

## THE IMPORTANCE OF LOADING FREQUENCY, RATE AND VIBRATION FOR ENHANCING BONE ADAPTATION AND IMPLANT OSSEOINTEGRATION

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### Abstract

Mechanical loading is one of the key factors that influence bone mass and the osseointegration of bone-anchored implants. From a clinical point of view, mechanical stimulation may be used to enhance bone strength and implant osseointegration. Among the many loading parameters that influence the response to mechanical loading, the effects of loading frequency and rate have been investigated in many studies. In this paper the most relevant animal studies that have addressed the effect of loading frequency, rate, and vibration on either bone adaptation or implant osseointegration are systematically reviewed. Apparently contradictory results are discussed and interpreted within the context of mechanotransduction and mechanoregulation of bone. A combined experimental and computational approach is suggested to address some of the remaining research questions.

**Keywords:** bone adaptation, loading frequency, strain rate, vibration, osseointegration, implant.

### Introduction

Bone as a living tissue has long been recognized to be capable of adapting its mass and structure in response to the demands of mechanical loading. The ability of bone to respond to mechanical signals has been repeatedly demonstrated in the past (Burr *et al.*, 1985; Lanyon and Rubin, 1984; Turner *et al.*, 1994) and also our understanding of how these signals are converted into an anabolic or catabolic response has increased considerably. Osteocytes, buried in the bone matrix, and the lacuno-canalicular porosity are believed to be the professional mechanosensory cells of bone and the structure that mediates mechanosensing respectively (Burger and Klein-Nulend, 1999; Cowin, 2002; Turner *et al.*, 1994; You *et al.*, 2001). The external mechanical stimuli are transduced into a cellular response through a process known as "mechanotransduction". Due to the difficulty of directly measuring the mechanisms *in vivo*, hypotheses on how bone may sense mechanical loading are to a large extent based on mathematical models. Physiological loading results in bone matrix deformation and creates pressure gradients within the fluid-filled lacunar-canalicular network, giving rise to the movement of extracellular fluid and osteocyte deformation. The (micro)mechanics by which fluid movement is coupled to cellular deformation can be quite complex and have been studied by means of mathematical models, such as the one from You *et al.* (2001). This model considers an osteocyte process in a canaliculus and its pericellular matrix that fills the space between the canalicular wall and the membrane of the cellular process. The model predicts that load-induced fluid flow leads to shear forces on the plasma membrane of the cellular process and drag forces on the fibrils in the pericellular matrix. Interestingly, the model also suggests that tissue strains may be amplified at the level of the cellular processes due to their microstructural organisation, providing further evidence of the importance of the osteocytes as the mechano-sensitive cells par excellence. If bone adaptation is driven by fluid flow, then adaptation should occur only in response to dynamic and not to static loads (Jacobs *et al.*, 1998), as the latter will not create fluid movement. Indeed, this was already noticed by Liskova and Hert (1971) in rabbits, which was later confirmed by Lanyon and Rubin (1984) by means of their well known isolated avian ulna model.

At a more phenomenological level, several mechanical parameters have been identified that influence the anabolic response to loading. Among these are strain rate, number of cycles (Kaspar *et al.*, 2002), strain distribution, local strain gradients (Judex *et al.*, 1997), and resting periods (LaMothe and Zernicke, 2004; Srinivasan *et al.*, 2002). Strain rate can be further decomposed into strain magnitude and loading frequency (see below for a more detailed explanation), suggesting that low-magnitude

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high-frequency mechanical stimuli may be as stimulatory as high magnitude low-frequency stimuli (Bacabac *et al.*, 2004). Low amplitude high frequency loading in bone has been shown to occur more often in normal daily activities *in vivo* (Fritton *et al.*, 2000) and might be used to promote adaptive bone formation.

The tissue's sensitivity to functional loading suggests an interesting potential pathway for therapeutic intervention. Mechanical stimuli could be capable of inhibiting or even reversing bone loss caused by diseases such as osteoporosis (Rubin *et al.*, 2001a; Rubin *et al.*, 2002b). It has also been postulated that the adaptive capacity of bone in response to specific loading regimes could be considered a means by which biological fixation of prostheses could be promoted. In contrast, unfavourable conditions of reduced load transfer at the bone-implant interface can initiate significant bone resorption ('stress shielding') and may contribute to eventual degradation of the long-term implant performance (Huiskes *et al.*, 1992; Pilliar *et al.*, 1979). In addition, both animal experimental and computational studies have demonstrated the importance of mechanical loading for the early healing response around implants (Duyck *et al.*, 2006; Geris *et al.*, 2008; Huiskes *et al.*, 1997; Prendergast *et al.*, 1997; Søballe *et al.*, 1992; Vandamme *et al.*, 2007). Similar to fracture healing, peri-implant tissue differentiation seems to be mediated by mechanics. Again, one could think of creating a mechanical environment that favours the differentiation towards bone, in order to accelerate implant osseointegration.

This paper is aimed at evaluating the possibility of using mechanical stimuli to enhance quality and quantity of bone, without or within the presence of an implant. More specifically a literature review was performed on *in vivo* animal studies in which loading frequency and rate were systematically varied within the context of bone adaptation or implant osseointegration. In addition, we also included studies that have explored the use of mechanical vibration to enhance bone mass. For the interpretation of these studies it is important to have a basic understanding of the relationship between commonly used loading parameters. Therefore, some basic mathematical relationships between these loading parameters will be presented first.

### Mathematical relationships between strain rate, amplitude and frequency

Strain rate, magnitude and loading frequency of a dynamic loading are interrelated (Turner, 1998). The relationships depend on the applied loading waveform (e.g. sinusoidal or trapezoidal). These concepts will be further elaborated below.

In an experimental system in which a bone is subjected to both stationary and cyclic loads, the applied load is given by:

$$F(t) = F_0 + F_1 \sin \omega t \quad (1)$$

where  $F_0$  is the magnitude of the stationary load,  $F_1$  is the amplitude of the cyclic load,  $\omega$  is the angular frequency ( $\omega=2\pi f$ , where  $f$  is the loading frequency) and  $t$  is the time. Considering bone tissue to be a linear elastic material, the local strain, encountered at a given location in the bone

tissue, will follow a similar time-dependency:

$$\boldsymbol{\varepsilon}(t, \mathbf{x}) = \boldsymbol{\varepsilon}_0(\mathbf{x}) + \boldsymbol{\varepsilon}_1(\mathbf{x}) \sin \omega t \quad (2)$$

where  $\boldsymbol{\varepsilon}(t, \mathbf{x})$  is the strain tensor (accounting for different normal and shear strain components),  $\mathbf{x}$  is a vector that gives the position of a point inside the bone tissue, and  $\boldsymbol{\varepsilon}_0$  and  $\boldsymbol{\varepsilon}_1$  are again the stationary and dynamic component of the strain tensor. For a simple geometry and loading condition the relation between the external load and the strain may be solved analytically. For complex geometries like bones, numerical techniques like the finite element (FE) method are needed to calculate detailed strain distributions. Examples for the study of well-controlled loading setups can be found in Gross *et al.* (2002) and Kotha *et al.* (2004) for cantilever-like bending of a mouse tibia and axial compression of a rat ulna respectively. Experimental validation of these numerical models is typically done by means of strain gauge measurements on the cortical bone surface. In addition, strain gauge measurements are widely used to calibrate an experimental set-up, i.e. to derive an empirical relation between the external load and the bone surface strain at a specific location (see e.g., Rubin and Lanyon, 1985 and Warden and Turner, 2004). Obviously, unlike an FE model, this only provides information at the point of strain gauge attachment, and only in the direction of the strain gauge element (at least for single element strain gauges).

