

REVIEW

Adaptive and injury response of bone to mechanical loading

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Bone responds to supraphysiological mechanical loads by increasing bone formation. Depending on the applied strain magnitude (and other loading parameters) the response can be either adaptive (mostly lamellar bone) or injury (mostly woven bone). Seminal studies of Hert, Lanyon and Rubin originally established the basic 'rules' of bone mechanosensitivity. These were reinforced by subsequent studies using noninvasive rodent loading models, most notably by Turner *et al.* More recent works with these models have been able to explore the structural, transcriptional and molecular mechanisms which distinguish the two responses (lamellar vs woven). Wnt/Lrp signaling has emerged as a key mechanoresponsive pathway for lamellar bone. However, there is still much to study with regard to effects of ageing, osteocytes, other signaling pathways, and the molecular regulation that modulates lamellar vs woven bone formation. This review summarizes not only the historical findings but also the current data for these topics.

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Introduction

The topic of this review is how bone responds to increased mechanical loading. Functional bone adaptation, the relationship linking mechanical loading and bone structure, was recognized by Roux and Wolff well over a century ago.¹ However, only since the 1970s and 1980s have advances in animal models and strain measurement techniques allowed researchers to explore this relationship with a controlled experimental approach. The key experimental models for advancing this field have been ones in which controlled external forces are applied to the skeleton of a live animal, and the local mechanical strains engendered by said forces are known. The primary functional outcomes are changes in local bone structure or dynamic indices of bone formation. Generally speaking, when the intensity of applied loading is greater than habitual loading, a bone formation (modeling) response is produced. Recent advances in biological factor detection (for example, gene expression) and genetic manipulation (for example, knockout mice) have facilitated examination of the biological mechanisms underlying the relationship between loading and bone.

An important distinction in all these studies is whether the loading stimulus engenders a lamellar or woven bone response. Increased lamellar bone formation may be considered an adaptive response to mild/moderate overloading, whereas woven bone formation may be considered an overloading/injury response. Although an appreciation of the lamellar-woven dichotomy is not new, it is unfortunate that many reports do

not show histology or even state which type of bone formation was stimulated.

Our objectives in this article are to briefly summarize key 'classic' findings related to bone and mechanical loading, and to review recent work on lamellar vs woven bone formation, aging and mechanisms by which bone responds to loading. We focus on increased loading, and do not address disuse/unloading. Moreover, although a wealth of knowledge has been gained from *in vitro* and *in silico* experiments, as well as *in vivo* exercise experiments, their inclusion is beyond the scope of this brief review. We have chosen to mention only results from *in vivo* animal experiments that used controlled loading parameters.

Key Early Findings

Seminal studies from Hert, Lanyon and Rubin led to the overarching paradigms of cortical bone mechanoresponsiveness. The following 'rules' relating mechanical loading and cortical bone formation are widely accepted. First, dynamic loading elicits a response but static loading does not.^{2,3} Second, there exists a minimum strain threshold. Applied loads that produce strains below this threshold induce no change in bone formation whereas loads above this threshold increase bone formation in a dose-dependent manner.⁴ The exact magnitude of the threshold is context-dependent and may vary based on factors such as species, age, sex and loading model. Third, the anabolic effects of adaptive loading plateau after a relatively low number of cycles (< 100 cycles per day).⁵

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Table 1 Summary of the three noninvasive loading models

Loading model	Limb tested	Stimulated bone types	Animals used	Physiological?	Complications	References
Four-point bending	Tibia	Cortical	Rat and mouse	No	Woven bone at loading points	Akhter <i>et al.</i> ⁹ ; Silva and Brodt ¹⁰
Cantilever bending	Tibia	Cortical	Mouse	No	Limited strain range	Gross <i>et al.</i> ¹¹
Axial compression	Tibia Fibula Ulna	Cortical and trabecular	Mouse Mouse Rat and mouse	Yes		De Souza <i>et al.</i> ¹² Fritton <i>et al.</i> ¹³ Moustafa <i>et al.</i> ¹⁴ Kotha <i>et al.</i> ¹⁵ ; Lee <i>et al.</i> ¹⁶ ; Torrance <i>et al.</i> ¹⁷

