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Modeling and remodeling in bone tissue / by Ronald Ruimerman. –
Eindhoven : Technische Universiteit Eindhoven, 2005.
Proefschrift. - ISBN 90-386-2856-0
NUR 954
Subject headings: bone tissue / bone adaptation / bone remodeling /
osteocytes / osteoblasts / osteoclasts / simulations

The work in this thesis has been carried out under the auspices of the research school IPA (Institute for Programming research and Algorithmics)
IPA dissertation series 2005-02

Cover design: JWL Producties

Printed by University Press Facilities, Eindhoven

Modeling and remodeling in bone tissue

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de
Technische Universiteit Eindhoven, op gezag van de
Rector Magnificus, prof.dr. R.A. van Santen, voor een
commissie aangewezen door het College voor
Promoties in het openbaar te verdedigen
op maandag 14 februari 2005 om 16.00 uur

door

Ronald Ruimerman

geboren te Apeldoorn

Dit proefschrift is goedgekeurd door de promotoren:

prof.dr. P.A.J. Hilbers
en
prof.dr.ir. R. Huiskes

Voor mijn ouders,

Falke en Sterre

Contents

Chapter 1

Introduction

Bone tissue, forming the skeleton, is a remarkable material. Two macroscopically different types are distinguished. The first is *cortical* or compact bone, which is a rather dense tissue although it is penetrated by blood vessels through a network of canaliculi. It is primarily found in the shaft of long bones. The second type is *trabecular* or cancellous bone. It is porous and primarily found near joint surfaces, at the end of long bones and within vertebrae. It has a complex three-dimensional structure consisting of struts and plates (Fig 1).

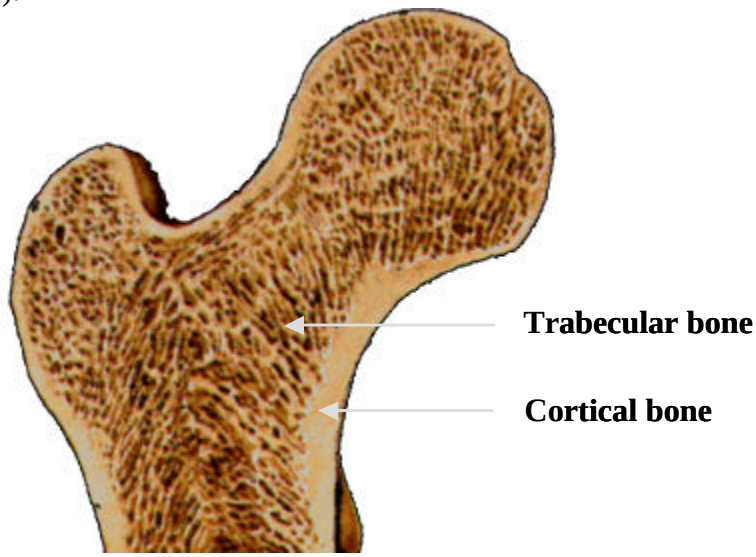

Fig 1: *A longitudinal section through the proximal femur shows trabecular bone enclosed by cortical bone.*

Already in 1892 Wolff found that the orientation of trabeculae coincides with the direction of the stress trajectories. He proposed that bone loading is somehow sensed and that the bone adapts its structure accordingly. This principle of functional adaptation is generally known as ‘Wolff’s Law’ (Wolff, 1892). It occurs in conditions of disuse, such as during immobility, space flight and long term bed rest, when bone is lost (Bauman et al., 1999; Vico et al., 2000; Zerwekh et al., 1998), and in overloading which causes a gain in bone mass (Suominen, 1993). It also occurs in growth, when the refined trabecular bone in childhood is changed to a coarser trabecular morphology in maturity (Tanck et al., 2000), after fracture healing (Wolff, 1892) and in relation with implant incorporation

(Guldberg et al., 1997). The ability of bone to adapt to mechanical loads is brought about by continuous bone resorption and bone formation. If these processes occur at different locations, the bone morphology is altered. Frost defined this as *modeling* (Frost, 1990a). In a homeostatic equilibrium resorption and formation are balanced. In that case old bone is continuously replaced by new tissue. This ensures that the mechanical integrity of the bone is maintained but it causes no global changes in morphology. Frost defined this as *remodeling* (Frost, 1990b).

Osteoporosis is a condition of reduced bone mass and increased bone fragility. The pathology of osteoporosis is poorly understood, but it is obviously caused by disturbed modeling and remodeling processes. Osteoporosis can express by bone fractures after minimal trauma. They particularly occur in the hip, lower forearm and in the vertebral bodies where trabecular bone is located. Early after menopause women undergo an accelerated phase of bone loss. This makes them more likely to become osteoporotic than men. Over 30% of women in the western world of over 50 years of age have osteoporosis (Melton et al., 2001). The lifetime risk of a bone fracture is 40% for women and 13% for men from age 50 and up (Melton et al., 1992). At those ages a bone fracture can be a catastrophic event. The one year mortality rate for hip fractures is 25% and the probability for an older patient to regain the previous level of function after a hip fracture is less than 30% (Magaziner et al., 1989; Chrischilles et al., 1991; Cooper et al., 1993). It can be expected that, with the increase in average age, osteoporosis can become a severe problem for society.

Proper understanding of the morphological degeneration in osteoporosis requires knowledge of the (re)modeling processes. These processes are conducted by specialized bone-resorbing cells (osteoclasts) and bone-forming cells (osteoblast). The activities of these cells are relatively well described, as discussed in the next section. The mechanisms that control their activities, however, are largely unknown.

Bone (re)modeling by osteoclasts and osteoblasts

The modeling and remodeling processes are not very different at the cellular level. They are based on the separate actions of bone resorbing cells called *osteoclasts* and bone forming cells called *osteoblasts*. The remodeling process begins at a quiescent bone surface with the appearance of osteoclasts. These are large multinucleated cells that form by fusion of mononuclear precursors of haemotopoetic origin (Vaananen & Horton, 1995). They attach to the bone tissue matrix and form a ruffled border at the bone/osteoclast interface that is completely surrounded by a “sealing” zone. Thus the osteoclast creates an isolated microenvironment. Subsequently the osteoclast acidifies the microenvironment and dissolves the organic and inorganic matrices of the bone (Vaananen et al, 2000). Briefly after this resorptive process stops, osteoblasts appear at

the same surface site. The osteoblasts derive from mesenchymal stem cells found in the bone marrow, periosteum and soft tissues. They deposit osteoid and mineralize it, so actually forming new bone. Some of the osteoblasts are encapsulated in the osteoid matrix and differentiate to osteocytes. Remaining osteoblasts continue to synthesize bone until they eventually stop and transform to quiescent lining cells that completely cover the newly formed bone surface. These lining cells are highly interconnected with the osteocytes in the bone matrix through a network of canaliculi (Lian & Stein, 2001).

It appears that osteoclasts and osteoblasts closely collaborate in the remodeling process in what is called a “Basic Multicellular Unit”, or BMU. This indicates that a coupling mechanism must exist between formation and resorption (Frost, 1964). The nature of this coupling mechanism, however, is not known. The organization of the BMU’s in cortical and trabecular bone differs, but these differences are mainly morphological rather than biological. In cortical bone the BMU forms a cylindrical canal of about 2000 μm long and 150-200 μm wide. It gradually burrows through the bone with a speed of 20-40 μm/day. In the tip, on the order of ten osteoclasts dig a circular tunnel (cutting cone) in the dominant loading direction (Petrtyl et al., 1996). They are followed by several thousands of osteoblasts that fill the tunnel (closing cone) to produce a (secondary) osteon of renewed bone (Parfitt, 1994). In this way, between 2% and 5% of cortical bone is remodeled each year. The remodeling process in trabecular bone is mainly a surface event. Due to the much larger surface to volume ratio, it is more actively remodeled than cortical bone, with remodeling rates that can be up to 10 times higher (Lee & Einhorn, 2001). Again osteoclasts come first in the process. They travel across the trabecular surface with a speed of approximately 25 μm/day, digging a trench rather than a tunnel, with a depth of 40-60 μm. Like in cortical bone they are followed by osteoblast bone formation. Active remodeling sites cover areas of varying sizes from as small as 50×20 μm up to 1000×1000 μm (Mosekilde, 1990). The trabecular BMU can be regarded as half a cortical BMU. The resulting structure that is formed is called a trabecular osteon or hemi-osteon (Frost, 1986; Eriksen and Kassem, 1992).

The cellular activities of osteoclasts and osteoblasts in modeling are basically similar to those in remodeling. However, in this case formation and resorption are not balanced, which causes changes in the micro-architecture. It can even occur that the activities of osteoclasts and osteoblasts are entirely uncoupled. Indeed, complete unloading may cause resorption not to be followed by formation (Mosekilde, 1990). That bone formation is not necessarily preceded by resorption can be concluded from the observation that lining cells at the bone surface can transform back to bone forming osteoblasts (Dobnig and Turner, 1995; Chow et al., 1998).

Chapter 1

How are mechanical forces expressed in osteoblast and osteoclast activities?

It is obvious that mechanical forces have a major influence on the bone modeling and remodeling processes in both cortical and trabecular bone, since their effects on bone morphology are obvious (Wolff, 1892). The pathways by which mechanical forces are expressed in osteoclast and osteoblast activity is currently one of the main unresolved issues in bone mechanobiology. The current concept is that the bone architecture is controlled by a local regulatory mechanism. This idea originates from Roux (1881), who proposed that bone remodeling is a self-organizing process. Frost captured these concepts in his 'mechanostat' theory (Frost, 1964, 1987). It assumes that local strains regulate bone mass. If strain levels exceed a so-called mechanical 'set-point', new bone is formed. If strain levels are below this set-point, bone is removed. It is a qualitative theory, but it forms the theoretical basis for several mathematical and computational theories that were developed to study bone adaptation (Cowin and Hegedus, 1976; Huiskes et al., 1987; Beaupré et al., 1990; Weinans et al., 1992; Mullender and Huiskes, 1995; Adachi et al., 2001).

