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REVIEW

Endogenous Glucocorticoids and Bone

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While the adverse effects of glucocorticoids on bone are well described, positive effects of glucocorticoids on the differentiation of osteoblasts are also observed. These paradoxical effects of glucocorticoids are dose dependent. At both physiologicaland supraphysiological levels of glucocorticoids, osteoblasts and osteocytes are the major glucocorticoid target cells. However, the response of the osteoblasts to each of these is quite distinct. At physiology levels, glucocorticoids direct mesenchymal progenitor cells to differentiate towards osteoblasts and thus increase bone formation in a positive way. In contrast with ageing, the excess production of glucocorticoids, at both systemic and intracellular levels, appear to impact on osteoblast and osteocytes in a negative way in a similar fashion to that seen with therapeutic glucocorticoids. This review will focus on therole of glucocorticoids in normal bone physiology, with particular emphasis on the mechanism by which endogenous glucocorticoids impact on bone and its constituent cells.

***Keywords*:** glucocorticoids; mechanisms of action; bone; osteoblasts; skeletal development; Wnt signaling

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Introduction

The adverse effects of high dose therapeutic glucocorticoids on bone are well described and include osteoporosis and osteonecrosis. These effects are primarily mediated through toxic effects on osteoblasts (the bone forming cells) and osteocytes (long-lived cells related to osteoblasts which are resident within bone tissue). By contrast, it is also clear that glucocorticoids have an important and often essential role in the differentiation of osteoblasts cultured *in vitro*. These data suggest that endogenous glucocorticoids could have a positive, rather than a negative, effect on bone development and metabolism. Resent *in vivo* studies have advanced our knowledge of the effects of endogenous glucocorticoids on bone and bone cells. The data obtained from experiments utilizing genetically modified mouse models demonstrate that glucocorticoids direct cell lineage commitment of early mesenchymal progenitors through effects on osteoblasts. These actions, at least in part, are regulated through the Wnt/β-catenin signaling pathway in mature osteoblasts. This glucocorticoid regulated signaling pathway within mature osteoblasts is essential for normal intramembranous (i.e. calvarial) bone development. During bone modeling and remodeling, glucocorticoids also appear to play an important positive role in maintaining bone structure. However, with ageing, these beneficial effects of endogenous glucocorticoidsare less evident and may in fact be detrimental e.g. causing bone loss and reduced levels of proteins associated with bone formation such as osteocalcin. These detrimental actions appear to result from a combination of a subtle increase in circulating glucocorticoid levels with age and an increased local production of glucocorticoids within osteoblasts with age. This review will discuss the effects of endogenous glucocorticoidson bone and contrast then to the well-established effects of exogenous, therapeutic glucocorticoids.

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The effects of therapeutic glucocorticoids on bone

Therapeutic glucocorticoids were first developed in the 1950s to treat rheumatoid arthritis (1). Since then the use of these potent, broad spectrum anti-inflammatory

agents has expanded dramatically and they are now utilized in almost all areas of medical practice. However, while the clinical benefits of glucocorticoids are well established, significant adverse outcomes with long term use frequently limit their clinical application. Glucocorticoid-induced musculoskeletal disorders, and in particular glucocorticoid-induced osteoporosis (GIO), have long been recognized as serious and sometimes devastating consequences of either endogenous hypercortisolism (as seen in Cushing's disease) (2), or of long-term treatment with exogenous glucocorticoids (3). Data obtained before the era of effective therapies for GIO estimated that 50% of all patients treated with oral glucocorticoids for 6 months or longer were affected by GIO. Similar detrimental effects of glucocorticoids on bone have been reported in a range of other species, including rodents (4-5).

Distinct from postmenopausal osteoporosis, which is associated with an increase in osteoclastic bone resorption, the predominant feature of GIO is a dramatic suppression of osteoblast activity and bone formation. At the same time, osteoclast numbers are either unchanged or slightly increased (6). There is also evidence that glucocorticoids directly prolong the lifespan of mature osteoclasts (7). However, both clinical (8-9) and experimental data (10-11) indicate that osteoblasts and osteocytes are the main target cells of glucocorticoid action in bone, and that glucocorticoid-induced bone loss is strongly associated with osteoblast and osteocyteloss of function and apoptosis and autophagy (6, 12-13). In contrast, the major event driving osteoclast numbers appears to be a change in the 'RANKL/OPG balance'. Although the precise mechanisms are still not fully understood, it is clear that the osteoclast development and survival are regulated through cells of the osteoblast lineage. In particular, glucocorticoids stimulate the production of the cytokine receptor activator of nuclear factor-κB ligand (RANKL) in cells of the osteoblast lineage, including osteoblasts and osteocyte (14), which acts as a key regulator of osteoclast recruitment, activation and survival. The same cells also produce osteoprotegerin (OPG) which is a decoy receptor for RANKL and inhibits its actions on osteoclasts. OPG production is also regulated by glucocorticoids but in this case negatively (15). Thus, the RANKL/OPG ratio is a central determinant of osteoclast-mediated bone resorption, and glucocorticoids tip the RANKL/OPG balance in favour of RANKL. Additionally, glucocorticoids cause an increase in macrophage colony-stimulating factor (m-CSF), another essential factor in osteoclastogenesis (10, 15-17).

