT-PCR of calvaria taken from 8- and 31-month-old C57BL/6 mice shows a 1.8-fold increase in 11β-HSD1, a tissue-specific amplifier of glucocorticoid action.

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In undecalcified longitudinal sections of the lumbar vertebrae taken from C57BL/6 mice, cancellous osteoblast apoptosis increased by 99% in the 31-month-old animals compared with 8-month-old animals (p <0.0005, ANOVA). Cortical osteocyte apoptosis also increased by 84% in these animals (p <0.001). Bone formation rate on a tissue area referent was decreased by 60% in 31-month old mice compared with 8-month-old mice (p <0.02). Strikingly, the bone formation rate was inversely related to the prevalence of osteoblast apoptosis (Fig. 5). Decreased bone formation and increased osteoblast and osteocyte apoptosis are hallmarks of glucocorticoid-induced osteoporosis (18). Taken together, these findings point to an important role for endogenous glucocorticoids and 11β-HSD1 in age-related decreases in bone strength and mass.

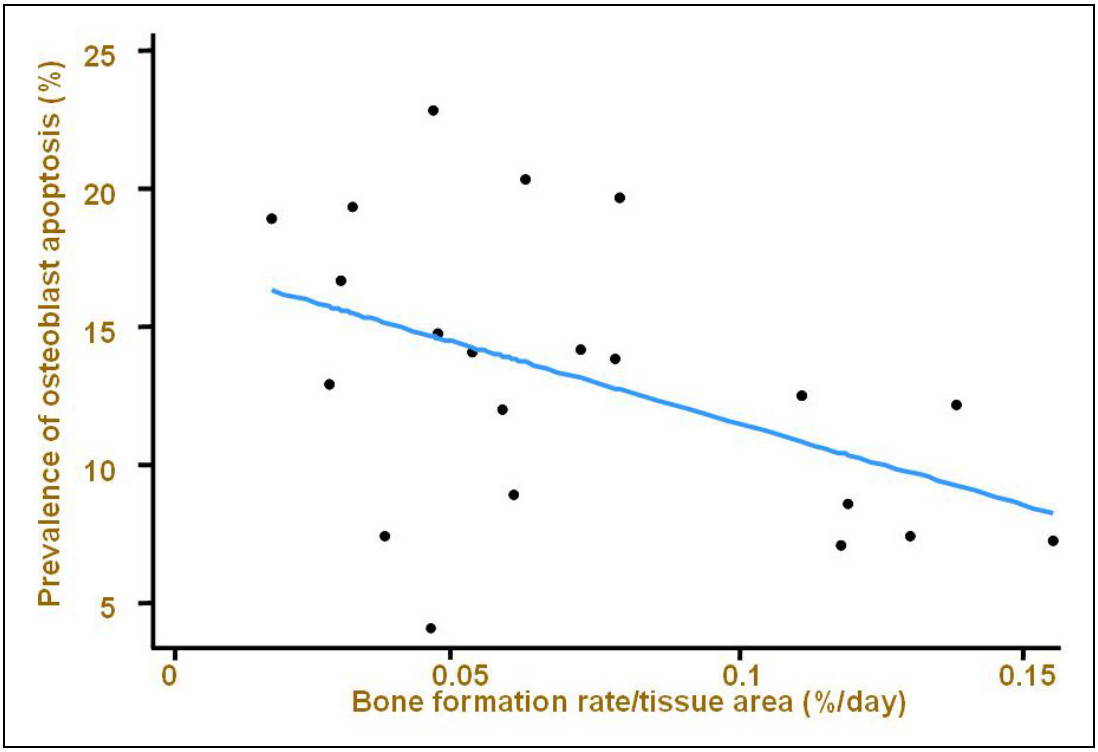

Figure 5: In the cohorts of older C57BL/6 mice, the prevalence of osteoblast apoptosis was inversely related to the bone formation rate on a tissue area referent (n = 21, r = –0.46, p <0.04).

Aging has been described as the universal, progressive, and intrinsic accumulation of deleterious changes that compromise the physiological effectiveness of an organism. No single factor can account for this progressive decline in bone fragility—*e.g.*, peak bone size and BMD, estrogen deficiency, increased oxidative damage, adverse effects of lipid peroxidation products, endogenous relative hypercortisolism, impaired mobility and mechanical stimulation, or drug therapy. To gather all patients with involutional osteoporosis into one group because of low BMD or fractures obscures the heterogeneity of the structural, cellular, and biomechanical basis of bone fragility as well as the varying contributions of age-related mechanisms responsible for the condition. The only way to understand skeletal aging is to manipulate it, and today, the technological ability to do this in transgenic animals has the potential to change the study of the biology of aging from a field concerned

primarily with description to one that is marked by intervention.

Conclusions: Take the E Train

The 11β-HSD shuttle is a vital pre-receptor control pathway regulating glucocorticoid action. Furthermore, the shuttle also represents a set of tools allowing for the dissection of the effects of excess glucocorticoid administration on the skeleton and of the role of endogenous glucocorticoids on the loss of bone mass and strength that occurs with aging. In the future, pharmacotherapeutic agents may be able to target 11β-HSD1 or 11β-HSD2 in specific tissues to prevent the deleterious effects of glucocorticoid administration while maintaining its advantages (1,2).

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