Once the dynamic strain (strain as a function of time) is known the strain rate  $\dot{\boldsymbol{\varepsilon}}$  can be obtained by taking the time derivative of (2). For the sake of simplicity we will only consider here one strain component at a certain bone location (as e.g., measured by a single element strain gauge), leaving out the directional and spatial dependency:

$$\dot{\boldsymbol{\varepsilon}}(t) = \frac{\partial \boldsymbol{\varepsilon}}{\partial t} = \omega \boldsymbol{\varepsilon}_1 \cos \omega t = 2\pi f \boldsymbol{\varepsilon}_1 \cos \omega t \quad (3)$$

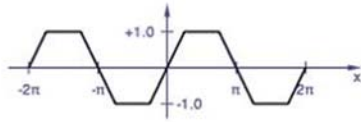
In which  $f$  is the loading frequency and  $\boldsymbol{\varepsilon}_1$  is the amplitude of the dynamic strain component (now a scalar value). Based on the experimental observations that bone adaptation seems to increase with increasing values of the dynamic strain amplitude (further denoted as  $\boldsymbol{\varepsilon}$  instead of  $\boldsymbol{\varepsilon}_1$ ) and loading frequency, Turner proposes the following governing stimulus (Turner, 1998):

$$E = k_1 \boldsymbol{\varepsilon} f \quad (4)$$

$E$  is called the strain stimulus and  $k_1$  is proportionality constant. It must be noted that this stimulus is not equal to strain rate (as this is a function of time as well, see (3)), but it is proportional to the amplitude of strain rate (which is equal to  $2\pi f \boldsymbol{\varepsilon}_1$  according to (3)).

In his review paper Turner proposes a generalisation of this stimulus for non-sinusoidal periodic loading conditions. Indeed, any kind of period function can be expanded to a Fourier series, which is an (infinite) sum of sines and cosines. As an example the Fourier series expansion of a trapezoidal loading waveform is given in figure 1.

In practice a finite number of terms is needed to obtain an approximation of the periodic waveform. Similar to an



$$F_{\text{trap}}(t) = \frac{8}{\pi^2} \sum_{n=1}^{\infty} \frac{\sin \frac{n\pi}{4} + \sin \frac{3n\pi}{4}}{n^2} \sin nt$$

**Figure 1:** Fourier series expansion of a trapezoidal loading waveform (trapezoidal waveform normalised to one, periodic on  $[-\pi, \pi]$ ) (taken from <http://www.till.com/articles/QuadTrapVCO/trapezoid.html>)

arbitrary periodic loading wave, the corresponding strain and strain rate can be written by means of a (finite) series expansion:

$$\varepsilon(t) = \sum_{n=1}^k \varepsilon_n \sin nt = \sum_{n=1}^k \varepsilon_n \sin 2\pi f_n t \quad (5)$$

$$\dot{\varepsilon}(t) = \sum_{n=1}^k \varepsilon_n n \cos nt = 2\pi \sum_{n=1}^k f_n \varepsilon_n \cos 2\pi f_n t \quad (6)$$

With  $\varepsilon_n$  and  $f_n = n/2\pi$  being the amplitude and frequency of the  $n$ -th term respectively in the expansion of the strain wave. Similar to a single sine-loading wave, Turner proposes that in the case of any periodic loading condition the strain stimulus can be defined in terms of the amplitudes and frequencies of the different terms (components) of the strain:

$$E = k_1 \sum_{n=1}^k \varepsilon_n f_n \quad (7)$$

Again, it must be noticed that this stimulus is different from strain rate. Instead, it is proportional to the sum of the strain rate amplitudes of the different components of the strain rate signal. This difference becomes important when experiments that apply either sinusoidal or non-sinusoidal (e.g. trapezoidal) periodic loading waves are interpreted. For a single sine wave the strain signal has only one frequency component, and therefore there will be only one term in the strain stimulus, as defined by (7). On the other hand, the strain rate is varying as a function of time, as it is a cosine function (equation (3)). For a trapezoidal loading wave a series of frequency components (frequency spectrum) is retrieved, leading to as many contributions to the strain stimulus in (7). On the other hand, the strain rate during loading and unloading is proportional to the slope of the loading wave (figure 1) and is therefore constant. In addition, as already mentioned by Turner (1998) sinusoidal loading allows to vary the loading amplitude, while keeping frequency constant. At the same time, strain rate (equation (3)) as well as the strain stimulus (equation (4)) will be varied as well. For a trapezoidal loading wave, a change of the height of the trapezoid (and therefore strain magnitude) can be obtained without changing the loading (and strain) rate, as long as the slope of the trapezoid remains unchanged. This was e.g. the case in the experiments by Rubin and Lanyon (1985) and Mosley *et al.* (1997). However, changing the height will lead to a different frequency spectrum and therefore a different value of the strain stimulus, defined in equation (7).

### Bone adaptation studies

The effects of loading frequency and rate have been assessed in several animal studies. We can distinguish

between experiments in which individual limbs/bones of the animal are exposed to mechanical loading and “Whole Body Vibration” in which the body or large part thereof of the animal under investigation is mechanically stimulated by means of vibrating platforms. Loading of individual limbs provides much more control on the loading characteristics (magnitude, frequency, waveform...) to which bones are exposed. In many of these studies information is also provided on the strain magnitude on the (cortical) bone surface experienced during load application (either measured by strain gauges or calculated by means of FE modelling). Well-known examples are the isolated avian ulna model (Lanyon and Rubin, 1984), the rat (Torrance *et al.*, 1994) and mouse (Lee *et al.*, 2002) ulnar compression model and the mouse tibial compression model (De Souza *et al.*, 2005), the latter three having the advantage of being non-invasive.

When using vibrating platforms, it is much less straightforward what is the mechanical stimulus (magnitude, frequency, waveform) to which the bones are exposed to. Studies that have used such devices often only provide information on the frequency and the maximum acceleration of the platform (see e.g. Flieger *et al.*, 1998, Oxlund *et al.*, 2003). Both loading parameters are coupled. Suppose a vibration motor results in a sinusoidal displacement  $x$  of the platform:

$$x(t) = A \sin \omega t = A \sin(2\pi f t) \quad (8)$$

with  $A$  being the displacement amplitude,  $\omega$  and  $f$  being the angular frequency and frequency respectively. The acceleration, being defined as the second derivative of the displacement is then given by:

$$a(t) = -A\omega^2 \sin \omega t = -4A\pi^2 f^2 \sin(2\pi f t) \quad (9)$$

The maximum acceleration is proportional to the square of the frequency. Unless the vibration motor also allows changing the displacement amplitude  $A$ , both parameters cannot be varied independently. In addition, as force transfer through the limbs may be very complex and among other factors may involve soft tissue dampening and muscle activation, one cannot readily derive what are the loading (strain) magnitude and frequency at the level of the bones (unless they are measured by strain gauges, see e.g., Rubin *et al.*, 2002a, 2002b).