Trabecular bone has received less attention because most loading models target cortical bone only. However, Chambers *et al.*⁶ developed a vertebral compression model in the 1990s, and several groups have used this model in recent years.⁷ Similar to cortical bone there is reported to be a context-dependent minimum force threshold for trabecular bone formation and a linear increase above the threshold.⁸ In contrast to cortical bone, there is conflicting/insufficient evidence as to whether static forces are effective and whether there is a plateau effect with respect to cycles and trabecular bone formation.

Noninvasive Loading Models

A limitation of the models used for the studies cited above is that they were surgically invasive, and thus may have introduced unintended side effects related to inflammation and soft tissue damage, as well as making them technically challenging.^{2–8} To address these concerns noninvasive loading models have been developed and implemented in rodents (**Table 1**). For more extensive descriptions of both invasive and noninvasive loading models see Robling *et al.*¹⁸ Results from the noninvasive models provided further support for the three rules described above.^{19–22} The noninvasive models have also facilitated investigation of other loading variables, given insights into what mechanisms differentiate adaptive vs injury responses (that is, lamellar vs woven bone), and provided evidence supporting the role of osteocytes as load-responsive cells. We have focused the remainder of this review on studies utilizing rats and mice because a multitude of reagents for and genetic manipulations of these animals are currently available. Furthermore, we excluded some studies where the type of bone formed was not adequately documented.

A noninvasive four-point bending model was created by Turner *et al.*²³ for use with rats, and later adapted for use with mice⁹ (**Figure 1**). The tibia rests on two stationary pads; two movable pads apply a transverse force to the lateral side of the limb such that a bending moment is created in the central portion of the diaphysis. Features of this loading method are that a defined strain gradient can be produced in cortical bone over a known area and that loading is applied in a non-physiological direction. The main limitation of this model is that direct pressure on the periosteal surfaces and overlying soft tissues often triggers a woven bone response, which occurs as an ‘all or nothing’ phenomenon,¹⁹ suggesting it is not proportional to the loading stimulus. The pressure-induced bone formation complicates interpretation of periosteal results^{19,10} especially in

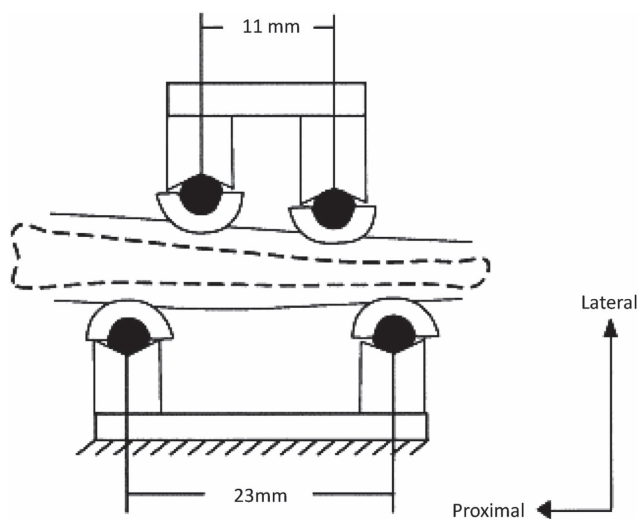


Figure 1 Schematic of four-point tibial bending. Bottom fixed points support the leg; top contact points displace downward. Reprinted from Turner *et al.*⁷⁹ with permission.

smaller animals such as mice. For this reason, use of this model has declined and we do not recommend it.

Another model that generates tibial bending is the cantilever model developed by Gross *et al.*¹¹ for use in mice. In this model, the knee is held rigid while a transverse load is applied to the distal end of the tibia, generating strains large enough to induce a periosteal bone formation response. A practical limitation of this model is that it is difficult to grip the knee and thus the peak force (and strain) is limited in magnitude and does not stimulate an endocortical response. Like the four-point bending model, the loading mode (direction) is non-physiological, which means that the applied loads generate a strain stimulus that is non-habitual in distribution/direction. It is unclear how the response to this novel stimulus differs from the response when loads are applied in a habitual direction, although these differences are likely to be in the degree of response rather than its fundamental nature.

