Published in final edited form as:
Endocr Dev. 2011 ; 20: 187–193. doi:10.1159/000321244.

Effects of Glucocorticoids on the Growth Plate

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Abstract

Glucocorticoids have a direct, inhibitory effect on the growth plate, as demonstrated by in vivo and organ culture studies. Glucocorticoids slow longitudinal bone growth by inhibiting chondrocyte proliferation, hypertrophy, and cartilage matrix synthesis. The molecular mediators of these effects are poorly understood. Glucocorticoids also delay growth plate senescence. The decreased rate of senescence appears to be a consequence of the growth inhibition and, in particular, may occur because glucocorticoids slow proliferation of stem-like cells in the resting zone and therefore conserve the limited proliferative capacity of these cells. This slowing of senescence appears to explain the phenomenon of catch-up growth following transient glucocorticoid exposure. After the exposure, the growth plate is less senescent, and therefore grows more rapidly than is normal for age. Glucocorticoids cause growth inhibition and subsequent catch-up growth not only in terms of longitudinal bone growth at the growth plate but also in terms of cross-sectional bone growth at the periosteum. Whether the underlying mechanisms are analogous to those at the growth plate is not known.

In mammals, longitudinal bone growth occurs at the growth plate, a cartilaginous structure present in tubular bones and vertebrae. The growth plate is composed of three principal layers: the resting zone, the proliferative zone, and the hypertrophic zone. The resting zone contains progenitor chondrocytes capable of forming new clones of proliferative chondrocytes [1]. In the proliferative zone, these clones of chondrocytes are arranged in columns parallel to the long axis of the bone. Within each column, the cells proliferate rapidly. The chondocytes that are located farthest from the end of the bone in these columns stop dividing and start enlarging to become hypertrophic chondrocytes. In addition, chondrocytes throughout the growth plate produce cartilage, the composition of which varies in the different zones. In isolation, this cell proliferation, cell hypertrophy, and cartilage matrix synthesis would lead to progressive widening of the cartilaginous growth plate. However, simultaneously, the hypertrophic zone is invaded by blood vessels and bone cells, which remodel the newly formed cartilage into bone tissue. The net result is that new bone is progressively created at the bottom of the growth plate, resulting in longitudinal bone growth.

The rate of longitudinal bone growth at the growth plate is governed by a system of endocrine signals including growth hormone, IGF-I, thyroid hormone, glucocorticoids, androgens and estrogens. A likely evolutionary purpose for this endocrine regulation is to conserve nutrients in times of adversity. If a child is undernourished, circulating IGF-I and thyroid hormone decline while glucocorticoid levels rise. In adolescents, undernutrition also

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causes decline in sex steroids. These endocrine changes suppress bone growth, presumably to conserve nutrients for vital function. However, as discussed below, during these times of adversity, growth is not simply lost irreversibly. Instead the growth plate is able to conserve much of its growth capacity until conditions improve, thus preserving, at least in part, the individual's adult stature.

Glucocorticoids provide an important component of this endocrine system that governs growth plate function. When glucocorticoid levels rise above physiological concentrations, longitudinal bone growth is potently inhibited. Although some of this effect may be mediated by indirect effects involving other endocrine signals, there is also an important direct, local effect of glucocorticoids on the growth plate. Evidence for a direct effect comes from studies in vivo and in vitro. In vivo, dexmethasone, a potent synthetic glucocorticoid, infused directly into the proximal tibial growth plates of young rabbits caused a 77% inhibition in growth compared to the contralateral, vehicle-treated growth plates [2]. In vitro, addition of dexamethasone to the culture medium caused a similarly severe inhibition of growth in cultured mouse fetal metatarsal bones and in rat fetal and postnatal metatarsal bones [3]. This inhibition results from decreased proliferation and hypertrophy. In addition, dexamethasone may stimulate apoptosis of terminal hypertrophic chondrocytes [4]. Whether this last effect contributes to the growth inhibition is unclear.

Because longitudinal bone growth results from chondrocyte proliferation, hypertrophy, and cartilage matrix secretion, the growth-inhibitory effects of glucocorticoids might result from inhibition of any of these three processes. In fact, there is evidence for an effect on each. Studies using fetal rat metarsal bones in organ culture indicate a suppressive effect on proliferation and hypertrophy [3]. Similarly, glucocorticoid hinders proliferative capacity of chondrocytes in vitro [5,6]. Furthermore, studies in primary cell culture systems suggest a stimulatory effect on proteoglycan synthesis at low concentration but suppressive effect at high concentration [7].

