REVIEW

Sensitivity of bone to glucocorticoids

Mark S. COOPER
Division of Medical Sciences, University of Birmingham, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH, U.K.

ABSTRACT

Glucocorticoids are used widely in a range of medical specialities, but their main limitation is an adverse impact on bone. Although physicians are increasingly aware of these deleterious effects, the marked variation in susceptibility between individuals makes it difficult to predict who will develop skeletal complications with these drugs. Although the mechanisms underlying the adverse effects on bone remain unclear, the most important effect appears to be a rapid and substantial decrease in bone formation. This review will examine recent studies that quantify the risk of fracture with glucocorticoids, the mechanisms that underlie this increase in risk and the potential basis for differences in individual sensitivity. An important determinant of glucocorticoid sensitivity appears to be the presence of glucocorticoid-metabolizing enzymes within osteoblasts and this may enable improved estimates of risk and generate new approaches to the development of bone-sparing anti-inflammatory drugs.

INTRODUCTION

The adverse effects of glucocorticoids on the skeleton were first recognized by Harvey Cushing [1] 70 years ago in the context of the disease that bears his name. However, the problem of GIOP (glucocorticoid-induced osteoporosis) has increased dramatically in recent decades with the introduction of synthetic glucocorticoids to treat a range of inflammatory diseases [2,3]. Physicians in most medical specialities are increasingly aware of the effects of therapeutic glucocorticoids on the skeleton, but the marked variation in susceptibility between individuals has made it difficult to predict individuals who are likely to have adverse effects with these drugs. The mechanisms underlying the effects of glucocorticoids on bone have been sought for many years, but no single effect has emerged as being of prime importance. In the last few years, key advances have been made in several areas, including the epidemiology of GIOP, the impact of glucocorticoids on bone cells in vitro and in vivo and the basis for variation in sensitivity between individuals. Additionally, the recent identification of glucocorticoid-modifying enzymes in bone cells suggests that endogenous glucocorticoids may make a greater contribution to bone metabolism than previously thought. This article will critically review these recent advances and will highlight areas where considerable uncertainty still exists.

RECENT ADVANCES IN THE EPIDEMIOLOGY OF GIOP

The epidemiology of GIOP has been difficult to define due to the wide variability in glucocorticoid doses used clinically, the impact of the underlying disease being treated and the large numbers of subjects needed to measure an effect on fracture risk. Recent epidemiological studies have overcome some, but not all, of these problems and have given us a more accurate estimation of the magnitude of fracture risk.

van Staa et al. [3] used information from the General Practice Research Database, a computerized medical records system that covers a large number of...
general practices in England and Wales, to estimate glucocorticoid usage in the U.K. It was determined that 0.9% of this population was continuously receiving prescriptions for glucocorticoids for more than 3 months, a figure which rose to 2.5% in the elderly. Over 90% of these prescriptions were for prednisolone with only 3% for hydrocortisone. Using this database, a group of 244,235 oral corticosteroid users were identified and individually matched with a similar number of control subjects who were receiving topical, but not oral, glucocorticoids [4]. The risk of developing a fracture before, during or after treatment was then examined in these subjects. Using this approach it was determined that fracture risk increased substantially at all skeletal sites examined with the greatest relative risk (2.6) seen at the spine.

Fracture risk at all sites increased with steroid dose with the largest increases again seen at the spine with relative risks of over 5 with high doses of steroids. A statistically significant increase in spine fracture risk was seen even with very low doses of prednisolone (relative risk 1.55 with doses <2.5 mg/day), suggesting that there may be no safe lower dose of oral glucocorticoid. These risks appeared to be more related to current than cumulative dose. What was a surprise was the time course of fracture risk. Fracture risk at all sites increased rapidly after starting glucocorticoids and then appeared to plateau even during treatment for up to 5 years. When steroid treatment was stopped, fracture risk fell rapidly back towards baseline. These results suggest that changes in BMD (bone mineral density) are unlikely to be mediating the changes in fracture risk. Loss of BMD during glucocorticoid treatment is often rapid in the first 6–12 months of treatment and then continues at a slower rate [5]. Additionally, although some gain in BMD might be expected on ceasing glucocorticoid treatment, it would be unlikely that the changes would be so rapid and complete as to account for the change in fracture risk. The origin of this rapid and reversible effect of glucocorticoids is likely to be the source of intense research interest in the future [6]. From a practical point of view, these results also suggest that attempts at prophylactic treatment should begin early during treatment, and probably at the time of commencing glucocorticoids. Furthermore, prophylactic measures probably do not need to be continued for prolonged periods once steroids have been ceased. In the U.K., these findings have been incorporated into excellent evidence-based guidelines for the management of GIOP [7].

The advantages of these studies were their large size and relevance to patients typical of the population at large. However, this, and related studies, do have some significant limitations. This approach cannot separate the impact of the underlying disease from that of glucocorticoids, although the effects seen appeared to be consistent across the various indications for glucocorticoid use (e.g. respiratory versus rheumatological). Furthermore, the data only reflected prescriptions that had been issued. There was no way of determining whether the glucocorticoids had actually been taken. More importantly, it is likely that many individuals in the low dose category were actually taking intermittent, but frequent, courses of glucocorticoids. These limitations, along with the use of a control group that were taking inhaled steroids, would tend to weaken the relationship and, thus, the data may actually underestimate the magnitude of the risks associated with glucocorticoids.