Most attention has focused on therapeutic glucocorticoids and their negative effects on bone cells. However, endogenous glucocorticoids are also important positive regulators of mesenchymal cell differentiation and function. While these catabolic and anabolic of glucocorticoids on bone were at time considered paradoxical, it is now clear that these differential actions are a function of dose and timing, depend on the particular target cell population. The clinical and mechanistic aspects of GIO have been reviewed elsewhere (18). This review will discuss the physiology of skeletal glucocorticoid actions, with particular emphasis on the mechanism by which endogenous glucocorticoids affect bone and its cells.

Mechanisms of glucocorticoid signaling

Endogenous glucocorticoids have a wide range of physiological functions and are essential to life. They control electrolyte and fluid homeostasis, systemic fuel metabolism, the immune system and the stress responses. These actions are mediated through activation of glucocorticoid receptor (GR) within target tissues. The GR is a ligand-inducible transcription factor that regulates gene transcription by several mechanisms. Although various forms of the GR exist (for instance a GR form proposed to be a dominant negative modulator of glucocorticoid action and the mineralocorticoid receptor is sometimes classified as a type of GR), most actions of glucocorticoids appear to be mediated through the 'classical' GR (sometimes referred to as GR) (19). The intracellular mechanisms by which the GR signals are diverse and not fully understood. These mechanisms include: binding (most often as a dimeric complex) to a consensus glucocorticoid response element (GRE) within DNA gene promoter elements; direct interaction (and interference) with other transcription factors (primarily those associated with proinflammatory signalling such as AP-1 and NF-κB) in a mechanisms that is independent of GR binding to the GRE; and interaction with, and binding to the GRE together with other transcription factors (19-21). In addition to these "classical" genomic mechanisms, the membrane-bound glucocorticoid receptor (mGR) has been reported to mediate non-genomic effects of therapeutic glucocorticoids. This may constitute an important additional mechanism of immunosuppression by these medications (22). The circulating level of endogenous glucocorticoid levels is regulated by the hypothalamic-pituitary-adrenal (HPA) axis, a classic endocrine negative feedback loop. However, it is now appreciated that glucocorticoid action depends not only on plasma and interstitial fluid glucocorticoid concentrations, but also on intracellular

glucocorticoid availability. Within specific tissues, this local glucocorticoid availability is critically determined by intracellular glucocorticoid metabolism (23-24). This local 'pre-receptor' regulation of glucocorticoid availability is mediated by the interconversion of hormonally active and inactive ligands by the two 11β-hydroxysteroid dehydrogenase (11βHSD) isoenzymes (23-24). The type 1 enzyme (11β-HSD1) predominantly catalyses the formation of active cortisol (in man) and corticosterone (in rodents) from inactive cortisone and 11-dehydrocorticosterone (11-DHC) respectively. The enzyme thus increases intracellular glucocorticoid concentrations. Various cytokines and growth factors are able to modulate this control on a local level (25-26). In contrast, 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) unidirectionally catalyses the conversion of active glucocorticoids to their inactive metabolites (Figure 1). Highest expression of 11βHSD2 is seen in mineralocorticoid target tissues such as the kidney (23, 27-28). As glucocorticoids have a similar affinity to the mineralocorticoid receptor (MR) to that of the main mineralocorticoid aldosterone, the primary biological function of 11βHSD2 in mineralocorticoid target tissues seems to be the protection of the MR against unwanted activation by glucocorticoids (25, 28-29).

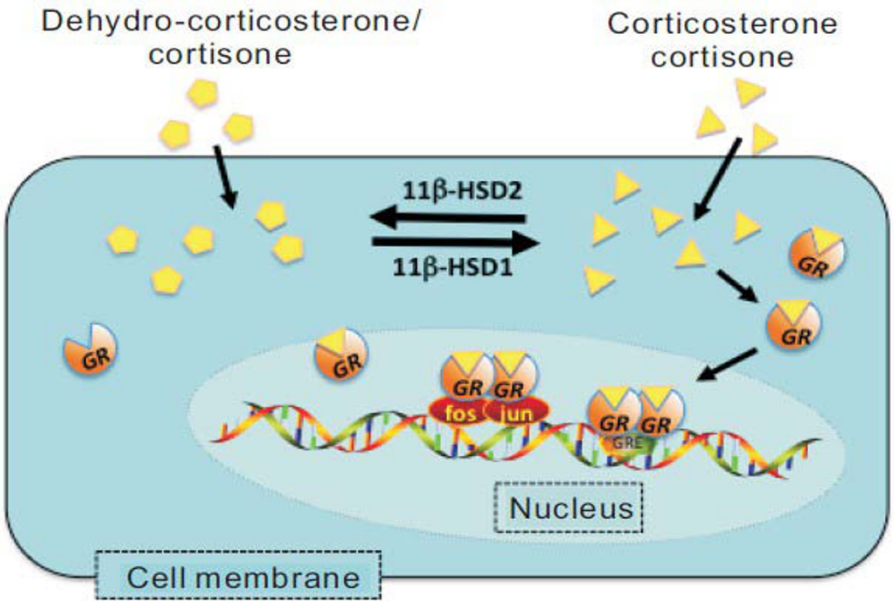

Figure 1 The mechanisms of action of glucocorticoids. Glucocorticoids, cortisone (in man) or dehydro-corticosterone (in rodents) are activated by 11β-HSD1 to cortisol or corticosterone which then binds to their receptor (GR), after which the activated ligand-receptor-complex travels to the nucleus. The activated GR can either bind to its specific response element (GRE) or bind to other transcription factors such as AP-1 (fos and jun) pathway.