Obviously, this complicates the comparison between loading studies on individual limbs and vibration studies. Another difference lies in the order of applied strains: typically, studies on individual limbs have investigated the effect of mechanical stimulation for strains that are orders of magnitude larger than for studies with vibrating platform (typically of the order of 100-1000  $\mu\epsilon$  versus 1-10  $\mu\epsilon$  respectively).

### Loading studies on individual limbs

Following our discussion on the relation between loading rate, frequency and amplitude we make a distinction here between sinusoidal (as e.g., used by Turner and co-workers) and trapezoidal (as e.g., used by Lanyon and co-workers). While the first loading wave is suitable for exploring the effect of loading frequency and amplitude independently (and within this context the role of the strain stimulus, defined in equation (4)), the second one allows applying a single strain rate value.

### Sinusoidal loading

Turner *et al.* (1994) measured the effect of applying sine waves at different loading frequencies (0.05, 0.1, 0.2, 0.5, 1 and 2 Hz) of the same magnitude (52 N) for 36 cycles per day on cortical bone formation rate in the right tibiae of rats. Either bending (through a four-point bending apparatus) or axial loading was applied as a sine wave. Histomorphometric indices (Parfitt *et al.*, 1987) on the periosteal surface in the mid-diaphyseal region of the left tibia were obtained and were expressed as relative values with respect to the contralateral unloaded limb. The analysis revealed that axial loading did not cause a significant increase in the relative bone formation rate over the bone surface (rBFR/BS) but that bending increased rBFR/BS for loading frequencies between 0.5 and 2 Hz. An explanation for this finding can be that for axial loading the induced strains were lower and less anabolic than for bending.

The anabolic response to bending was further explored for the same animal model, by varying the load magnitude (0, 18, 36 and 54 N) and keeping the frequency constant (2 Hz) (Turner *et al.*, 1995). They found that relative bone formation rate (rBFR) on the endocortical surface was proportional to load magnitude. By combining the bending results of both studies and plotting rBFR with respect to the strain stimulus (equation (4)), Turner (1998) found similar linear relations for both studies, therefore providing evidence for the strain stimulus being the governing stimulus (at least for frequencies up to 2 Hz).

Hsieh and Turner (2001) further examined the validity of the strain stimulus for frequencies equal to 1, 5, or 10 Hz. A sinusoidal compressive loading was applied on the right ulna of rats for 360 cycles/day for 10 days with peak loads ranging from 4.3 to 18 N. They found that the relative periosteal bone formation rate (given by rBFR/BS) was proportional to peak strain at each of the three loading frequencies tested. The proportionality was different for the three test groups, with similar strains being the most anabolic at 5 Hz and the least anabolic at 1 Hz. When plotting periosteal bone formation rate against the strain stimulus (equation (4)) regression analysis revealed two distinct curves, one for the 1 Hz test results and one for the combined 5 and 10 Hz results. Using the same ulna-loading model (but adapted to the mouse), Warden and Turner (2004) studied bone adaptation to sinusoidal load with frequency of 1, 5, 10, 20 and 30 Hz for C57BL/6 mice. The histomorphometry response of the right ulna was studied when loaded at one of the five frequencies and one of two load magnitudes (1.5 and 2 N). Relative bone formation rate (rBFR/BS) in the periosteal surface at

each frequency and peak load revealed that there were no differences among frequencies when loading at 1.5 N. However, when loading at 2 N, cortical bone adaptation increased with increasing loading frequency up to 5-10 Hz. No additional bone adaptation with loading frequencies beyond 10 Hz was found. Similar to the previous study, it reveals that the proposed strain stimulus, which accounts for strain amplitude and frequency, is not able to fully describe the response to mechanical loading.

Recently, Castillo *et al.* (2006) investigated the effect of low-amplitude broad-frequency vibration, superimposed on a 2Hz sinusoidal loading wave, for the same mouse ulnar compression model (again for C57BL/6 mice). Two studies were completed wherein several loading waveforms, with or without an additive low-amplitude, broad-frequency (0-50 Hz) vibration signal, were applied to the right ulnae. In a first study, mice were loaded short-term (30 s/day, 2 days) with either a sine wave signal alone (1 or 2 N), vibration signal alone (0.1 N or 0.3 N) or combined sinusoidal and vibration signal. In a second study, mice were loaded long-term (30 s/day, 3 days/week, 4 weeks) with a sinusoidal signal alone, static signal alone, vibration signal alone (0.02 N, 0.04 N, 0.08 N or 0.25 N) or combined sinusoidal and vibration signal. After loading, cortical bone formation parameters were assessed by histomorphometry. Data from the first study indicated that vibration increased cortical bone formation when superimposed to the sine wave. Animals that were loaded with the 2 N sine waveform alone plus 0.3 N vibration signal had significantly greater bone formation rates than animals loaded with the 2N sine wave alone. However, further review of these data indicated that the enhanced response could be explained by increased load and strain levels rather than by the vibration signal itself. This confounding factor may also explain the enhanced osteogenic response for a similar combined waveform, reported by Tanaka *et al.* (2003). Also in this study broad frequency noise was added to a 2Hz sine wave, without compensating for the load and strain magnitude. In the second study of Castillo *et al.* (2006) the magnitude of the sinusoidal waveform was reduced to counterbalance the increase in magnitude introduced by the superimposed vibration signal. Data from the second study showed that cortical bone formation was not significantly altered by vibration. These findings suggested that low-amplitude, broad-frequency vibration superimposed onto an osteogenic waveform or vibration alone did not enhance cortical bone adaptation at the frequencies, amplitudes and loading periods tested.

### Trapezoidal loading

Mosley and Lanyon (1998) used a trapezoidal loading waveform to test the anabolic potential of the strain rate by applying an axial compression load between 1 and 20 N on the left ulna of Sprague Dawley rats, corresponding to about 4000  $\mu\epsilon$  at the medial midshaft (compressive strain). Three levels of strain rate (i.e. slope during loading and unloading) were tested: low ( $\pm 0.018/\text{sec}$ ), moderate ( $\pm 0.030/\text{sec}$ ) and high ( $\pm 0.100/\text{sec}$ ). The frequency (referring here to the number of trapezoidal cycles per second, which is different from the frequency spectrum,

as retrieved from a Fourier series expansion, see fig. 1) of 2 Hz was kept constant by altering the low dwell period ('rest period') between trapezoids. Periosteal bone formation was evaluated at different positions along the midshaft and compared to the contralateral (non-stimulated) right ulna. For all three test groups a similar site-specific response was found, with the highest load-related osteogenic response (compared to contralateral control) at 2-3 mm distal from the midshaft. When comparing test groups, the highest osteogenic response was found for the highest strain rate group, the lowest for the lowest strain rate group. The positive effect of the insertion of the rest periods in affecting the potential of the mechanical loading was not taken into account. Although this has been shown to have an effect in other studies (Robling *et al.*, 2001; Srinivasan *et al.*, 2002), it may not have been relevant for this study due to the short (less than half a second) rest periods.

