

**Pre-clinical models for skeletal research: How commonly used species mimic (or don't)  
aspects of human bone.**

Matthew R. Allen<sup>1,2,3</sup>

<sup>1</sup>Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis  
IN, United States

<sup>2</sup>Department of Medicine/Division of Nephrology, Indiana University School of Medicine,  
Indianapolis IN, United States

<sup>3</sup>Department of Biomedical Engineering, Indiana University Purdue University of Indianapolis,  
Indianapolis, IN, United States.

Send Correspondence to:

Matthew R. Allen, PhD  
Indiana University School of Medicine  
635 Barnhill Dr.  
Indianapolis, IN 46202  
Tel: 317-274-1283  
Email: [matallen@iupui.edu](mailto:matallen@iupui.edu)

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## **ABSTRACT**

Preclinical studies play an indispensable role in exploring the biological regulation of the musculoskeletal system. They are required in all drug development pipelines where both small and large animal models are needed to understand efficacy and side effects. This brief review highlights four aspects of human bone, longitudinal bone growth, intracortical remodeling, collagen/mineral interface, and age-related changes and discusses how various animal models recapitulate (or don't) these aspects of human skeletal physiology.

**Keywords:** animal model, remodeling, longitudinal growth, mineral/collagen, aging

## **Introduction**

The value of preclinical models in biological research is clear. Most discussions of preclinical models are divided into rodent (rats/mice) and large animal (such as rabbit, dog, pig) classifications. The vast majority of pre-clinical studies across physiological systems utilize rodents, due to a number of factors, the most prominent of which is the ability for genetic manipulation to answer mechanistic questions. Pre-clinical studies of the musculoskeletal system also make heavy use of rodent models. This is despite the fact that there are clear limitations to rodents with respect to key aspects of skeletal biology. The goal of this brief review is to provide an overview of four aspects of human bone and then to discuss how various animal models recapitulate (or don't) these aspects of human skeletal physiology.

## **Longitudinal bone growth**

Bones are formed through either intramembranous or endochondral ossification processes (Burr and Allen, 2014). Endochondral ossification of long bones and vertebra is a multi-stage process that allows the transformation of a cartilaginous template into a mineralized structure. While much of the initial mineralization process is completed prenatally, the ends of the bone retain a highly active growth plate that allows continued longitudinal growth of a mineralized structure. Humans, many non-human primates, and common large animal research models such as dogs and rabbits experience closure (mineralization) of these growth plates in the years following puberty at which point longitudinal growth is no longer possible (Reinwald and Burr, 2008).

Rodents (rats and mice) do not undergo growth plate closure and therefore experience continued longitudinal growth throughout life. The size and characteristics of the growth plate cartilage change and the rate of growth slows with age yet the cells remain active and growth occurs at a rate of several microns per day (Roach et al., 2003). This has two main implications when it comes to using rodents as research models. First, any manipulation (such as genetic or

pharmacological) that affects chondrocytes will affect rodents in ways that would not occur in humans (Ogawa et al., 2002). Second, any intervention that is imparted on a rapidly growing animal (as is prominent in the literature) has the potential drastically affect the results. Take for example bisphosphonate drug treatment. The robust growth of rats was used early on as a tool to assess the efficacy of bisphosphonates, as inhibition of remodeling could easily be seen in the metaphysis over a relatively short time course (Schenk et al., 1973). Studies have shown that treatment of young, growing animals (2-5 weeks of age) can experience nearly a 4-fold increase in trabecular bone volume (Zhu et al., 2014). Visualization of the metaphyseal region shows the robust amount of mineralized tissue, yet this is mostly retention of the calcified cartilaginous scaffold formed during longitudinal bone growth. Even in older animals (16-24 weeks of age) robust increases in trabecular bone have been noted as these animals are still growing (Aref et al., 2016). In contrast, assessment of trabecular bone changes in humans (Recker et al., 2007), non-human primates (Smith et al., 2003), and dogs (Allen et al., 2006b) that all have ceased longitudinal growth show quite modest changes in trabecular bone following bisphosphonates. Given the coordination between longitudinal growth and activity not only on the trabecular bone, but in the cortex to shape the metaphysis, it is likely that effects of altering growth extend to the cortex too although those data are more challenging to capture.

### **Intracortical remodeling**

Bone remodeling serves as a tissue level mechanism for bone renewal (Robling et al., 2006). Through the coordinated action of osteoclast resorption followed by osteoblast formation, regions of bone are removed and replaced. Although stochastic (random) remodeling is thought to exist (Li et al., 2001; Martin, 2002), the majority of remodeling is believed to be targeted. Remodeling is known to target regions of bone containing damage, both linear microcracks and diffuse damage (regions of small submicron cracks) (Bentolila et al., 1998; Burr et al., 1985). There is also evidence that regions containing non-viable osteocytes are targeted by remodeling

(Tatsumi et al., 2007) and it's likely that other signals (such as hypermineralization or altered collagen properties) could signal for osteoclast-driven remodeling.

