The pathogenesis of glucocorticoid-induced osteoporosis

R.S. Weinstein

Department of Internal Medicine, Division of Endocrinology/Metabolism, The Center for Osteoporosis and Metabolic Bone Diseases, and the Central Arkansas Veterans Healthcare System, University of Arkansas for Medical Sciences, Little Rock, Arkansas, USA.

Please address correspondence and reprint requests to: Robert S. Weinstein, MD, University of Arkansas for Medical Sciences, 4301 West Markham Street, Slot 587, Little Rock, Arkansas 72205-7199, USA. E-mail: weinsteinroberts@ exchange.uams.edu

Key words: Glucocorticoids, osteoporosis, apoptosis, bone cells, osteonecrosis.

ABSTRACT
Decreased bone formation, diminished intestinal calcium absorption and urinary calcium reabsorption, secondary hyperparathyroidism and hypogonadism are some of the proposed mechanisms of the deleterious effects of glucocorticoid excess on bone. Recent evidence suggests that extraskeletal mechanisms may not be essential in steroid-induced osteoporosis and that the major mechanisms of the condition may be a direct result of glucocorticoid administration on the suppression of osteoblastogenesis and osteoclastogenesis and the increased apoptosis of osteoblasts and osteocytes.

Introduction
"Spontaneous fractures occurred from time to time, involving sternum, clavicle and ribs. The autopsy showed skeletal osteoporosis with spinal curvature, the bones being easily cut with a knife . . . the spongy part of the bone having largely disappeared"

Harvey Cushing, 1932

When Cushing recognized the adverse effects of hypercortisolism on bone over 60 years ago, the disease only occurred endogenously (1). Today, the iatrogenic situation has become far more common than the syndrome that bears his name. Glucocorticoid-induced osteoporosis is now the third leading cause of osteoporosis, following the postmenopausal and senile varieties, and is the most common cause of drug-related osteoporosis (2). The majority of the bone loss is early and rapid (3). During the first year approximately 12% losses in bone mineral density (BMD) have been documented, followed by slower annual decrements of about 3% (4). Therefore, chronic glucocorticoid treatment leads to atraumatic fractures in 30-50% of patients and most of these fractures occur during the first year of therapy (5). Osteoporosis due to glucocorticoid excess is diffuse, affecting both cortical and cancellous bone, but there is a distinct predilection for the axial skeleton, a manifestation of the dis-

ease well appreciated by Cushing (1). In addition to these fractures, a devastating accompaniment of long-term glucocorticoid therapy is osteonecrosis, also known as aseptic, avascular or ischemic necrosis, which causes collapse of the femoral neck or proximal humerus in as many as 25% of patients who receive high-dose or long-term therapy (6).

The pursuit of a unified theory that accounts for glucocorticoid-induced bone disease has been slow because of the heterogeneity of the diseases treated with glucocorticoids and the wide variations in the dose and duration of treatment. To complicate matters further, glucocorticoid therapy is often used in disorders and situations that independently increase the risk of fracture, such as rheumatoid arthritis, myeloma and transplantation. In addition to these constraints, the lack of a reliable animal model of glucocorticoid-induced osteoporosis has confounded elucidation of the mechanisms responsible for the condition and, consequently, therapy to protect the skeleton has remained empirical.

Today, new information about bone remodeling and glucocorticoid-induced bone disease indicates that the balance between bone resorption and bone formation, which maintains adult skeletal mass, is controlled not only by changes in the production of osteoclasts and osteoblasts but also by alterations in the duration of the lifespan of these cells through programmed cell death, or apoptosis (Fig. 1). Like anemia, glucocorticoid-induced bone disease can result from primary disorders of the production or survival of cells.

