Mechanisms of Glucocorticoid Action in Bone

Ernesto Canalis, MD

Address
Department of Research, Saint Francis Hospital and Medical Center, 114 Woodland Street, Hartford, CT 06105-1299, USA.
E-mail: ecanalis@stfranciscare.org

Current Osteoporosis Reports 2005, **3**:98–102
Current Science Inc. ISSN 1544-1873

Glucocorticoids induce rapid bone loss and increase the risk for osteoporotic fractures. The mechanisms include a phase of increased bone resorption, probably a result of the increased expression of receptor activator of nuclear factor-κ-B ligand and colony stimulating factor-1, followed-up by a decrease in bone formation. This effect is central to the actions of glucocorticoids in bone and it is secondary to the loss of bone forming cells, caused by an inhibition of cell differentiation and an increase in the apoptosis of mature osteoblasts and osteocytes. Glucocorticoids also inhibit the function of mature osteoblasts and suppress the synthesis of insulin-like growth factor-I, an agent that enhances bone formation. Glucocorticoids alter the growth hormone/insulin-like growth factor axis in cartilage and, as a consequence, suppress linear growth.

Introduction

Osteoporosis is a frequent complication of the chronic exposure of skeletal tissue to glucocorticoids, and glucocorticoid-induced osteoporosis (GIO) is a common form of secondary osteoporosis. Glucocorticoids cause rapid bone loss leading to osteoporotic fractures [1•]. GIO is a complex disorder since patients receiving corticosteroids often have an underlying disease that carries a significant risk for osteoporosis. Frequently, the disease is chronic and debilitating, such as rheumatoid arthritis, chronic obstructive pulmonary disease, and chronic inflammatory bowel disease. These disorders have an underlying inflammatory component, leading to the release of cytokines, with the potential to enhance bone resorption. These, as well as the direct and indirect effects of glucorticoids on the skeleton, play an important role in the bone loss observed in GIO.

The direct effects of glucocorticoids on skeletal cells appear to be the most significant determinants of their impact on skeletal metabolism, and glucocorticoids alter the fate, life-span, and function of cells of the osteoblast and osteoclast lineages [2•]. As a consequence, corticosteroids regulate bone formation and bone resorption. Patients receiving glucocorticoids undergo an initial phase of increased bone resorption, but as the disease progresses there is an inhibition of bone formation, possibly leading to a state of decreased bone remodeling. The increased bone resorption is probably responsible for the rapid bone loss that comes after the initiation of therapy with corticosteroids. This is reflected by a rapid decline in bone mineral density. The decreased bone remodeling is secondary to the loss of osteoblasts, and is probably responsible for a steady, but slower rate of bone loss. This is reflected by a stabilization of bone mineral density. It is important to note that because of the complexity of the effects of glucocorticoids on the skeleton, the increased risk for fractures is not reflected by marked changes in bone mineral density.

Effects of Glucocorticoids on Bone Resorption

Glucocorticoids have direct effects on bone resorbing cells. Glucocorticoids inhibit calcium absorption in the gastrointestinal tract and enhance renal losses of calcium. The exact mechanisms are not clear, but impaired gastrointestinal calcium transport is secondary to vitamin D resistance, since vitamin D levels are normal [3]. Because of the decrease in calcium absorption and increased renal losses of calcium, secondary hyperparathyroidism has been postulated, but it does not appear to be a major determinant of bone resorption or skeletal loss in GIO [4]. Glucocorticoids increase the expression of parathyroid hormone (PTH)/PTH-related peptide receptors, and it is possible that enhanced sensitivity to PTH plays a role in the observed bone resorption. If mechanisms leading to a hyperparathyroid state are present in GIO, one should expect consistent elevations of serum PTH and a pattern of bone loss that mirrors that seen in hyperparathyroidism. However, this is not the case and acute or chronic use of glucocorticoids is not associated with serum levels of PTH that are in the hyperparathyroidism range [5]. Bone densitometric studies also suggest lack of involvement of PTH in GIO. In primary hyperparathyroidism there is preferential bone loss in the cortical skeleton with preservation of cancellous bone, whereas in GIO an opposite pattern is found with preferential loss of cancellous bone and an increased risk for vertebral fractures [4,6]. Histomorphometric analysis of bone biopsies confirms that hyperparathyroidism and GIO are distinct disorders. In primary hyperparathy-

roidism, there is enhanced bone turnover with preservation of osteoblast number. In contrast, in GIO there is suppression of bone turnover and eventual loss of osteoblasts. Because of these reasons, it appears that PTH does not play a major role in the pathogenesis of GIO. Hypogonadism can play a role in the bone loss observed in GIO since glucocorticoids inhibit gonadotropin hormone secretion, and often patients receiving glucocorticoid therapy are postmenopausal.