LaMothe *et al.* (2005) tested the hypothesis that high strain rate enhanced the adaptive response by applying a 1 Hz trapezoidal waveform for 60 s, 5 days per week, for 4 weeks in C57BL/6 mice. The proximal right tibia was immobilized, and loads were applied to the distal lateral tibia so that the tibia was in cantilever bending. Unlike Mosley and Lanyon (1998), the frequency (again defined as number of trapezoidal cycles per second) was kept constant by altering the high dwell period (i.e., period of maximum load). Relative mineral apposition rate and bone formation rate were evaluated for three different levels of strain rate (0.004/s, 0.02/s, and 0.100/s) on the periosteal and endocortical surface. For the three test groups significant increases (with respect to contralateral control) for almost all histomorphometrical parameters were found. The difference between the test groups was less pronounced, although for some of the parameters the highest strain rate led to a significantly higher increase than the other two strain rates.

### Vibration studies

Experiments performed on sheep (Rubin *et al.*, 2001a; Rubin *et al.*, 2002a; Rubin *et al.*, 2002b), rats (Flieger *et al.*, 1998; Judex *et al.*, 1997; Oxlund *et al.*, 2003; Rubin *et al.*, 2001b) and mice (Xie *et al.*, 2006) tested the ability of high-frequency low-magnitude vibrations in promoting the anabolic response in trabecular and/or cortical bone.

Rubin *et al.* (2001, 2002a, 2002b) evaluated the hypothesis that extremely low level (0.3g), high frequency (30 Hz) mechanical strains are anabolic. The hindlimbs of adult female sheep were stimulated for 20 minutes/day during one year, using a non-invasive vertical oscillation sufficient to induce approximately 5 microstrains (peak-to-peak 30 Hz oscillations) on the cortex of the tibia (as measured by strain gauges). Femoral, tibial and (non-stimulated) radial bone was analysed by means of dual X-ray absorptiometry (DXA), peripheral quantitative computer tomography (pQCT) and histomorphometry (Rubin *et al.*, 2001a, 2001b, 2002a). *In vivo* DXA measurements revealed an increase of bone mineral density in the tibia (whole bone measurement) of test versus control group, which was significant only at week 29. In order to

rule out any systemic effects, the same measurements were also done on the distal radius, where no significant differences were found between test and control groups. Post mortem pQCT of the proximal femur revealed no differences in bone mineral density for the cortical bone, but a 34.2% increase in trabecular bone density in the test group. Histomorphometry on undecalcified sections of the same trabecular bone region revealed a 45% increase in trabecular number and a 36% decrease in trabecular spacing. An increase in bone formation rate and mineralising surface could also be detected, although these differences were much less significant. In contrast, no histomorphometrical differences between test and control group could be found in the trabecular bone of the distal radius, again ruling out any systemic effect. In addition trabecular bone from the distal femoral condyle was analysed by means of microfocus computer tomography ( $\mu$ CT) for the same experiment (Rubin *et al.*, 2002b). In the experimental animals bone mineral content was 10.6% greater, the trabecular number was 8.3% higher, while the trabecular spacing decreased with 11.3%, indicating that the higher bone quantity was related to a higher number of trabeculae and a higher trabecular thickness. All together, these findings indicated that low magnitude high frequency vibrations can be effectively transmitted to the bone (at least to the level of cortical bone) and that they can enhance bone mass in trabecular bone in a location-dependent, non-systemic way. The results also demonstrate that the study outcome may depend on the analysed anatomical location (e.g. proximal versus distal femur) and the applied analysis techniques (e.g. DXA, pQCT,  $\mu$ CT and histology), which may strongly vary in terms of their discriminative power.

Adult female Sprague-Dawley rats were used by Rubin *et al.* (2001b) in order to study the ability of extremely low-magnitude (0.25g), high-frequency (90 Hz) whole body vibration to restore bone metabolism in animals, exposed to disuse (tail suspension). After 28 days, bone formation rate over bone volume (BFR/BV) and mineralizing surface over bone surface (MS/BS) in the trabecular bone of the proximal tibia increased for mechanically stimulated rats compared to controls (not subjected to vibration). In contrast, those parameters were found to decrease in the disuse group. Disuse interrupted by 10 min per day of vibration brought the histomorphometric parameters to normal values.

Similarly, Xie *et al.* (2006) investigated the effect of whole body vibration in the growing skeleton, by exposing 8 week old BALB/cByJ mice to whole body vibration at 45 Hz (0.3 g) for 15 min/day during 3 weeks. *In vivo* strain gauging indicated that the mechanical signal induced 45 Hz strain oscillations of approximately 10 microstrain (peak-to-peak) on the periosteal surface of the proximal tibia, demonstrating that the high-frequency mechanical signal could be transmitted to the bone. Whole body vibration only affected bone formation rates (BFR/BS) on the endocortical surface of the metaphysis and osteoclastic resorption in metaphyseal and epiphyseal trabecular bone, resulting in a respective increase and decrease with respect to age-matched controls. For all other histomorphometrical

and chemical properties investigated no effect could be determined for either epiphyseal, metaphyseal and middiaphyseal bone.

In other studies (Flieger *et al.*, 1998; Oxlund *et al.*, 2003) the influence of low intensity whole body vibration on the prevention of bone loss due to postmenopausal osteoporosis in rats was investigated. Flieger *et al.* (1998) found that early bone loss after ovariectomy (OVX), as assessed by DXA, was prevented by stimulating the animals with low intensity vibrations (frequency = 50Hz, acceleration = 2g) for 30 min/day for 5 days/week. Whole body vibration led to significantly higher BMD values at 5 weeks after ovariectomy for both the femur and the tibia. No effect of vibration was found in sham-operated animals. Oxlund *et al.* (2003) used the ovariectomized (OVX) rat model to study the effect of whole body vibrations on bone mass and strength (tibia and femur) and muscle mass. The animals were subdivided in groups for evaluating the effect of vibration at different magnitudes and frequencies (0.5g, 17 Hz; 1.5g, 30 Hz; 3g, 45 Hz) given 30 min/day for 90 days. Histomorphometrical analyses of the tibia revealed a significantly higher periosteal mineral apposition rate for all vibration-stimulated OVX groups, while periosteal bone formation rate was only significantly higher for the 45 Hz-stimulated OVX group (compared to non-stimulated OVX control group). Endocortical bone formation rates were not significantly different in OVX groups with or without vibration. Three-point bending of the tibia revealed no significant differences in stiffness and strength between the OVX test and control groups. Compression of the distal femoral metaphysis resulted in a significantly lower strength for all OVX groups (non-stimulated and stimulated) versus sham-operated controls. Only the 45 Hz-stimulated OVX group led to significantly higher strength compared to the non-stimulated OVX control group. No differences in muscle mass were seen for all test and control groups, indicating that vibration was likely to cause a direct mechanical effect on bone, rather than indirect through an increase of muscle mass. As maximum acceleration and frequency were coupled and changed at the same time (see equation 9) it cannot be concluded whether the highest response, observed for the 45 Hz-stimulated OVX group, was related to the higher frequency or the higher acceleration and therefore potentially higher force transfer and engendered strains at the level of the bone. As to the latter, no attempt was made to measure cortical bone surface strains.

