

Nonruminant Nutrition 8: Skeletal Tissue:
Applications from a Physiology/Endocrine Functional Perspective

The Regulation of Growth Plate Cartilage Turnover¹

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ABSTRACT: The advances made in the areas of genetics and nutrition during this century have resulted in improved growth rates for livestock. However, one drawback has been the increased prevalence of long bone growth problems, such as rickets, avian tibial dyschondroplasia, and osteochondrosis. Growth plate cartilage, which regulates long bone development, must maintain a tightly controlled balance between cartilage synthesis and degradation as well as chondrocyte proliferation and apoptosis. This paper will briefly review the various nutritional

factors, cell signals, and proteins that help regulate growth plate chondrocytes. Some of the growth plate diseases will be discussed with an emphasis on how a breakdown in growth plate metabolism is related to the observed problems. The author's intention is that readers will gain an appreciation for the complexity of this relatively small tissue and for why a better understanding of its physiology will be important in the years to come for the prevention of skeletal problems related to long bone growth.

Key Words: Growth, Reviews, Osteochondritis, Dyschondroplasia, Growth Factors

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Overview of Growth Plate Cartilage Biology

Growth Plate

In rapidly developing bones, such as the tibia and femur, growth plate cartilage determines the rate of longitudinal growth as well as the ultimate length of bones. Active chondrocytes progress through primarily three phases during their life cycle in the growth plate: proliferation, differentiation/hypertrophy, and apoptosis. Growth plate chondrocyte turnover, as defined for this paper, is the rate at which chondrocytes go through these phases; it varies depending on the species and stage of development of the animal. Chondrocytes can move from the top to the bottom of the growth plate in less than 24 h in broiler chickens, whereas in rabbits the process takes closer to 4 d (Sissons, 1953; Thorp, 1988). Vascularization from

metaphyseal arteries in subchondral bone occurs in the lacunae of apoptotic chondrocytes, bringing endothelial cells and osteoblasts into the region to initiate bone formation. The cell products and signals involved in the various phases of chondrocyte development will be briefly summarized (see Table 1).

Proliferation. The growth plate consists primarily of three zones: the resting, proliferative, and hypertrophic zones. Proliferating chondrocytes are likely recruited from the resting zone. Besides undergoing cell division, chondrocytes produce extracellular matrix macromolecules, including Types II, IX, and XI collagen and proteoglycan, specifically aggrecan, that give the growth plate its structure. Type II collagen is the predominant fibrillar collagen in the matrix. Type XI collagen is enmeshed in collagen II fibrils and might help regulate fibril diameter size (Mendler et al., 1989). Type IX collagen is unique in that it contains a proteoglycan moiety. It is covalently cross-linked to the surface of collagen II fibrils and presumably interacts with other extracellular proteins (van der Rest and Mayne, 1988; Smith and Brandt, 1992). Aggrecan, a large aggregating proteoglycan, is

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Table 1. Cellular signals and proteins found in the growth plate

| Phase of Maturation | Cellular signaling molecules ^a | Proteins synthesized by growth plate chondrocytes |
|-----------------------------|---|---|
| Proliferation | bFGF | Syndecan-3 |
| | IGF-1 | Aggrecan |
| | PDGF | Type II Collagen |
| | TGF β | Type IX Collagen |
| | GH (other than avian) | Type XI Collagen |
| | Thyroxine (with IGF-1) | Heat Shock Protein (HSP) 110 |
| | C-myc | Gelatinase A (MMP-2) |
| Differentiation/Hypertrophy | C-myc | HSP 28,70 |
| | Vitamin A | Cartilage Matrix Protein |
| | Vitamin D | M-calpain (Calpain II) |
| | Calcium | Type X Collagen |
| | PTHrP | Fibronectin |
| | Indian Hedgehog | Collagenase-3 (MMP-13) |
| | bmp-6 | Cathepsin K |
| | C-raf-1 | Alkaline Phosphatase |
| | FGF | Osteocalcin |
| | Transferrin | Osteopontin |
| | Endothelial Cell Stimulating Angiogenesis Factor (ESAF) | Bone Sialoprotein |
| Apoptosis | Nitric Oxide (??) | Transglutaminase |

^abFGF = basic fibroblast growth factor; PDGF = platelet-derived growth factor; TGF β = transforming growth factor- β ; PTHrP = parathyroid hormone-related peptide; bmp-6 = bone morphogenic protein-6.