Another noninvasive loading approach, axial compression, has become the gold standard for studying mechanically induced bone formation in rats and mice. Axial compression is meant to mimic physiological loading through the joints. Its first application by Torrance *et al.*¹⁷ on rat forelimbs successfully utilized the natural curvature of the ulna to create bone-stimulating strain on the medial and lateral periosteal surfaces without soft tissue

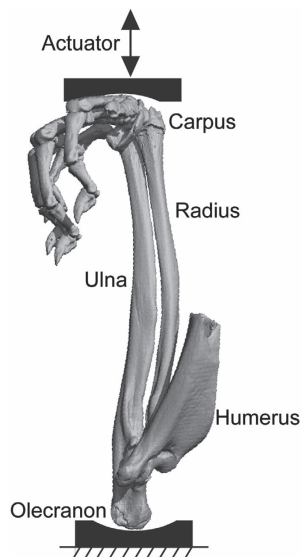


Figure 2 Micro computed tomography of rat forelimb positioned for axial compression loading. The olecranon sits in a fixed cup, whereas the flexed wrist is displaced downward with each load cycle. Reprinted from Uthgenannt *et al.*³⁶ with permission.

damage or ectopic periosteal reaction (**Figure 2**). Approximately two-thirds of the total forelimb force is carried by the ulna.¹⁵ The forelimb compression model has since been applied to mouse ulna.¹⁶ In both mice and rats, forelimb compression is used to study cortical responses in the central diaphysis of the ulna (although the radius could be examined as well).

The axial compression approach was later developed for the mouse hindlimb, with a focus on the tibial response^{12,13} (although the fibular response can also be examined¹⁴). The greatest bone formation is on the periosteal surface experiencing maximum strain, although depending on the force applied, endocortical responses are also seen. Axial compression of the tibia also permits examination of the trabecular bone response because loads are transmitted through the proximal metaphysis. We are not aware of axial tibial compression being used in rats.

Key Mechanical Parameters Affecting the Loading Response

For all noninvasive models three independent parameters of dynamic loading are commonly modulated, each of which may influence the amount of new bone formation: magnitude, frequency and rest-insertion.

Magnitude refers to the peak applied force or strain, as they are linearly coupled (that is, increasing loading force linearly increases strain). Typically, peak strain is controlled as this tightly correlates to the amount of bone production.^{22,24,25} Also, application of the same force magnitude to all animals may result in different bone strains across experimental groups, depending on differences in bone geometry and material properties. However, once the force required to produce a target strain is established for an individual animal, the same force can safely be applied to species-, sex-, age-, weight- and genotype-matched animals and can be assumed to impart similar strains. For details on this procedure see Saxon and Lanyon.²⁶ The range of strain magnitudes that stimulate a bone formation

response depends on the animal variables listed above as well as the other loading variables. Generally, local peak strains in the range 1200–2000 microstrain ($\mu\epsilon$) have been shown to elicit a lamellar bone formation response.^{22,26} Turner *et al.*¹⁹ noted a switch from lamellar to woven when the applied peak force (and hence peak strain) exceeded a threshold, although the particular strain threshold they reported (1900 $\mu\epsilon$) was for the rat tibial bending model and may not apply to other models. An important caveat in all loading studies is that the strain magnitude is only controlled for at the start of the study. Over time, as adaptation occurs, the values of strain may differ.