Judex *et al.* (2007) subjected OVX rats to whole body vibrations (0.15 g) at 45 Hz or 90 Hz for 10 min/day for 28 days and compared to OVX controls. In vivo strain gauge measurements were done on the proximal tibia. The 45 Hz regime resulted in an average strain of 2.12  $\mu\epsilon$  and an average peak strain rate of 312  $\mu\epsilon/s$ , while for the 90 Hz regime this resulted in 0.74  $\mu\epsilon$  and 194  $\mu\epsilon/s$  respectively. Fast Fourier Transform (FFT) analysis revealed the presence of a dominant frequency component equal to the applied frequency. Bone formation rates in the metaphysis of the proximal tibia were significantly larger in the 90 Hz test group when compared to controls, while for the 45 Hz test group results were not different

from the control group. This result was found for both trabecular and cortical bone. In addition,  $\mu$ CT analysis of the distal femur showed that 90 Hz stimulated rats had significantly more trabecular bone volume and thicker trabeculae in the epiphyseal, but not in the metaphyseal area (compared to control group and 45 Hz test group). The fact that the 90 Hz loading regime led to significantly lower strain and strain rate levels, suggests that for this experiment loading frequency was the dominating factor that determined the osteogenic response.

### Implant osseointegration studies

While several studies have investigated the effect of loading frequency, rate and vibration on bone adaptation, few attempts have been made to address their effect on implant osseointegration. Rubin and McLeod (1994) used the turkey ulna model of disuse osteopenia to evaluate if mechanical loading could be used actively to stimulate bony ingrowth into a porous surface of a cylindrical implant placed across the dorsal and ventral cortices of the bone diaphysis. The relative bony ingrowth was quantified by backscatter electron microscopy after eight weeks of: disuse alone, disuse plus 100 seconds per day of a 1 Hz sinusoidal load, and disuse plus 100 seconds per day of a 20 Hz load. In this model mechanical loading of the tissues surrounding the implant was achieved by loading the host bone in bending, instead of applying a load directly to the implant. The strains generated in the cortex immediately adjacent to the implant were quantified by means of strain gauges and equalled 150  $\mu\epsilon$ . The results showed that disuse alone caused loss of bone away from the implant surface, while a 1 Hz load regimen prevented resorption and stimulated some growth of bone into the implant. Finally the 20 Hz loading regime led to the most favourable response, with new bone being formed not only at the interface, but also at both the periosteal and endosteal surfaces.

De Smet and co-workers performed a number of studies on the effect of mechanical loading on implant osseointegration (De Smet *et al.*, 2006, 2007). All studies were performed for bicortically fixed screw-type metallic implants that were directly loaded (in contrast with Rubin and McLeod, 1994) in bending by means of an eccentrically applied implant force. Implants were placed in the tibial diaphysis of the guinea pig, and the amount of peri-implant bone was quantified histologically and compared to contralateral (unstimulated) controls. Both amplitude and frequency were varied for sinusoidal loading waves, the main research hypothesis in all studies being that the peri-implant bone response is proportional to the strain stimulus, as defined in equation 4 (i.e. proportional to strain rate amplitude). In a first study the strain rate amplitude was varied between 1620 and 12000  $\mu\epsilon/s$  (De Smet *et al.*, 2006). In total 6 different sinusoidal loading regimes were evaluated (3 at 3 Hz, 3 at 30 Hz), with strain amplitudes varying between 160 and 1000  $\mu\epsilon$  (as measured by means of a strain gauge attached to the cortical bone at 1.3 mm from the implant neck). Loading started one week after implant installation at 1800 cycles/day, 5 days/week, for 4 weeks. After 4 weeks post implantation more peri-

implant bone was seen for the mechanically stimulated implants with respect to control implants for all test groups, although the difference was not significant. The largest increase was seen for the lowest strain rate amplitude (1620  $\mu\epsilon/s$ , corresponding to 3 Hz x 540  $\mu\epsilon$ ). When plotting the increase in bone surface at the distal medullary cavity (the area that exhibited the largest increase) with respect to strain rate amplitude, an inverse correlation was even obtained. Therefore, this study did not support the hypothesis that the peri-implant osteogenic response is governed by the strain rate amplitude. This finding was further substantiated in another study with the same animal model, but for a different screw-type implant (De Smet *et al.*, 2007). Three different test groups were defined, all having the same strain rate amplitude of 1600  $\mu\epsilon/s$ , corresponding to a frequency of 3-10-30 Hz and a strain amplitude of 533-160-53  $\mu\epsilon$  respectively. The load duration was taken equal to 10 minutes per day in all test groups. A significant increase in bone surface area (with respect to contralateral control) was only evident for the 3Hz-loading regime, while for the higher (10-30 Hz) frequency no differences could be found.

## Discussion

### Loading studies on individual limbs

Based on the findings of Hsieh and Turner (2001) and Warden and Turner (2004) it seems that the osteogenic response to dynamic loading is not proportional to the strain rate amplitude (equation 4) within a broad frequency range (1-30 Hz). Instead, a non-linear response was retrieved, with the highest response encountered at 5-10 Hz (Warden and Turner, 2004). One may speculate about the causes of this non-linearity. As in the mouse ulna loading model the applied force needs to be transferred through the soft tissues (skin, cartilage) of the joints, dampening may render the force transfer at higher frequencies less efficient, leading to lower strains for the same force magnitude. Indeed, Hsieh *et al.* (1999) found such an effect for compressive loading of the rat ulna and four point bending loading of the rat tibia, both loading models leading to a logarithmic decrease of strain (per unit of force amplitude) with increasing frequency. The authors contributed this to the viscoelastic properties of the soft tissues and concluded that these loading systems acted like a low-pass filter, which filtered out the higher frequency loading components. In contrast, Warden and Turner (2004) could not confirm this effect in the mouse ulna model. Their strain gauge measurements revealed a constant strain per unit of load for loading frequencies between 1 and 30 Hz. Therefore, for their study dampening can be ruled out as a possible explanation.

Instead the authors believe that the non-linearity may be related to a non-linear mechanical response of one of the components of the mechanotransduction chain. Assuming that flow-induced deformation of osteocytes is the mechanism by which cells sense external loading, either the relation between bone matrix deformation and fluid flow or the relation between fluid flow and cell deformation (or both) may be frequency or rate dependent, leading to a

less efficient mechanotransduction process above a certain frequency or rate threshold. This idea is not supported by the theoretical findings of You *et al.* (2001). Their model predicts that strains are amplified from the tissue to the cell level and that the strain amplification ratio (i.e., ratio of cellular strain versus tissue strain) is increased with increasing load frequency, suggesting a more efficient mechanotransduction process with increasing loading frequency. Although the increase of this ratio becomes less substantial for higher frequencies (up to 20 Hz), they could not establish a decrease of the ratio beyond a certain frequency.