Glucocorticoid-induced osteoporosis
Current concepts of the pathophysiology of glucocorticoid-induced bone disease involve interactions with calcium and bone metabolism at multiple levels with both direct and indirect effects, which ultimately impact on osteoblasts and osteoclasts. A commonly invoked explanation for this impact is that glucocorticoids suppress bone formation, cause
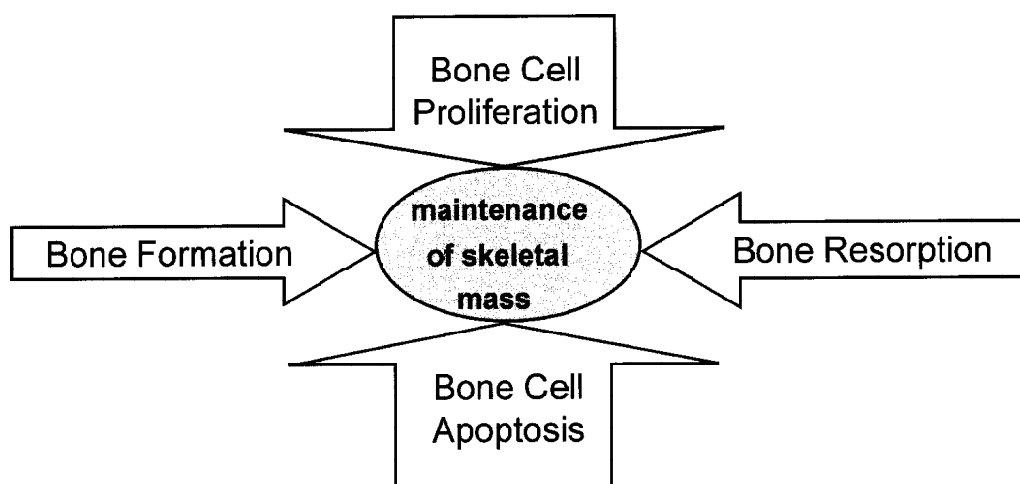
```
                    ┌─────────────┐
                    │  Bone Cell  │
                    │ Proliferation│
                    └─────────────┘
         ┌──────────────┐     ╱ maintenance ╲     ┌──────────────┐
         │ Bone Formation│───▶│ of skeletal │◀───│Bone Resorption│
         └──────────────┘     ╲    mass    ╱     └──────────────┘
                    ┌─────────────┐
                    │  Bone Cell  │
                    │  Apoptosis  │
                    └─────────────┘
```

Fig. 1. A balance between bone formation, bone resorption, bone cell proliferation and bone cell apoptosis maintains normal adult skeletal mass.

malabsorption of calcium in both the intestine and renal tubule resulting in secondary hyperparathyroidism with increased bone resorption, and reduce sex steroid levels (7). Considerable evidence, however, indicates that elevated levels of parathyroid hormone (PTH) or subnormal vitamin D metabolite concentrations are not typical of patients receiving glucocorticoid therapy (8, 9) and that concentrations of bone resorption markers, such as serum intact PTH, serum acid phosphatase and urinary excretion of the N-telopeptide of type I collagen show either no change or decrease with long-term glucocorticoid therapy (10, 11). Furthermore, the decreased sex steroid levels attributed to glucocorticoid therapy may be due to the morbidity of the underlying disease rather than to glucocorticoid treatment. A recent prospective study of infertile but otherwise healthy men given 50 mg/d of prednisolone for one to six months to reduce antisperm antibodies revealed that there was no change in the testosterone/sex steroid-binding globulin ratio (11). Moreover, the fertility of these men was, as intended, increased by the prednisolone treatment.

The most corroborated part of the commonly invoked explanation is suppressed bone formation. Histomorphometric studies in patients receiving long-term glucocorticoid treatment consistently show decreases in the percentage of cancellous bone covered by plump, cuboidal osteoblasts (the osteoblast perimeter) and in the rate of bone formation (5, 12). Further evidence of the decreased work pro-duced by teams of osteoblasts subjected to glucocorticoid excess is the reduced wall thickness of the newly formed bone structural units (13, 14). Additional confirmation of reduced osteoblast effort is supplied by the demonstration that glucocorticoids rapidly decrease serum levels of osteocalcin, an osteoblast product that reflects the rate of bone formation (15). Glucocorticoids decrease osteoblasts by two disastrous, additive effects: decreased production and shortened lifespan.

Decreased cell production
The development of clonal assays for bone progenitor cells has allowed the investigation of these cells as the progenitors proliferate, differentiate and eventually undergo apoptosis. These assays have revealed that osteoporosis may result from a reduced rate of osteoblast production or a reduction in osteoblast lifespan, or both mechanisms. New insight into the pathogenesis of glucocorticoid-induced bone disease has been provided by the evidence that the long term skeletal impact is due to the suppressive effects of glucocorticoids on both osteoblastogenesis and osteoclastogenesis (Table I) (16-21).

Mice receiving glucocorticoids for 4

Table I. Cellular changes in glucocorticoid-induced osteoporosis.