The mechanostat does not specify the cellular level mechanisms behind the (re)modeling process. In other words, it does not describe how local mechanical signals are detected, nor how they are translated to bone formation and resorption. Osteocytes may play an important role here. Several studies revealed that these cells respond to mechanical stimulation (Skerry et al., 1989; Klein-Nulend et al., 1995). Together with the lining cells they form a system that seems well equipped for signal transduction (Cowin et al., 1991). It could be that mechanically induced osteocyte signals are transferred through the canaliculi to the bone surface where they control osteoclast and osteoblast activity (Burger and Klein-Nulend, 1999). Whether this is true remains to be proven.

Thesis outline

Bone modeling and remodeling were subjects of extensive studies in many fields of research. Much of this research to obtain insight in bone cell biology is based on reduction, i.e. isolating the various components to unravel their individual (and often very complex) behavior. The behavior of self-organizing systems like bone, however, is not necessarily fully understood, however, when the smallest elements involved are revealed. The objective of our studies is to reveal how mechanical forces are sensed in the bone, and how these mechanical forces are translated to structural adaptation of the internal tissue architecture. For that purpose we attempt to develop a coherent theory for bone modeling and remodeling as modulated by mechanical forces. For this dissertation we applied computer simulations to study the processes involved. This is a suitable approach to study self-organization, but requires abstraction from details. The use of computer

simulations to study bone adaptation became popular during the nineties. The first models were empirical. They completely abstracted from the underlying cellular processes, but related density changes in bone directly to local strain magnitudes. These models were capable of predicting density distributions in the bone as an effect of mechanical loads. The computational theories became more refined thereafter, i.e. they became more mechano-biologically oriented. We published a theory for bone modeling and remodeling in which the most important relationships of the mechanobiological cellular processes are captured (Huiskes et al., 2000; Ruimerman et al., 2001). It assumes that osteocytes are mechanosensitive cells capable of controlling resorption and formation at the trabecular surface. Applying 2D computer simulations we showed that trabecular-like structures are formed aligned to the mechanical loading direction, based on this regulation scheme. Hence, the theory provides a qualitative explanation for modeling and remodeling of trabecular bone as controlled by mechanical forces. In Chapter 2 we describe the developments of the earliest empirical models of bone adaptation towards our present theory.

The study described in Chapter 3 was performed to validate the theory with morphological data of actual trabecular bone. For that purpose we developed a 3D computer simulation model and investigated whether the theory predicts the development and maintenance of 3D trabecular bone structures with realistic characteristics in terms of morphology and metabolic activity.

A basic assumption for our theory is that osteocytes respond to mechanical stimulation. In the original theory we assumed that the anabolic osteocyte signals are triggered by the local mechanical loading variables they experience directly. These osteocyte signals, however, might also be triggered by fluid flow in the osteocytic network, as was suggested by others (Klein-Nulend et al., 1995; Burger & Klein-Nulend, 1999). This may have a considerable effect on the behavior of our theory as the distribution of fluid flow and deformation may differ considerably. We address this issue in Chapter 4 where we describe the effects of different mechanical loading variables on the morphological predictions of our computational model.

Post-menopausal osteoporosis, occurring in women, is caused by estrogen deficiency. It leads to increased remodeling rates, i.e. both osteoclasts and osteoblasts are more active, and decreased bone mass due to loss of trabecular connectivity. The thickness of the remaining trabeculae is mainly preserved, or even slightly increases (Aaron et al., 1987; Barger-Lux and Recker, 2002). It is obvious that the decreased estrogen levels deregulate the remodeling processes. For Chapter 5 we applied our (re)modeling theory in order to search for pathways by which estrogen deficiency might affect the remodeling process and cause the phenomena that are typical for postmenopausal osteoporosis.

Cortical and trabecular bone experience the same cycle of activation, resorption

and formation. This suggests that similar cellular communication pathways control the activity of osteoclasts and osteoblasts in both cortical and trabecular BMU's (Frost 1986, Eriksen et al, 1986, Parfitt, 1994). For Chapter 6 we refined our computational theory in order to investigate whether the proposed regulatory mechanisms can also explain osteoclast and osteoblast activity in the single remodeling BMU's themselves. More in particular, we investigated whether the theory explains that osteoclasts in the cutting cone resorb bone in the dominant loading direction and that osteoblasts follow in such a coordinated fashion.

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Chapter 2

Development of a unifying theory for mechanical adaptation and maintenance of trabecular bone

R Ruimerman and R Huiskes

Abstract

Trabecular bone is a tissue with a complex 3-dimensional structure, consisting of struts and plates, which attains its mature morphology during growth in a process called ‘modeling’. In maturity, the tissue is renewed continuously by local bone resorption and subsequent formation in a process called ‘remodeling’. Both these metabolic activities are executed by bone-resorbing osteoclastic and bone-forming osteoblastic cells. It is known that bone mass and trabecular orientation are adapted to the external forces and that alternative loading conditions lead to adaptations of the internal tissue architecture. The question is how the characteristics of external loads are sensed in the bone, and how they are translated to structural adaptation of the tissue. The time scale of the underlying processes is on the order of months or even years. This aspect makes bone a complex research topic. In this paper we discuss the application of computer simulation to investigate the remarkable adaptive processes. We describe our developments of empirical models in the past 15 years, able to predict bone adaptation to external loads from a macroscopic level towards a cell-based level, in which the most important relationships of the cellular processes are captured. The latest model explains the morphological phenomena observed in trabecular bone at a microscopic level.

Accepted for publication in: Theoretical Issues in Ergonomics Sciences

Chapter 2

Introduction

Bone tissue, which forms the skeleton, is a remarkable material. Two different types of bone tissue are distinguished. The first type is cortical or compact bone, which is a low porosity solid material, mainly found in the shaft of long bones. The second type is trabecular bone. It has a complex three-dimensional structure consisting of struts and plates and is mainly found near joint surfaces, at the end of long bones and within vertebrae. It is capable of optimizing its internal tissue structure under the influence of external forces to fulfill its primary function, mechanical load transfer. This paradigm was originally known as Wolff's Law [Wolff, 1892]. How the bone actually produces its internal architecture to fulfill its task in an optimized sense is currently unknown, but it is obvious that mechanical feedback must be involved. Trabecular orientation is in the direction of the principal mechanical loads. High loads, such as in intensive exercise, increase bone mass [Courteix et al., 1998], while reduced loads as in inactivity or disuse lead to decreased bone mass [Zerwekh et al., 1998]. Excessive loss of bone (osteoporosis) leads to increased fracture risk. As the populations get older, metabolic bone diseases such as osteoporosis are becoming increasingly problematic. It often leads to bone fractures, most commonly in femoral neck, vertebrae or distal forearm. This results in immobility, social and emotional problems, and high societal costs. Improved understanding of bone cell biology is an important issue in order to prevent osteoporosis and to improve physical and pharmaceutical treatment methods, as well as prosthetic designs. Of course it also involves many complex biochemical processes of which much is unknown.

One approach to obtain insight in bone cell biology is by isolating the different components to unravel their individual (and often very complex) behavior. Although this cannot fully explain the behavior of bone as a whole organ, it can provide valuable information. It is, however, difficult to take all aspects of bone biology into account in realistic conditions. In vivo experiments are hard to control and they still have limited life spans, insufficient to study anything but the earliest stages of adaptive responses, as the time scales at which the relevant processes occur are on the order of months, or even years. An alternative approach to gather knowledge of the processes involved in bone remodeling is by computer simulation. In this paper we describe an historical overview of computer simulations in bone biomechanics, that starts with an empirical model that was able to predict density changes in bone under the influence of external forces, and its subsequent expansion towards a more mechanobiologically oriented model that is cell based and explains bone behavior and morphology on the scale of individual trabeculae as effects of force transmission. For a wider historical survey we refer to Hart and Fritton (1997).

Methods

Bone density controlled by mechanical factors

With increasing computer capacity, computer simulation of bone adaptation in whole bones became in reach in the late 1980's. The early models, based on finite element analysis (FEA), calculated mechanical signals within the bone that were assumed to initiate changes in density or material properties, for which process numerical formulations of adaptive bone remodeling theories were applied. The models were empirical and related mechanical signals, like stress or strain, to bone adaptation, without direct consideration of the underlying cel-biological mechanisms. They were applied to complete bones and simulated adaptation of bone tissue on a macroscopic level.

In the work of Fyhrie and Carter (1986a) the bone tissue was considered as a continuum, characterized by particular apparent density value distributions. The bone was assumed to be a self-optimizing material with the objective to adapt its apparent density r to an 'effective stress' s_{eff}. Based on strength or strain optimization criteria they derived

$$r = A s_{eff}^{a}, \qquad (1)$$

where A and a are constants, and the effective stress is determined from either a failure or an elastic energy criterion. In later publications (Carter et al., 1987; and Fyhrie and Carter, 1986b) they assumed $a = 0.5$ and for the effective stress they postulated

$$s_{eff}^{2} = 2EU, \qquad (2)$$

where E is the apparent elastic modulus and U the apparent strain energy density (SED). By assuming a modulus-density relation of $E = cr^3$ (Carter and Hayes, 1977), with c a constant, the optimization function transforms to

$$r = c'U, \qquad (3)$$

where c' is a constant. Applying this optimization criterion made it possible to predict a density distribution of the proximal femur assumed optimal (Carter et al., 1987; Fyhrie and Carter, 1986b). To test the theory they visually compared the resulting density patterns to those in radiograms of a real femur, and observed some similarity in it.

