Growth plate studies earn Kappa Delta Award

By Maureen Leahy

MULTIDIMENSIONAL ANALYSIS PROVIDES INSIGHT INTO BONE ELONGATION

What began as a study of osteochondrosis 30 years ago has resulted in the awarding of the 2010 Kappa Delta Elizabeth Winston Lanier Award to Cornelia E. Farnum, DVM, PhD, and Norman J. Wilsman, DVM, PhD. They summarized their decades of research in their award-winning paper "Analyzing the Growth Plate in Four Dimensions: A Thirty-year Perspective on Growth Plate Dynamics."

Osteochondrosis, which is associated with maturation of articular and growth plate cartilage in children, is also significant in several animal species. It was the contrast between articular cartilage and growth plate cartilage—specifically the dynamic nature of growth plates—that first captivated Dr. Farnum.

"As veterinarians, we are interested in diseases that affect domestic animals, but we are also interested in how those diseases parallel diseases in humans," explained Dr. Farnum. "This was the first time I really had the opportunity to look at growth plates and realized that they are a rapidly growing organ that is also transitory. I was very interested in trying to figure out how cellular activity within the growth plates works and how it leads to bone elongation."

Over the years, her research focused on the kinetic analysis of growth plate chondrocytes using primarily morphologic- and physiologic-based techniques.

Evolving methodology

When she began her studies, researchers were still debating what the cells of the growth plate looked like in situ because preservation of chondrocytes is particularly tricky, said Dr. Farnum. As a veterinarian, she was in a unique position to study this in fairly large animal models. For example, the growth plates and articular cartilage in swine closely parallel how these tissues look and act in growing children.

Cornelia E. Farnum, DVM, PhD

Advances in imaging technology over the years enabled Drs. Farnum and Wilsman to continue these parallel analyses. As imaging modalities evolved, the researchers were also able to take advantage of advances in stereology, a branch of the mathematical sciences that enhances the accuracy of the quantitative aspects of their imaging. As a result, they've been able to make analyses that are more consistent with what is thought to be happening in vivo.

Adding the dimension of time has also been key for converting two-dimensional images of growth plates into the dynamic tissues that they are, according to the authors.

"We were always able to examine the tissue in four dimensions, even when it was preserved, because labeling techniques enabled us to measure growth over a 24-hour period," said Dr. Farnum. "We could bring time into the analysis, but it was not real time."

By using multi-photon microscopy, they've been able to look at the entrance of systemically introduced molecules into the growth plates of live, anesthetized mice, in real time from three different vascular interfaces. In his lab at the University of Wisconsin, with the help of Mark Markel, DVM, PhD, and orthopaedic surgeon **Kenneth Noonan, MD,** Dr. Wilsman placed miniature recording devices over the growth plates of live, unrestrained lambs. The devices recorded in micrometers—every 3 minutes—how much the growth plate had actually increased bone elongation.

Norman J. Wilsman, DVM, PhD, with his Running Walker hound Kramer.

This evolution—from analyzing growth plates on preserved tissues to analyzing growth plates on living tissues—has been a significant aspect of their research, according to Dr. Farnum.

Animal studies demonstrate differentiation cascade

By incorporating volumetric and time dimensions into the analysis of growth plate morphology, Drs. Farnum and Wilsman hope to more effectively demonstrate the dynamics of the chondrocytic differentiation cascade that are not apparent from standard histological images. They've used the techniques in a variety of animal experimental systems.

The percentage contribution of the proximal or distal growth plate to total length of a given bone is remarkably consistent across a range of species, and the importance of synchronicity is clearest for the four growth plates in the radius and ulna of the forearm. Because growth plates not only develop at different rates but also close at different times (differential growth), the researchers used time-based stereologic analysis of multiple independent variables to determine chondrocytic dynamics in the proliferative and hypertrophic cell zones.

A significant finding was that "cellular enlargement, although always the most significant contributor to quantitative elongation, is more significant the faster the growth plate is elongating." This is important, the authors point out, for making inferences about chondrocytic dynamics in children based on results from animal models, because animals mature so much faster than humans.

To more fully understand how chondrocytic dynamics within the differentiation cascade are translated into bone elongation, the researchers used the following models:

1. *Animal models of compromised growth resulting in spontaneously occurring chondrodysplasias and dwarfisms:* In a study of young Scottish Deerhound dogs with pseudoachondroplasia (PSACH), similar to the disease in children, the researchers found that "appropriate shape of chondrocytes in each differentiative stage is critical for achieving maximum elongation potential for a given cellular volume increase." They further determined

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BOTTOM LINE

- No one factor can account for differential growth in growth plates.
- The faster growth plates elongate, the more significant cellular enlargement is.
- Studies of chondrocytes and growth plate development in animals provide keys for treating human growth plate disorders.

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that "final height/width axial ratios in hypertrophic cells that are low compared to controls indicate that a greater than normal component of the volume increase is being translated into increase in width rather than height." This may explain the finding of metaphyseal flaring in multiple different chondrodysplasias.

2. *Nutritionally based experiments with rodents to manipulate the rate of bone elongation:* To study the effect of nutritional status on the rates of elongation, including catch-up growth, the researchers analyzed growth plate dynamics in two different rodent models with compromised postnatal nutrition. Responses from the two models were markedly different, providing insight into the complex nature of growth rate responses.
3. *Experimental manipulation with rodents to mimic therapeutic strategies used by orthopaedic surgeons to correct growth plate abnormalities in growing children:* The researchers studied the effect of stapling across the proximal tibial growth plate of young rats to analyze the chondrocytic kinetic responses initiated by a mechanical description. The results again demonstrated that proliferative and hypertrophic responses act together to decrease bone elongation.