Major insights into the role of endogenous glucocorticoids within the skeleton have been derived from experiments utilizing genetic modified mouse models. Thus, although not expressed naturally in bone cells, the glucocorticoid-inactivating enzyme, 11βHSD2, has been used as a tool to examine the effects of endogenous glucocorticoids on specific bone cells. Given the intrinsic activity of the enzyme, transgenic overexpression of 11βHSD2 would be expected to confer resistance to glucocorticoid action (30). As such, a number of transgenic mouse lines have been generated using bone cell-specific transgenic expression of 11βHSD2 to disrupt intracellular glucocorticoid signaling. The range of osteoblast specific promoters (Figure 2) used to drive either 11βHSD2 gene expression or to delete the GR gene by Cre expression in the bone lineage ranges from those that target early multi-potential cells (Dermo1-Cre; $GR^{fl/fl}$) (31-32) to early committed osteoblastic progenitors (Runx2-Cre; $GR^{fl/fl}$) (33), pre-osteoblasts (Col3.6-11βHSD2) (34), mature osteoblasts (Col2.3-11βHSD2) (35-40), and later stage mature osteoblast to osteocytes (OG2-11βHSD2) (11). Findings utilizing these models will be discussed throughout this review.

Role of glucocorticoids and Wnt signaling in mesenchymal cell differentiation

The effects of glucocorticoids on mesenchymal cell differentiation have primarily been explored *in vitro*. *In vivo* studies were limited due to mice with global GR deletion or mesenchymal targeted-deletion of GR being unable to survive post-natally (32, 41). However, even

targeted deletion of the GR in mesenchymal cells, using the Dermo1-Cre approach (Dermo1-Cre; $GR^{fl/fl}$ mice), results in mice with postnatal lethality (soon after born) due to a failure of lung-maturation (32). The phenotype of these mice is very similar to the one seen in mice with global GR deletion (41). In addition, a large proportion of Dermo1-Cre; $GR^{fl/fl}$ embryos presented with a defect in ventral abdominal wall formation (32), indicating that mesenchymal GR signaling plays a critical role in embryonic lung and suggests an additional role in abdominal wall development (32). However, Dermo1-Cre $GR^{fl/fl}$ embryos appear to form normal bone and cartilage suggesting that at least during embryonic/fetal development there is no absolute requirement for glucocorticoids signaling within mesenchymal cells to form these tissues (32).

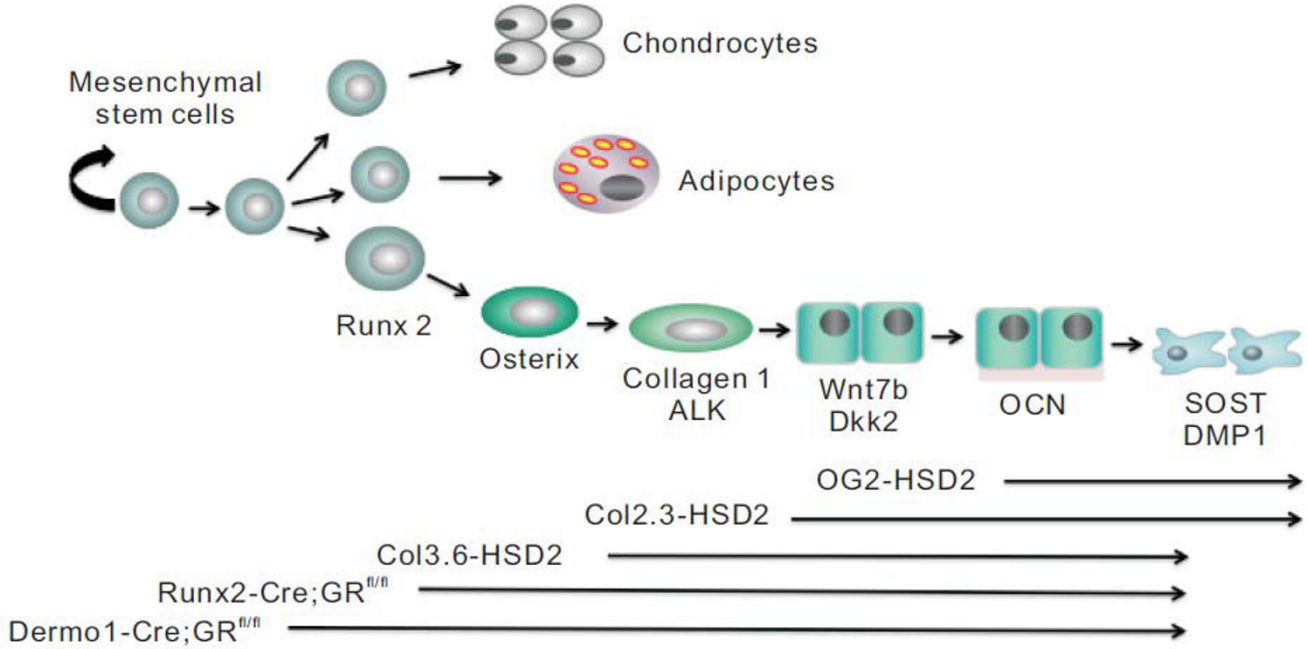

Figure 2 Model of osteoblast differentiation and the putative stage of transgene expression. Diagram showing the expression of transgenes under the control of various promoters active along the mesenchymal/osteoblastic lineage. Dermo1 promoter (31-32) is activated in early multi-potential cells. Runx2 (33, 88-89) and Osterix (89-90) are the two well characterized promoters activated in early committed osteoblastic progenitors; Col3.6 promoter (91-92) is expressed after osterix expression in pre-osteoblasts at the stages in which cells express type I collagen (Collagen 1) and subsequently alkaline phosphatase (ALP); Col2.3 promoter (91-92) is expressed in mature osteoblasts which express Wnt7b and then Dkk2 which switch on mineralization (47); Osteocalcin promoter (OG2) is active at the later stage of mature osteoblasts when mineralization has been initiated (11, 57-58, 93). Both Col2.3 and OG2 promoters are expressed in osteocytes.