composed of a protein core to which approximately 100 chondroitin sulfate and 20 to 30 keratan sulfate chains are attached. Several aggrecan molecules bind to hyaluronic acid, forming high molecular weight complexes (MW 1 to 2 million). Aggrecan, being highly negatively charged and hydrophilic, absorbs biomechanical forces in the cartilage. The aggrecan/hyaluronic acid complex can modulate the response of chondrocytes to its microenvironment via interactions with cell surface receptors (Dowthwaite et al., 1998). Epiphyseal arteries provide proliferating chondrocytes nutrients, oxygen, and growth factors. Insulin-like growth factor 1, basic fibroblast growth factor (**bFGF**), transforming growth factor- β (**TGF β**), platelet-derived growth factor (**PDGF**), and thyroxine are some of the factors that have mitogenic activity in chondrocytes primarily isolated from the proliferative zone of avian growth plate (Rosselot et al., 1994). Growth hormone has been shown to upregulate IGF-1 expression in rat rib growth plate chondrocytes (Isgaard et al., 1988). In avian postembryonic chondrocytes GH had no direct effect on cell proliferation or proteoglycan synthesis (Rosselot et al., 1994). Most of the data on the effect of growth factors on chondrocyte proliferation has been obtained from in vitro culture systems. The relative importance of each factor in vivo as well as their interactions are not well

understood. Relatively high levels of bFGF can be detected in the proliferative zone of the avian growth plate after partial enzymatic digestion of the matrix (Twal et al., 1996).

Differentiation. Proliferating chondrocytes eventually undergo differentiation and hypertrophy. Hypertrophic chondrocytes regulate the rate of longitudinal growth and facilitate the interactions with subchondral bone. During differentiation, chondrocytes begin expressing several new proteins and can grow to ten times their original size. Some of these proteins include cartilage matrix protein, fibronectin, and collagen X (Schmid and Linsenmeyer, 1985b; Chen and Linsenmeyer, 1993; Chen et al., 1996). The function of cartilage matrix protein and fibronectin in the growth plate are not well understood. Type X collagen, short chain and non-fibrillar, likely helps facilitate either mineralization or matrix degradation near the chondro-osseous junction (Schmid et al., 1986; Schmmid et al., 1991). Many proteins involved in mineralization of the extracellular matrix are expressed in hypertrophic chondrocytes, including osteocalcin, osteopontin, bone sialoprotein, and alkaline phosphatase. Proteolytic enzymes such as m-Calpain (Calpain II) and collagenase-3 (**MMP-13**) are expressed so that the pericellular matrix can be degraded and chondrocytes can expand (Yasuda et al.,

1995; Johansson et al., 1997). Calcitonin and bone morphogenic protein-6 (**bmp-6**) are involved in regulating differentiation. Recently, Indian hedgehog and parathyroid hormone-related peptide (**PTHrP**) have been shown to help control the rate of differentiation (Vortkamp et al., 1996). In culture, as chondrocytes increase in size they increase their production of $TGF\beta$, suggesting it might also be involved in chondrocyte differentiation (Gelb et al., 1990). As chondrocytes approach subchondral bone, certain angiogenic factors, such as bFGF, endothelial cell stimulating angiogenesis factor, and transferrin are expressed (McFarland et al., 1990; Twal et al., 1996; Carlevaro et al., 1997). Some vitamins and minerals can impact chondrocyte differentiation. Vitamin A (retinoic acid) in cell culture will stimulate chondrocyte differentiation and matrix calcification (Iwamoto et al., 1993; Wu et al., 1997). A Vitamin A-deficient diet will lead to a poorly differentiated, enlarged growth plate, while Vitamin A toxicity will prematurely close the growth plate. A deficiency of Vitamin D, phosphorus, or calcium will lead to rickets, which is characterized by an elongation of the growth plate due to a significant increase in the number of hypertrophic chondrocytes. The complexity of long bone growth is suggested by the number of dietary factors, growth factors, and other cellular signals involved in chondrocyte differentiation.