Internal and external remodeling

Frost (1964) also suggested that internal and external remodeling [1)] should be distinguished. Internal remodeling is the adaptation of the density of bone tissue, while external or surface remodeling is the apposition or removal of bone tissue on the bone surface. The two forms were actually separated by Cowin and associates and by Huiskes

[1] Although 'modeling' and 'remodeling' of bone were clearly defined by Frost (1964) as change in shape (as in growth) and the subsequent localized resorption and formation of bone, the term 'remodeling' is often used in biomechanics as if to mean 'adaptation'. In fact, 'modeling' is closer to adaptation than 'remodeling'.

and coworkers. Cowin et al. (1981, 1985) used the strain tensor as the mechanical signal to be the driving factor of bone adaptation, and assumed a quadratic relationship between strain and the rate of adaptation (Firoozbakhsh and Cowin, 1981). Huiskes and coworkers (1987) used the strain energy density (SED) U [J/mm^3] as the signal that controls remodeling of the bone, with

$$U = \frac{1}{2} e_{ij} s_{ij} . \tag{4}$$

The relation between adaptive rate and SED was linear. For external surface remodeling the bone can either add or remove material, according to

$$\frac{dX}{dt} = C_x (U - U_n) , \tag{5}$$

where $\frac{dX}{dt}$ is the rate of bone growth perpendicular to the surface, U the SED, U_n a homeostatic SED, and C_x a proportionality constant. For internal remodeling the bone could adapt its density value. Assuming that the elastic modulus relates to the apparent density one can write

$$\frac{dE}{dt} = C_e (U - U_n) , \tag{6}$$

where E [MPa] is the local elastic modulus, and C_e a proportionality constant.
Carter (1984) suggested that bone is 'lazy' in terms of reacting to mechanical signals. The concept of a so-called 'lazy zone', meaning that there are thresholds to be exceeded before bone adaptation could occur (fig. 1), were incorporated by Huiskes et al. (1987). The SED was compared to a homeostatic one, U_h. For $U > (1+s)\, U_h$ or $U < (1-s)\, U_h$ adaptive activity is initiated, whereby s is the threshold level that marks the borders of the lazy zone. The remodeling rule transforms to a new set of equations for internal remodeling

$$\frac{dE}{dt} = \begin{cases} C_e (U - (1+s) U_n) & \text{for} \quad U > (1+s) U_n , \\ 0 & \text{for} \quad (1-s) U_n \le U \le (1+s) U_n , \\ C_e (U - (1-s) U_n) & \text{for} \quad U < (1-s) U_n , \end{cases} \tag{7}$$

and a similar set of equations for external remodeling.

This theory was applied to predict the density distribution in the normal proximal femur. Starting from an initial uniform density distribution the simulation produced a configuration that was similar to the one obtained by Fyhrie and Carter (1986b), showing some similarity to the natural density distribution in the femur. The theory was also applied to predict cortical adaptation after hip-prosthetic implantation in a geometrically idealized model (Huiskes et al., 1987). The bone was represented as a hollow cylinder in

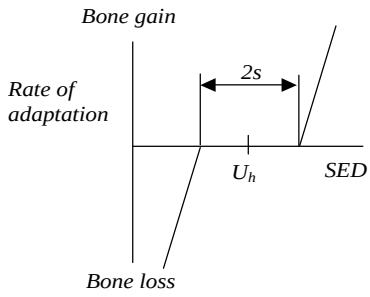

Fig 1: *The assumed local adaptation as a function of the strain energy density (SED). There is no adaptive response in the "lazy zone".*

which a cylindrical stem was placed and the effects of "stress shielding" due to load transfer through the stem could be predicted. The results illustrated the potential applicability of computer simulations for peri-prosthetic bone adaptation and encouraged further investigation. Later the theory was successfully applied to evaluate bone adaptation in a realistic 3-dimensional femur model after implantation of prostheses. Three-dimensional finite element models were constructed from an experimental animal configuration, in which smooth press fitted stems were applied. The adaptive remodeling procedure was integrated with the model and predicted similar amounts of proximal bone loss and distal bone densification as found in animal experiments (Rietbergen et al., 1993; Weinans et al., 1993).

Discontinuous end configurations

In the remodeling rules discussed in the previous section bone adapts towards reference states of deformation caused by single loads. To incorporate variable loading, a stimulus S was defined that took account of individual loading configurations by averaging their individual contributions (Carter et al., 1989), as

$$S = \frac{1}{n}\frac{1}{r}\sum_{i=1}^{n} U_i \,, \tag{8}$$

here U_i is the SED values for loading case i, n the total number of loading cases and r the apparent density. In order to estimate the actual SED value in the trabeculae the remodeling signal was approximated by U/r as the strain energy per unit of bone mass (Carter et al., 1989). In 1992 Weinans et al. applied this theory to a two dimensional finite element model of a proximal femur. Bone was represented as a continuum, capable of adapting its apparent density due to mechanical stimulation. The stimulus value S was measured per element, and the apparent density r was adapted per element according to:

$$\frac{dr}{dt} = B(S-k) \,, \tag{9}$$

with B and k constants and S the stimulus value in the element. This theory produced

density distributions that showed good resemblance with those in a real proximal femur. However, only if these distributions were locally averaged from discontinuous density patterns in the femoral head, where the trabecular bone is located (fig. 2).

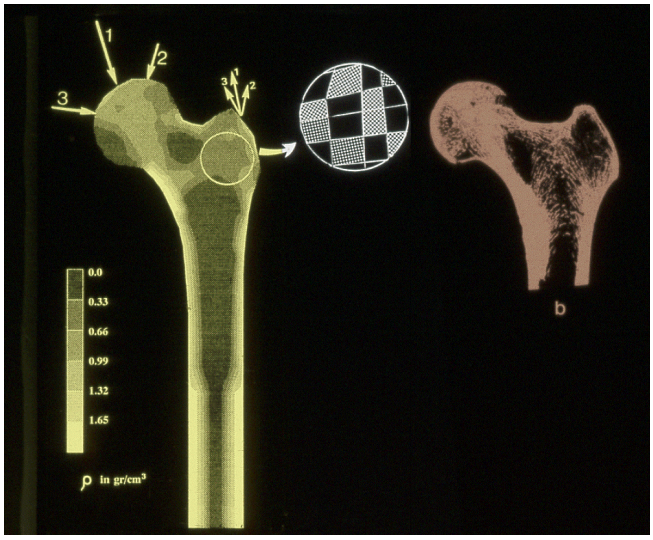

Fig 2: *Applied to a femur model and starting from a uniform density distribution the simulation model of Weinans et al. (1992) produced density distributions (a), that show some similarity to the density distribution in a real femur (b).*

In fact, the underlying patchwork of either full or empty elements is inadmissible relative to the mathematical basis of FEA. However, Weinans et al (1992) also showed that it is in the nature of the differential equations used to mathematically describe the adaptive remodeling process that the simulation produces discontinuous end configurations, a phenomenon called “checker boarding”. Using an analytical two-unit model they showed that the differential equations have an unstable uniform solution and that the slightest non-uniformity causes stress shielding by the stiffer elements. Hence, in the ongoing process the stiffer elements will even become stiffer, while the others loose density, until they are virtually empty, so that a “checkerboard” results (fig. 2). This phenomenon is the result of capturing both sensation of the local mechanical stimulus and actual adaptation of bone mass in that same location in one equation, which make the elements -which are artifacts for biology- ‘work for themselves’.

Osteocytic mechanosensation

Besides mathematically unstable, the previous theory was also biologically naïve, because osteoblasts and osteoclasts, the bone producing and removal cells, do not reside within the bone, but only appear at the trabecular surface as an effect of mechanical signals, which must be sensed within the bone matrix. Hence, the sensation of mechanical

stimuli and adaptation of material properties should be separated in the theory, as was done by Mullender et al. (1994) and Mullender and Huiskes (1995). Osteocytes within the tissue matrix were assumed to be mechanosensitive and capable of translating signals to the bone surface to attract so-called basic multicellular units BMU's (i.e. osteoclasts and osteoblasts, the actor cells of bone biology) which control the net apposition or removal of bone tissue. The signal sent to the surface by an osteocyte was assumed to decay exponentially with increasing distance *d* [mm], according to

$$f(x,x') = e^{-d(x,x')/D}, \tag{10}$$

where *x* is the location in which the signal strength is determined, *x'* is the location of the osteocyte concerned and *D* [mm] determines the decay in signal strength.

The relative density *m* [-] at location *x* was regulated by the BMU's. Relative to the total amount of stimulus *P* in that location they adapt the bone density according to

$$\frac{dm(x,t)}{dt} = tP(x,t), \tag{11}$$

where *t* [$MPa^{-1} \cdot s^{-1}$] is a rate constant. All osteocytes *N* located within the region surrounding *x* contribute to the stimulus *P*, so that its value in *x* is determined by

$$P(x,t) = \sum_{i=1}^{N} f(x,x_i)(S(x_i,t) - k). \tag{12}$$

Here x_i is the location of osteocyte *i*, $S(x_i,t)$ is the mechanical signal this osteocyte senses, and *k* is a reference value.

Although hypothetical, the mechanosensory role of osteocytes is not controversial. Osteocytes are shown to be sensitive to mechanical loading. They derive from osteoblasts that are encapsulated in the tissue matrix they produce. They are interconnected by a network of canaliculi, that seems perfectly equipped for signal transmission (see also Burger and Klein-Nulend, 1999).