In vitro studies have demonstrated that glucocorticoids play an important role in osteoblast differentiation, with dose-dependent effects on both osteoblast and adipocyte lineage commitment (42-44). Glucocorticoids appear essential for the differentiation of mesenchymal cells (usually derived from bone marrow) into mature osteoblasts (43-45). Although additional glucocorticoids administration is usually not required for differentiation of cultures of cells derived from calvaria, it is important to realize that glucocorticoids are contained in all osteogenic culture media. For example, 10% fetal bovine serum (FBS), one of the most commonly used osteogenic culture additives, contains significant amounts of cortisol at concentration of approximately $8x10^{-9}$ $mol{\cdot}L^{-1}$ (37). Work involving selective abrogation of glucocorticoid signaling has indicated that the requirement for additional glucocorticoids to induce differentiation of mesenchymal cells into osteoblasts may depend on the proportion of mature osteoblasts contained within the isolates. Calvarial cell cultures derived from Col2.3-11βHSD2 transgenic mice exhibit greatly reduced osteoblastogenesis (37, 46) and predominant adipogenesis (37) when compared with wild-type cultures. This phenotypic shift in mesenchymal progenitor cell commitment is accompanied by a reduction in Wnt7b and Wnt10b mRNA and β-catenin protein levels and an increase in mRNA expression for sFRP1, a Wnt signaling pathway inhibitor, in Col2.3-11βHSD2 transgenic versus wild-type cultures. It thus appears that if glucocorticoid signaling is attenuated in osteoblasts and osteocytes the Wnt/β-

catenin pathway becomes suppressed. This leads to activation of PPARγ, a critical factor in adipocyte development, and thus mesenchymal progenitors are pushed towards differentiating into adipocytes. Of note, transwell co-culture of Col2.3-11βHSD2 transgenic mesenchymal progenitor cells with wild-type osteoblasts restored commitment to the osteoblast lineage, as did treatment of transgenic cultures with exogenous Wnt3a (37). The ability of wild-type osteoblasts to restore commitment to the osteoblast lineage was blocked by sFRP1. These studies demonstrate that glucocorticoids not only act on mature osteoblast through regulation of Wnt/β-catenin signaling but also play an essential role in directing cell lineage commitment of early mesenchymal progenitors via indirect involvement of osteoblasts (37).

Expression of Wnt7b and Wnt10b in mature osteoblasts is modulated by corticosterone (the main glucocorticoid in rodents), in a biphasic fashion with 3 to 3.5-fold up-regulation at 10^{-8} mol·L^{-1} corticosterone, and 50% down-regulation at 10^{-7} mol·L^{-1} corticosterone. Corticosterone at 10^{-7} mol·L^{-1} also increased expression of the Wnt inhibitors, sFRP-1 and DKK-1 by 2- to 3-fold. These dose dependent biphasic actions may explain the observations that glucocorticoids both enhance and impair osteoblast differentiation from mesenchymal progenitor cells, and indicate that this is at least in part through the regulation of Wnt expression in osteogenic cultures (39). This suggests that the contrasting anabolic and catabolic effects of glucocorticoids on bone are, at least in part, mediated through the regulation of Wnt expression and its inhibitors in mature osteoblasts. The biphasic effects of glucocorticoids in regulating Wnt expression and its inhibitors via osteoblasts may provide a mechanistic basis for the seemingly disparate actions of glucocorticoids on bone and could explain their phenotypic consequences.

In the calvarial cell culture system, calvarial dissociations consist of heterogeneous cell populations containing immature mesenchymal precursor cells, pre-osteoblasts, mature osteoblasts and osteocytes. Among these cells, mature osteoblasts appear to be the dominant source of Wnt proteins, as they express a wide range of Wnt molecules at significantly higher level than early mesenchymal precursor cells (37-39, 47), especially Wnt7b, Wnt10b and Wnt9a, all of which are important regulators of osteoblastogenesis (47-52). Furthermore, the glucocorticoid-induced upregulation of Wnt signaling is observed only in differentiated osteoblasts with no observable effect of glucocorticoids on precursor cells (39).

These findings are consistent with the ability of mature osteoblasts to produce Wnt proteins as signaling molecules, while the precursor cells are primarily recipient cells that rely on paracrine signals to control cell fate specification (Figure 2). Further evidence for this mechanism has been derived from *in vivo* studies examining cranial bone development (38).