The second parameter, frequency, also has a threshold response. Similar to static loading, very low frequencies (<1 Hz) produce little response. With increasing frequency bone formation increases until a peak is reached around 10–20 Hz.^{17,27–29} There is some evidence that frequencies over ~20 Hz are dampened by the overlying soft tissues and joints, thus resulting in lower applied strain on the bone surface and no additional benefit.²⁷ A frequency of 2–4 Hz is commonly used, which matches the range of stride frequency reported for rats during locomotion.²⁴

The third parameter, rest insertion, is based on the observation that increased lamellar bone formation in response to loading was saturated after relatively few cycles.⁵ Essentially, cells become desensitized to any additional stimulation. Two methods of restoring mechanosensitivity are to either break the total cycles up into shorter bouts separated by several hours^{25,30} or insert a short (~10 s) rest in between each cycle.³¹ For example, Robling *et al.*²⁵ saw a >50% increase in bone formation if 360 cycles of axial compressive loading were divided into four 90-cycle bouts separated by as few as 3 h rather than administered in one bout. Likewise, Srinivasan *et al.*³¹ enhanced the response to tibial cantilever loading by adding a 10-s hold between each cycle.

Strain rate is another parameter shown to modulate bone formation.^{32,33} But if loading magnitude and frequency are prescribed, then strain rate is not an independent factor. In practice, it is difficult to decouple frequency and strain rate effects. Moreover, to achieve different strain rates while keeping number of cycles and total loading time similar across study groups, a dwell or rest has to be inserted between cycles. The dwell effectively mimics a rest insertion and introduces another variable.

Depending on the combination of the three independent loading parameters and the end point (for example, number of cycles, loss of stiffness and increase in displacement) you can not only ‘switch on’ quiescent bone cells in as little as one bout of mechanical loading but also control whether the response is lamellar or woven bone.³⁴ The concept of a single bout being sufficient to induce lamellar bone formation was established by Forwood *et al.*³⁵ They noted that new bone formation is histologically evident 5–8 days after a bout of loading, and that subsequent loading bouts incrementally increase the magnitude of the bone formation response in a ‘quantum’ manner, a finding consistent with the later rest insertion studies.

Damage as a Stimulus for Woven Bone Formation and Intracortical Remodeling

Woven bone formation is triggered under a variety of loading conditions. It may occur after relatively few loading cycles when

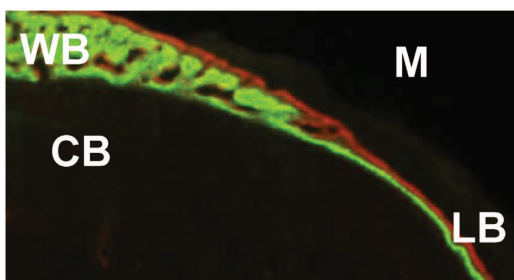


Figure 3 Woven–lamellar transition. Transverse section of the periosteal surface of a rat ulna, collected 14 days after fatigue loading.³⁶ Calcein green was administered on day 7 and alizarin red on day 12. CB, original cortical bone; LB, new lamellar bone; M, muscle; WB, new woven bone.

the applied strain magnitude exceeds some threshold;¹⁹ it is unclear if this reflects a damage response or an extreme on the strain-adaptive continuum. More clearly, under conditions where loading produces discrete bone damage (for example, a stress fracture) robust woven bone formation ensues.^{34,36–39} Although it looks disorganized, woven bone formation is a well-regulated response to certain extreme conditions. A woven bone response occurs when there is a ‘need’ to accrue bone at a faster rate than can be accomplished by lamellar bone formation.

Despite the obvious differences in histological organization, lamellar and woven bone can be forming at the same time on contiguous segments of the bone surface. To estimate the maximal rate for lamellar bone apposition, we analyzed bone sections where a lamellar–woven transition was evident (**Figure 3**). Transverse, undecalcified sections ($n = 100$) were obtained from a previous study in which damaging fatigue loading of the rat ulna was used to stimulate woven bone formation.³⁶ Samples were included from different timepoints, damage levels and longitudinal locations. The average mineral apposition rate for lamellar bone just adjacent to woven bone was $3.5 \pm 1.1 \mu\text{m}$ per day (data not previously reported). This ‘upper bound’ for lamellar mineral apposition rate likely depends on a number of factors, but these observations indicate that there is a maximal rate at which a single team of osteoblasts can deposit lamellar bone.