A two-dimensional computational model was developed for this theory and applied at a microscopic level to a square plate of 2x2 mm^2, devided in 80x80 elements. The separation of sensation and density adaptation into two different functions prevented the so-called checker-boarding phenomenon. The theory produced configurations with characteristics that resemble actual trabecular bone structures, with average density correlating to the loading magnitude and trabecular orientation directly related to the external loading direction (fig. 3). The influence parameter *D* in equation (10) had a prominent role and variations affected the resulting morphology. The smaller the influence distance *D*, the more refined the resulting architectures, with thinner trabeculae. The model was able to realign its trabeculae to alternative loads and so explained both modeling and adaptation. After the structure was optimized or adapted to the external loads the activity of the BMU's stopped.

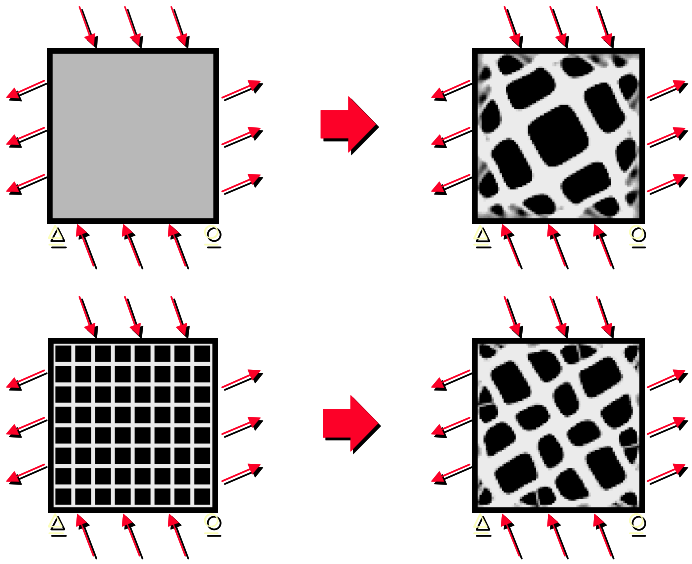

Fig 3: *Trabecular like patterns are formed by the simulated remodeling process starting from different initial configurations. The osteocyte influence parameter D = 100 mm, the reference SED k was .02 MPa and the orientation of the external load imposed is* $a = 30^o$ *(Mullender and Huiskes, 1995).*

Separation of osteoclastic and osteoblastic activity

In actual trabecular bone however, the activity of both osteoclasts and osteoblasts continues throughout life. In the growth stage, or during adaptation, these cells actually shape the bone structure (modeling), while in homeostatic conditions (or adulthood) they maintain the bone structure by tissue renewal through constant osteoclastic resorption and subsequent refilling of the cavities by osteoblasts (remodeling). In metabolic diseases (e.g. osteoporosis) it is the activities of these cells that are deregulated. How are the separate actions of these cells controlled? How is coupling between activity of these cells established, and how does this relate to bone maintenance? In order to answer such questions a more refined theory was required, in which the separate activities of osteoclasts and osteoblasts were incorporated. Such a theory was developed by Huiskes and associates (see Huiskes et al., 2000; Ruimerman et al., 2001). The new theory is based on the regulation scheme depicted in figure 4 and the relationships involved were quantified. Tissue adaptation on a specific location x at time t was supposed to be the result of osteoclastic bone resorption and osteoblastic bone formation, hence

$$\frac{dm_{tot}(x,t)}{dt} = \frac{dm_{cl}(x,t)}{dt} + \frac{dm_{bl}(x,t)}{dt}. \qquad (13)$$

What exactly initiates resorption is not known. In the theory it was assumed that osteoclasts are attracted towards the bone surface by microdamage, and that microdamage occurs spatially random, so that bone resorption is determined according to

$$\frac{dm_{cl}(x,t)}{dt} = -r_{cl}, \qquad (14)$$

where r_{cl} [mm^3/day] represents a stochastic function. Osteoblastic activity was controlled by an osteocytic bone formation signal P [mol·mm^{-2}·day^{-1}]. If the stimulus exceeds a certain threshold value k_{tr} [mol·mm^{-2}·day^{-1}] there is assumed to be tissue formation at the trabecular surface according to

$$\frac{dm_{bl}(x,t)}{dt} = t\,(P(x,t) - k_{tr})\,, \tag{15}$$

where t [mm^5·mol^{-1}] is a proportionality factor that determines the formation rate. The formation stimulus P in location x is determined by all osteocytes N located within the influence region, the exact signal R [J·mm^{-3}·s^{-1}] sensed by each osteocyte i, with mechanosensitivity m_i [mol·mm·J^{-1}·s·day^{-1}] and location x_i:

$$P(x,t) = \sum_{i=1}^{N} f(x,x_i)\,m_i R(x_i,t)\,, \tag{16}$$

where $f(x, x_i)$ [-] determines the decay in signal strength according to equation (10). Until now computational models of bone adaptation used static loads to evaluate the mechanical signals. It is, however, known that bone only reacts to dynamic loads. In the new theory the stimulus sensed by osteocytes is assumed to be a 'typical' strain energy density rate (SED-rate) $R(x, t)$ in a recent loading history. Cyclic loading conditions, characterized by frequency and magnitude, were imposed and it was assumed that osteocytes react to the maximum SED-rate during the loading cycle. It was shown that the maximal SED-rate is related to the SED value for some substitute static load and that it could be calculated by static finite element analysis (Ruimerman et al., 2001; Appendix A)

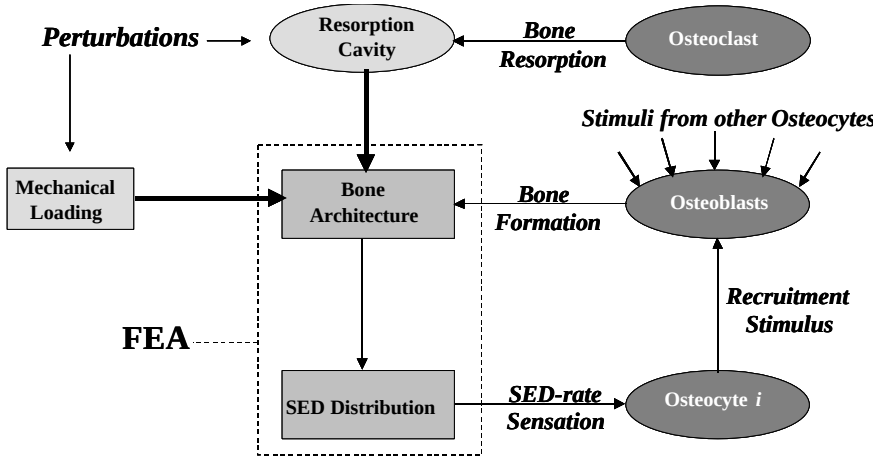

Fig 4: *Scheme of the regulation mechanism proposed: (i) Osteoclasts are assumed to resorp bone on locations where microdamage occurs which is thought to be on stochastic locations on the trabecular surface. (ii) Osteocytes locally sense a mechanical signal due to external load transfer through the architecture and locally recruit (iii) osteoblasts to form bone tissue (Huiskes et al., 2000).*

The relationships were incorporated in a 2-dimensional computer simulation model. Starting from different initial configurations the structures remodeled toward similar homeostatic configurations, in which the process of resorption and formation continued, but no more architectural changes occurred (fig. 5). Trabeculae were aligned to the loading direction. The regulatory mechanism was able to adapt the structure to alternative loading conditions. Increasing the magnitude led to apposition of bone tissue while decreasing it led to bone loss. After rotating the direction of the external load, the trabeculae realigned their orientations accordingly (fig. 5). From the simulation model it was concluded that the theory was able to explain both modeling (formation and adaptation) as well as remodeling (maintenance) of trabecular-like architectures as governed by external forces. Several additional conclusions could be drawn from the theory. Osteoblasts and osteoclasts are often assumed to work together in so-called BMU's. In our theory, coupling between these cells is established only implicitly through mechanics. The resorption cavities produced by osteoclasts cause local stress and strain concentrations. Consequently, osteocytes within the tissue matrix sense elevated mechanical signals and locally recruit osteoblasts to form bone tissue until the mechanical signal has decreased to normal levels, i.e. until the cavity is refilled (fig. 6). This does not imply that biochemical pathways are irrelevant (Udagawa et al., 1999), but it does show that mechanical feedback can be a potent coupling factor for the relevant biochemical processes to take place. Another conclusion came from the observation that both modeling (growth and adaptation) and remodeling (maintenance), can be explained by one unifying theory. Indeed both processes are based on the same cellular mechanisms and it could be concluded that both modeling and remodeling are similar in nature and just different stages of the metabolic cascade.