**IMPACT OF GLUCOCORTICOIDS ON BONE CELLS IN VITRO**

The effect of glucocorticoid excess on the skeleton may be mediated by indirect effects on skeletal metabolism, such as reduction in sex steroid levels, reduction in muscle mass and strength, and secondary hyperparathyroidism due to impairment in calcium absorption or increased calcium excretion [8,9] (Figure 1). These effects may be very important in various clinical settings, but the dominant effect of glucocorticoids appears to be due to direct effects on bone cells [10,11]. The three main cell types within bone are osteoblasts, osteocytes and osteoclasts. Osteoblasts are cells which form bone matrix and are essential for its mineralization. Osteocytes are terminally differentiated osteoblasts that have been incorporated into bone, whereas osteoclasts are derived from the monocyte lineage and resorb bone. In various settings, glucocorticoids have potentially important impacts on all of these cell types.

**Osteoblasts in vitro**

Osteoblasts have long been thought to be the most important target of glucocorticoids in bone. The histological hallmark of GIOP is a rapid and profound decrease in the capacity to form bone [12–14] and...
dramatic reductions in biochemical markers of bone formation and osteoblastic activity are seen in a clinical setting [15–18]. Correspondingly, glucocorticoids have dramatic effects on osteoblasts in a range of experimental settings. Most studies have focussed on the role of glucocorticoids in the commitment of cells to the osteoblast lineage or on the function of mature osteoblasts. More recently the impact of glucocorticoids on the death of osteoblasts has been explored.

Glucocorticoids appear to be important in the commitment of cells to the osteoblast lineage [10]. In cells of human origin, glucocorticoids appear to be essential for this stage. Glucocorticoids also induce the expression of a range of genes that are characteristic of mature osteoblasts, such as osteocalcin and type I collagen. By contrast, glucocorticoids can also suppress features of the osteoblast phenotype in mature cells. Various studies have examined the impact of glucocorticoids on a range of intracellular and extracellular signalling pathways that may be important in bone metabolism. Glucocorticoids decrease the stability of mRNA for the bone specific transcription factor cbfa-1 (core-binding factor-1) [19] and the activity of several intracellular kinases may also be reduced [20]. PTH (parathyroid hormone) action on osteoblasts may be reduced by glucocorticoids [21], whereas BMP (bone morphogenic protein) generation is increased [22]. The expression of Notch receptors (which are important in BMP signalling) may be increased by glucocorticoids [23]. Multiple aspects of the GH (growth hormone)/IGF (insulin-like growth factor) system may be affected [9,24] and glucocorticoids may reduce TGF-β (transforming growth factor-β) activity [19]. Despite the potential importance of all these effects, the impact on bone physiology in vivo remain unclear.

More recently, a significant impact of glucocorticoids on osteoblast apoptosis has been noted. In some systems, glucocorticoids induce apoptosis [25], whereas in others glucocorticoids protect against apoptosis [26,27]. However, in primary osteoblasts and osteoblasts in vivo, it is likely that high doses of glucocorticoids have primarily an apoptotic action [25,28].

In vitro experiments continue to be plagued by problems with interpretation. There are clearly contrasts between transformed cells and primary cultures, e.g. it is difficult to interpret studies examining proliferation and apoptosis in transformed cells. There is also difficulty in defining the impact of glucocorticoids at various stages of differentiation especially when trying to extrapolate to the in vivo situation. Additionally, the presence of multiple effects on a range of genes potentially important in bone cell function suggests that no one pathway is likely to explain the clinically relevant actions of glucocorticoids. In vivo, bone is remodelled at distinct sites with controlled coordination of groups of osteoclasts and osteoblasts as part of the ‘bone remodelling unit’. In vitro models are currently unable to reproduce this level of complexity. An example of where in vitro experiments may give insight into the pathogenesis of GIOP is the capacity of intermittent PTH to protect against apoptosis in osteoblast cell lines that are normally sensitive to glucocorticoid-induced apoptosis [29]. These studies have the capacity to stimulate in vivo and clinical studies.

**Osteocytes in vitro**

Osteoblasts have a number of fates. They can die via apoptosis, become quiescent osteoblasts lining the bone surface or become incorporated into bone matrix and undergo differentiation into osteocytes. The function of osteocytes remains poorly defined, but roles in mechanosensing, the detection of microfractures and the regulation of bone repair have been proposed. This has recently raised the possibility that osteocyte damage may be important in the pathogenesis of glucocorticoid-induced osteonecrosis [25,30]. Progress in the understanding of osteocyte function has been limited by the inability to study viable human osteocytes in vivo. An important advance has been the development of a rodent cell line that demonstrates many features typical of osteocytes, such as high osteocalcin levels, expression of connexin43 and a dendritic morphology [31]. Although the function of osteocytes remains poorly defined, it is difficult to assess the functional impact of glucocorticoids on osteocytes except in the extreme example of osteocyte death. More evidence of the role of glucocorticoids on osteocyte function has come from in vivo, rather than in vitro, experiments.