Endogenous glucocorticoids are important in cranial bone development *in vivo*

Neonatal Col2.3-HSD2 transgenic mice exhibit a distinct phenotype characterised by hypoplasia and osteopenia of the cranial skeleton, increased suture patency, ectopic differentiation of cartilage in the sagittal suture and an increased amount of parietal cartilage (38, 53). Another mouse model in which the 11βHSD2 transgene is expressed in the osteoblast lineage from pre-osteoblasts onwards using a 3.6 kb collagen type I (Col3.6) promoter (Col3.6-11βHSD2 transgenic) showed a very similar phenotype to the Col2.3-11βHSD2 transgenic mice (34). During cranial bone development, it appears that endogenous glucocorticoids stimulate the expression and secretion of Wnt proteins in mature cranial osteoblasts (38). The ensuing canonical Wnt signaling cascade induces: mesenchymal progenitor cells to differentiate away from the chondrocyte lineage towards the osteoblast lineage and thus form bone; osteoblasts to initiate MMP14-mediated remodelling of the collagenous matrix surrounding osteoblasts; and parietal cartilage chondrocytes to initiate MMP14-mediated cartilage degradation (Figure 3). These concurrent and tightly interconnected pathways establish a novel role for both glucocorticoids and osteoblasts in the intricate process of intramembranous bone development. In young animals, the sutures serve as growth centres, where mesenchymal cells reside as a reservoir for postnatal osteogenesis and new bone formation. In this process, Wnt/β-catenin signaling is required to suppress chondrogenesis and to allow osteoblasts to form (54-55). Of note, the phenotype in Col2.3-11βHSD2 transgenic mice was associated with a dramatic reduction in β-catenin protein accumulation in calvarial osteoblasts and progenitor cells located in the sutures, indicating that canonical Wnt signaling was attenuated. The impaired cranial cartilage degradation in Col2.3-11βHSD2 transgenic mice is similar to the calvarial cartilage phenotype seen in MMP14 (also named MT1-MMP) knockout mice (56). As expected, expression of MMP14 is markedly reduced in parietal bone and cartilage of Col2.3-11βHSD2 transgenic mice. Expression of Wnt9a and Wnt10b is significantly reduced in osteoblasts with disrupted glucocorticoid signaling,

and accumulation of β-catenin, the upstream regulator of MMP14 expression, is decreased in osteoblasts, chondrocytes and mesenchymal progenitors of transgenic mice. The reduced expression of osteoblastic Wnts and MMP14 is also accompanied by a pronounced disorganisation of the resident osteoblasts and the collagenous bone matrix in Col2.3-11βHSD2 transgenic calvaria suggesting the normal calvarial bone formation is also dependent on an autocrine Wnt/β-catenin-MMP14 signaling loop. Supra-calvarial injection of Wnt3a protein not only rescued the abnormal cartilage phenotype, but also significantly improved parietal bone formation, mineralization and, consequently, suture narrowing, further confirming the role of glucocorticoid regulated paracrine and autocrine Wnt signaling in cranial bone development.

Col2.3-11βHSD2 transgenic mice were characterized by delayed intramembranous (i.e. calvarial) bone development in the presence of normal endochondral formation (long bones). Day and colleagues have shown that intramembranous ossification requires high levels of β-catenin, through up-regulation by Wnt signaling, to promote osteoblast differentiation. In contrast, during endochondral ossification (i.e. long bone), β-catenin protein levels are kept low by inhibition of canonical Wnt signaling inside mesenchymal condensations to ensure that only chondrocytes can form initially (55). This difference may explain why long bone development is not affected in postnatal Col2.3-11βHSD2 transgenic mice, but altered when the mice are at the age of 3 weeks onwards (40).

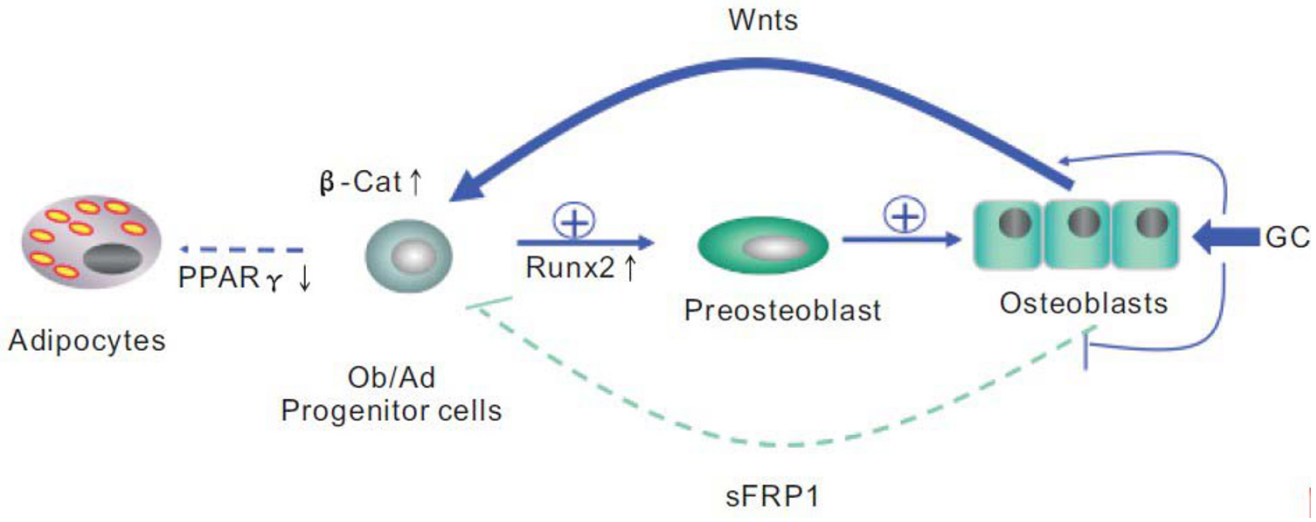

Figure 3 Glucocorticoids control of lineage commitment of mesenchymal progenitors through mature osteoblast via Wnt signaling. Glucocorticoids stimulate osteoblasts to increase secretion of Wnts and to decrease secretion of the Wnt inhibitor, sFRP1; both actions would advance canonical Wnt signaling in mesenchymal progenitor cells which in turn, causes β-catenin to enter the nucleus which increases Runx2 expression while inhibiting PPARγ expression. These actions promote osteoblastogenesis but inhibit adipogenesis.