Apart from theoretical models, *in vitro* mechanotransduction experiments on bone cells may help to address the non-linearity observed by Turner and co-workers (obviously considering the limitations of *in vitro* models to explain an *in vivo* response). Interestingly, Jacobs *et al.* (1998) found a decreased responsiveness (increase in intracellular calcium) of human foetal osteoblastic cells to oscillatory and pulsatile fluid flow with increasing frequency, although in a frequency range (0.5 to 2 Hz) that is still lower than the 5-10 Hz “threshold” established by Warden and Turner. More recently, Faure *et al.* (2008) demonstrated that the expression of vascular endothelial growth factor (VEGF) in osteoblastic cells, which is important for bone angiogenesis, is regulated by stretch frequency. Interestingly, regulation seemed to be different for soluble and matrix-bound isoforms. While expression of soluble VEGF isoforms was found to be upregulated at low (0.05 Hz) frequencies, the matrix-bound VEGF isoforms was upregulated at high (5 Hz) frequencies. The implication of these *in vitro* findings for the *in vivo* response of bone to loading frequency remains to be investigated.

### Vibration studies

When confronting the results from some of the vibration studies with those of loading studies on individual limbs, it seems hard to explain how such extremely low strains can have an anabolic (and/or antiresorptive) effect. Indeed, many studies on individual limbs have clearly shown that a certain threshold needs to be exceeded to cause a net (positive) change in bone mass. This has led to the idea of error-driven adaptation, meaning that abnormal strains drive bone adaptation, which in its simplest form can be captured by the following equation:

$$\frac{dm}{dt} = C(\mathbf{r})[\phi(\mathbf{r}, t) - \phi_0(\mathbf{r})] \quad (10)$$

Where  $m$  is bone mass,  $C$  is a rate constant,  $\phi$  is a strain-related mechanical stimulus (which is dependent on time and space),  $\phi_0$  is a stimulus reference value (threshold),  $r$  is a spatial coordinate and  $t$  is time. If the stimulus is taken to be strain itself, the threshold is typically of the order of 100-1000  $\mu\epsilon$  in order to induce bone formation. Also, strain (or stimulus) values that cause an effect are typically related to vigorous, abnormal activity, which may only occur a few times per day (Rubin and Lanyon, 1984). Based on the concept that only large, “unusual” strains can have an osteogenic effect, we cannot explain the osteogenic response observed in vibration studies. Alternatively,

Rubin and co-workers have hypothesised that low magnitude high frequency loading events, which occur multiple times per day throughout the entire skeleton, may be as important for bone architecture as high magnitude, which are related to vigorous activity and which occur only a few times per day (Rubin *et al.*, 2002a). Fritton *et al.* (2000) quantified the daily strain history for different bones and animals by means of *in vivo* strain gauge measurements (12-24 hours monitoring) and indeed demonstrated that that large strains (more than 1000  $\mu\epsilon$ ) occur relatively few times a day, while very small strains (< 10  $\mu\epsilon$ ) occur thousands of times a day. They could establish a power law relation between frequency and average strain magnitude (during 12 hours) that was similar for the different bones and animals tested:

$$\bar{\epsilon}(f) = \frac{a}{f^b} \quad (11)$$

in which  $f$  is the frequency,  $\bar{\epsilon}(f)$  is the average strain at a certain frequency and  $a$  and  $b$  are constants. Average strain values were quite low, ranging from 1-10  $\mu\epsilon$  at 0.2 Hz to 0.05-0.5  $\mu\epsilon$  at 10 Hz. By applying vibration for 10-30 minutes per day at 17-90 Hz (as in the studies reported here) there will be a “disruption” of the power law relation, shifting it towards (relatively) higher (average) strains for the high frequency loading. Rubin hypothesised that this shift may be responsible for the adaptive (anabolic) response.

A further comparison can be made between the different vibration studies and the studies of Tanaka *et al.* (2003) and Castillo *et al.* (2006), who also applied high frequency low magnitude stimulation, although not by means of a vibrating platform, but by means of the (much more controlled) mouse ulna compressive model. Neither of the two studies could find an osteogenic effect of the high frequency signal alone, although strain gauge measurements could clearly demonstrate that high frequency components (up to 50 Hz) were transferred to the bone. It is likely that this mechanical stimulation regime disrupted the power law relation of equation (11) and therefore, according to the earlier mentioned hypothesis, this should have led to an osteogenic response. One may speculate why this was not observed and whether it could be related to the shorter daily load application (30 seconds per day versus 10-30 minutes per day for vibration studies), the different wave form (broad frequency noise versus sinusoidal loading for vibration studies) or the different mode of load application (the much more “constrained” axial compression versus “unconstrained” vibration). A final (maybe most important) difference between Tanaka *et al.* (2003) and Castillo *et al.* (2006) versus vibration studies was the fact that in the former two studies the low magnitude strains were actually not that low (at least not compared to vibration studies) and reached peak-to-peak values of several hundreds of microstrain.

One may wonder whether bone cells, like osteocytes are able to sense such extremely low strains or the corresponding fluid flows. Again, the theoretical model of You *et al.* (2001) may suggest some explanation. Interestingly, the strain amplification ratio is predicted to

not only increase with increasing load frequency, but also with decreasing load magnitude. Therefore, low-magnitude high frequency bone strains are most efficiently amplified. In the study amplification ratios of 19 and 122 are given for a 1Hz –1000  $\mu\epsilon$  regime and a 20 Hz – 50  $\mu\epsilon$  regime respectively. The paper does not give an answer to the question whether extrapolation of these data to even higher frequencies and lower bone strains may lead to cellular strains that can be sensed by osteocyte cellular processes. Apart from explanations based on a disruption of the power law relation between frequency and strain magnitude, increased mechanosensitivity or mechanotransduction towards higher frequencies, Rubin and co-workers (Rubin *et al.*, 2002a) and Turner and co-workers (Tanaka *et al.*, 2003; Castillo *et al.*, 2006) have suggested that a phenomenon called stochastic resonance may play a role as well. It refers to the ability of a non-linear system to detect and transmit a weak, otherwise undetectable signal by adding a random signal (such as noise) to it. Castillo *et al.* (2006) could however not detect such an effect for the investigated combination of sinusoidal carrier signals and added noise signals. At this stage it is unclear whether it could be an explanation for the observed osteogenic effect of vibration (also given the fact that loading regimes applied by vibrating platforms are sinusoidal and therefore not random). At least, based on the sheep studies from Rubin and co-workers (Rubin *et al.*, 2001a, 2001b, 2002a), the effect of vibration does not seem to be systemic, but limited to the limbs that have been mechanically stimulated.