The role of glucocorticoids on osteoblasts that line the bone surface is also obscure. These cells may be important in the initiation of bone remodelling via the expression of enzymes capable of resorbing the thin layer of unmineralized matrix which has to be removed to allow access of osteoclasts to the bone surface [32,33]. Glucocorticoids may increase expression of these enzymes [34], and this effect could account for some of the increased resorption of bone seen during treatment with glucocorticoids.

**Osteoclasts in vitro**

The other cell type within bone that may be a target for glucocorticoids is the osteoclast. Osteoclasts are multinucleated cells derived from the mononuclear cell lineage. The formation and function of osteoclasts is regulated by a range of factors, many of which are derived from osteoblasts. A critical step in the differentiation of mononuclear cells to osteoclasts is signalling via the RANK (receptor activator of NF-κB, where NF-κB is nuclear factor κB) pathway by RANKL (RANK ligand) [35]. RANKL is a cell-surface receptor expressed on a range of osteoblasts and some immune cells. In addition to being required for osteoclast formation, RANKL also stimulates the activity of mature osteoclasts. A
naturally occurring inhibitor of RANKL signalling, OPG (osteoprotegerin) is also secreted by osteoblasts and, thus, the local RANKL/OPG ratio is likely to determine the degree of osteoclast formation.

Several protocols are now available for the in vitro generation of osteoclasts. In all of these in vitro systems, high doses of synthetic glucocorticoids, primarily dexamethasone, are required. In contrast with osteoblasts and osteocytes, high doses of glucocorticoids do not appear to induce osteoclast apoptosis. Glucocorticoids appear to have an important role in the formation and function of osteoclasts via effects on osteoblasts [36]. Glucocorticoids up-regulate the expression of RANKL on the surface of a range of osteoblasts. Additionally, glucocorticoids reduce the production of OPG by osteoblasts. This increase in the RANKL/OPG ratio would be expected to increase the generation and activity of osteoclasts and this may account for some of the early bone resorption seen with glucocorticoids. Glucocorticoids may also have direct effects on osteoclasts. In osteoclasts cultured in vitro, glucocorticoids stimulate intracellular signalling pathways which are associated with inflammation. In this setting, glucocorticoids exert their stimulatory effect on osteoclast formation not by directly stimulating the activity of the RANK signalling pathway, but rather by inhibiting the production of IFN-κβ (interferon-κβ) [37]. IFN-κβ exerts an important inhibitory action on the RANKL pathway and, thus, this effect of glucocorticoids may be due to their relief of IFN-κβ-mediated inhibition of RANK signalling. The importance of these effects in vivo during glucocorticoid treatment or in inflammatory disease has yet to be explored.

Factors regulating sensitivity of bone cells to glucocorticoids in vitro

When performing in vitro studies it is usual to try to create a reproducible and uniform model system. Such an approach may mask variations in the sensitivity of bone cells to glucocorticoids. Sensitivity to glucocorticoids could be regulated at a number of levels (Figure 2). Individuals could differ in the absorption, distribution or metabolism of steroid, the number of and affinity of GRs (glucocorticoid receptors), or amount and binding of nuclear cofactors. Additionally, all of these factors could change in a clinical setting during inflammation or prolonged glucocorticoid exposure.

For glucocorticoids to act, steroids need to diffuse into a target cell, bind to specific receptors and alter gene transcription either by binding to DNA or interfering with other signalling pathways, such as NF-κB or AP-1 (activator protein-1). GRs are expressed in a range of bone cells, including osteoblasts and osteocytes [38,39], and this topic has previously been reviewed in Clinical Science [40]. The number of GRs varies between tissues, but whether this has a role in determining differences in glucocorticoid sensitivity is unclear. Some GR variants have been described. An asparagine to serine change at codon 363 results in increased suppression of serum cortisol levels in response to low dose dexamethasone. Heterozygous individuals have a trend to decreased BMD at the lumbar spine, but the overall effect appears small [41]. GR phosphorylation status has also been implicated in regulation of GR function [42], but its physiological significance remains unclear. Glucocorticoids can also bind to MRs (mineralocorticoid receptors) in tissues that do not express the 11β-HSD2 (11β-hydroxysteroid dehydrogenase 2) enzyme (see below). MRs are expressed within bone cells and may be involved in glucocorticoid signalling [39]. Nuclear corepressors and coactivators are likely to be important in tissue-specific responses to glucocorticoids. Whether expression of these cofactors is likely to explain any differences in sensitivity between individuals is currently unclear.