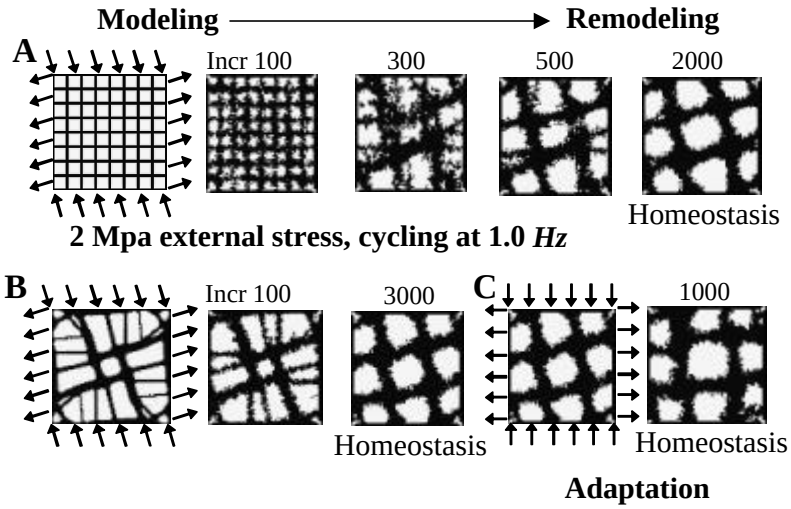

Fig 5: *Development of bone architecture in the simulation model, started from different initial configurations (A, B), and adaptation to an alternative loading direction (C), (Huiskes et al., 2000).*

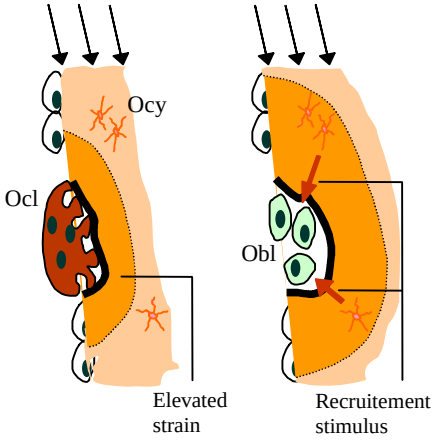

Fig 6: *In the theory osteoblast-osteoclast coupling is established as illustrated. Load transfer around osteoclastic resorption cavities results in elevated mechanical signals as sensed by osteocytes. Consequently osteoblasts are recruited to form bone tissue until the mechanical signals have decreased to normal levels, i.e. when the resorption cavity is filled. During bone formation some osteoblasts are entrapped in the tissue matrix they produce and differentiate to osteocytes. The remaining osteoblasts become inactive lining cells that completely cover the trabecular surface.*

Towards 3-dimensional structures

Applied in a 2-dimensional computer simulation model the theory could explain morphological adaptations observed in trabecular bone as related to load-induced cell activities. However, only in a conceptual sense. To actually validate the theory in a quantitative sense, and to apply it for valid predictions that are useful and falsifiable, its application in a 3-dimensional geometry is required. Computer capacity is a limiting factor for this requirement and it is currently not possible to simulate bone modeling and remodeling in complete bones at the trabecular level. In order to test whether our theory can produce trabecular-like structures in three dimensions, with morphological characteristics in a quantitative realistic range, for actual trabecular bone, we developed a 3-dimensional computer simulation model that can be applied to a small domain of bone tissue. Preliminary results show that the theory mimics the realistic morphological expressions of bone cell metabolism in a robust way, in growth, adaptation and remodeling, when applied to a small cube ($2x2x2mm^3$) of bone tissue with external loads imposed. When started from an initial porous configuration, the model produced structures with trabecular alignment in the loading direction (fig. 7), while volume fraction correlated to the magnitude of the external loads. The model described the growth as well as adaptation of complex 3-dimensional structures that are similar to actual trabecular bone. The architectures produced are remarkably stable, while the remodeling process proceeds.

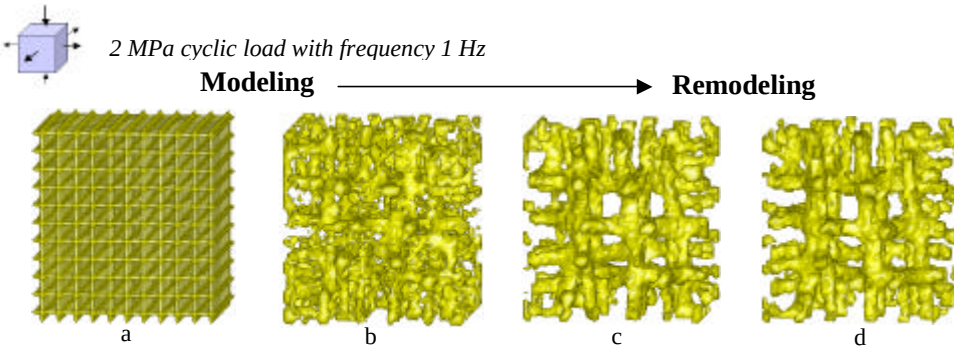

Fig 7: *Development of a 3-dimensional trabecular architecture as governed by external forces, starting from the porous initial configuration illustrated in (a), and the configuration after 10, 30 and 200 iterations (b-d).*

Discussion

Initially, theories for bone adaptation related bone density changes directly to mechanical, strain derived variables as effects of external forces. The computational simulation models based on these theories proved useful tools for prosthetic design. They were empirical models on a macroscopic level and the underlying biological processes involved were not taken into account. In the past decade the focus has shifted from biomechanics to mechanobiology, as it is likely that actual intervention in metabolic processes, to cure bone diseases, will be based on understanding the relationship between mechanics and cell biology. Understanding of bone biology has improved enormously in the past 15 years. This combined with increased computer capacities and efficient finite element algorithms has enabled the application of realistic theories, including the main aspects of bone biology. The theory relates cellular activity in bone modeling and remodeling to external forces and explains adaptive behavior of trabecular bone at a microscopic level. Although the most important relationships are captured in the theory, it should still be considered as a framework. The relationships are captured in simple mathematical equations, but actually encompass complex biological processes, the biochemical components of which are largely unknown. Nevertheless, the computational models based on the theory will enable us to investigate the morphological consequences of alternative loading conditions, metabolic disorders as well as their pharmaceutical interventions. They are useful tools for the development and investigation of hypotheses and for efficient design of experiments. Potentially they are useful for prosthetic design, as the adaptation of bone tissue to alternative mechanical environments can be pre-clinically tested. They can also be used in the development of treatment methods of osteoporosis, either based on physical exercise or in combination with pharmaceutics that alter cell activity. In the near future the 3D computational model will be used in order to investigate questions like: can the morphological changes seen in senile osteoporosis be explained by reduced physical activity alone? Can anti-resorptive drug administration

prevent loss of bone tissue and preserve trabecular connectivity, and at what stage should such a treatment start? Another area where the model can be applied is in postmenopausal osteoporosis. Can the theory mimic the phenomena observed in postmenopausal osteoporotic bone (loss of bone density and trabecular connectivity at a higher remodeling rate) and what is the role of mechanical coupling between osteoclasts and osteoblasts for this condition? Can osteoporosis be an effect of reduced osteocyte mechanosensitivity? Can the morphology of osteopetrotic bone be predicted as an effect of dis-functioning osteoclasts? Can the loss of trabeculae in older ages be prevented? Could the effects of physical activity be replaced by high frequency vibrations? Can trabecular and cortical (osteonal) bone modeling and remodeling processes be described with the same theory, and can the theory explain the transformation of trabecular into cortical bone from mechanobiological stimuli, as it occurs in growth? These are all important questions in bone biology and pharmacology.

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Appendix A

Remodeling effects of dynamic loading can be analyzed with static finite element analysis

We assume that the osteocytes sense, as a mechanical signal, the typical *strain energy density rate* (SED-rate) in a recent loading history. We propose that this SED-rate can be determined in a static finite element analysis. In this particular case, we take a uniformly distributed, periodic stress $s_e(t)$ on the external boundary of a bone specimen, cycling between zero and s [MPa], according to

$$s_e(t) = \tfrac{1}{2}\, s\,(1 + \cos wt), \tag{A1}$$

where $\omega = 2\pi f$, with f the frequency [Hz].

The external stress produces stresses and strains within the bone, which, at a given location, are fully characterized by the principal stresses s_i and principal strains e_i (i = 1, 2, 3). Assuming linear elastic material behavior, and neglecting inertia effects, these principal stresses and strains are directly proportional to the externally applied stress, and can be written as

$$s_i(t) = \tfrac{1}{2}\, c_i s\,(1 + \cos wt) \quad \text{and} \quad e_i(t) = \tfrac{1}{2}\, e_i s\,(1 + \cos wt), \tag{A2}$$

where c_i and e_i are principal stress and strain variables independent of time. For the strain energy density (SED) it follows, by definition

$$S(t) = \tfrac{1}{8} K s^2 (1 + \cos wt)^2, \quad \text{with } K = \sum_{i=1}^{3} c_i e_i \tag{A3}$$

The SED-rate is then calculated as

$$R(t) = \frac{dS}{dt} = -\frac{K s^2}{4}(1 + \cos wt) w \sin wt. \tag{A4}$$

The osteocytes are assumed to react to the maximal absolute value of the SED-rate. This maximum occurs for $wt = \pi/3$, and its value is found from

$$R_m = |R(t)|_{max} = a s^2 w K, \quad \text{where } a = \frac{3\sqrt{3}}{16} \approx 0.325. \tag{A5}$$

When the same bone specimen would have been loaded with a constant external stress s', the principal stresses and strains at a given location in the bone would have been

$$s_i(t) = c_i s' \quad \text{and} \quad e_i(t) = e_i s' \tag{A6}$$

The SED is then calculated as

$$S' = \tfrac{1}{2} K [s']^2 \tag{A7}$$

From (A5) and (A7) it follows that the dynamic SED-rate R_m equals the static S' if (with $w = 2\pi f$)

$$s' = s\,(4apf)^{\frac{1}{2}} \approx 2sf^{\frac{1}{2}} \tag{A8}$$

Chapter 3

A theoretical framework for strain-related trabecular bone maintenance and adaptation

R Ruimerman, P Hilbers, B van Rietbergen, R Huiskes

Abstract

It is assumed that density and morphology of trabecular bone is partially controlled by mechanical forces. How these effects are expressed in the local metabolic functions of osteoclast resorption and osteoblast formation is not known. In order to investigate possible mechano-biological pathways for these mechanisms we have proposed a mathematical theory [Huiskes et al., *Nature* (2000) 405:704-706]. This theory is based on hypothetical osteocyte stimulation of osteoblast bone formation, as an effect of elevated strain in the bone matrix, and a role for micro-cracks and disuse in promoting osteoclast resorption. Applied in a 2-D Finite Element Analysis model, the theory explained the formation of trabecular patterns. In this article we present a 3-D FEA model based on the same theory and investigated its potential morphological predictability of metabolic reactions to mechanical loads. The computations simulated the development of trabecular morphological details during growth, relative to measurements in growing pigs, reasonably realistic. They confirmed that the proposed mechanisms also inherently lead to optimal stress transfer. Alternative loading directions produced new trabecular orientations. Reduction of load reduced trabecular thickness, connectivity and mass in the simulation, as is seen in disuse osteoporosis. Simulating the effects of estrogen deficiency through increased osteoclast resorption frequencies produced osteoporotic morphologies as well, as seen in post-menopausal osteoporosis. We conclude that the theory provides a suitable computational framework to investigate hypothetical relationships between bone loading and metabolic expressions.