Role of endogenous glucocorticoids in long bone development and maintenance

Although endogenous glucocorticoids may not be required in endochondral ossification they appear to be required for normal bone growth and maintenance. Deletion of the GR in osteoblast progenitors (Runx2-Cre; $GR^{fl/fl}$, also named $GR^{Runx2Cre}$) results in mice with a mild but highly significant decrease in bone density (33). With targeted overexpression of 11βHSD2 in mature osteoblasts and osteocytes, Col2.3-11βHSD2 transgenic mice exhibit vertebral (but not femoral) trabecular osteopenia (35), while femoral cortical bone parameters were decreased in both female and male mice (36). These data suggest that glucocorticoid signaling in osteoblasts may be required to maintain normal bone mass in sexually mature mice but this may be modulated by sex hormone levels. To determine the role of endogenous glucocorticoids in bone growth and maintenance, and its dependence on age, sexual maturity, gender and skeletal site, 3-week-old (sexually immature) and 7-week-old (sexually mature) male and female Col2.3–11βHSD2 transgenic mice and their wild-type littermates were compared (40). Lower bone volume, lower trabecular number and higher trabecular separation in tibial trabecular bone were observed in 3 and 7-week-old Col2.3-11βHSD2 transgenic mice, indicating that the biological effect of disrupted glucocorticoid-

signalling was independent of sexual maturity (40). However, this was not the case for the vertebral bones, where significant differences between Col2.3-11βHSD2 transgenic and wild-type mice were seen in 7-week-old but not in 3-week-old animals, suggesting that the effects of the transgene at this site may be modulated by age and/or changes in circulating sex hormone levels (40). Col3.6-11βHSD2 transgenic mice showed a very similar phenotype to the Col2.3-11βHSD2 transgenic mice (34), further confirming that glucocorticoid signaling plays an important role in the osteoblast lineage differentiation. Over-expression of 11β-HSD2 has also been achieved utilizing the murine osteocalcin gene 2 (OG2) promoter which is active only in very mature osteoblasts and osteocytes (11). Under the control of this promoter, expression of the 11β-HSD2 transgene did not appear to affect normal bone development or turnover as demonstrated by identical bone mineral density (BMD), strength, and histomorphometry in adult transgenic and wild type animals, suggesting that endogenous glucocorticoid action in osteocalcin-expressing cells is not required for normal skeletal development (11).