The molecular mechanisms by which glucocorticoids suppress longitudinal bone growth are not well understood. Several studies have explored the possibility that glucocorticoids may act by modulating local components of the growth hormone-IGF-I system. In cultured rat growth plate chondrocytes, glucocorticoids can downregulate expression of growth hormone receptor, IGF-I, and IGF1R [8]. However, other studies suggest that glucocorticoids increase expression of IGF1R mRNA [9] in porcine cultured chondrocytes and increase GHR mRNA in vivo in rabbit growth plate [10]. These disparate findings could reflect species differences but may be related to the experimental system chosen; when growth plate chondrocytes are removed from the context of the growth plate and placed in isolated cell culture, their behavior may change substantially [11] presumably because the chondrocytes are no longer subject to the complex array of paracrine signals from neighboring cells or to the normal cell-matrix interactions. Addition of IGF-I to the culture medium can partially compensate for the growth impairment of glucocorticoids, but the cellular mechanisms appear to be different with glucocorticoids inhibiting proliferation primarily and IGF-I stimulating hypertrophy primarily [12]. Thus, it is not clear to what extent glucocorticoids might inhibit growth plate function by modulating the local components of the GH-IGF-I system.

Other studies of the molecular mechanisms have taken an unbiased approach, using microarray to assess global changes in gene expression due to glucocorticoid exposure [13,14]. In cultured growth plate chondrocytes, addition of dexamethasone to the culture medium significantly changes expression of hundreds of genes including many cytokines and growth factors [14]. However, the functional role of these changes remains to be established as does the applicability to the growth plate in vivo.

Glucocorticoids above physiological levels inhibit not only growth plate function but also growth plate senescence. Growth plate senescence is a developmental process that occurs during juvenile postnatal life [15]. With increasing age, the growth plate progressively involutes. The overall height of the growth plate decreases because of a decrease in the number of resting, proliferative, and hypertrophic chondrocytes [16]. Individual hypertrophic chondrocytes do not grow as large in an older animal and the chondrocyte columns become more widely spaced, with less intervening matrix [16]. In addition, growth plates undergo a progressive loss of function with age, primarily because of a decrease in the rate of chondrocyte proliferation [16]. Thus, growth plate senescence explains why linear growth progressively slows in postnatal life and eventually ceases.

Glucocorticoid excess slows the programmed senescence of the growth plate in young rabbits [17]. This slowing of senescence has also been observed with growth inhibiting conditions, including hypothyroidism [18] and tryptophan deficiency (unpublished data), suggesting that senescence is driven by the process of growth, rather than age itself. Each of these experiments involved a similar conceptual design in which growth was inhibited transiently in young animals and then the growth inhibiting condition was withdrawn. After the animals had recovered, the growth plates were examined to determine whether the preceding period of growth inhibition had delayed the program of senescence. In these experiments the growth-inhibiting conditions did delay the progression of multiple structural markers of senescence, such as the decline in growth plate height, functional markers such as the decline in proliferation in the proliferative zone, and molecular markers, such as the decline in IGF2 mRNA expression.

The mechanism by which growth drives growth plate senescence is not known. One possibility is that chondrocytes in the growth plate might contain a cell-cycle counter. This counter could be achieved if some characteristic of the cell were progressively increased or decreased with each successive cell division and this characteristic affected the subsequent behavior of the cell. While it is unclear whether a cell-cycle counter exists in the growth plate, there is evidence suggesting that a counter exists in some other mammalian tissues. For example, removal of pancreatic progenitor cells in mice during early development results in a smaller pancreas [19], implying that these progenitor cells undergo a defined number of cell divisions before proliferation ceases. If such a cell-division counter is responsible for growth plate senescence, it seems likely that it would reside in the resting zone chondrocytes. Cell-cycle counting would not occur in hypertrophic chondrocytes because these cells are terminally differentiated and do not proliferate. Cell-cycle counting in the proliferative zone would not readily provide an explanation for progression of senescence. Proliferative zone chondrocytes replicate transiently but then undergo hypertrophic differentiation, then apoptosis. Thus these cells remain in the growth plate only transiently and thus are unlikely to store long-term information about the growth history of the growth plate. In contrast, the resting zone chondrocytes, which serve as progenitor cells, are thought to persist in the growth plate for the long term. This line of reasoning suggests that senescence might be a function of the number of cell divisions undergone by the resting zone chondrocytes [20].