Accepted for publication in: Journal of Biomechanics

Chapter 3

Introduction

Trabecular bone has a complex 3-dimensional structure, consisting of struts and plates, which attains its mature morphology during growth in cell-based processes called 'modeling' (Frost, 1990a). In maturity, local bone resorption and subsequent formation in a process called 'remodeling' continuously renew the structure (Frost, 1990b). These metabolic activities are executed by bone-resorbing osteoclasts and bone-forming osteoblasts, cells that are recruited from the bony environment. It is known that bone mass and trabecular orientations are adapted to the intensity and directionality of external forces. Physical exercise increases bone mass (Courteix et al., 1998), while inactivity or microgravity reduces it (Zerwekh et al., 1998). It is also assumed that alternative loading directions cause adaptations in the spatial orientation of the internal trabecular architecture (Wolff, 1892). In osteoporotic bone, at older age, characterized by loss of bone mass, micro-structural deterioration and increased fragility, the regulatory mechanisms are obviously disturbed. 'Disuse osteoporosis', as it occurs in males and females, is attributed to lack of mechanical loading. But 'postmenopausal osteoporosis' is caused by a lack of estrogen. Hence a systemic, rather than a mechanical environmental disturbance. The generic question is, how are the effects of mechanical forces on bone expressed in the local balance of osteoclast resorption and osteoblast formation?

In the past we have investigated mathematical theories that might explain and predict development of bone density (Huiskes et al., 1987; Weinans et al., 1992; Kerner et al., 1999) and trabecular architecture (Mullender et al., 1994) as effects of external forces, using Finite Element Analysis (FEA) computer simulation models. Mechanical adaptation of trabecular density and architecture could be explained with a theory assuming mechano-sensory and signaling functions for the osteocytes (Mullender & Huiskes, 1995; Mullender & Huiskes, 1997; Mullender et al., 1998). These studies involved the net local bone-metabolic functions of Bone Multi-cellular Units (BMU), according to the conceptual theories of Frost (1990a, 1990b). In concept, our theory could explain the effects of forces in bone 'modeling', as it occurs in growth and (mechanical) adaptation. The theory did not, however, consider 'remodeling' (turnover, maintenance) - the production of resorption cavities by osteoclasts and their subsequent repair by osteoblasts - because it only described net adaptations of mass and form. For our present theory we left this restriction (Huiskes et al., 2000). We consider bone mass and form, at any time, as a balance between osteoclast resorption and osteoblast formation, modulated by external loads through osteocytic sensing and signaling. This new theory was made computationally operational in a 2-dimensional FEA model, to test its viability (Huiskes et al., 2000; Ruimerman et al., 2001). We have now developed a three-dimensional version of the FEA model, which allowed us to test its predictions relative to trabecular-bone metabolism as it occurs in reality. The results of these validation studies are reported here.

The precise mechano-biological pathways of strain-induced bone metabolism are not really known. Hence, the theory is partly based on assumptions. According to our theory bone mass is, at any time, determined by the mass balance of osteoclast resorption and osteoblast formation. Osteoblasts are recruited from the bone environment or by reactivation of lining cells (Dobnig and Turner, 1995; Chow et al., 1998). They form bone relative to osteocyte signals sent through the osteocytic canalicular network to the surface (Burger & Klein-Nulend, 1999; Cowin et al., 1991; Klein-Nulend et al., 1995; Knothe & Schaffler, 2002; Nicolella & Lankford, 2002; Skerry et al., 1989). The strength of an osteocyte stimulus is determined by the strain-energy density rate in its environment, created by external forces, depending on their magnitudes and frequencies. The osteocyte signal is transducted to the bone surface and is subject to exponential decay by distance. Osteoclasts are assumed to be recruited by osteocyte apoptosis due to micro-damage or cracks (Bronckers et al., 1996; Noble et al., 1997; Verborgt et al., 2000). Micro-cracks and damage are assumed to occur spatially random (Fazzalari et al., 2002), e.g. they can occur anywhere at any time. The (biochemical) 'coupling factor' (Frost, 1964) between osteoclast resorption and osteoblast formation is assumed to have a mechanical origin (Rodan, 1991). The osteoclast resorption cavity – called a 'notch' in mechanics – produces a stress concentration around itself (McNamara et al., 2002). We assume that this creates an enhanced biochemical recruitment signal for the osteoblasts from the vital osteocytes in that area (Fig. 1). In this way, mechanics and biology are thought to be closely interwoven in one and the same regulatory process. Our theory assumes osteoblast recruitment pathways to be similar in modeling and remodeling, and that the biochemical 'coupling' factor between osteoclasts and osteoblasts is circumstantial to the mechanical environment (Rodan, 1991; Chambers, 1998).

For the present validation studies we applied our 3-dimensional FEA model of bone modeling and remodeling. It represents a relatively small cube of trabecular bone tissue, which can be loaded at its faces. We tested the following hypotheses: Does the proposed theory produce a mature 3-D trabecular bone-like morphology - with more or less realistic trabecular thickness, volume fraction and orientation - from a morphology representing early childhood (Tanck et al., 2001; Fig. 2)? Is this homeostatic, mature configuration characterized by realistic physiological parameters like remodeling spaces, osteoclast activation frequencies and remodeling rates? Are architectural adaptations to alternative loads realistic? Can the theory explain disuse osteoporosis, characterized by reduced density and trabecular thickness, due to reduced external loads? Can the theory explain postmenopausal osteoporosis due to estrogen deficiency and subsequent increases in osteoclast resorption frequencies (Chambers, 1998), characterized by reduced trabecular connectivity, thickness and strength?

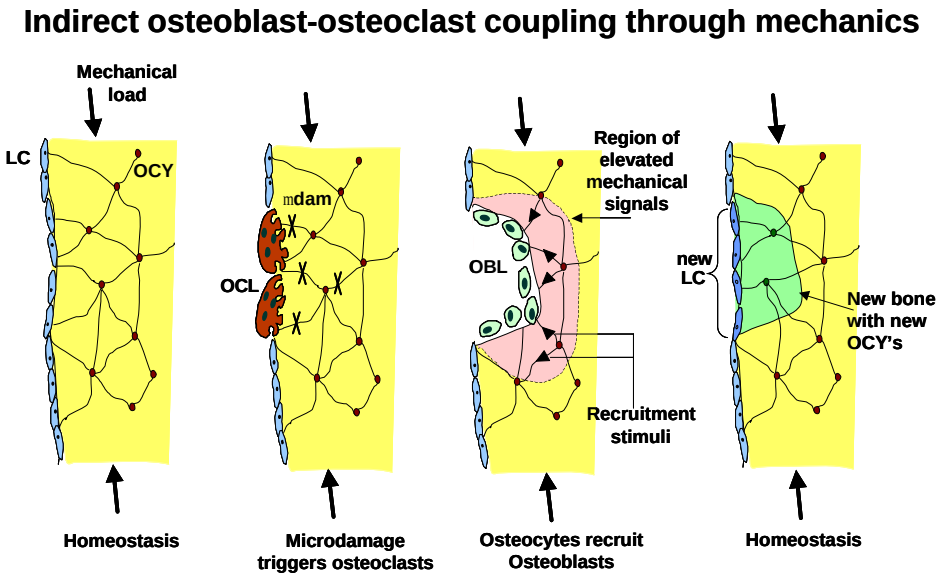

Fig 1: *During remodeling osteoclasts are attracted towards the trabecular surface due to microdamage. They produce a resorption lacunae resulting in local stress and strain concentrations surrounding the resorption cavity due to mechanical load transfer. Consequently osteocytes sense higher mechanical signals and recruit osteoblasts that form bone until the resorption cavity is filled. Some of the osteoblasts are encapsulated in the tissue matrix and differentiate to osteocytes. Other osteoblasts become inactive lining cells that cover the bone surface. Finally the homeostatic configuration is restored.*

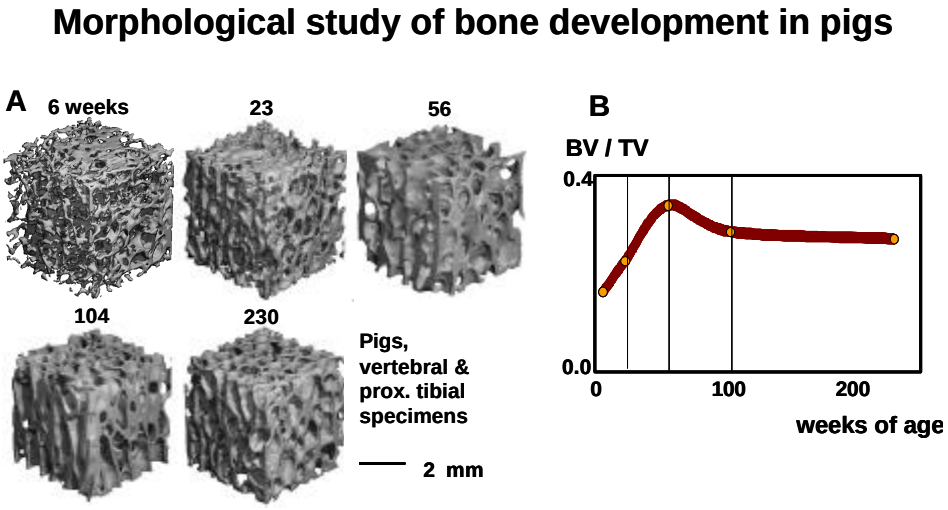

Fig 2: Results of a morphological study of bone development in pigs show similar behavior to what we found in our simulations. (A) The initial configuration is porous and fine and becomes coarser and obtains more directionality when it matures. (B) Initially the volume fraction (VF) rises sharply, overshoots and then stabilizes (Tanck et al., 2001). (Note that maximal VF is not equivalent to peak bone mass).