To better understand bone’s responses to damaging loading, the ulnar axial loading protocol of Torrance *et al.*¹⁷ was adapted to create a model of controlled, fatigue damage.^{37,38,40} Basically, by cyclically loading the forelimb until a certain loss of stiffness or increase in displacement is reached, one can control the amount of bone damage (for example, loss of strength, micro- and macro-cracks) and examine both the periosteal modeling (formation) response and the intracortical remodeling response. Using this model, we have shown that the amount of periosteal woven bone formation directly parallels the amount of induced fatigue damage³⁶ (**Figure 4**). In this setting, woven bone formation is clearly not an ‘all or nothing’ response but is well modulated in location and extent. Additional support for the concept that bone damage induces a proportional woven bone response came from a follow-up experiment.⁴¹ In this study, creep loading (that is, progressive displacement under a static force) was applied until prescribed levels of bone damage were produced. Even in the absence of dynamic loading, damage alone resulted in significant woven bone formation in a dose-dependent manner.

Apart from the periosteal response, microdamage produced by fatigue loading triggers intracortical osteoclastic

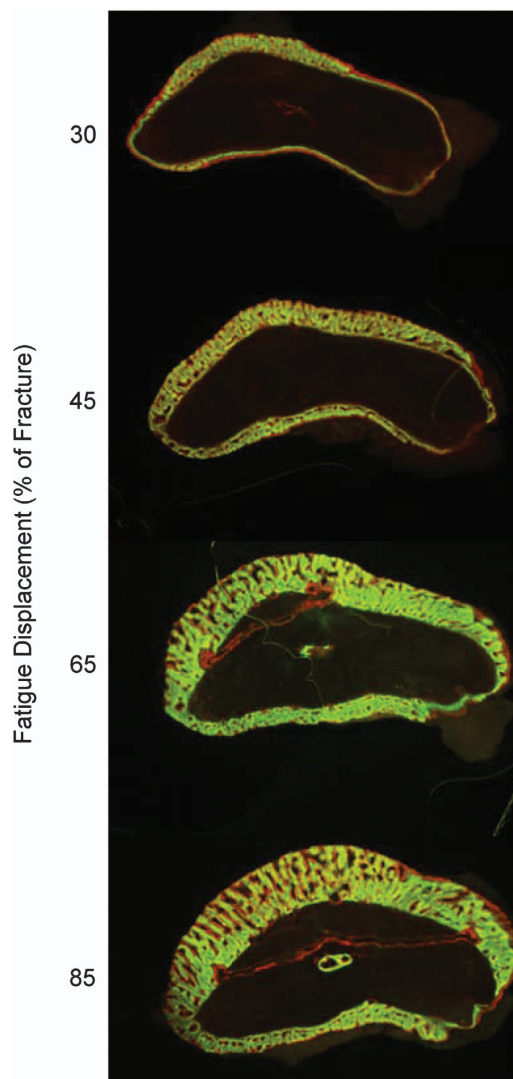


Figure 4 Woven bone-dose response. Transverse sections of the periosteal surface of rat ulnas, collected 14 days after fatigue loading. Calcein green was administered on day 7 and alizarin red on day 12. The amount of periosteal woven bone increases with increasing bone damage. Damage is controlled by the applied fatigue displacement. Modified from Uthgenannt *et al.*³⁶ with permission.

remodeling.^{37,39,40} Kennedy *et al.*⁴² have demonstrated that microcracks cause local osteocyte apoptosis and that the adjacent, non-apoptotic osteocytes produce osteoclastogenic factors such as RANKL. Additional support for this mechanism of targeted bone remodeling came from experiments wherein apoptosis was inhibited and local resorption was diminished.⁴³ Recently Herman *et al.*⁴⁴ demonstrated that damage severity governs osteocyte apoptosis, osteoclast recruitment and resorption. Increased apoptosis and osteoclast resorption were found in the areas containing and proximal to microcracks, but not areas with less severe (diffuse) damage.