Specifically, resting zone chondrocytes might have a finite proliferative capacity which is gradually exhausted, causing depletion of these cells. In vivo labeling experiments in rabbits support this concept. Resting zone chondrocytes do become depleted with age, both quantitatively depleted, that is, the number of these cells decreases with age, and also qualitatively depleted, that is, the replication rate of these cells declines with age [20]. Furthermore, administration of dexamethasone to juvenile rabbits slows the replication rate of the resting zone chondrocytes and slows their numerical depletion [20]. Thus, taken together, the findings support the concept that glucocorticoids slow senescence by inhibiting

resting zone proliferation and consequently slowing the depletion of these stem-like cells. If this model is correct, then resting zone chondrocytes are not true stem cells, because one of the defining characteristics of a stem cell is the ability to proliferate indefinitely, or at least as long as the life span of the organism. Consequently, these resting zone chondrocytes have been labeled, stem-like cells [15].

The effect of glucocorticoid excess on growth plate senescence may provide an explanation for the phenomenon of catch-up growth. Catch-up growth is defined as a growth rate greater than is normal for chronological age following a period of growth inhibition [21]. Catch-up growth has been observed after a variety of growth-inhibiting conditions, including glucocorticoid excess, in humans and other mammals [21]. It has been suggested that catch-up growth might be caused by a neuroendocrine mechanism within the central nervous system that senses the child's body size, compares that actual body size to an age-appropriate set point, and then adjusts the growth rate accordingly [22]. However, more recent data are not compatible with this neuroendocrine hypothesis. Dexamethasone was infused locally into the growth plates of juvenile rabbits for four weeks causing growth inhibition [23]. After the dexamethasone infusion ended, local catch-up growth was observed solely within the dexamethasone-treated growth plates. This anatomic specificity is not readily explained by any systemic mechanism but instead suggests that the mechanism responsible for catch-up growth resides within the growth plates themselves [23]. Furthermore, the growth pattern in the growth plates that had previously received dexamethasone suggested that the catch-up growth occurred because of a delay in the normal senescent decline in the growth rate. Similar patterns have been reported in catch-up growth in rabbits following systemic glucocorticoid treatment [17], in rats following hypothyroidism [18], and in children with celiac disease placed on a gluten-free diet [24]. In each case, once the growth-inhibiting condition resolved, the ensuing growth rate appeared to be right shift of the normal senescent decline in growth rate. Furthermore, in the animal studies, the magnitude of the time shift was similar to the delay in the other markers of growth plate senescence. Thus, taken together, the findings support the hypothesis that catch-up growth occurs because growth-inhibiting conditions slow growth plate senescence. Consequently, after the growth inhibiting condition resolves, the growth plates are less senescent and therefore behave like younger growth plates, growing more rapidly and for a longer period of time than is normal for age, causing catch-up growth.

Catch-up growth following glucocorticoid exposure occurs not only in longitudinal bone growth but also in cross-sectional growth [25]. Dexamethasone administration to juvenile rabbits slows periosteal bone formation, resulting in a thinner bone shaft. However, after the dexamethasone treatment was stopped the rate of periosteal bone formation in these rabbits exceeded that of controls, resulting in catch-up growth in bone width [25]. Whether this catch-up growth at the periosteal surface involves mechanisms analogous to those at the growth plate is unknown.

In summary, glucocorticoid in excess of physiological levels can inhibit longitudinal bone growth by a direct, local action at the growth plate. This slowing of growth appears to involve an inhibition of chondrocyte proliferation, hypertrophy, and cartilage matrix secretion. Glucocorticoid excess also slows growth plate senescence. Senescence is slowed by other growth-inhibiting conditions, suggesting that senescence is not a function solely of age but rather of growth itself. One attractive hypothesis is that stem-like cells in the resting zone have a finite proliferative capacity, and, as this capacity is exhausted, growth in the growth plate progressively slows. Glucocorticoid excess therefore may slow senescence by slowing proliferation in these cells, thereby conserving their proliferative capacity.

Acknowledgments

This work was supported by the Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH.

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