Cellular changes	Explanations
Osteoblastogenesis	Decreased Cbfa1 and TGF- type I receptor; decreased BMP2 and IGF1 action
Osteoclast numbers*	
early	Rapid, transient increase in the RANK ligand/OPG ratio with early prolongation of osteoclast lifespan†
late	Decreased osteoblast progenitors with a resulting fall in osteoclast production.
Bone marrow adipocytes	Increased PPAR 2 and LPL expression
Lifespan of osteoblasts	Decreased Bcl-2/BAX ratio
Lifespan of osteocytes	Decreased Bcl-2/BAX ratio

* Osteoclast numbers increase transiently during the early stages of steroid therapy; but later markedly decrease.
† The early increase in osteoclast numbers occurs in spite of a decrease in the production of new osteoclasts, suggesting a glucocorticoid-induced increase in the lifespan of the osteoclasts that were present at the onset of glucocorticoid administration.
Adapted from Manolagas and Weinstein: Perspective: New developments in the pathogenesis and treatment of steroid-induced osteoporosis. *J Bone Miner Res* 1999; 14: 1061-6.

Table II. Bone histomorphometry in patients with glucocorticoid-induced osteoporosis.

Histomorphometric determination	Patient results (mean ± SEM)	Normal values* (mean ± SEM)
Bone area / Tissue area (%)	9.5 ± 2.0	22.4 ± 1.2
Trabecular width (μm)	78 ± 23	161 ± 9
Wall width (μm)	33.0 ± 4.0	57.6 ± 2.1
Osteoid area / Bone area (%)	1.0 ± 0.5	2.1 ± 0.3
Osteoid width (μm)	2.0 ± 0.2	7.6 ± 0.4
Osteoid perimeter / Bone perimeter (%)	10.5 ± 3.0	15.9 ± 1.9
Osteoblast perimeter / Bone perimeter (%)	2.3 ± 0.1	7.6 ± 0.4
Osteoclast perimeter / Bone perimeter (%) ¥	0.4 ± 0.2	0.9 ± 0.2
Reversal perimeter / Bone perimeter (%) ¥	15.4 ± 2.5	6.9 ± 0.7
Mineralizing perimeter / Bone perimeter (%)	4.2 ± 3.5	10.6 ± 1.4
Mineral appositional rate (μm/d)	0.57 ± 0.30	0.90 ± 0.03
Bone formation rate / Bone perimeter ($μm^2$/(m/d)	0.05 ± 0.02	0.095 ± 0.012

†Bone biopsies were obtained during 1978 to 1995 from 5 patients receiving long-term glucocorticoid treatment for nephrotic syndrome, myasthenia gravis, allergies and pulmonary disease.
* Normal values are from Weinstein RS, Bell NH: Diminished rate of bone formation in normal black adults. *N Engl J Med* 1998; 319: 1689-701.
¥ If the osteoclast and reversal perimeters are measured together as the eroded perimeter, the determination no longer faithfully reflects bone resorption because of the inclusion of the empty Howship's lacunae. For example, glucocorticoid (17) or calcitonin (32) treatment increases the eroded perimeter while decreasing the osteoclast perimeter.

weeks - a period equivalent to about 3 to 4 years in humans when prorated by lifespan - exhibited an early rapid and later slow loss of bone mineral density (BMD), a two-phase time course similar to that found in the clinical studies. This loss was associated with a dramatic reduction in cancellous bone area, trabecular width and the rate of bone formation, and a decrease in the number of osteoblast and osteoclast progenitors in *ex vivo* cultures taken from the bone marrow. The number of osteoblast progenitor colony-forming units per femur (CFU-OB) decreased by 86%, while the number of osteoclast progenitors decreased by 65%. The greater decline in osteoblast precursors than in osteoclast precursors accounts for part of the decrease in the cancellous bone area and trabecular width.

Although a reduction in bone formation will not by itself cause bone loss, the decrease in trabecular width – the major structural change observed with chronic glucocorticoid treatment – is usually the result of incomplete cavity repair due to inadequate osteoblast production. The decrease in osteoblastogenesis also explains the histomorphometric findings of a moderate increase in the erosion or eroded perimeter, which is the sum of the osteoclast and reversal perimeters (Table II). Increased eroded perimeter can merely represent the accumulation of empty Howship's lacunae (the reversal phase) resulting from a glucocorticoid-induced defect or delay in the coupling of bone resorption and bone formation, rather than a real increase in osteoclasts. The reduction in osteoblastogenesis is also of sufficient magnitude to explain the decrease in the rate of bone formation, and thereby further contributes to the declining trabecular width. These changes were not confounded by drug-induced changes in weight, food intake, hepatic fatty infiltration or gonadal function, which plague use of the rat, dog, rabbit and ewe as models of the disease (17). Furthermore, histological evidence of secondary hyperparathyroidism was absent. Thus, the direct inhibitory effect of glucocorticoids on early bone cell progenitors in the bone marrow can account for many of the *in vivo* observations.