Apoptosis. In order for vascularization and bone formation to occur, hypertrophic chondrocytes adjacent to the chondro-osseous junction undergo apoptosis (Farnum and Wilsman, 1987; Farnum and Wilsman, 1989). The transition from a hypertrophic to apoptotic chondrocyte currently is not well characterized. Expression of the bcl-2 protein in cells will prevent apoptosis. In growth plate chondrocytes, PTHrP up-regulates bcl-2 expression as part of its mechanism to control the rate of chondrocyte turnover (Amling et al., 1997). PTHrP-deficient mice have accelerated chondrocyte apoptosis that coincides with decreased bcl-2 mRNA (Lee et al., 1996). The penetrating vasculature from subchondral bone could initiate apoptosis. Recently, mice deficient in gelatinase B (**MMP-9**) were shown to have delayed apoptosis in hypertrophic chondrocytes (Vu et al., 1998). Gelatinase B is expressed in cells at the vascularization front of the chondro-osseous junction and might be involved in releasing growth factors or other signal-transducing molecules from the extracellular matrix of hypertrophic cartilage. The biology involved in apoptosis of growth plate chondrocytes needs to be further clarified.

Current Research Using an In Vitro Model for Cartilage Degradation

An explant culture system has been developed to study apoptosis and extracellular matrix degradation using tibiae of d-12 chick embryos (Cole et al., 1992, 1993). The tibiae consist of developing cartilage, a bony sheath, and marrow. The cartilage at both ends of the tibia contain a proliferative and hypertrophic zone similar to that found in posthatch growth plate cartilage (Schmid and Linsenmeyer, 1985a). Also, the explant culture maintains the interactions of bone and marrow that are present in vivo. Cole et al. (1992) provided evidence that bone and marrow are required for complete cartilage degradation. When stimulated by lipopolysaccharide (**LPS**) or other catabolic agents, tibiae in culture produce proteolytic enzymes that degrade the two major structural components of the extracellular matrix, proteoglycan followed by collagen. Metalloproteinases released from the tibiae peak in the conditioned media simultaneously with collagen release (Cole et al., 1993). Based on histology, we have found that a majority of chondrocytes within the proliferative zone first undergo hypertrophy and then later apoptosis. This explant culture system can provide important information in understanding growth plate cartilage turnover. In vivo, a chondrocyte's life cycle is short and apoptotic chondrocytes can be scarce because they quickly are resorbed by nearby phagocytic cells. In the explant culture, we can follow chondrocyte development from the proliferative to apoptotic stage over an extended period of time (10 to 14 d). Apoptotic cells remain in the cartilage until the surrounding matrix has been degraded.

One of our long-term objectives is to identify molecule(s) that regulate apoptosis and extracellular matrix degradation in the growth plate. One potential molecule is nitric oxide, which has been shown to increase the expression of gelatinase B, suppress proteoglycan synthesis, and induce chondrocyte apoptosis in articular chondrocytes (Häuselmann et al., 1994; Blanco et al., 1995; Sasaki et al., 1998). In our cultures, proteoglycan release from the cartilage in the tibiae coincides with a peak in nitric oxide production (Figure 1). Pyrrolidine dithiocarbamate, a known inhibitor of nitric oxide synthase expression, inhibited LPS-induced nitric oxide production and cartilage degradation (Figures 2 and 3). Furthermore, the increased nitric oxide production coincides with the transition of chondrocytes from hypertrophy to apoptosis during the culture period (Chlebik-Brown, 1996). Other catabolic factors induced by LPS (i.e., interleu-

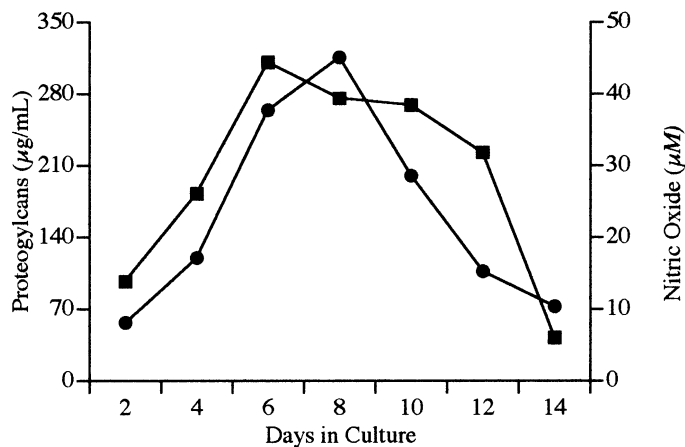


Figure 1. Nitric oxide (●) and proteoglycan (■) concentrations in conditioned media from embryonic chick tibiae cultured in the presence of lipopolysaccharide (LPS) to induce cartilage degradation.

kin-1, tumor necrosis factor) could be responsible for proteoglycan degradation. We are also in the process of looking at the effects of nitric oxide donors on apoptosis in our explant culture.