Effect of Aging

Does age alter the mechanoresponsiveness of the skeleton? If so, this may be one factor contributing to age-related bone loss. However, the few published studies that directly address this question have not reached consensus (**Table 2**). Two earlier

Table 2 *In vivo* studies comparing young and old animals using direct loading models

Authors	Loading model	Animals	Age	Findings
Rubin <i>et al.</i> ⁴⁵	Isolated ulnar compression	Turkey	1 and 3 years	Aged animals unresponsive to protocol that is anabolic in younger animals
Turner <i>et al.</i> ⁴⁶	Tibial four-point bending	Rat	9 and 19 months	Markedly diminished endocortical response in older animals
Srinivasan <i>et al.</i> ^{47,48}	Tibial cantilever bending	C57Bl/6 mouse	4 and 22 months	Diminished response in older animals; rescued by concurrent Cyclosporin A treatment
Silva <i>et al.</i> ⁴⁹	Tibial axial compression	BALB/c mouse	2, 4, 7 and 12 months	All ages responsive; bone accrual moderately greater at 4 months
Brodt and Silva ⁵⁰	Tibial axial compression	BALB/c mouse	7 and 22 months	Aged animals have equivalent periosteal response, greater endocortical response

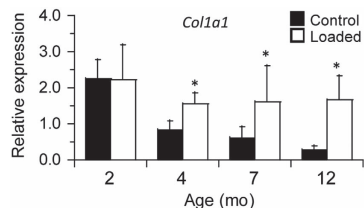


Figure 5 Relative expression of type I collagen mRNA in tibial samples from control and loaded limbs of mice aged 2–12 months. Right hindlimbs were loaded daily by axial tibial compression for 1 week. With aging there is a natural decline in expression of this bone matrix gene (relative to the reference gene *cyclophilin*), indicating reduced bone formation. Mechanical loading increases expression in older animals, offsetting the age-related decline. (Modified from Silva *et al.*⁴⁹) *loaded vs control, $P < 0.05$.

studies reported that aged animals had a negligible response to loading protocols that were previously shown to be strongly anabolic in younger animals.^{45,46} However, these studies used loading models with some of the previously noted limitations (invasive,⁴⁵ periosteal contact⁴⁶). More recently, Srinivasan *et al.*^{47,48} reported that aged (22 month) C57Bl/6 mice have a markedly diminished periosteal response to cantilever tibial bending compared with young–adult (4 month) mice loaded to the same peak periosteal strain. We have examined this question using axial tibial compression in BALB/c mice at different ages, with strain matching across the age groups. We recently reported that 4-month-old mice added more cortical bone than 2-, 7- and 12-month-old mice after 6 weeks of daily tibial loading.⁴⁹ Interestingly, loading increased the expression of osteogenic genes in older mice, offsetting the normal declines that occurred with aging (**Figure 5**). In a separate experiment comparing 7-month- (adult) vs 22-month-(old) mice, we found no deficit in the bone formation response to loading in the old mice.⁵⁰

Based on available evidence, our current view is that under some conditions old animals can respond robustly to loading with a re-activation of bone modeling. Although the magnitude of the response may be diminished compared with younger animals, old bones are clearly mechanoresponsive. Additional work is needed to better define the loading and animal factors that might contribute to age effects, and to determine a mechanistic basis for any differences between young and old animals.

Osteocytes as Mechanosensors

Osteocytes are widely believed to be the primary mechanosensing cell in bone. Their abundance and interconnectivity make

them prime candidates for this function, although direct *in vivo* evidence is still quite limited.^{51,52} Tatsumi *et al.*⁵³ reported that mice in which osteocytes were acutely ablated were relatively resistant to bone loss with hindlimb unloading, although the absence of osteocytes did not affect bone gain during reloading. Recently, Kwon *et al.*⁵⁴ used the same transgenic mice and reported diminished loss of cortical bone with unloading when compared with wild type. However, when mice with osteocyte ablation were subjected to an anabolic, intramedullary pressure stimulus, they had no deficit in their response. We are unaware of comparable experiments performed using a direct loading model (for example, axial compression). Perhaps the best evidence for the role of osteocytes in mechanoresponse comes from studies on the role of osteocytes in Wnt signaling (reviewed below).