Glucocorticoids have important direct effects on cells of the osteoclast lineage, which can explain the increased bone resorption. The receptor activator of nuclear factor-κB ligand (RANKL) and osteoprotegerin play a central role in osteoclast recruitment, differentiation, and bone resorption [7]. RANKL binds and activates its receptor, RANK, on the surface of osteoclast precursors and in association with colony stimulating factor-1 (CSF-1), induces osteoclastogenesis. Osteoprotegerin binds RANKL, acting as a decoy receptor preventing RANKL from binding to its osteoclast receptor. Glucocorticoids increase the expression of RANKL and CSF-1, and decrease osteoprotegerin expression in surrounding osteoblasts and stromal cells [8,9]. As a consequence, there is an increase in osteoclast formation and bone resorption. Glucocorticoids also alter the lifespan of osteoclasts, although increased and decreased apoptosis have been reported [10,11]. Glucocorticoids oppose bisphosphonate effects on osteoclastic apoptosis. Eventually, glucocorticoids deplete the population of osteoblasts and stromal cells, which are necessary to maintain osteoclastogenesis. When this occurs, patients should exhibit a state of decreased bone remodeling.

Effects of Glucocorticoids on Bone Formation

Effects on cell differentiation

Bone histomorphometric studies suggest that decreased bone formation is the most significant event leading to bone loss after chronic glucocorticoid exposure [12]. A significant consequence of skeletal exposure to glucocorticoids is a decrease in the number of cells of the osteoblastic lineage [13]. Cell genesis and death are the ultimate determinants of the pool of osteoblasts available to form bone and glucocorticoids inhibit osteoblastogenesis and induce the apoptosis of osteoblasts and osteocytes [13,14]. Both events contribute to a decreased number of mature osteoblasts. Some investigators have reported that glucocorticoids induce osteoblastic cell differentiation [15]. Although effects of glucocorticoids may be dependent on the stage of cell differentiation and culture conditions, an increase in osteoblastogenesis is inconsistent with the loss of cells of the osteoblastic lineage observed after glucocorticoid exposure. Recent research has confirmed that glucocorticoids impair the differentiation of mesenchymal cells toward cells of the osteoblastic lineage and prevent the terminal differentiation of osteoblastic cells [16]. This results in a decrease in the number of mature osteoblasts. Glucocorticoids decrease osteoblastogenesis by suppressing the differentiation of osteoblasts and by shifting the differentiation of mesenchymal cells away from osteoblasts and toward adipocyctes [16]. The shift in the differentiation of stromal cells toward the adipocyte lineage involves the regulation of nuclear factors of the CCAAT/enhancer binding protein (C/EBP) family, and of peroxisome proliferator activated receptor γ2 (PPARγ2) [16,17]. C/EBP α, β, and δ play essential roles in adipogenesis and mice carrying null mutations of C/EBP α, β, and δ exhibit impaired adipocyte differentiation and decreased adipose tissue [17]. Furthermore, overexpression of C/EBP homologous protein (CHOP) or DNA damage-inducible transcript 3 (DDIT 3), a transdominant negative inhibitor of classic C/EBPs, prevents adipogenesis and induces osteoblastic cell maturation, suggesting that there is a shift in the population of differentiating cells, which could play a role in the effect of glucocorticoids [18]. The effect of glucocorticoids on adipocyte differentiation involves additional signals, including the induction of PPARγ2 and the transcriptional repression of preadipocyte factor-1 (pref-1), a factor that inhibits the differentiation of preadipocytes to adipocytes [19]. It is of interest that null mutations of pref-1 exhibit not only accelerated adipocity, but also retarded growth and skeletal malformations [20].