Recently, the potential importance of glucocorticoid-modifying enzymes in a range of tissues has become apparent. 11β-HSDs are intracellular enzymes that catalyse the interconversion of the inactive hormone cortisone and hormonally active cortisol [43]. Even though cortisol has been used for over 50 years as an orally active glucocorticoid, it requires conversion (predominantly on first-pass metabolism in the liver) into cortisol (referred to as hydrocortisone when given as a pharmaceutical) to have bioactivity [44]. Two distinct 11β-HSD enzymes have been described in man [43]. 11β-HSD1 is bidirectional, but in most tissues examined to date is primarily a glucocorticoid activator converting cortisone into cortisol. This enzyme is found in tissues which express high levels of GR, such as liver, adipose and gonad. 11β-HSD2 is unidirectional, solely inactivating cortisol to cortisone, and is found in tissues which express MR, such as the kidney. Cortisol and aldosterone can both bind
the MR with similar affinity, but the circulating level of cortisol is substantially higher than that of aldosterone. 11β-HSD2 is thus required in mineralocorticoid target tissues to reduce the intracellular level of cortisol to enable specific binding of aldosterone to the MR.

The expression of glucocorticoid-modifying enzymes has recently been examined in bone cells. Enzyme activity and mRNA expression was first studied in a range of rat and human osteosarcoma cells [45,46]. Only 11β-HSD2 activity was detected, and the direction of activity was glucocorticoid inactivation. By contrast, 11β-HSD1, but not 11β-HSD2, activity was seen in primary cultures of human, mouse or rat osteoblasts, and these cells generate active from inactive glucocorticoids [46]. Osteoblasts from the CD-1 mouse strain have exclusive 11β-HSD1 expression and activity [47], as do osteoblasts derived from rat vertebrae [48]. Interestingly, osteoblasts from the C57Bl/6 mouse strain exhibit 11β-HSD1 activity, but also express 11β-HSD2 mRNA [49]. The presence of functional 11β-HSD1 activity (cortisone to cortisol activation) within osteoblasts is also indicated by the ability of cortisone to influence the formation of bone nodules by bone marrow derived from CD-1 mice and Wistar rats [50], an effect that could be blocked by the 11β-HSD1 inhibitor carbadoxone. The functional consequences of 11β-HSD enzyme expression have been explored in osteosarcoma cells stably transfected with cDNAs for the human enzymes [51,52]. In cells expressing 11β-HSD2, cortisol was unable to induce alkaline phosphatase activity in contrast with control empty vector cells. In cells expressing 11β-HSD1, alkaline phosphatase activity was induced normally by cortisol, but these cells were rendered sensitive to cortisone despite this steroid having no effect in control cells. These experiments suggest that circulating cortisone can behave as an active glucocorticoid in osteoblasts expressing 11β-HSD1.

Although the metabolism of cortisol (hydrocortisone) is likely to be of relevance in normal physiology, the predominant glucocorticoids used therapeutically are prednisolone (used in the U.K.) and prednisone (the most frequently used oral glucocorticoid in the U.S.A.) [3]. Prednisolone and prednisone are synthetic derivatives of cortisol and cortisone respectively and, like cortisone, prednisone requires activation by 11β-HSD1 for bioactivity. The metabolism of these synthetic steroids has been examined in primary osteoblasts and other cells expressing 11β-HSD1 [53]. The enzyme kinetics of the conversion of prednisone into prednisolone were indistinguishable from those of cortisone into cortisol. This suggests that local glucocorticoid metabolism will also be relevant to these clinically important synthetic glucocorticoids.

The expression of 11β-HSD1 and the ability to generate cortisol from cortisone in human osteoblasts increases substantially when cells are exposed to the pro-inflammatory cytokines TNF-α (tumour necrosis factor-α) or IL-1β (interleukin-1β) [54]. Glucocorticoid levels are thus likely to be higher near sites of inflammation and may contribute to the periarticular bone loss seen in patients with long-standing rheumatoid arthritis [55]. Additionally, glucocorticoids themselves can increase 11β-HSD1 activity and mRNA expression in these cells [53]. These effects are likely to have implications for the regulation of local cortisol generation in various clinical settings.

Other levels of potential interest will be variation in enzyme expression with skeletal site, especially in view of the marked sensitivity of the bones of the axial skeleton (e.g. vertebrae and ribs) compared with that of the peripheral skeleton. Although bone found throughout the body has similar physical properties, there are considerable regional differences in developmental origin and mechanisms by which various bones are formed [56]. During the earliest phases of development, templates for bones are formed from condensations of mesenchymal cells. These cells have distinct origins with the craniofacial skeleton formed by cranial neural crest cells [57], the axial skeletal elements from paraxial (somatic) mesoderm [58] and the limb elements from lateral plate mesoderm [59]. There is a suggestion that expression of 11β-HSD1 might vary with skeletal site, with variability of activity being less in osteoblasts derived from the calcaneus compared with other sites in the lower limb [53], but further work needs to be done in this area. Along similar lines, it is also clear that osteoblasts in the periosteum (the outer layer of the bone that is important in apposition growth) differ in some ways from their endosteal (the inner part of the bone) counterparts. Changes in the periosteum are likely to be important in determining skeletal structure during aging [60]. GRs are expressed in osteoblasts at both periosteal and endosteal sites [61], but functional differences in the response of these osteoblasts to glucocorticoids have not been examined. An additional under-appreciated area is the potential differences in the behaviour of bone cells involved in either bone modelling (the establishment of the basic skeletal structure) or remodelling (the ongoing repair and renewal of the skeleton) [62]. These differences are extremely difficult to examine in vitro and new approaches will be needed.