It is thought that osteocytes indirectly sense strain via the increased fluid movement through the lacunar/canalicular system that occurs when pressure gradients are created by functional loading, although it is also possible that osteocytes sense the bone strain directly.^{51,52} Although not the focus of this review, a wealth of *in vitro* information exists that attests to the mechanosensitivity of osteocytes to fluid movement and substrate strain. For recent and detailed reviews see Chen *et al.*⁵¹ and Jacobs *et al.*⁵²

Owing to the tight correlation between local strains experienced by osteocytes and formation of new bone, conventional wisdom says that osteocyte signaling remains local. Thus, adaptation is limited to the highly strained regions of loaded bones. However, arguments have arisen suggesting a systemic response to loading regulated by the nervous system whereby loading stimulates neuronal signaling causing systemic bone formation and also affecting local bone formation.^{55,56} Although there is compelling evidence to support this claim, it has only been shown in the setting of loading-induced woven bone formation, which, as will be discussed below, involves many more biological processes and pathways than lamellar bone formation. Furthermore, this claim has been specifically refuted by other studies.^{34,57} The importance of systemic effects in loading-induced bone formation remains to be determined, although most evidence indicates that local effects dominate the response.

Mechanoresponsive Pathways

Early efforts to identify the pathways activated by mechanical loading focused on prostaglandin E_2 ,⁵⁸ nitric oxide⁵⁹ and cyclooxygenase-2.⁶⁰ All three factors were noted to be released

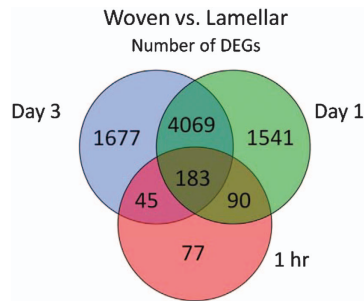


Figure 6 Gene transcription profiles are greatly different for woven bone formation compared with lamellar bone formation. Venn diagram shows the number of differentially expressed genes (DEGs) in woven vs lamellar loading groups based on microarray analysis of rat ulnae collected 1 h, 1 and 3 days after mechanical loading. From McKenzie *et al.*⁶⁵

within minutes following loading, and pharmacological inhibition diminished bone formation. Many *in vitro* and *ex vivo* studies have supported these findings and investigated other pathways (for example, Papachristou *et al.*⁶¹). However, *in vitro* and *ex vivo* studies will never truly replicate the complex *in vivo* scenario. Unfortunately, interpretation of many early *in vivo* studies is challenging because they failed to clearly differentiate between lamellar and woven bone formation.

Recent technical advances provide many opportunities for extending our knowledge of *in vivo* bone mechanobiology. Quantitative PCR and microarray technologies allow extensive probing of gene responses, while genetically modified mice allow studies into biological mechanism. *In vivo* microarray studies have shown that during lamellar bone formation, genes relating to cell signaling, movement, proliferation and metabolism have a modest peak in transcriptional activity shortly after loading (4–8 h),^{62–64} most of which return to basal levels by 24 h.^{63–65} Somewhat surprisingly, another peak was reported to occur around 12–16 days for genes related to solute carrying, matrix production, transforming growth factor- β signaling and Wnt/ β -catenin signaling.⁶⁴ The importance of estrogen signaling in mechanoresponsiveness of bone has also been established by a number of *in vivo* studies (recently reviewed elsewhere Melville *et al.*⁶⁶). A key result is that loss of circulating estrogen does not appear to strongly alter loading responses, whereas loss of signaling through the estrogen receptor alpha diminishes responses to loading.