### Implant osseointegration studies

While Rubin and McLeod (1994) found a higher amount of bone ingrowth for a 20 Hz compared to a 1 Hz loading regime, De Smet and co-workers found the largest osteogenic effect for the lowest strain rate and frequency (De Smet *et al.*, 2006, 2007). The contradiction may be related to the different modes of load application: while in Rubin and McLeod (1994) the host bone was stimulated, in the studies of De Smet and co-workers the implant was directly loaded. Therefore, it is likely that the strain distribution in the host bone and peri-implant tissues were very different for these loading set-ups. Our group has developed a methodology to create highly accurate, individualised finite element models of bones and implants, based on high-resolution  $\mu$ CT images (Jaecques *et al.*, 2004). This could be a way to evaluate the different local mechanical conditions to which peri-implant tissues are exposed in case of direct versus indirect implant loading. In case load application starts immediately or early after implant installation (as was the case in the studies of De Smet and co-workers), it is important to realise that the mechanical environment will interact with a biological system that can be very different from mature bone tissue. Davies (2003) described peri-implant healing as a process that recapitulates some of the events that also occur during bone fracture healing. It involves blood clot formation and platelet activation, migration, proliferation and differentiation of osteoprogenitor cells, and *de novo* bone formation. The implant surface characteristics (chemistry, topography) can interact with all of these processes. Given



these differences compared to mature bone, mechanoregulation of peri-implant healing will rely on mechanisms that are different from adaptation of mature bone. For bone adaptation it was hypothesised that the osteocytes are the mechanosensory cells. In the differentiating tissues around an implant, osteoprogenitor cells surrounded by their provisional matrix may be influenced by mechanical loading. A substantial number of both *in vitro* and *in vivo* studies have indeed demonstrated that the differentiation of mesenchymal precursor, progenitor, or stem cells is influenced by mechanical signals (see Estes *et al.* (2004) for a review). In parallel, computational models have been developed to describe the mechanoregulation of tissue differentiation (Prendergast *et al.*, 1997, Carter *et al.*, 1998, Claes and Heigele, 1999). Our group has validated the model developed by Prendergast and co-workers (Prendergast *et al.*, 1997, Lacroix and Prendergast 2002) for the quantitative description of peri-implant bone formation in an *in vivo* bone chamber that enables to control the mechanical environment (Geris *et al.*, 2008). An interesting feature of the mechanoregulation model by Prendergast and co-workers is the fact that it treats the differentiating tissues as biphasic (a mixture of solid and fluid constituents). Therefore, when loaded, cells and tissues will be exposed to deformation and fluid flow. In the model, both signals are considered as governing stimuli for tissue differentiation. For a biphasic material deformation and flow will not only depend on the load magnitude, but also the frequency or rate. As such, this model forms an attractive tool to explore how loading rate or frequency may influence tissue differentiation in the gap tissues around an implant.

### Concluding remarks

Mechanical loading is a key factor for bone architecture. Based on the many animal experimental studies that were included in this review, it is clear that loading frequency and rate are important parameters that influence the response to mechanical loading. Much less is however known about the mechanisms that govern the response to loading frequency and rate.

In terms of the effect of loading frequency, results from “high strain” loading studies on individual limbs seem to be in contradiction with results from “low strain” vibration studies. “High strain” individual limb loading studies suggest the existence of an optimal frequency around 5-10 Hz, beyond which loading becomes less osteogenic. “Low strain” vibration studies have reported an osteogenic effect for frequencies within the range of 17-90 Hz. Most of these studies considered only one frequency. The few studies that looked at different frequencies within the same animal model also varied other loading parameters, so that at this moment, it is unclear whether also for vibration studies there may be a frequency “threshold”. “High strain” individual limb loading studies that have used broad frequency noise (that contains higher frequency components up to 50 Hz and higher) could not establish an osteogenic effect. It is unclear whether this apparent discrepancy with respect to high frequency loading is related to the different loading set-ups (“constrained”

loading versus “free” vibration) or the fact that mechanotransduction efficiency is different at high strains versus low strains (as suggested by theoretical models that have studied strain amplification from the tissue to the cell level). As strain gauge measurements have demonstrated that high frequency loading components can be transduced to the (cortical) bone for both set-ups, it is unlikely that dampening has been an important confounding factor.

At a more phenomenological level, it has been suggested that the effect of extremely low magnitude high frequency strains may be related to a shift of the power law relation between frequency and strain (equation 11), which may lead to an adaptive response. At a more mechanistic level, theoretical model suggest that high frequency, low amplitude loading may be amplified the most.

Addressing these questions clearly requires further research:

- \* In order to further explore the difference between “constrained” loading setups (i.e. the loading models that were used in individual limb loading studies) and vibrating platforms, it would be interesting to investigate the effect of both loading modes within the same animal for similar strain magnitudes, loading frequencies and waves. This should be coupled to a thorough mechanical analysis of load transfer in both models, requiring the combination of experimental and computational tools.

- \* More fundamental research is needed on how load magnitude and frequency influence mechanotransduction. The word mechanotransduction should be interpreted here as the transduction of mechanical signals over multiple length scales (e.g. organ-tissue-cell) as well as the transduction of the mechanical signals into a biochemical response. Again, a combined computational and experimental approach is desirable. Computational models are needed that consider the coupling bone deformation and bone fluid flow (such as poromechanical models, see Cowin, 1999) and/or that are able to bridge the multiple length scales (hierarchical or multiscale models). *In vivo* and *in vitro* experiments on the molecular effects of (high frequency, low amplitude) loading should be able to increase our fundamental understanding of the pathways involved.

Extrapolation of bone adaptation studies to mechanoregulation studies of implant osseointegration does not seem to be obvious, given the different biology of peri-implant healing compared to bone adaptation. The number of studies that have looked at the effect of loading frequency and rate on implant osseointegration is still limited, and opposite results have been reported, although for different loading modes (direct versus indirect loading). Again, a number of suggestions for further research can be made:

- \* The evaluation of direct versus indirect loading within the same animal, in combination with image-based finite element modelling to quantify the strain distribution, would certainly aid in resolving this apparent contradiction.

\* The effect of loading frequency and rate on tissue differentiation in the peri-implant tissues should be combined with finite element models that consider these tissues as biphasic (or poroelastic). In this way the effect of frequency and rate on fluid flow and possible flow-mediated differentiation can be addressed.

\* The effect of vibration (high frequency, extremely low amplitude loading) on osseointegration has not yet been properly addressed. Further *in vivo* work is needed to fill that gap.

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## Discussion with Reviewers

**L. Vico:** As for whole body vibration, the “Rubin’s school” claimed for signals below 1g which is not the case for other studies of other teams who have tested accelerations greater than 1g. What do the authors think about the acceleration level?

**Authors:** The specific parameter settings in Whole Body Vibration studies that result in the strongest anabolic bone response are still a matter of debate. The identification of an acceleration level leading to an “optimal” strain environment able to induce bone cell response is a complex issue. As discussed in this paper, Whole Body Vibration studies often report on the acceleration level and frequency of the vibration loading; these are not independent parameters, but linked through the displacement amplitude of the platform (equation 9). It also has to be realized that the relationships between local tissue strains and global loading are only poorly understood, since force transfer through the limbs may involve soft tissue dampening and muscle activation (see introductory paragraph of the “Bone adaptation studies” section). Hence, more fundamental research is required for analyzing first, the transmission of this acceleration level from the body level through the organ level and tissue level down to the cell level, and second, to further enhance our knowledge on how the local mechanical environment around the cell affects cell response.