There are also species differences in the response to glucocorticoids. Many transformed cell lines and primary osteoblasts derived from mice can spontaneously mineralize without the need for additional glucocorticoids [63–65]. Human cell lines and primary cultures of osteoblasts in contrast appear to need supplemental glucocorticoids to induce mineralization [66–68]. This raises important questions about the applicability of murine cell lines to potentially important features of osteoblast behaviour such as matrix composition and the ability to mineralize this matrix.
In contrast with osteoblasts, little is known about the variation in glucocorticoid sensitivity of osteoclasts or osteocytes. In osteoclasts, the capacity of cells to differentiate in vitro in response to the synthetic glucocorticoid dexamethasone does not appear to differ between males and females [69]. Furthermore, sensitivity to dexamethasone does not change with age [69]. Dexamethasone is not metabolized to any great extent by glucocorticoid-modifying enzymes, thus differences might still be seen with endogenous glucocorticoids if enzyme expression was to change in these settings. As a consequence of the inability to culture human osteocytes in vitro, any differences in sensitivity due to age, sex, skeletal site or disease status have not been examined, but would have relevance to glucocorticoid-induced apoptosis and osteonecrosis.

**IMPACT OF GLUCOCORTICOIDS IN VIVO**

**Animal models**

Many animal models of GIOP have been suggested, but most have important limitations. The response of various bone cells to glucocorticoids can differ considerably between species. In the rat, bone density increases with glucocorticoid treatment, an effect that appears to be due to glucocorticoid-induced osteoclast apoptosis [70]. Additionally, the organization of cortical bone structure in rodents is different from that in humans and other primates. Once cortical bone is formed in rodents, it does not undergo further remodelling with age. By contrast, human cortical bone has an osteonal (Haversian) organization that undergoes remodelling throughout life, a process that removes bone that has sustained microfracture or become hypermineralized [71]. This limits rodent models in their ability to mimic changes that occur with aging or that focus primarily on cortical bone. Osteonal modelling also occurs in sheep and this animal has thus been proposed as a model of GIOP [72]. The changes in biochemical bone markers and bone histology in sheep exposed to glucocorticoids are similar to those seen in humans. The sheep model is likely to be of particular value in studying the impact of glucocorticoids on biomechanical properties of bone and the interaction with oestrogen deficiency. Despite the limitations of rodent models, the most important animal model to emerge recently is the mouse. Mice appear to develop changes in the skeleton which are broadly similar to those seen in humans [25]. The ease of use, short breeding time and the capacity to generate genetically modified animals gives the mouse model a number of strengths, and recent studies have generated data that are now testable in humans and of potential relevance to clinical bone disease in man.

Several studies have now been reported from the Weinstein’s group using mice treated with subcutaneous prednisolone pellets [25,73–76]. These animals have changes in bone histomorphology analogous with those seen in man [25]. These studies have shed light on the importance of the rates of birth and death of bone cells. Glucocorticoids appear to increase the rate of osteoblast and osteocyte apoptosis. Additionally, glucocorticoids reduce the generation of early osteoblasts as assessed by the capacity of bone marrow to generate colony forming units containing mineralized matrix. The capacity of bone marrow to generate osteoclasts was also reduced. The survival of mature osteoclasts was, however, maintained by glucocorticoid treatment [74]. Intermittent PTH injections were able to reduce the level of osteoblast apoptosis in these studies [29]. More recently, evidence has been presented suggesting that glucocorticoids can reduce vertebral bone compression strength independently of bone density [75].

The expression of glucocorticoid-metabolizing enzymes has also been defined in mice. An 11β-HSD1 gene-ablation mouse model has been produced [77], but it has not been examined whether there are differences in the response of these animals to therapeutic glucocorticoids [49]. Murine osteoblasts, as in humans, express 11β-HSD1 and can generate active from inactive glucocorticoids. An interesting approach to the study of the effects of glucocorticoids on bone has been the development of mice that have targeted overexpression of the glucocorticoid-inactivating enzyme 11β-HSD2 within osteoblasts [47]. These animals were generated using the well characterized Col2.3 type 1 collagen promoter [78]. Hydrocortisone inhibited collagen synthesis in calvaria from wild-type mice, but this effect was blunted in mice with osteoblastic 11β-HSD2 expression. A similar mouse has been generated with 11β-HSD2 expression under an osteocalcin promoter [75]. These animals were protected against many of the skeletal changes induced by short-term prednisolone treatment. The authors conclude that the effects of glucocorticoids on bone are thus direct on osteoblasts in this model rather than being mediated by changes in factors such as sex steroid levels or calcium homeostasis. The applicability of enzyme expression to mice models of GIOP will depend on the levels of circulating glucocorticoids during glucocorticoid treatment. Prednisolone and prednisone levels have not yet been reported in these models.

Studies in mice have contributed a great deal to our understanding of the potential mechanisms underlying GIOP. Future models should allow the impact of inflammatory conditions on the response to glucocorticoids to be defined and thus be better able to mimic clinical scenarios.