Although there is a significant correlation between the severity of the bone loss and the extent of the reduction in bone formation (17), some of the early bone loss is due to a rapid but transient increase in bone resorption, as demonstrated by the increase in the percentage of cancellous bone covered by osteoclasts (the osteoclast perimeter) detected by histomorphometric examination of murine vertebral cancellous bone after only 7 days of steroid treatment (17). Preliminary evidence indicates that the increase in osteoclast numbers occurs in spite of a decrease in the production of new osteoclasts in the bone marrow, suggesting that glucocorticoid administration may have prolonged the lifespan of the pre-existing osteoclasts (22). However, after 4 weeks of prednisolone administration, bone resorption decreases to or below normal (17). This biphasic response of bone resorption to steroid treatment may be explained by the rapid, transient effect of glucocorticoids to down-regulate the mRNA for osteoprotegerin [OPG; also known as OCIF (osteoclastogenesis inhibitory factor)] (23), a soluble decoy receptor for RANK (receptor activator of NF- B) ligand, while increasing the level of RANK ligand*. The increased RANK ligand/OPG ratio will facilitate the RANK ligand, expressed on committed preosteoblastic cells (24), to increase osteoclastogenesis by unopposed binding to a specific receptor, RANK, on the surface of hematopoietic osteoclast progenitor cells (25). In other words, glucocorticoid-induced increments in the RANK ligand/OPG ratio will enhance the osteoclast support role of preosteoblasts (Table I). The transient nature of this early increase in bone resorption is further guaranteed by the glucocorticoid-induced decrease in osteoblastogenesis and resulting decline in the RANK ligand-producing, osteoclast-supporting preosteoblasts.

The same decrease in osteoclast perimeter seen in the mice receiving glucocorticoids for 4 weeks is found in biopsies from patients receiving glucocorticoid therapy for years (Table II). Murine histomorphometric studies have demonstrated that the mouse, unlike several other laboratory animals, is a faithful model of the glucocorticoid-induced bone loss in humans (17). Furthermore, understanding the murine model has facilitated appreciation of the role of osteo-

*RANK ligand is also known as ODF (osteoclast differentiation factor), TRANCE (tumor necrosis factor-related activation-induced cytokine) or osteoprotegerin ligand (OPGL).

blast and osteocyte apoptosis in the pathogenesis of glucocorticoid-induced bone disease.

Decreased cell lifespan

Glucocorticoid administration caused a three-fold increase in the prevalence of osteoblast apoptosis in murine vertebral cancellous bone and induced apoptosis in 28% of the osteocytes in metaphyseal cortical bone. Precise inventory of the fraction of apoptotic cells in bone varies depending on the identification technique and the duration of the histologically recognizable features of a cell undergoing apoptosis, but demonstration of only a small fraction of apoptosis in a particular tissue can represent a huge proportion of cells destined to undergo programmed cell death. A three-fold increase in osteoblast apoptosis could result in the loss of up to 90% of the mature osteoblasts (20,21). This means that small changes in the prevalence of osteoblast apoptosis can result in enormous changes in bone formation.

The model is clinically relevant, given the similarity of the apoptosis responses in the mice to those associated with glucocorticoid-induced bone disease in humans. TUNEL-positive osteoblasts and osteocytes with the characteristic morphometric features of apoptosis, such as nuclear fragmentation and condensation of chromatin, have been clearly identified in transiliac bone biopsies taken from patients receiving chronic prednisone treatment, but are absent from sections taken from age-matched controls (Figs. 2 and 3) (17). Compared to osteoblast apoptosis in cancellous tissue, cortical osteocyte apoptosis is far more prevalent because of the unique unavailability of these osteocytes for phagocytosis due to their anatomic isolation from scavenger cells and the need for extensive degradation to small molecules to dispose of the cells through the narrow canalicular system. Therefore, the process in cortical bone is prolonged and affected osteocytes accumulate – a set of conditions that may contribute to osteonecrosis.