We are also investigating the role of m-calpain as a regulator of chondrocyte apoptosis and extracellular

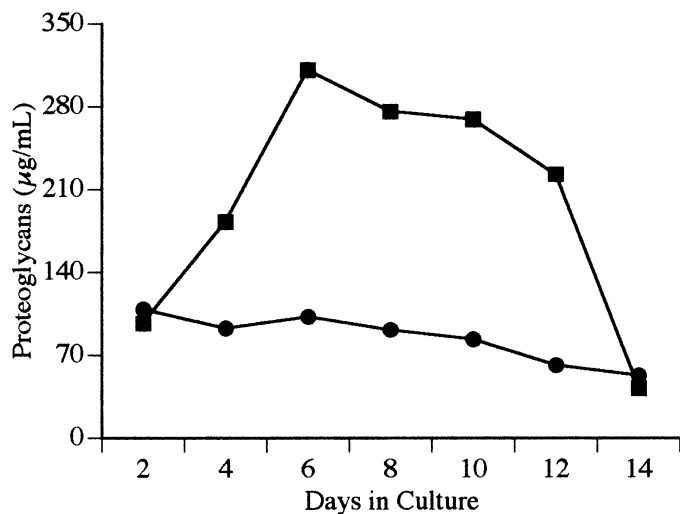


Figure 2. The effect of pyrrolidine dithiocarbamate (PDTC) on proteoglycan concentrations in conditioned media from cultured embryonic chick tibiae. Tibiae were cultured in the presence of either no PDTC (■) or 100 µM PDTC (●). The decreased concentrations of proteoglycans in the media from tibiae treated with PDTC is indicative of decreased cartilage degradation.

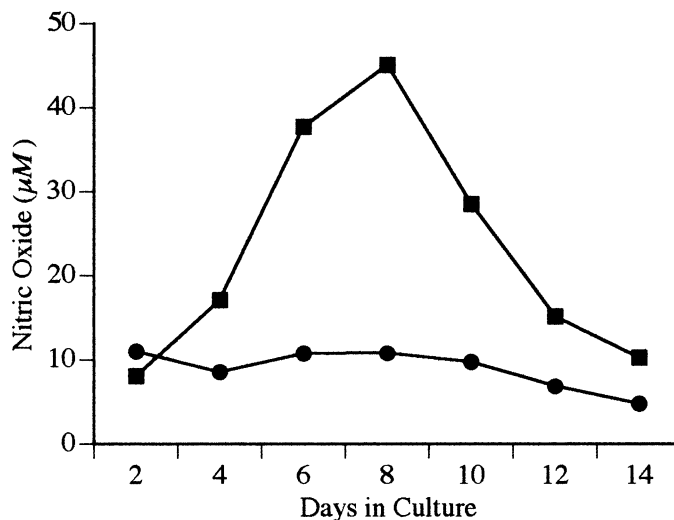


Figure 3. The effect of pyrrolidine dithiocarbamate (PDTC) on nitric oxide concentrations in conditioned media from cultured embryonic chick tibiae. Tibiae were cultured in the presence of either no PDTC (■) or 100 µM PDTC (●).

matrix degradation. M-calpain is a cysteine protease that requires millimolar concentrations of Ca for activation (Suzuki et al., 1992). The hypertrophic region of cartilage formed from rat growth plate chondrocytes in vitro contains m-calpain (Shimizu et al. 1991; Yasuda et al., 1995). Calpain is also an upstream regulator of thymocyte apoptosis (Squier and Cohen, 1997). Inhibitors of calpain block the LPS-induced production of nitric oxide in macrophages, suggesting that it could regulate the expression of nitric oxide synthase by activating nuclear factor-kB (Griscavage et al., 1995). Thus, m-calpain and nitric oxide in the growth plate might help regulate cartilage turnover (Figure 4).