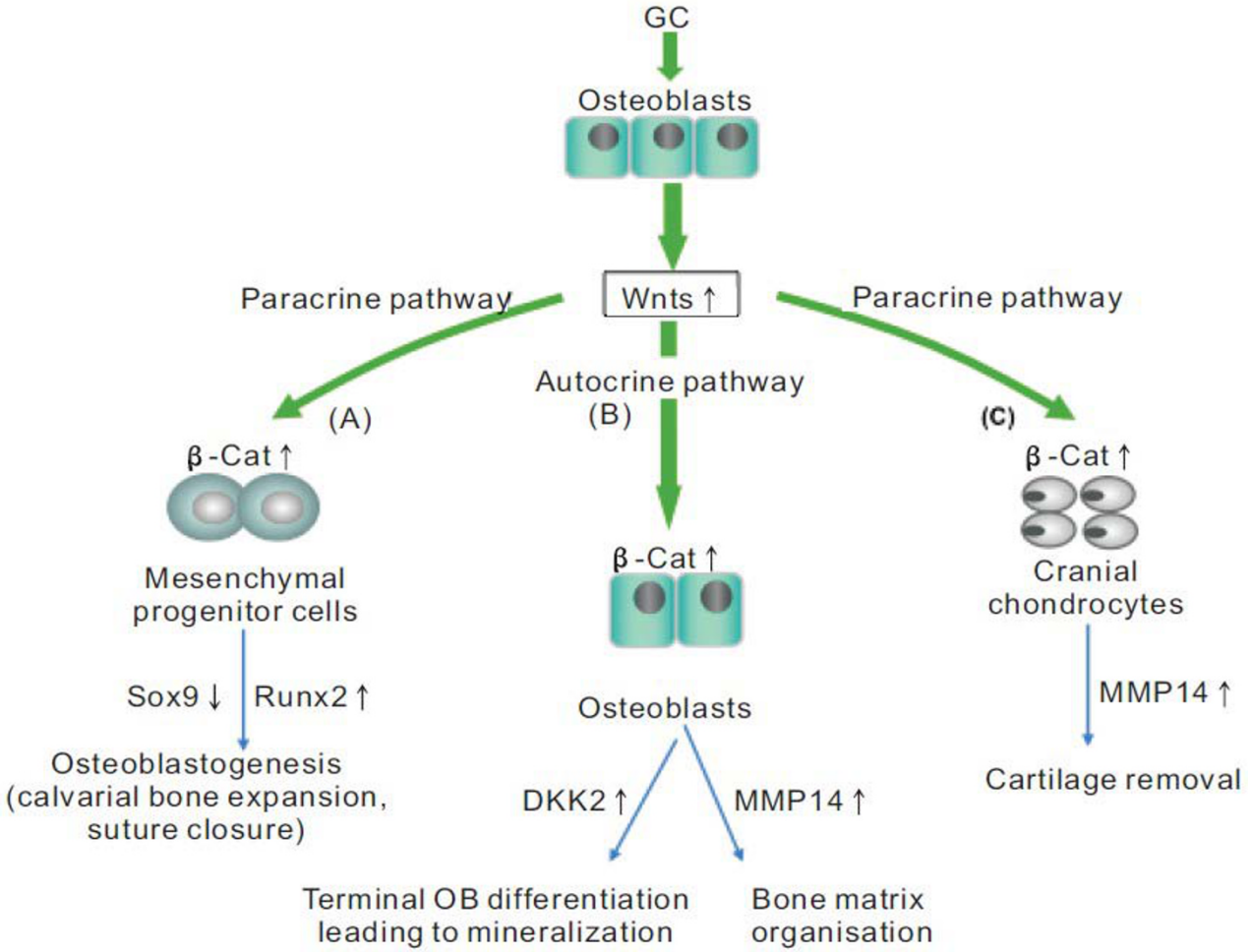

Figure 4 Model of glucocorticoid dependent canonical Wnt signaling in cranial development. Glucocorticoids stimulate mature osteoblasts to produce canonical Wnt proteins, which activate the β-catenin signaling cascade in: (A) cranial mesenchymal progenitor cells, through up-regulation of Runx2 and down-regulation of Sox9 expression, to differentiate towards osteoblasts and away from chondrocytes; (B) osteoblasts, through up-regulation of DKK2 terminating osteoblast differentiation and leading to mineralization; up-regulation of MMP14 to initiate the remodelling of the collagenous matrix surrounding the osteoblast; (C) cranial chondrocytes, through up-regulation of MMP14 to initiate the cranial cartilage degradation.

One explanation for discrepancy in results using different promoters could be that osteocalcin is only expressed during the latest stage of osteoblast differentiation, the bone matrix becomes mineralized (57-58). Expression of Wnt7b, however, peaks during the maturation phase of osteoblastic development and then falls during mineralization (47). It is conceivable that during later stages of osteoblast differentiation, disruption of glucocorticoid signalling has little or no effects on Wnt7b expression, hence the absence of a distinct phenotype.

Role of endogenous glucocorticoids in bone during aging

There is good evidence that evening (i.e. nadir) as well as mean cortisol levels increase with age in humans (59-62). In addition, studies in humans suggest that there is an age-related decline in the negative feedback response of the HPA axis, leading to progressively greater exposure of tissues tocortisol (61-62). Age-related changes in mice are similar to those observed in humans. Ageing in mice is associated with an increase in adrenal production of corticosterone (63), the predominant glucocorticoids in rodents. Two factors might facilitate this process: age-related decrease in brain corticosteroid receptors leads to a decreased HPA axis sensitivity to negative feedback from glucocorticoids; and repeated cortisol (or corticosterone)-generating stress challenges. Animal studies indicate that ageing may be accompanied by a reduction in the number of hippocampal glucocorticoid receptors. This would result in an age-dependent decrease of the negative feedback effect of cortisol on the HPA axis and cause an increased cortisol response to each stressful event. In humans, studies have examined the physiological regulation of HPA axis responses and demonstrated that the cortisol response to stressors can be exaggerated in the elderly. In addition, there is a reduced negative feedback, such that circulating cortisol levels stay elevated longer (64-65). Furthermore, a gender effect was also suggested, with a greater age-related increase in HPA axis reactivity to challenge in women, relative to men (64-65).

In addition to the increase in systemic glucocorticoid levels, 11β-HSD1 expression in certain tissues is also increased. It has been shown that 11β-HSD1 expression and activity are positively correlated with donor age in human skin tissue (66) and in *ex vivo* cultured human osteoblasts (67). Similar findings have also been observed in mouse bone (63). The vertebral bone expression of 11β-HSD1 is increased in aged (25-31 months old) male and female compare to 4-month-old animals (63).

Although excessive glucocorticoids exposure is a well-recognized cause of osteoporosis, little is known about the role of age-related excessive endogenous glucocorticoids in determining bone mass. Only a few epidemiological studies have examined the relationship between the levels of cortisol in the circulation and current bone density in community-dwelling elderly women and men. 684 men and women aged 70–79 were participants in the MacArthur Study of Successful Aging over a 7-year period (68). When all participants were considered together, increasing overnight urinary free cortisol was associated with increasing risk of fracture. Greendale *et al* showed that evening salivary cortisol was significantly elevated in elderly men and women as compare to younger control individuals. Evening salivary cortisol levels were found to be negatively correlated with spine BMD in elderly women, whereas morning salivary cortisol was negatively correlated with lumbar BMD in elderly men (69). In a study of the HPA axis in response to an injection of synthetic ACTH, the relationships between cortisol secretion and bone mass was examined in a cohort of 247 elderly men and women. The peak cortisol response following injection of ACTH correlated negatively with baseline BMD at the femoral neck in women, with loss of BMD at the femoral neck in women and loss of BMD at the lumbar spine in men. Although these data were in consistent in terms of the time of day, subject gender and the method used to ascertain cortisol levels, these data provide evidence that circulating endogenous glucocorticoids influence the rate of bone loss in healthy elderly individuals.