To further investigate these findings, we examined the prevalence of osteocyte apoptosis in whole femoral heads obtained from patients with glucocorticoid-

induced osteonecrosis who had undergone resection of the femoral head and prosthetic hip replacement (26). Control specimens were obtained from patients with osteonecrosis due to other clinical disorders, including femoral specimens from patients with sickle cell disease, after traumatic fracture or with alcoholism. Apoptotic osteocytes and cells lining cancellous bone were plentiful in the proximal femoral heads resected from patients with glucocorticoid-induced osteonecrosis, whereas apoptotic bone cells were absent from specimens removed

because of trauma or sickle cell disease and were rare in alcohol-induced femoral necrosis. Furthermore, the apoptotic osteocytes were most abundant adjacent to the subchondral fracture crescent. Empty osteocytic lacunae, once thought to be the cardinal sign of bone necrosis, were infrequent and may merely represent loss of the condensed, apoptotic osteocytes during tissue fixation and processing. Reduced cancellous bone area, increased marrow adipocytes and decreased hematopoietic marrow were also noted in the specimens of the patients re-

Fig. 2. The effect of chronic prednisone treatment on human cancellous bone. Apoptotic osteoblasts (**arrows**) are identified by the dark staining and nuclear condensation. Normal osteoblasts (*) are seen adjacent to the apoptotic cells. *In situ* detection of DNA fragmentation, a marker of apoptosis, was done with the TUNEL reaction (transferase-mediated biotin-dUTP nick end-labeling). The section was viewed by Nomarski differential interference contrast microscopy, original magnification x400.
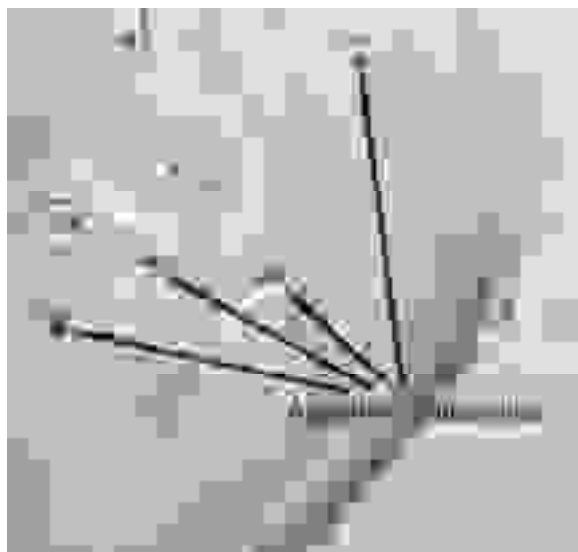

Fig. 3. The effect of chronic prednisone treatment on human cortical bone. Apoptotic osteocytes (**arrows**) are identified by the dark staining and nuclear condensation. Normal osteocytes (*) are nearby. (TUNEL reaction viewed by Nomarski differential interference contrast microscopy, original magnification X400.)

ceiving glucocorticoid. Increased apoptosis of osteocytes may account for the osteonecrosis associated with glucocorticoid excess.

In the past, glucocorticoid-induced osteonecrosis has been attributed to fat emboli, compression of the blood vessels of the femoral head by marrow fat, or fluid retention and poorly mending fatigue fractures (6). Glucocorticoid-induced osteonecrosis is a misnomer because the cell swelling and inflammation that characterizes necrosis in soft tissues does not occur (6, 26). Glucocorticoid-induced osteonecrosis may actually be osteocyte apoptosis, a cumulative and unrepairable defect that would uniquely disrupt the mechano-sensory role of the osteocyte-canalicular network and thus promote collapse of the femoral head. Glucocorticoid-induced osteocyte apoptosis would explain the correlation between total steroid dose and the incidence of osteonecrosis and its occurrence after glucocorticoid administration has ceased (17).