The differentiation of cells of the osteoblastic lineage is determined by bone morphogenetic proteins (BMP) and the Wnt family of secreted glycoproteins [21•,22•]. Wnts play a role in cell fate and abnormal Wnt signaling is implicated in osteoporosis and disorders of increased bone mass [22•]. Wnt signals by diverse mechanisms, but the canonical Wnt signaling pathway is the most widely studied. After the binding of Wnt proteins to their specific Frizzled transmembrane receptors and co-receptors, low-density lipoproteins-related proteins (LRP) 5 and 6, there is stabilization and nuclear translocation of β-catenin and association of β-catenin with members of the lymphoid enhancer binding factor/T cell specific factor (LEF/TCF) family of transcription factors [22•]. Wnt activity is regulated by multiple intracellular and extracellular signals and two of these, Dikkopf and Notch, have been implicated in mechanisms of glucocorticoid action in bone. Dikkopf inhibits Wnt signaling by binding to LRP 5/6 and Krem resulting in the removal of the complex from the cell membrane by endocytosis [22•]. A way that glucocorticoids inhibit osteoblast cell differentiation is by inducing Dikkopf expression and inhibiting Wnt signaling [23]. Notch are transmembrane receptors that mediate cell to cell interactions controlling cell fate decisions [24]. Their ligands, Delta and Serrate/Jagged, are single-pass transmembrane proteins that induce the proteolytic cleavage of Notch, leading to the release of the Notch intracellular domain (NotchIC) and its translocation to the nucleus, where it complexes with specific DNA binding proteins. Activated Notch receptors prevent osteoblast differentiation and chondrocyte maturation [25•,26]. Notch interacts with Wnt at diverse levels of signaling. Wnt binds to the Notch extracellular

domain and the Wnt-dependent Dishevelled, which stabilizes β-catenin, binds to NotchIC [27,28]. In addition, glygogen synthase kinase 3β, which inactivates β-catenin, modulates Notch stability [29]. Furthermore, presenilins regulate β-catenin degradation and play a role in the activation of Notch [27]. Studies from this laboratory have demonstrated that Notch1 overexpression decreases Wnt/β-catenin signaling in stromal cells resulting in impaired osteoblastic maturation [25•]. Notch1 transcripts are increased by glucocorticoids in osteoblasts [30]. Consequently, the upregulation of Notch1 expression may play a role in the inhibition of osteoblastogenesis by glucocorticoids.

Glucocorticoids induce apoptosis of mature osteoblasts and osteocytes, which, in association with the impairment of cell differentiation, causes a decrease in the number of bone forming cells [14]. The induction of apoptosis may also involve indirect mechanisms, such as the suppression of insulin-like growth factor (IGF) -I transcription by glucocorticoids, since IGF-I prevents apoptosis [31].

Effects on osteoblast function and local growth factors

Selected actions of glucocorticoids are secondary to the regulation of the growth hormone/IGF axis [31]. IGF-I enhances osteoblastic function and bone collagen synthesis, and its actions are opposite to those of glucocorticoids. However, IGF-I does not determine cell differentiation. Glucocorticoids suppress IGF-I transcription through a C/EBP recognition site adjacent to the third start site of transcription of exon 1 [31]. The involvement of C/EBPs in the regulation of IGF-I expression and adipogenesis reveals convergence on the effects of glucocorticoids on specific cellular signals.

Glucocorticoids have the potential to regulate IGF-I activity through their actions on the synthesis of IGFBPs. The six known IGFBPs are synthesized by osteoblasts, and glucocorticoids decrease the expression of IGFBP-3, -4, and -5 messenger RNAs (mRNA) and stimulate IGFBP-6 synthesis in osteoblasts [32]. IGFBP-5 has been reported to have anabolic effects on the skeleton and the inhibitory effect on IGFBP-5 transcription was considered relevant to the mechanism of action of glucocorticoids in bone. However, transgenic mice overexpressing IGFBP-5 under the control of the osteocalcin promoter are osteopenic, suggesting that IGFBP-5 is not anabolic in the skeleton [33]. Consequently, its suppression by corticosteroids should not be relevant to their catabolic actions in bone.