As discussed above, selective overexpression of 11βHSD2 in mature osteoblasts and osteocytes driven by the osteocalcin gene 2 (OG2) promoter (OG2-11βHSD2) has no apparent effect on the bone phenotype at 5 months of age (11). Interestingly, however, positive effects of the transgene are found in aged OG2-11βHSD2 mice (63). Like humans, mice also exhibit an age-related decrease in bone mass and strength. These declines are associated with an increase in adrenal production of glucocorticoids as well as 11βHSD1 expression in bone cells. OG2-11βHSD2 transgenic mice had similar levels of serum glucocorticoids and osteoblastic 11βHSD1 expression compared to wild-type animals, however, the adverse effects of aging on osteoblast and osteocyte apoptosis, bone formation rate and microarchitecture, crystallinity, vasculature volume, interstitial fluid, and strength seen in wild-type animals were prevented. Interestingly, the expression of osteocalcin, a gene down-regulated by glucocorticoids, is higher in OG2-11βHSD2 transgenic mice when compare to age and gender matched wild-type animals (63). There is now mounting evidence that osteocalcin is involved in the regulation of normal fuel metabolism (70-76) and recent research suggests that the peptide plays a central role in the pathogenesis of glucocorticoid-induced dysmetabolism (77). Up to date, it is not known whether the age-related changes in body composition and fuel metabolism involve the action of endogenous glucocorticoid on osteoblast function, specifically via osteocalcin. At least, body

weight did not appear to differ between the 21-month-old male wild-type and OG2-11βHSD2 transgenic mice (63).

Role of endogenous glucocorticoids in bone resorption

As discussed above, most studies indicate that the main cellular target of glucocorticoid action is indeed the osteoblast (10-11, 15, 40, 77-78). The bone effects of endogenous and exogenous glucocorticoids appear a consequence of their direct actions on the osteoblast. In contrast, using the same strategy to delineate the effects of endogenous glucocorticoids on osteoclasts, osteoclast-specific expression of 11βHSD2 did not affect skeletal development, adult bone mass or the number of osteoclasts and osteoblasts in mice (7). It therefore appears that the changes in osteoclast function in response to endogenous glucocorticoids in bone are usually of a secondary nature.

Possible impact of local glucocorticoid metabolism on bone

The use of ectopic expression of 11βHSD2 to examine the effects of glucocorticoids on bone has been discussed in depth above. However, it should also be borne in mind that 11βHSD1, the activator of endogenous glucocorticoids, is normally expressed within bone, and in particular, within osteoblasts. An age-related increase in expression within bone has been reported in both human and mouse osteoblasts (63, 67, 79). This would be expected to impact on bone formation (reducing it) but could also impact on bone resorption (to increase it) through a glucocorticoid mediated increase in RANKL expression by osteoblasts (15). 11βHSD1 global knockout mice have no obvious bone phenotype suggesting a limited role for 11βHSD1 in normal bone development (80). However, aged mice have not been examined. In elderly humans there is a strong correlation between plasma levels of cortisone and osteocalcin with a high cortisone level predicting a low osteocalcin level (81). These relationships were independent of the level of cortisol in the circulation suggesting that the reduction in osteocalcin with high cortisone levels is a reflection of direct production and action of locally generated cortisol via the 11βHSD1 enzyme within osteoblasts. Additional support for a role of 11βHSD1 in age related bone loss comes from two independent gene association studies (82-83). These demonstrated significant associations between polymorphisms in HSD11B1, the gene for 11βHSD1, and bone mineral density (BMD) and fracture risk. It was suggested that polymorphisms that predicted low activity of the 11βHSD1 enzyme were associated with a higher BMD and a reduced risk of fracture.

There is a limited amount of data available regarding chemical inhibition of 11βHSD enzymes in humans. The main study was only of one week duration but demonstrated a significant reduction in markers of bone resorption suggesting a possible clinical application in the treatment of post-menopausal osteoporosis (79). Further clarification of the possible application will come from studies examining specific inhibitors of the 11βHSD enzyme which have recently been developed primarily to target obesity and insulin resistance.

The expression of 11βHSD1 is dramatically up regulated by pro-inflammatory cytokines such as TNFα and IL-1β (84). As such, the amount of glucocorticoids generated in bone is likely to increase in response to local or systemic inflammation. Bone loss in inflammatory disorders is known to be due to an increase in bone resorption but also to a relative reduction in bone formation. The mechanisms underlying this 'uncoupling' of bone formation from resorption are not fully understood but local production of glucocorticoids is likely to contribute (85). Indeed, the global 11βHSD1 knockout mouse, despite not having a resting bone phenotype, develops an osteophytic reaction in response to experimental arthritis (86). This is in keeping with an abnormal maintenance or exaggeration of bone formation in the face of increased inflammation. These data might suggest that inhibitors of 11βHSD1 activity might be useful in preserving bone formation in patients with inflammation associated bone loss. However, 11βHSD1 activity is also important in the immune response so care would be needed to ensure that inflammation is not increased by such treatment (87).

Conclusion

It is now apparent that osteoblasts and osteocytes are the major glucocorticoid target cells in bone for both endogenous levels of glucocorticoid and the levels seen during therapeutic glucocorticoid treatment. However, the response of the osteoblasts to each of these is quite distinct. At physiology levels, glucocorticoids direct mesenchymal progenitor cells to differentiate towards osteoblasts and thus increase bone formation via up-regulation of Wnt/β-catenin signaling in mature osteoblasts in a positive way. In contrast with ageing, the excess production of glucocorticoids, at both systemic and intracellular levels, appear to impact on osteoblast and osteocytes in a negative way in a similar fashion to

that seen with therapeutic glucocorticoids.

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