Compared with lamellar bone formation, the number of differentially regulated genes occurring in the context of loading-induced woven bone formation is markedly greater.⁶⁵ Inflammation, cytoskeletal remodeling, cell adhesion and developmental pathways are all affected, with inflammatory genes being particularly notable 1 and 24 h after loading (**Figure 6**). Angiogenesis occurs with woven but not lamellar bone formation.³⁴ Processes associated with injury and anabolism are dominant early after damaging loading, while the expression of factors related to bone remodeling/resorption are activated later, showing increases from 1 to 7 days.^{34,39,65,67}

Importance of WNT/Lrp5 Pathway

The WNT/Lrp pathway has emerged as a key regulator of skeletal anabolism, and has been implicated as an important mechanoresponsive pathway in bone (reviewed in Bonewald

and Johnson⁶⁸). In lamellar bone formation, WNT signaling has recently attracted attention due to: (1) the complete lack of response to loading in mice lacking LRP5 (a key receptor of WNT ligands)⁶⁹ and (2) an increased, dose-dependent response in constitutively active LRP5 mice.⁷⁰ Sclerostin, the protein product of the *Sost* gene, is an LRP5 antagonist and has been identified as mechanoresponsive. *Sost* expression in osteocytes decreases with increased mechanical strain^{71–73} Alternatively, if *SOST* levels cannot decrease, which has been accomplished in mice by periostin knockout⁷² or *SOST* over expression,⁷³ new lamellar bone will not form.

The effect of WNT/Lrp signaling on bone formation is thought to be through downstream effects on β -catenin. Canonical WNT signaling blocks β -catenin degradation allowing increased translocation to the nucleus and transcription of osteogenic target genes. But, *in vitro* assays have shown multiple ways to affect β -catenin levels without modulating WNT signaling.⁷⁴ More recently, *in vivo* knockout of factors such as Stat3,⁷⁵ midkine⁷⁶ and HIF-1 α ⁷⁷ have significantly modulated load-induced lamellar bone formation. It is hypothesized that each of these factors affects β -catenin levels through non-WNT mechanisms.

The role of WNT/Lrp/ β -catenin signaling on bone responses to damaging loading remains to be determined. One recent study has shown a decrease in osteocytic sclerostin levels in bones loaded with a protocol that induced woven bone formation.⁷⁸ Lastly, we reported marked downregulation of *Sost* expression after damaging fatigue loading and before woven bone formation,⁶⁵ suggesting that osteocytes might be orchestrating the woven bone response to bone damage thru the Wnt/Lrp pathway.

Conclusions

In conclusion, several loading models can be used to apply precisely controlled mechanical loads to bone to study adaptive and injury responses. Noninvasive models are necessary to study the molecular mechanisms and resulting gene regulation. Three such models have been developed and are commonly used: four point bending, cantilever bending and axial compression. All three types of loading stimulate osteogenesis, but not all mimic physiological loading and thus may cause off target effects. Furthermore, all the models are limited to appendicular long bones. This precludes or limits the study of mechanical loading on flat bones and trabecular bone. The most physiological model is compressive axial loading, which can be applied to either the ulna or the tibia. Depending on how parameters such as strain magnitude, cycle frequency, rest insertion and test end point are applied, the response outcome can be tuned from adaptive (mostly lamellar) to damage/injury (mostly woven). Strikingly, both are dose-dependent and can be provoked in as little as one bout of loading with few cycles.

The mechanisms elicited in lamellar and woven bone formation pathways are still not fully understood. Nonetheless, controlled *in vivo* studies of gene expression and knock out have indicated a role for cell signaling, cell metabolism and WNT/Lrp signaling in lamellar and woven bone formation, with the addition of inflammation and angiogenesis in woven bone formation. At this time, it is unclear if the importance of these pathways is universal to all loading-induced bone formation, or if different pathways will be more/less important as a function of loading method, animal age and other factors. Nonetheless, with current understanding of how to control formation outcomes

and advancement of genetic models, researchers are poised to clarify known mechanisms and discover new ones.

Conflict of Interest

The authors declare no conflict of interest.

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