Human bone undergoes remodeling on trabecular and endocortical bone surfaces (those adjacent to marrow), as well as within the cortical bone (intracortical remodeling). The latter is the basis of forming secondary osteons, which play a critical role in bone mechanics. Several animal models used in skeletal research, such as non-human primates, dogs, rabbits, have normal intracortical remodeling (Jowsey, 1966; Reinwald and Burr, 2008). Rodents, rats and mice, lack intracortical remodeling under normal circumstances (Jowsey, 1966), although remodeling in the cortex can be stimulated by things like induction of damage (Bentolila et al., 1998), ablation of osteocytes (Tatsumi et al., 2007), or removal of endogenous estrogen (Kubek et al., 2010). There is also evidence in several genetic mouse strains of intracortical remodeling (Jilka et al., 2014) although the underlying mechanism of such activity has not been explored in great detail.

The absence of intracortical remodeling in rodents has important implications when studying effects on mechanical properties of bone. First, the role of secondary osteons in determining bone mechanical properties is well-established. This means the mechanical properties of rodent bone are unlikely to mimic human bone in conditions of interventions/manipulations that alter remodeling. As one example, a commonly used anti-remodeling drug class, bisphosphonate, exerts a potent suppression of remodeling (Allen, 2008). Large animal studies (mainly dog) have shown potent remodeling suppression (trabecular and intracortical) with bisphosphonates (Allen et al., 2006b; 2006a). Intracortical suppression is associated with numerous tissue-level changes in cortical bone such as higher levels of microdamage, alterations in collagen crosslink, and lower levels of mineral heterogeneity (Allen and Burr, 2007). These tissue-level change have all been shown in multiple human studies, but have not

been shown to manifest in rodents. Furthermore, changes in large animal models that have reductions in intracortical remodeling have associated these changes with reduced mechanical properties (Burr et al., 2015) – changes that also have not manifested in most rodent studies. Collectively, these distinct differences illustrate the importance of having a model that undergoes intracortical remodeling when studying cortical bone mechanical property changes to interventions.

### **Collagen/mineral interface**

Bone is a hierarchical structure which at its core is a composite material made up of hydroxyapatite mineral that is impregnated within a collagen matrix (Garnero, 2015). The mineral/collagen interface represents a fruitful area of study in part because it has, in general, been unexplored (Stock, 2015). Collagen has over a half-dozen described orientations (Warshaw et al., 2017), with rodent cortical bone having mainly rapidly-formed fibrolamellar collagen. This contrasts with more slowly-formed intracortically arranged collagen that occurs within secondary osteons. Whether these differences in collagen orientation affect collagen/mineral interface, or other aspects of the skeletal biology needs to be studied in more detail (Stock, 2015). Recent studies have shown that soaking in high concentrations of sodium fluoride (Silva and Ulrich, 2000) or raloxifene (Bivi et al., 2016; Gallant et al., 2014) have clear effects on bone mechanics thought to manifest through altering the bone mineral interface.

### **Age-related bone loss**

Skeletal aging is a complex process (Manolagas and Parfitt, 2010). A main driver of age-related bone loss in humans is changes in sex steroids, the most prominent of which is loss of estrogen with menopause although changes in sex steroids also contribute to age-related bone loss in men. Other factors, such as decreased activity and concomitant disease contribute to loss of bone with age.

Surgical ovariectomy is the most commonly used preclinical model used to study age-related skeletal changes (Jilka, 2013). Reduction of endogenous estrogen levels through ovariectomy results in bone loss in non-human primates, sheep, rabbits, and rodents (both rats and mice) but fails to do so in dogs in part due to their low basal estrogen levels (Priemel et al., 2002; Reinwald and Burr, 2008). Loss of trabecular bone, through increased osteoclast activity, has been shown in numerous rodent studies while increased cortical porosity has been shown in some mouse strains (Bonucci and Ballanti, 2014; Priemel et al., 2002).

Pure aging studies (aging the animal without any intervention) also show skeletal changes which mimic those in humans (Waarsing et al., 2006; Willingham et al., 2010; Wronski et al., 1989). Commonly used genetic mouse strains experience age-related bone loss, as do rats although these studies are not all that common as they take considerable time (a year in mice and many years in rats) (Jilka, 2013). There exists a number of premature aging mouse models, driven by genetic mutations, all of which show loss of bone that appears to mimic the human aging process (Watanabe and Duque, 2016). Pure aging studies in larger animals are limited, likely because of the time and expense (Black et al., 2001; DeRousseau, 1985; Detenbeck and Jowsey, 1969; Martin et al., 1981).

We know that human aging is complex and multifactorial given the numerous conditions that have increased risk of developing with age. Unfortunately, most aging studies take an isolationist approach, that is they study skeletal changes in an otherwise healthy animal. This type of 'healthy aging', while useful for studying the independent effects of age on the skeletal system, most certainly limits translation given the high prevalence of diseases such as atherosclerosis, renal disease, diabetes, obesity, osteoarthritis, and other diseases that could directly or indirectly affect the skeleton during aging. The field would greatly benefit from studies

in which integrative aging effects on the skeleton area assessed in models with multiple age-related conditions are modeled.

### **Conclusions**

Rodent have a long and storied history as models in biomedical research. Their utility in the study of the musculoskeletal system is undeniable, yet it is important to always remember the key physiological differences between rodents and humans. Continued longitudinal bone growth and lack of intracortical remodeling could have significant effects on how interventions affect the bone and thus must be considered when thinking about translation to humans.

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