Methods

The theory is summarized in Fig. 3. Mathematical equations were introduced to quantify relationships (Huiskes et al., 2000; Ruimerman et al., 2001). The change in bone mass at a particular trabecular surface location x at time t is determined by osteoblast bone formation minus osteoclast bone resorption, hence

$$\frac{dm_{tot}(x,t)}{dt} = \frac{dm_{bl}(x,t)}{dt} - \frac{dm_{cl}(x,t)}{dt}. \quad (1)$$

Osteoblast activity at the trabecular surface is controlled by osteocytic bone formation stimuli. For a total stimulus P [mol·mm^{-2}·day^{-1}] that exceeds a certain threshold value k_{tr} [mol·mm^{-2}·day^{-1}] we assume osteoblast tissue formation according to

$$\frac{dm_{bl}(x,t)}{dt} = t\,(P(x,t) - k_{tr}), \quad (2)$$

where t [mm^{5}·mol^{-1}] is a proportionality factor that regulates the formation rate relative to the formation stimulus. The bone formation threshold k_{tr} and the formation rate t affect VF and remodeling space. Their values were chosen empirically. All osteocytes N within the influence region contribute to the bone formation stimulus P on trabecular surface location x, depending on their mechanosensitivity m_i [mol·mm·J^{-1}·s·day^{-1}], their distance d to surface location x and the signal R [J·mm^{-3}·s^{-1}] the osteocytes sense in their location x_i, hence

$$P(x,t) = \sum_{i=1}^{N} f(x,x_i) m_i R(x_i,t), \quad \text{with} \quad f(x,x_i) = e^{-d(x,x_i)/D} \quad (3)$$

where $f(x, x_i)$ describes the decay in signal intensity relative to distance d and decay parameter D.

Osteoclast bone resorption is described by

$$\frac{dm_{cl}(x,t)}{dt} = -r_{cl}, \quad (4)$$

where r_{cl} [mm^3/day] represents a function that removes specified portions of tissue V_r at random locations at the bone surface.

The signal sensed by osteocytes is assumed to be a 'typical' strain energy density rate (SED-rate) $R(x,t)$ [J·mm^{-3}·s^{-1}] in a recent loading history that is calculated using FEA, for which the domain was divided in cubic voxels. Each voxel was considered as a hexahedral element, containing eight integration points. An element-by-element solver (Rietbergen et al., 1996) was used to solve the force-displacement equations.

Material properties are assumed isotropic, linear elastic with elastic modulus E [MPa] depending on the relative density value m, according to (Currey, 1988)

$$E = E_{\max} \cdot m^{g}, \quad (5)$$

where the maximal elastic modulus E_{max} [MPa] and exponent g are constants.

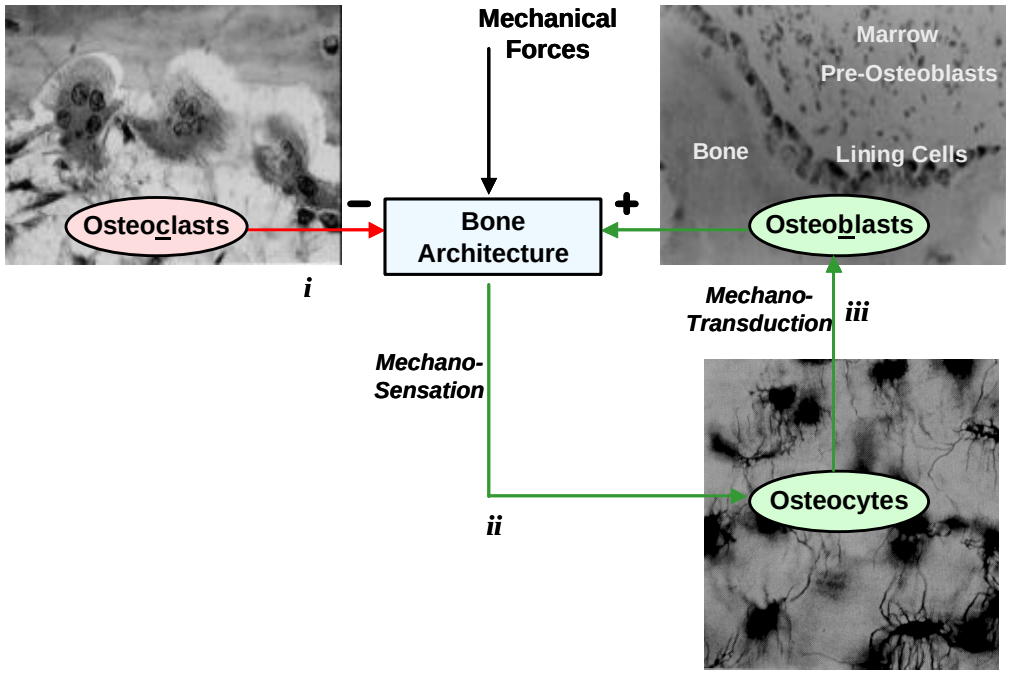

Fig 3: *Proposed regulation mechanism: (i) Osteoclasts are assumed to resorb bone on locations where microdamage occurs which is thought to be on stochastic locations on the trabecular surface. (ii) Osteocytes locally sense a mechanical signal due to external load transfer through the architecture and locally recruit (iii) osteoblasts to the bone surface to form bone tissue.*

The mathematical equations were implemented in a computer-simulation model that was applied to a cubic domain of 3.3×3.3×3.3 mm^3, divided in 100×100×100 cubic voxels. To impose external forces, plates (thickness of one voxel) were added at the surfaces of the cubic domain, disconnected at the ribs of the cube in order to avoid stress shielding (Ruimerman et al., 2001). The plates do not participate in the remodeling process and were given material properties as fully mineralized bone tissue. The iteration time step was set to 2 weeks, i.e. on the order of the time period necessary for osteoclasts to produce a resorption lacuna (Parfitt, 1994). Harmonic loads were imposed that were compressive in vertical and tensile in horizontal directions (frequency 1 *Hz*, magnitude 2 *MPa*). Whether the loads are compressive or tensile does not affect the resulting morphology, as local SED values in compression and tension are equal. Osteocytes were randomly distributed in the tissue at a density of 44.000 mm^{-3}. Their influence distance *D* was 100 *mm* so that approximately 90 osteocytes contribute to the total bone formation stimulus received at one particular bone surface location.

Osteocytes were assumed susceptible to the maximal SED-rate during one loading cycle. This value is calculated using a substitute static stress of 4 *MPa* (Ruimerman et al., 2001). Other parameter settings were as specified in Table 1.

variable	**symbol**	**unit**	**value**
Osteocyte density	n	mm^{-3}	44.000 [1]
Osteocyte mechanosensitivity	m	$nmol \cdot mm \cdot J^{-1} \cdot s \cdot day^{-1}$	1.0
Osteocyte influence distance	D	mm	100 [2]
Formation threshold	k_{tr}	$nmol \cdot mm^{-2} \cdot day^{-1}$	$5.0 \cdot 10^{6}$
Proportionality factor	t	$mm^{5} \cdot nmol^{-1}$	$1.4 \cdot 10^{-10}$
Resorption amount per cavity	V_r	mm^{3}	$5.6 \cdot 10^{-5}$ [3]
Osteoclast recruitment frequency	f_{ocl}	$voxel^{-1} \cdot day^{-1}$	$7.1 \cdot 10^{-4}$
Maximal elastic modulus	E_{max}	GPa	5.0 [2]
Poison ratio	n	-	0.3 [2]
Exponent gamma	g	-	3.0 [4]
Loading frequency	f	Hz	1
Loading magnitude	A	MPa	2.0

Table 1: *Parameter settings for the simulations. [1] Mullender et al. (1996), [2] Mullender and Huiskes (1995), [3] Eriksen and Kassem (1992) and [4] Currey (1988).*

Three simulation series were performed to answer our research questions. The first series tested whether the theory produced trabecular-like 3D configurations. The simulation started from a conceptual initial configuration, representing bone in the post-mineralized fetal stage, and was prolonged until no more gross architectural changes occurred, representing the homeostatic mature stage. The developments in VF and SED distribution, as sensed by osteocytes within the bone were monitored, and the morphological characteristics were determined. The 'remodeling space' – the total volume of unfilled resorption cavities - in the homeostatic configuration was determined by prolonging the simulation with osteoclast resorption completely inhibited. The simulation was repeated, starting from a uniform-density and a chaotic (random-density) configuration to test the influence of the initial configuration and with an external load that was rotated by 20 degrees in order to test mesh dependency.

The second simulation series was conducted from the homeostatic structure of the first series, to investigate whether the structure adapts to alternative loading conditions, in trabecular thickness and directionality. The orientations of the external loads were rotated by 20 degrees to test whether the trabeculae realign. The magnitudes of the external forces were increased by 50%, to test whether trabecular thickness – hence volume fraction – would increase. The external loads were reduced by 50% and 75% to test whether, and to what extent, trabecular thickness and volume fraction would

decrease. Finally, the horizontal (x- and y-) loads were decreased by 75%, while the vertical (z-) loads were preserved. The latter simulates a simplified loading pattern, characterized by a lack of horizontal 'error' loads, which is thought to produce a condition of disuse osteoporosis, common in the elderly (van Rietbergen et al., 2003; Homminga et al., 2004).