**Human studies**

Studies in humans have also been performed. The hallmark of GIOP is a rapid reduction in bone formation markers, such as osteocalcin, alkaline phosphatase and
the propeptides of type 1 collagen [15]. By contrast, there is no change in markers of bone resorption. Bone density decreases and this can occur very rapidly [5,79,80]. This loss of bone appears to be due to sustained resorption in the face of reduced formation. The ability of bisphosphonates, which inhibit bone resorption, to reduce fracture risk at the spine in patients taking glucocorticoids [79,81,82] also suggests that resorption plays an important role in GIOP. At a histological level, studies have again supported a dominant role of reduced bone formation, but, in post-menopausal women, increased resorption also appears to be a feature [13]. In addition to the features described above, a decrease in the viability of osteocytes has been observed in trans-iliac biopsies from patients with rheumatoid arthritis treated with prednisolone [28].

In humans, in contrast with animals, mechanistically based approaches to treatment can be evaluated in genuine clinical settings. The potential of intermittent PTH to protect against osteoblast apoptosis in isolated cells and in animal models was discussed earlier. The use of PTH in a clinical setting has been examined in a trial of women taking prednisone and HRT (hormone-replacement therapy) [83]. Women randomized to PTH showed an increase in spine BMD compared with women who did not receive the drug. There was no early change in femoral neck BMD, but a follow-up study demonstrated a delayed increase [84]. Using QCT (quantitative computerized tomography) it was apparent that these increases were predominantly in trabecular bone. The study was not powered to examine changes in fracture risk, but the changes in BMD seem likely to be protective. Very few studies have been able to look at the material properties of glucocorticoid-treated bone, but the profound changes in matrix protein expression are unlikely to be without consequence. The recent increased focus on bone quality [85,86] should allow a fuller examination of this area.

Although the commonest skeletal problems associated with glucocorticoid use is fracture, the complication perhaps most feared by physicians is osteonecrosis. Osteonecrosis (also termed avascular necrosis) involves the rapid and focal deterioration in bone quality and primarily affects the femoral head [87]. It can lead to pain and ultimately collapse of the bone often requiring hip replacement. It can affect individuals of all ages and can occur with relatively low doses of glucocorticoids (e.g. during corticosteroid-replacement therapy for adrenal failure) [88]. The pathogenesis and basis for susceptibility between individuals remains unknown, but recent data implicate glucocorticoid-induced osteocyte death in the condition. These conclusions are based on the relative frequency of osteocyte apoptosis observed in animal models and the increased rate of local osteocyte apoptosis in femoral heads removed for this reason [25,30]. The lack of a direct role for an interrupted blood supply suggests that the term osteonecrosis is preferable in this context to avascular necrosis. Even if osteocyte apoptosis is the mechanism underlying glucocorticoid-induced osteonecrosis, there is still no explanation for why this occurs in only a few individuals and there is no way of predicting the risk of the complication occurring in an individual.

**Local and systemic metabolism of glucocorticoids in vivo**

Until recently, very little attention was paid to the pharmacology of commonly used glucocorticoids. The effects of glucocorticoids were thought to be broadly similar and effects on bone were an inevitable consequence of anti-inflammatory potency. Pharmaceutical companies have recently driven attempts to develop dissociated glucocorticoids [89]. These compounds would ideally maintain their anti-inflammatory action (thought to be via transrepression, GR binding to transcription factors, such as NF-κB and AP-1) whilst lacking their negative impact on bone (thought to be via transactivation, binding of GR to DNA). Although promising candidates have been developed, none have demonstrated a steroid-sparing effect on bone in vivo [90,91].

What has been a surprise is the realization that the pharmacology of the most commonly used oral glucocorticoids may be more complex than previously thought. Furthermore, there may be simple ways in which the negative impact on bone could be reduced. As discussed above, cortisone and prednisone are biologically inactive and thus little attention has been paid to these steroids compared with their active counterparts cortisol and prednisolone. The recent identification of glucocorticoid-modifying enzymes within bone cells suggests that these inactive steroids may have a functional role in bone. Even in normal individuals there is interconversion of cortisol and cortisone with shuttling of these steroids between liver and kidney (Figure 3) [43]. Circulating cortisone derives primarily from the kidneys where the enzyme 11β-HSD2 is expressed [92]. Cortisone can be converted into cortisol in the liver or any other tissue expressing 11β-HSD1. Both cortisone and prednisone can be given orally since there is almost complete first-pass hepatic conversion into their active counterparts. However, in a similar manner to cortisone, prednisolone can be metabolized to prednisone by renal 11β-HSD2 [93]. The levels of prednisone generated in normal individuals after a 5 mg dose are of the order of 60 nmol/l, which are similar to those of cortisone [53]. At these concentrations, sufficient substrate should be available for conversion into prednisolone in tissues expressing 11β-HSD1. This suggests that the effects of glucocorticoids may be direct via circulating active glucocorticoids or by local conversion of inactive glucocorticoids into their active counterparts.
Figure 3  Systemic and local metabolism of glucocorticoids by 11β-HSD enzymes

Endogenous and exogenous glucocorticoids undergo extensive interconversion between active and inactive forms in vivo with inactivation occurring primarily in the kidney and reactivation in the liver and other tissues such as bone that express 11β-HSD1.