Recently, the researchers have focused on live animal-based experimental systems that enable them to monitor growth plate dynamics over significantly decreased time scales. "The unexpected nature of results using these approaches has convinced us that adding the fourth dimension of time, on multiple scales, was and is essential for understanding responsive capabilities of the growth plate," they wrote.

Clinical relevance

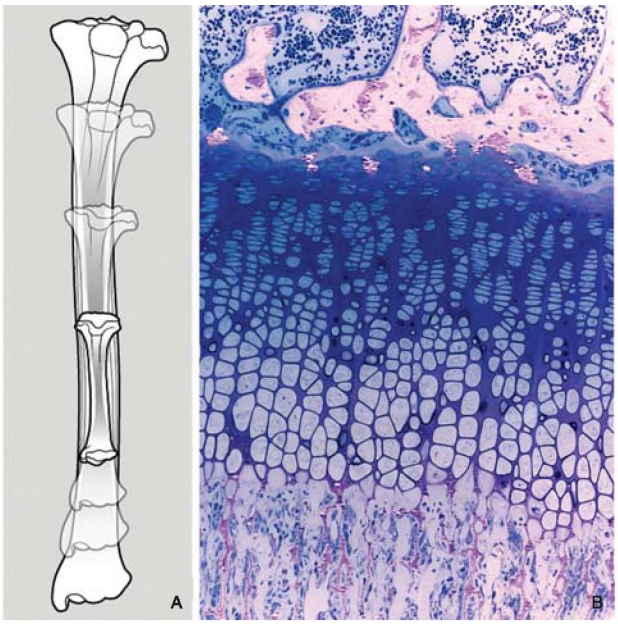
Such research can have specific clinical implications, particularly for pediatrics. "Putting what I study as a basic scientist into a clinically relevant context is very important," said Dr. Farnum. "During our analysis of how growth plates function we used a variety of models that have direct parallels to bone elongation problems in young children—chondrodysplasias, growth hormone deficiencies, nutritional deficiencies. Our research goes a long way to increasing the understanding of the biology behind what is going on with those diseases."

Dr. Noonan, who worked with Dr. Wilsman for 5 years at the University of Wisconsin, agrees.

"I began my collaboration with Dr. Wilsman as a scientific voyeur to observe growth plate function. As time progressed, I was able to propose ideas that may have clinical importance," said Dr. Noonan. "As my role expanded from collaborator to co-investigator, we began to study the efficacy of different growth plate implants on the ability to correct limb deformity. Dr. Wilsman still provides mentorship in these ongoing studies; his philosophy is that results are for all to study, critique, and build on for the benefit of all living creatures."

"Partnering with practicing orthopaedic surgeons enables us to balance our scientific perspectives with very clinical perspectives," explained Dr. Farnum, "and to increase the knowledge of the basic biology of the growth plate, which has the potential to lead to advances in how patients are treated."

Dr. Wilsman added, "We call these collaborations 'One Medicine' and we credit our departments, colleges, and institutions (Cornell University and the University of Wisconsin-Madison) for fostering them as seamlessly as possible." NOW



Courtesy of Cornelia E. Farnum, DVM, PhD

Fig. 1 In a histologcial section, a postnatal growth plate is seen as organized columns (or clones) of chondrocytes between epiphyseal and metaphyseal bone. The morphology of growth plate chondrocytes changes from flattened disc-shaped cells on the epiphyseal side (A) to enlarged egg-shaped cells on the metaphyseal side (B). This morphological change mirrors the two principle activities of chondrocytes–cellular proliferation that controls the number of cells and cellular enlargement referred to as hypertrophy. Matrix synthesis in both zones also contributes to elongation.

Dr. Farnum will present her award-winning paper on Tuesday, March 9, in Auditorium A of the Morial Convention Center, as part of the Orthopaedic Research Society Annual Meeting; the Kappa Delta Elizabeth Winston Lanier Award will be presented to the authors during the Opening Ceremonies of the AAOS Annual Meeting, on Wednesday, March 10, in the La Nouvelle Ballroom, Morial Convention Center.

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biomechanical changes. "More material of lesser quality is produced when activity level is increased," according to Dr. Soslowsky.

Surgery to repair injury to the rotator cuff will result in reduced range of motion, but immobilization after surgery will not result in permanent loss of motion or increase in joint stiffness. "Because immobilization does improve the repair properties at the insertion site where repairs often fail, immobilization should be considered in the postsurgical management of rotator cuff tears," said Dr. Soslowsky.

Additionally, his research shows that early passive motion during immobilization is detrimental to shoulder mechanics; when or whether passive motion should start is a subject that is still being investigated.

Early repair, however, is supported by Dr. Soslowsky's findings. "Repair tension—the force required at repair to reappose the tendon to its original insertion site on the humerus—rapidly increases after the injury, followed by a progressive, but less dramatic, increase with additional time. These findings suggest that rotator cuff tears should be repaired early, when possible, in the clinic."

Clinical impact

Dr. Soslowsky is well aware of the clinical applications that may result from his research. "Based on the fundamental nature of the information obtained to date, future investigations, by my team and by others, are now poised to investigate true mechanisms of injury and healing at the molecular, cellular, and tissue level in this and other model systems," he said. "We plan to continue this important basic science and clinically relevant research to address this common and disabling problem." NOW

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