Although the direct effects of glucocorticoids on bone cell proliferation and apoptosis can account for the majority of glucocorticoid-induced bone disease, the molecular mediators of these direct cellular changes have not been clearly established. Nonetheless, glucocorticoid-induced changes in hepatic or local insulin-like growth factor (IGFs) production, IGF-binding protein (IGFBP) synthesis or proteases that control the activation of IGFBP may be involved in the decreased bone formation (27). Glucocorticoid administration also directly suppresses bone morphogenetic proteins and Cbfa1 [core-binding factor a1; also known as Osf2 (osteoblast specific transcription factor 2)], factors required to induce osteoblast differentiation, giving the coup de grâce to osteoblastogenesis, and increases the production of peroxisome proliferator-activated receptor 2 (PPAR 2), a transcription factor that induces terminal adipocyte differentiation while suppressing osteoblast differentiation (28-30). The possibility that glucocorticoid excess increases marrow fat at the cost of mature, matrix-secreting osteoblasts and cancellous bone is also suggested by the murine model. In preliminary work, we found that glucocorticoid

treatment caused a 2-fold increase in murine femoral cancellous adipocyte area when compared with the placebo group, and the production of adipocytes in *ex vivo* bone marrow cell cultures was also significantly increased (21).

A possible molecular moderator that may account for the apoptosis is suggested by the prevention of the pro-apoptotic effect of glucocorticoids on osteoblasts by overexpression of the B-cell lymphoma/leukemia-2 gene (Bcl-2). Thus, glucocorticoid-induced suppression of Bcl-2 or the Bcl-2/BAX* ratio may also be important (31).

References

1. CUSHING H: The basophil adenomas of the pituitary body and their clinical manifestations (pituitary basophilism). *Bull Johns Hopkins Hosp* 1932; 50: 137-95.
2. FITZPATRICK LA: Glucocorticoid-induced osteoporosis. *In* MARCUS R (Ed.): *Osteoporosis*. Boston, Blackwell, 1994: 202-26.
3. REID IR: Pathogenesis and treatment of steroid osteoporosis. *Clin Endocrinol* 1989; 30: 83-103.
4. LoCASCIO V, BONUCCI E, IMBIMBO B *et al*.: Bone loss in response to long-term glucocorticoid therapy. *Bone Miner* 1990; 8: 39-51.
5. LUKERT BP, KREAM BE: Clinical and basic aspects of glucocorticoid action in bone. *In* BILEZIKIAN JP, RAISZ LG, RODAN GA (Eds.): *Principles of Bone Biology*, San Diego, CA, Academic Press 1996: 533-48.
6. MANKIN HF: Nontraumatic necrosis of bone (osteonecrosis). *N Engl J Med* 1992; 326: 1473-9.
7. REID IR: Preventing glucocorticoid-induced osteoporosis. *N Engl J Med* 1997; 327: 420-421.
8. HAHN TJ, HALSTEAD LR, BARAN DT: Effects of short term glucocorticoid administration on intestinal calcium absorption and circulating vitamin D metabolite concentrations in man. *J Clin Endocrinol Metab* 1981; 52: 111-5.
9. PAZ-PACHECO E, FULEIHAN GE-H, LEBOFF MS: Intact parathyroid hormone levels are not elevated in glucocorticoid-treated subjects. *J Bone Miner Res* 1995; 10: 1713-8.
10. PRUMMEL MF, WIERSINGA WM, LIPS P, SANDERS GTB, SAUERWEIN HP: The course of biochemical parameters of bone turnover during treatment with corticosteroids. *J Clin Endocrinol Metab* 1991; 72: 382-6.
11. PEARCE G, TABENSKY DA, DELMAS PD, BAKER HWG, SEEMAN E: 1998 Corticosteroid-induced bone loss in men. *J Clin Endocrinol Metab* 1998; 83: 801-6.
12. BRESSOT C, MEUNIER PJ, CHAPUY MC, LEJEUNE E, EDOUARD C, DARBY AJ: Histomorphometric profile, pathophysiology and reversibility of corticosteroid-induced osteoporosis. *Metab Bone Dis Rel Res* 1979; 1: 303-11.

*BAX is a Bcl-2 homolog that can heterodimerize and inactivate Bcl-2, thereby promoting apoptosis.