The relative contributions of these two pathways has been examined in a clinical trial of healthy subjects taking prednisolone [18]. All subjects taking 5 mg of prednisolone twice daily demonstrated decreases in the bone formation markers osteocalcin and N-terminal propeptide of type I collagen. The extent of the fall was highly correlated with baseline 11β-HSD1 activity measured by the urinary ratio of cortisol/cortisone metabolites. This study suggests that local glucocorticoid generation makes a considerable contribution to the effects of glucocorticoids on bone formation markers. However, the urinary ratios of steroid metabolites are likely to reflect enzyme activity occurring in several tissues, including liver, fat and kidney, in addition to bone. Although predictive of changes in bone metabolism in healthy subjects, it remains to be determined if the same relationships apply in subjects during illness, since inflammation and glucocorticoid treatment may lead to tissue-specific dysregulation of steroid metabolism in this setting [44].

This area of research suggests new ways in which tissue selectivity of glucocorticoids can be achieved. If a steroid can maintain its effects on immune tissue, but is unable to be reactivated locally via 11β-HSD1 in osteoblasts, it is likely to have a reduced impact on bone. It is interesting that local glucocorticoid metabolism may explain some of the findings with deflazacort, an oxazoline derivative of prednisolone. This steroid was promoted as a relatively bone-sparing glucocorticoid when compared with prednisone/prednisolone. Several studies suggested a reduced effect on bone compared with prednisolone when used in equivalent anti-inflammatory doses, whereas others found no difference or that higher doses of deflazacort were needed to maintain an anti-inflammatory effect equivalent to that of prednisolone. These studies were done at a time when the potential significance of tissue-specific metabolism of glucocorticoids was not appreciated. Prednisolone is inactivated to prednison in the kidney by 11β-HSD2 and thus the intrarenal levels of prednisolone are selectively reduced. In mice given an intraperitoneal injection of prednisolone the prednisolone/prednison ratio in the kidney was 0.8 after 60 min but 7 in the plasma [93]. Tissues that express 11β-HSD1 can convert prednison back into prednisolone, thus the corresponding prednisolone/prednison ratio in the liver was 43. In contrast with prednisolone, deacetyl-deflazacort, the active metabolite of deflazacort formed by first-pass hepatic metabolism, is a relatively poor substrate for 11β-HSD1 [94] and, thus, renal levels would not be expected to be significantly lower than in other tissues. Additionally, the metabolites of deflazacort are poor substrates for reactivation by 11β-HSD1 [95].

It is thus not a surprise that the most dramatic studies supporting a bone-sparing effect of deflazacort were seen in patients with renal disease [96,97]. Two double-blind studies randomized patients with nephrotic syndrome or renal transplantation to prednisone or deflazacort assuming a 1:1.2 relative potency. Bone loss was significantly reduced in patients taking deflazacort compared with prednisone in both of these trials with equivalent levels of immunosuppression. However, when the relative potencies of deflazacort and prednisolone were assessed in patients with polymyalgia rheumatica, higher doses of deflazacort were required for disease control (equivalent to a ratio of 1:1.4) [98]. The equivalent doses of prednisolone and deflazacort needed for effects in various tissues in healthy subjects has been examined. For suppression of eosinophils and deflazacort needed for effects in various tissues in healthy subjects has been examined. For suppression of eosinophils the dose ratio was 1.12, for osteocalcin the ratio was 1.54 and for cortisol suppression the ratio was 2.27 [99]. On this basis it would appear that deflazacort is superior to prednisone for treating renal inflammation. It is also possible that deflazacort may have reduced effects on bone due to a lack of reactivation by 11β-HSD1 in osteoblasts. This is likely to be the case, but the clinical significance will be dependent on whether 11β-HSD1 is expressed in the tissue being targeted therapeutically. 11β-HSD1 can be induced in a range of tissues during inflammation [100–102] and this has implications for the design of anti-inflammatory medications that have selective enzymic reactivation. It is possible that glucocorticoid therapy in the future may be optimized on the basis of the underlying disease being treated and the capacity of various tissues to undergo enzymic modification of the particular glucocorticoid.

SENSITIVITY TO ENDOGENOUS GLUCOCORTICOIDS

Endogenous corticosteroids may play a role in normal bone physiology. Previous studies have examined natural
variation in serum cortisol levels and found weak correlations with BMD. In healthy men, integrated 24 h cortisol levels correlated with lumbar spine BMD ($r = 0.37, P < 0.05$) and femoral neck BMD ($r = 0.31, P = 0.06$), although the relationships were removed by adjustment for BMI (body mass index) [103]. Peak, trough or integrated serum cortisol levels in men do not correlate with biochemical bone markers, but trough cortisol levels predicted bone loss at the spine and hip over a 4 year period after adjustment for adiposity [103]. No associations were seen between these variables in women. Other investigators have found weak associations of serum cortisol and lumbar spine BMD in men [104], but not women [105]. A further study examined the association of salivary cortisol with lumbar spine BMD. Evening salivary cortisol levels were found to be negatively correlated with spine BMD in elderly women, whereas morning salivary cortisol was negatively correlated with spine BMD in elderly men [106]. In the MacArthur Study of Successful Aging [107], the relationship between baseline urinary free cortisol excretion, measured on an overnight urine sample, and fracture risk was examined in 684 community-dwelling men and women aged 70–79 over a 7 year period. Increasing quartiles of urinary free cortisol were associated with increasing risk of fracture, an effect that was seen primarily in men. These results support a relationship between free cortisol and bone metabolism, but other factors are possible, such as an impact of cortisol on muscle mass and propensity to fall.