Effects of glucocorticoids on matrix proteins

The bone matrix is composed primarily by type I collagen, which is synthesized by osteoblasts and degraded by proteases secreted by skeletal cells. Glucocorticoids inhibit type I collagen synthesis by transcriptional and post-transcriptional mechanisms, and regulate the synthesis of collagenases, which are matrix metalloproteinases (MMP) that cleave collagen fibrils, and regulate matrix breakdown [34,35]. Collagenases also play a role in bone resorption, and mutations of the collagenase 3 cleavage site of the type I collagen molecule and null mutations of the collagenase 3 gene result in impaired bone resorption. Glucocorticoids increase collagenase 3 mRNA and protease levels in osteoblasts by post-transcriptional mechanisms and this effect may contribute to their effects on bone resorption [35]. The decrease in IGF-I transcription by glucocorticoids may contribute to their effects on matrix proteins in bone, as they do in muscle, since IGF-I increases type I collagen synthesis and suppresses collagenase 3 transcription [36].

Indirect Actions of Glucocorticoids on Bone—Effects on Cartilage and Muscle

Glucocorticoids also have important effects on the growth hormone (GH) IGF-I axis on the epiphyseal growth plate [37]. Glucocorticoids decrease IGF-I expression in liver cells, the main source of circulating IGF-I, but serum levels of IGF-I and GH are not suppressed [38]. Glucocorticoids impair IGF-I secretion in chondrocytes, as they do in osteoblasts, and blunt chondrocytic responses to GH and IGF-I [37]. This would suggest that the direct effects of glucocorticoids on IGF-I expression and actions in target tissues are more relevant than their effects on systemic IGF-I levels. The decreased IGF-I synthesis and cellular sensitivity to IGF-I in chondrocytes contribute to the actions of glucocorticoids and explain the impaired linear growth observed in children with GIO [39].

Rapid loss of muscle protein is the result of decreased protein synthesis and increased degradation, and glucocorticoid induced myopathy and muscular atrophy may alter bone mass indirectly. The mechanism may involve suppression of IGF-I synthesis and signaling by glucocorticoids in myocytes since IGF-I increases protein synthesis and prevents proteolysis in muscle cells [40,41]. IGF-I suppresses the expression of the E3 ubiquitin ligand atrogin-1/muscle atrophy F box (MAFbx), which activates protein degradation in the proteosome by ubiquitination [41]. Glucocorticoids not only suppress IGF-I synthesis and actions, but also increase atrogin-1/MAFbx expression directly, counteracting the effects of IGF-I. These actions should result in increased protein degradation and muscle atrophy, and indirectly contribute to the bone loss that follows glucocorticoid exposure.

Target Tissue Regulation of Glucocorticoid Action

Pre-receptor steroid regulation is critical to steroid action and is regulated by 11β-hydroxysteroid dehydrogenases (11β-HSDs), which are isoenzymes that catalyze the interconversion of hormonally active cortisol and inactive cortisone [42]. 11β-HSD1, a low affinity NADP(H)-dependent enzyme, displays primarily reductase activity and converts cortisone to cortisol, and can act as a pivotal determinant of steroid responses in bone by amplifying glucocorticoid

signaling in osteoblasts. 11β-HSD1 is expressed in glucocorticoid target tissues, including bone, and can facilitate glucocorticoid action in target tissues. The activity and synthesis of 11β-HSD1 in osteoblasts is glucocorticoid dependent, so that it may serve as a positive local mechanism to amplify the effect of glucocorticoids.

Conclusions

Glucocorticoids enhance bone resorption and have important effects on cells of the osteoblastic lineage, impairing osteoblastic cell maturation, function, and survival. Eventually, this leads to a state of decreased bone formation and remodeling, and to osteoporosis.

Acknowledgments

Work from the author's laboratory was supported by grant DK 45227 from the National Institute of Diabetes Digestive and Kidney Diseases. The author thanks Ms. Nancy Wallach for secretarial assistance.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1.• Saag K: **Glucocorticoid-induced osteoporosis.** *Endocrinol Metab Clin North Am* 2003, **32:**135–157.

An excellent clinical review on the subject of GIO.

2.• Canalis E, Bilezikian JP, Angeli A, *et al.*: **Perspectives on glucocorticoid induced osteoporosis.** *Bone* 2004, **34:**593–598.

An excellent review on the subject of GIO.

3. Hattersley AT, Meeran K, Burrin J, *et al.*: **The effect of long- and short-term corticosteroids on plasma calcitonin and parathyroid hormone levels.** *Calcif Tissue Int* 1994, **54:**198–202.