13. DEMPSTER DW, ARLOT MA, MEUNIER PJ: Mean wall thickness and formation periods of trabecular bone packets in corticosteroid-induced osteoporosis. *Calcif Tissue Int* 1983; 35: 410-7.
14. DEMPSTER DW: A prospective: Bone histomorphometry in glucocorticoid-induced osteoporosis. *J Bone Miner Res* 1989; 4: 137-41.
15. JOWELL PS, EPSTEIN S, FALLON MD, REINHARDT A, ISMAIL F: 1,25-dihydroxyvitamin D3 modulates glucocorticoid-induced alteration in serum bone GLA protein and bone histomorphometry. *Endocrinology* 1987; 120: 531-6.
16. JILKA RL, WEINSTEIN RS, BELLIDO T, PARFITT AM, MANOLAGAS SC: Osteoblast programmed cell death (apoptosis): Modulation by growth factors and cytokines. *J Bone Miner Res* 1998; 13:793-802.
17. WEINSTEIN RS, JILKA RL, PARFITT AM, MANOLAGAS SC: Inhibition of osteoblastogenesis and promotion of apoptosis of osteoblasts and osteocytes by glucocorticoids: Potential mechanisms of the deleterious effects on bone. *J Clin Invest* 1998; 102: 274-82.
18. JILKA RL, WEINSTEIN RS, TAKAHASHI K, PARFITT AM, MANOLAGAS SC: Linkage of decreased bone mass with impaired osteoblastogenesis in a murine model of accelerated senescence. *J Clin Invest* 1996; 97:1732-1740.
19. JILKA RL, WEINSTEIN RS, BELLIDO T, ROBERSON P, PARFITT AM, MANOLAGAS SC: Increased bone formation by prevention of osteoblast apoptosis with PTH. *J Clin Invest* 1999; 104: 439-46.
20. MANOLAGAS SC, WEINSTEIN RS: Perspective: New developments in the pathogenesis and treatment of steroid-induced osteoporosis. *J Bone Miner Res* 1999; 14: 1061-6.
21. WEINSTEIN RS, MANOLAGAS SC: Apoptosis and osteoporosis. *Am J Med* 2000; 108: 153-64.
22. WEINSTEIN RS, CRAWFORD JA, SWAIN FL, MANOLAGAS SC, PARFITT AM: Early bisphosphonate treatment prevents long term adverse effects of glucocorticoid-induced osteoporosis in mice. *J Bone Miner Res* 2000; 15 (Suppl. 1): S172.
23. VIDAL NOA, BRÄNDSTRÖM H, JONSSON KB, OHLSSON C: Osteoprotegerin mRNA is expressed in primary human osteoblast-like cells: Down-regulation by glucocorticoids. *J Endocrinol* 1998; 159: 191-5.
24. LACEY DL, TIMMS E, TAN HL *et al*.: Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* 1998; 93: 165-76.
25. HOFBAUER LC, KHOSLA S, DUNSTAN CR, LACEY DL, BOYLE WJ, RIGGS BL: The roles of osteoprotegerin and osteoprotegerin ligand in the paracrine regulation of bone resorption. *J Bone Miner Res* 2000; 15: 2-12.
26. WEINSTEIN RS, NICHOLAS RW, MANOLAGAS SC: Apoptosis of osteocytes in glucocorticoid-induced osteonecrosis of the hip. *J Clin Endocrinol Metab* 2000; 85: 2907-12.
27. JONES JI, CLEMMONS DR: Insulin-like growth factors and their binding proteins: Biological actions. *Endo Rev* 1995; 16: 3-34.
28. LECKA-CZERNIK B, GUBRIJ I, MOERMAN EJ *et al*.: 1999 Inhibition of Osf2/Cbfa1 expres-

sion and terminal osteoblast differentiation by PPAR 2. *J Cell Biochem* 1999; 74: 357-71.

29. CHANG DJ, JI C, KIN KK, CASINGHINO S, MC-CARTHY TL, CENTRELLA M: Reduction in transforming growth factor beta receptor I expression and transcription factor Cbfa1 on bone cells by glucocorticoids. *J Biol Chem* 1998; 273: 4892-6.

30. SHI XM, CHANG ZJ, BLAIR HC, MCDONALD JM, CAO X: Glucocorticoids induce adipogenesis of stromal cells by transcriptionally activating PPAR 2. *J Bone Miner Res* 1998; 12: S454.

31. BELLIDO T, HAN L, MOCHARLA H *et al.*: Overexpression of Bcl-2 renders osteoblastic cells refractory to glucocorticoid-induced apop-

tosis. *Bone* 1998; 23: S324.

32. BARON R, VIGNERY A, LANG R: Reversal phase and osteopenia: Defective coupling of resorption to formation in the pathogenesis of osteoporosis. *In:* DELUCA KF, FROST HM, JEE WSS, JOHNSTON CC, PARFITT AM (Eds): *Osteoporosis*. Baltimore, OH, University Park Press, 1981; 311-20.