There are technical difficulties with examining any relationships between endogenous cortisol and bone health such as problems in measuring free cortisol levels rather than total [primarily CBG (corticosteroid binding globulin)-bound] cortisol, and the complicating factors of stress responses and diurnal variation. Additionally, urinary free cortisol measurements are heavily influenced by individual differences in cortisol metabolism with only approx. 1 % of the total cortisol synthesized excreted as ‘free’ cortisol [108].

The presence of $11\beta$-HSD1 within bone suggests that endogenous ‘inactive’ steroids may play a role in normal bone physiology via local reactivation to their active counterparts. Such an effect would not be reflected in circulating levels of cortisol. The circulating concentration of cortisone in healthy individuals is approx. 50 nmol/l [109]. Since cortisone has much less binding to CBG than cortisol, the free concentrations of cortisol and cortisone are likely to be very similar and the levels of cortisone would be sufficient to have an impact in tissues if they express sufficient $11\beta$-HSD1. An independent impact of cortisone on bone has yet to be shown and it is unclear whether this impact would be anabolic (by virtue of increased commitment of immature cells to the osteoblasts lineage) or catabolic (by suppressing the function of mature osteoblasts). Enzyme expression is likely to vary across differentiation and, thus, the impact is likely to depend on the stage of osteoblast differentiation at which $11\beta$-HSD1 is expressed.

Changes in the capacity of human osteoblasts to generate active glucocorticoids locally may also occur with age [53,110]. There is considerable variation in the activity of $11\beta$-HSD1 in osteoblasts cultured from different individuals [53]. Additionally, significant correlations were seen between cortisol generation and donor age with osteoblasts from older individuals able to generate 2–3 times more cortisol than osteoblasts from younger donors. Were such an effect to occur in vivo then osteoblasts within older subjects would be exposed to much higher levels of active glucocorticoids than those of younger individuals. It is thus possible that part of the dramatic increase in fracture risk that occurs with age [111] is due to a localized form of GIOP without changes in the circulating levels of glucocorticoids. This hypothesis requires further testing in vivo.

Insights into the role of endogenous steroids have been derived from mice which overexpress $11\beta$-HSD2 in osteoblasts and osteocytes. Reduced vertebral bone density was seen in female mice expressing $11\beta$-HSD2 under a type 1 collagen promoter, suggesting an anabolic effect of glucocorticoids at this site [47]. Currently, it is unclear whether this is due to blocking circulating or locally generated corticosteroids. An $11\beta$-HSD1 knockout model has been examined and displays subtle abnormalities in trabecular structure, suggesting that local glucocorticoid generation may have a role in bone development [49]. Analysis of the bones of this animal is complicated by the alterations in HPA (hypothalamo–pituitary–adrenal) axis function that occur in these mice [112] and the selection of a mouse strain (C57/Bl6) which expresses $11\beta$-HSD2, as well as $11\beta$-HSD1, in osteoblasts. The animal models described above are not ideal when examining the changes that occur in the human skeleton with aging and there is a need for further clinical studies in this area.

**OUTSTANDING QUESTIONS**

The reasons for the rapid and transient nature of the increase in fracture risk during glucocorticoid treatment remains an important area of research. Proposed explanations such as osteocyte viability cannot explain the rapid reduction in fracture risk that occurs with glucocorticoid withdrawal. A more plausible explanation is that glucocorticoids have a subtle impact on the material properties of bone. No study has yet examined in depth the material properties of glucocorticoid-treated bone, but the profound reductions in alkaline phosphatase, osteocalcin and other enzymes during glucocorticoid treatment are unlikely to be without consequence. New techniques such as NMR and infrared spectroscopy are becoming more accessible and will be used to examine this issue. An area of considerable clinical importance
is the impact of recurrent short-term high dose glucocorticoid exposure on the skeleton. The impact on fracture risk has yet to be determined in this setting, although large numbers of individuals, primarily with respiratory disease, are treated in this manner. Further clarification of the basis for differences in sensitivity between individuals is also needed. If this is due primarily to differences in local glucocorticoid metabolism, the clinical utility of measures of glucocorticoid metabolism needs to be determined. There is also the prospect that some individuals will have aberrant expression of glucocorticoid-metabolizing enzymes and are thus exposed to abnormally high glucocorticoid levels within bone and thus may develop a localized form of GIOP. Blocking local glucocorticoid production is likely to have a beneficial effect in these individuals.

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