4. Rubin MR, Bilezikian JP: **The role of parathyroid hormone in the pathogenesis of glucocorticoid-induced osteoporosis: a re-examination of the evidence.** *J Clin Endocrinol Metab* 2002, **87:**4033–4041.

5. Paz-Pacheco E, Fuleihan GE, Leboff MS: **Intact parathyroid hormone levels are not elevated in glucocorticoid-treated subjects.** *J Bone Miner Res* 1995, **10:**1713–1718.

6. Reid IR, Evans MC, Stapleton J: **Lateral spine densitometry is a more sensitive indicator of glucocorticoid-induced bone loss.** *J Bone Miner Res* 1992, **7:**1221–1225.

7. Suda T, Takahashi N, Udagawa N, *et al.*: **Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families.** *Endocr Rev* 1999, **20:**345–357.

8. Hofbauer LC, Gori F, Riggs BL, *et al.*: **Stimulation of osteoprotegerin ligand and inhibition of osteoprotegerin production by glucocorticoids in human osteoblastic lineage cells: potential paracrine mechanisms of glucocorticoid-induced osteoporosis.** *Endocrinology* 1999, **140:**4382–4389.

9. Rubin J, Biksobing DM, Jadhav L, *et al.*: **Dexamethasone promotes expression of membrane-bound macrophage colony-stimulating factor in murine osteoblast-like cells.** *Endocrinology* 1998, **139:**1006–1012.

10. Weinstein RS, Chen JR, Powers CC, *et al.*: **Promotion of osteoclast survival and antagonism of bisphosphonate-induced osteoclast apoptosis by glucocorticoids.** *J Clin Invest* 2002, **109:**1041–1048.

11. Dempster DW, Moonga BS, Stein LS, *et al.*: **Glucocorticoids inhibit bone resorption by isolated rat osteoclasts by enhancing apoptosis.** *J Endocrinol* 1997, **154:**397–406.

12. Carbonare LD, Arlot ME, Chavassieux PM, *et al.*: **Comparison of trabecular bone microarchitecture and remodeling in glucocorticoid-induced and postmenopausal osteoporosis.** *J Bone Miner Res* 2001, **16:**97–103.

13. Pereira RMR, Delany AM, Canalis E: **Cortisol inhibits the differentiation and apoptosis of osteoblasts in culture.** *Bone* 2001, **28:**484–490.

14. Weinstein RS, Jilka RL, Parfitt AM, *et al.*: **Inhibition of osteoblastogenesis and promotion of apoptosis of osteoblasts and osteocytes by glucocorticoids.** *J Clin Invest* 1998, **102:**274–282.

15. Bellows CG, Aubin JE, Heersche JNM: **Physiological concentrations of glucocorticoids stimulate formation of bone nodules from isolated rat calvaria cells in vitro.** *Endocrinology* 1987, **212:**1985–1992.

16. Pereira RC, Delany AM, Canalis E: **Effects of cortisol and bone morphogenetic protein-2 on stromal cell differentiation: correlation with CCAAT-enhancer binding protein expression.** *Bone* 2002, **30:**685–691.

17. Wu Z, Bucher NLR, Farmer SR: **Induction of peroxisome proliferator-activated receptor g during the conversion of 3T3 fibroblasts into adipocytes is mediated by C/EBPb, and glucocorticoids.** *Mol Cell Biol* 1996, **16:**4128–4136.

18. Pereira RC, Delany AM, Canalis E: **CCAAT/Enhancer binding protein homologous protein (DDIT3) induces osteoblastic cell differentiation.** *Endocrinology* 2004, **145:**1952–1960.

19. Smas CM, Chen L, Zhao L, *et al.*: **Transcriptional repression of pref-1 by glucocorticoids promotes 3T3-L1 adipocyte differentiation.** *J Biol Chem* 1999, **274:**12632–12641.

20. Moon YS, Smas CM, Lee K, *et al.*: **Mice lacking paternally expressed Pref-1/Dlk1 display growth retardation and accelerated adiposity.** *Mol Cell Biol* 2002, **22:**5585–5592.

21.• Canalis E, Economides AE, Gazzerro E: **Bone morphogenetic proteins, their antagonists and the skeleton.** *Endocr Rev* 2003, **24:**218–235.

An excellent review on bone cell biology.