Implications in the Livestock Industry

Proper development of a long bone requires that growth plate cartilage maintain a delicate balance of cartilage synthesis and degradation followed by bone formation. In the animal industry, the emphasis on maximizing the growth rate of livestock through genetics and nutrition has led to more abnormalities associated with the growth plate and long bone growth. As an example, within the past 30 yr the growth rate of birds reared for meat production has increased dramatically (Lilburn, 1994). A problem that developed because of the accelerated growth is

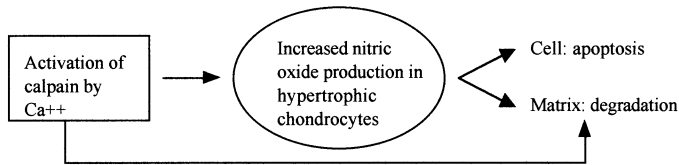


Figure 4. The proposed model for the role of calpain and nitric oxide in growth plate chondrocyte turnover.

tibial dyschondroplasia (**TD**), a disorder in which growth plate cartilage accumulates in the proximal region of the tibia and femur. It was an insignificant health concern over 30 yr ago, whereas currently it is found in 50% of broiler chickens (Leach, 1996). Problems associated with TD include lameness, increased fractures in the fibula, and an increased susceptibility to osteomyelitis. In turkeys, it increases the incidence of breast blisters. All of these problems can lead to significant economic losses at the processing plant, exceeding \$200 million in a single year (Edwards, 1983). Some experimental conditions and compounds that have been found to induce TD include fusarochromanone, thiram, antabuse, excessive dietary levels of cysteine and homocysteine, Cu deficiency, metabolic acidosis, and some environmental conditions (Orth and Cook, 1994). However, the etiology of TD in the field has not been clarified. The problem is that growth plate cartilage in birds with TD is not properly degraded and accumulates at the expense of bone formation (Freedman et al., 1985; Orth et al., 1991). Chondrocyte differentiation and hypertrophy are interrupted before cell maturation is reached (Hargest et al., 1985; Bashey et al., 1989). A recent finding links impairment of apoptosis to TD. The researchers compared histological sections from normal and dyschondroplastic growth plates and found that relatively few apoptotic chondrocytes were present in dyschondroplastic cartilage (Ohyama et al., 1997). Thus, understanding the regulatory molecules in chondrocyte differentiation and apoptosis could be critical to alleviating or preventing this growth plate disorder. Also, birds reared for meat production can develop a rachitic growth plate very quickly if the proper ratio and amounts of Ca and P are not in the diet.

Osteochondrosis (**OCD**) is a developing cartilage disorder with similarities to TD that occurs in domesticated animals. It is characterized by a failure of endochondral ossification in the articular/epiphyseal and growth plate cartilage in the weight-bearing region of long bones. Osteochondrosis can be a significant economic problem (Hill, 1990; Jeffcott,

1991). In pigs, growth rate and hormonal imbalance have been implicated in the disease. The use of recombinant porcine somatotropin by either injection or a slow-release implant increased the incidence of OCD in Yorkshire pigs (He et al., 1994). Growth rate was not a factor in this experiment since pigs that received a slow-release implant grew at the same rate as control animals. Excessive levels of growth hormone, and possibly other growth factors such as IGF-1, might disrupt the balance between cartilage synthesis and degradation. Osteochondrotic cartilage contains decreased percentages of uronic acid (component of proteoglycans), collagen, and collagen cross-links when compared to unaffected age-matched cartilage (He et al., 1994; Wardale and Duance, 1994). Abnormal proteoglycans were found in osteochondrotic lesions isolated from horses (Lillich et al., 1997). Others have observed problems with the cartilage canal blood supply in both horses and pigs (Carlson et al., 1991, 1995). Necrotic chondrocytes were located around necrotic blood vessels and thus the cells could not fully differentiate. In another study, lesions contained small rounded chondrocytes that suggested a disruption in the progression from the proliferative to hypertrophic phase (Henson et al., 1997). Some causative factors of OCD in horses that have been reported include diets high in digestible energy, P, Zn, and a deficiency in Cu (Lillich et al., 1997).

Because of the susceptibility of developing cartilage to problems during growth, good skeletal structure should be considered when selecting livestock for production. Identification of biological markers that can be used to monitor long bone growth could facilitate the selection process. Unfortunately, selection for good bone growth or structure currently does not appear to correlate well with overall growth performance. Boars selected for both gait and growth performance had a 24% reduction in growth performance relative to boars selected only for growth performance (Steenbergen et al., 1990). Turkeys selected for walking ability had reduced breast widths relative to commercial lines (Ye et al., 1997). Thus, the challenge for geneticists and nutritionists will be to maintain and, in some cases, improve skeletal structure while improvements to lean tissue growth rates and feed conversion are being pursued.

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