Extremely low-level (< 1 g) high-frequency vibrations were found by the Rubin’s school as the “optimal” combination for promoting bone response, whereas other studies (Flieger *et al.*, 1998; Oxlund *et al.*, 2003) reported (relatively) higher magnitude (2-3 g) high-frequency vibrations to be osteogenic. One may speculate that these different combinations of parameters would induce similar strain environments at the cell level. Indeed, You *et al.* (2001) in their mathematical model affirmed that higher “amplification ratios” (i.e., ratio of cellular strain versus tissue strain, see also second subparagraph in the “Discussion” section) were obtained for lower tissue strains (evaluated by You *et al.* down to 50  $\mu\epsilon$ ) and higher frequencies (evaluated up to 20 Hz). Hence, it may be that acceleration levels, either smaller than 1g (causing tissue strains of the order of 1-10  $\mu\epsilon$ , as quantified by Rubin and co-workers) or larger than 1 g (2-3 g, and therefore expected to causing larger tissue strains) would generate similar cellular strain levels and therefore similar osteogenic responses.

**L. Vico:** One important difference versus free vibrations might be the fact that controlled loading studies in turkey, rat, or mouse limb bones mainly showed a cortical modelling response while whole body vibrations seems to alter primarily the trabecular compartment. Do the authors have an explanation or a hypothesis?

**Authors:** Loading studies on individual limbs have shown that relatively high magnitude (order of 1000  $\mu\epsilon$ ) loading is needed for a net positive change in cortical bone mass. The studies reviewed in our paper simply did not investigate the trabecular bone response (among others because the applied animal loading models were not

suitable for this) and therefore they do neither confirm nor reject the hypothesis that also trabecular bone responds to such stimuli. Indeed, De Souza *et al.* (2005) developed a non-invasive axial loading model in the mouse tibia, which enabled them to study both cortical and trabecular bone response in the same limb. They demonstrated that mechanical loading induced magnitude-related increase in bone cortical formation (for cortical strains between 1150 and 2000  $\mu\epsilon$ ) and changes in trabecular bone architecture in mice.

For their sheep experiments, Rubin and co-workers only found a response to whole body vibration in trabecular bone, and not in cortical bone (Rubin *et al.*, 2001a, 2002a, 2002b). However, other vibration studies did report a cortical bone response as well. Xie *et al.* (2006) demonstrated that 45 Hz-0.3g vibration affected bone formation rates (BFR/BS) on the endocortical surface of the metaphysis in the proximal tibia of 8-week-old BALB/cByJ mice (in addition to trabecular bone effects). Judex *et al.* (2007) showed that 90 Hz-0.15 g vibration enhanced bone formation rates in the proximal tibia of OVX rats for both cortical and trabecular bone. These findings do not support the hypothesis that whole body vibration only affects trabecular bone mass.

Cortical and trabecular bone present very distinct architectures, and therefore it is likely that the same externally applied loading regime will result in different mechanical stimuli at tissue and cell level. In addition, trabecular bone may be metabolically more active by virtue of a larger specific surface area that is available for modeling and remodeling. Architectural, mechanical and biological differences between cortical and trabecular bone may be related to a different response to the same mechanical loading regime.

**M. Bohner:** A question related to the non-linear effect of loading frequency: since load signaling to bone cells depends on the fluid flow in canaliculi, and as the size of these canaliculi is likely to be known, is there no article attempting to calculate the fluid flow as a function of loading frequency and amplitude? (is that what the authors suggest in the second last sub-paragraph?)

**Authors:** A non-linear effect of loading frequency on bone response was reported in the controlled loading study of Warden and Turner (2004). They found bone adaptation to increase with increasing loading frequency up to 5-10 Hz, while a decrease was found for higher frequencies. They hypothesized that with frequencies higher than 10 Hz, the mechanotransduction process becomes less efficient, leading to reduced cellular stimulation and a resultant reduction in bone formation.

To our knowledge, no experimental study has been performed that directly measured the load-induced fluid flow in the canaliculi. Alternatively, mathematical models have been developed with the aim to understand the mechanical stimuli experienced by bone cells. In these models the fluid flow in the canaliculi is assumed to follow Darcy’s law, meaning that fluid velocity is related to the pressure gradient along the canaliculi (Weinbaum *et al.*, 1994; Zeng *et al.*, 1994). In one study, Zeng and coworkers (1994) attempted to couple the small-scale fluid mechanics

with the larger scale deformation of the entire osteon, when assuming bone as a poroelastic material. The canalicular geometry was idealized to a tube containing a centrally positioned osteocyte process. The velocity profile in the fluid annulus and the shear stresses acting at the exterior surface of the osteocytic process were derived from the pressure gradients generated by a sinusoidal loading applied to the osteon. You *et al.* (2001) improved this model by including pericellular matrix fibers anchoring and centering the cell process in its canaliculus and in turn coupling them to the intracellular actin cytoskeleton. The mechanical signal provided by the drag forces exerted on these fibers and transmitted to the cell process, appeared to be more important than shear forces on the cell membrane in stimulating and activating the cellular response. As discussed in our paper You *et al.* investigated the effect of loading frequency, but did not find a theoretical evidence for the hypothesis that the mechanotransduction process becomes less efficient for frequencies higher than 10 Hz.

The limitations of this mathematical model need to be mentioned. The geometry of the lacuno-canalicular network was idealized to a single canaliculus that drains towards the Haversian canal and, in addition, the annular region between the cell process and the canalicular wall was modeled as a cylindrical canal. The knowledge on the actual geometry with all its “irregularities” is indeed critical for estimating the flow in the canaliculi, as demonstrated by Anderson and Knothe Tate (2008). In their computational model the actual geometry of the pericellular

space was modeled, based on transmission electron micrographs (TEM), and magnitude and spatial variation of fluid velocity, pressure as well as shear stresses on the cell surface were predicted. Although the dependency of the fluid profile in canaliculi on loading frequency was not investigated, this study has the merit to evaluate the effect of the idealization of the pericellular fluid space geometry and dimensions. Indeed, the study demonstrated that this idealization underestimated fluid pressures, flow velocities as well as shear stresses. However, although this study took into account more complex situations from a geometrical point of view, it neglected all the compliant cell structures (cytoskeleton, cell membrane, transmembrane proteins and cytosol), which may be very important for mechanotransduction.

### Additional References

Anderson EJ, Knothe Tate ML (2008). Idealization of pericellular fluid space geometry and dimension results in a profound underprediction of nano-microscale imparted by fluid drag on osteocytes. *J Biomech* **41**: 1736-1746.

Weinbaum S, Cowin SC, Zeng Y (1994). A model for the excitation of osteocytes by mechanical loading induced bone fluid shear stresses. *J Biomech* **27**: 339-360.

Zeng Y, Cowin SC, Weinbaum S (1994). A fiber matrix model for fluid flow and streaming potentials in the canaliculi of an osteon. *Ann Biomed Eng* **22**: 280-292.