22.• Westendorf JJ, Kahler RA, Schroeder TM: **Wnt signaling in osteoblasts and bone diseases.** *Gene* 2004, **341:**19–39.

An excellent clinical review on bone cell biology.

23. Ohnaka K, Taniguchi H, Kawate H, *et al.*: **Glucocorticoid enhances the expression of dickkopf-1 in human osteoblasts: novel mechanism of glucocorticoid-induced osteoporosis.** *Biochem Biophys Res Comm* 2004, **318:**259–264.

24. Weinmaster G: **Review: the ins and outs of notch signaling.** *Mol Cell Neurosci* 1997, **9:**91–102.

25.• Sciaudone M, Gazzerro E, Priest L, *et al.*: **Notch 1 impairs osteoblastic cell differentiation.** *Endocrinology* 2003, **144:**5631–5639.

New signals involving mechanisms of glucocorticoid action in bone.

26. Crowe R, Zikherman J, Niswander L: **Delta-1 negatively regulates the transition from prehypertrophic to hypertrophic chondrocytes during cartilage formation.** *Development* 1999, **126:**987–998.

27. DeStrooper B, Annaert W: **Where Notch and Wnt signaling meet: the presenilin hub.** *J Cell Biol* 2001, **152:**F17–F20.

28. Axelrod JD, Matsuno K, Artavanis-Tsakonas S, *et al.*: **Interaction between Wingless and Notch signaling pathways mediated by Disheveled.** *Science* 1996, **271:**1826–1832.

29. Foltz DR, Santiago MC, Berechid BE, *et al.*: **Glycogen synthase kinase-3b modulates Notch signaling and stability.** *Curr Biol* 2002, **12:**1006–1011.

30. Pereira RMR, Delany AM, Durant D, *et al.*: **Cortisol regulates the expression of Notch in osteoblasts.** *J Cell Biochem* 2002, **85:**252–258.

31. Delany AM, Durant D, Canalis E: **Glucocorticoid suppression of IGF I transcription in osteoblasts.** *Mol Endocrinol* 2001, **15:**1781–1789.

32. Okazaki R, Briggs BL, Conover CA: **Glucocorticoid regulation of insulin-like growth factor-binding protein expression in normal human osteoblast-like cells.** *Endocrinology* 1994, **134:**126–132.
33. Devlin RD, Du Z, Buccilli V, *et al.*: **Transgenic mice overexpressing insulin-like growth factor binding protein-5 display transiently decreased osteoblastic function and osteopenia.** *Endocrinology* 2002, **143:**3955–3962.
34. Delany A, Gabbitas B, Canalis E: **Cortisol down-regulates osteoblast a1 (I) procollagen mRNA by transcriptional and post-transcriptional mechanisms.** *J Cell Biochem* 1995, **57:**488–494.
35. Rydziel S, Delany AM, Canalis E: **AU-Rich elements in the collagenase 3 mRNA mediate stabilization of the transcript by cortisol in osteoblasts.** *J Biol Chem* 2004, **279:**5397–5404.
36. Hock JM, Centrella M, Canalis E: **Insulin-like growth factor I (IGF-I) has independent effects on bone matrix formation and cell replication.** *Endocrinology* 1988, **122:**22–27.
37. Canalis E: **Inhibitory actions of glucocorticoids on skeletal growth. Is local insulin-like growth factor I to blame?** *Endocrinology* 1998, **139:**3041–3042.
38. Reid IR, Gluckman PD, Ibbertson HK: **Insulin-like growth factor I and bone turnover in glucocorticoid-treated and control subjects.** *Clin Endocrinol (Oxf)* 1989, **30:**347–353.
39. Bachrach LK: **Bare-bones fact – children are not small adults.** *N Engl J Med* 2004, **351:**924–926.
40. Singleton JR, Baker BL, Thorburn A: **Dexamethasone inhibits insulin-like growth factor signaling and potentiates myoblast apoptosis.** *Endocrinology* 2000, **141:**2945–2950.
41. Dehoux M, Van Beneden R, Pasko N, *et al.*: **Role of the insulin-like growth factor I decline in the induction of atrogin-1/MAFbx during fasting and diabetes.** *Endocrinology* 2004, **145:**4806–4812.
42. Tomlinson JW, Walker EA, Bujalski IJ, *et al.*: **11b-hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response.** *Endocr Rev* 2004, **25:**831–866.