The third simulation series was conducted to simulate mild and severe postmenopausal osteoporosis. We started from a homeostatic morphology and mimicked the effects of estrogen deficiency as in postmenopausal osteoporosis by increasing the osteoclast recruitment frequency with 100 and 200 percent, respectively (Kanis, 1997; Garnero et al., 1996).

Results

Starting from the initial stage, representing bone tissue in the post-mineralized fetal stage (Fig. 4A), the morphology developed to a (homeostatic) equilibrium architecture with trabeculae aligned to the loading orientation (Fig. 4F). After this stage no more gross architectural changes occurred. The homeostatic morphology was maintained, with balanced bone resorption and formation continuing; the turnover rate was such that the net total bone mass would be replaced in approximately 4 years (Han et al., 1997).

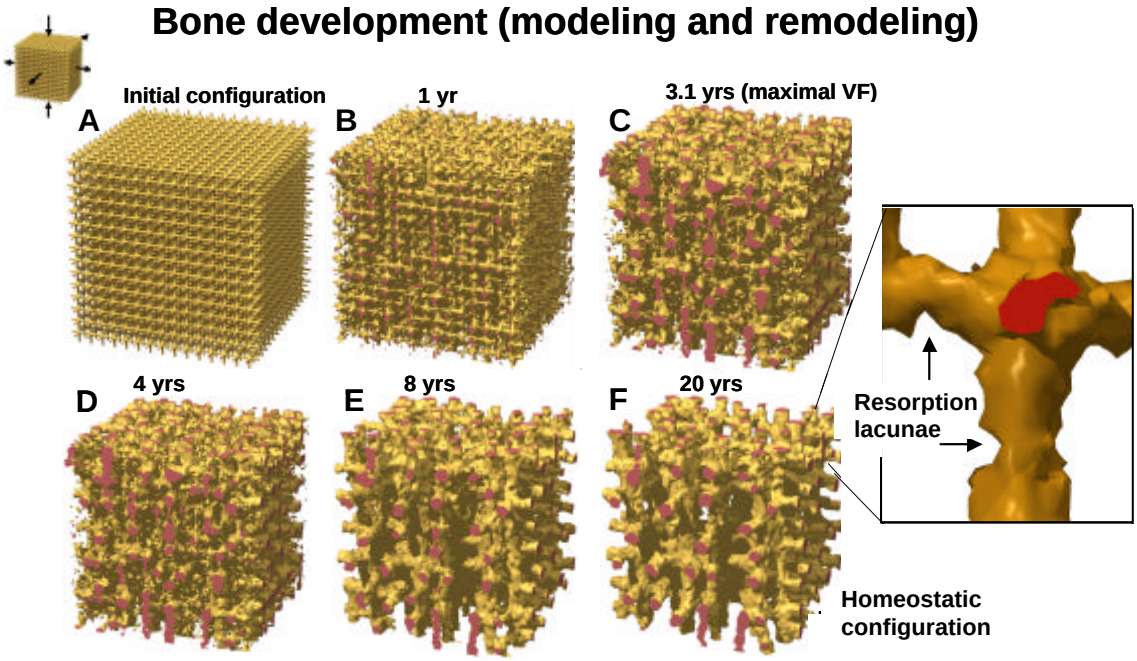

Fig 4: *Starting from a porous initial configuration representing bone in the post mineralized fetal stage (A) the structure developed in approximately 8 years to a mature structure (E) (modeling). From this point the structure was maintained (F), while no more large architectural changes occurred (homeostasis or remodeling).*

Initially (Fig. 4A) the trabeculae were thin (33 *mm*), while their numbers were high (Tr.N = 3.00 mm^{-1}). After homeostasis was reached (Fig. 4F) the number of trabeculae was reduced considerably (Tr.N = 1.01 mm^{-1}), while trabecular thickness increased to an average of 172 *mm*, close to the value of 151 *mm* found for the trabecular thickness in pigs (Mullender et al., 1996). Bone volume fraction (VF) rose sharply initially (Fig. 5A), overshot towards a maximum of 0.191 and then dropped back to fluctuate around approximately 0.175. Similar phenomena were seen in the development of real (pig) bone (Fig. 2).

As found by Liberman et al. (1995), the largest increase in bone mass after completely inhibiting bone resorption in a homeostatic configuration was seen early after inhibition. Afterwards the VF increased gradually until finally all cell activity stopped and the remodeling spaces were entirely filled. Although on the large side, its value (8%) is not unrealistic for actual trabecular bone (Kanis, 1997). The remodeling space became smaller when the osteoblast bone formation rate was increased.

In the early stage of development, tissue loading (measured by SED), as assumed to be sensed by the osteocytes within the structure, was high (mean value $6.0 \cdot 10^5$ Nm^{-2}) and its values varied in a wide range (Fig. 5B). Gradually the structure adapted to the external loads, the average osteocyte SED decreased to $2.6 \cdot 10^5$ Nm^{-2} and its variation decreased significantly (Fig. 5C). The spatial SED-distribution obtained (Fig. 5C) was similar to those found for actual trabecular bone in the femoral head (van Rietbergen et al., 2003) and in trabecular bone of vertebral bodies (Homminga et al., 2004).

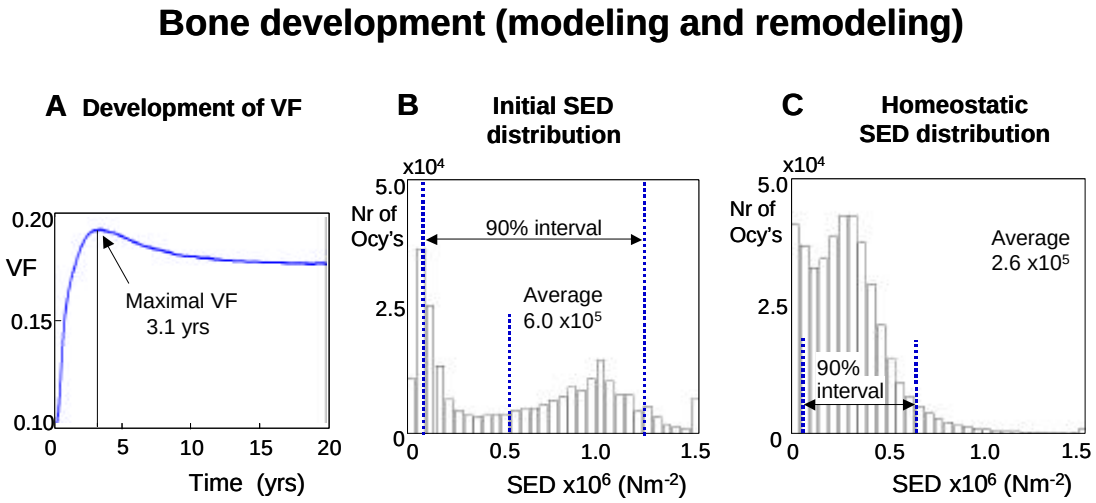

Fig 5: *(A) Development of the volume fraction. (B) The initial mechanical signals as sensed by osteocytes have a high average value and a large 90% interval. (C) In the homeostatic situation the average value and 90% interval have reduced considerably.*

Starting from alternative initial configurations or loading orientations led to similar results: Structures were formed with trabecular orientations aligned to the load orientations, and quantitative characteristics in a similar range (Fig. 6). This implies that the final morphology was relatively insensitive to the initial configuration.

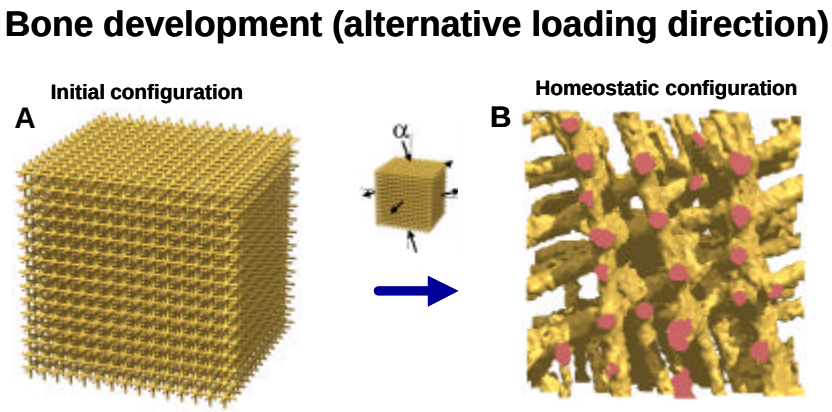

Fig 6: *Bone development (modeling) under influence of an alternative loading direction ($a = 20^0$).*

In the second simulation series the homeostatic morphology (Fig. 4F) was used to test whether the regulatory mechanism can adapt the internal tissue architecture to new loading conditions. Enlarging the loading magnitude by 50% increased the volume fraction from 0.175 to 0.262 at an average osteocyte SED of $2.6 \cdot 10^5$ Nm^{-2}. Trabecular thickness increased to 203 *mm*, but no new trabeculae were formed (Fig. 7). Reducing external loads by 50% reduced bone mass and trabecular thickness (Tr.Th = 140 *mm*), to a VF of 0.090 and some trabeculae were lost (Tr.N reduced to 0.64 mm^{-1}). Average osteocyte SED was $2.8 \cdot 10^5$ Nm^{-2}. Decrease of the loads by 75% led to a reduced VF of 0.051, with Tr.Th = 121 *mm* (Fig. 7), and severe loss of trabecular connectivity (Tr.N = 0.42 mm^{-1}) with an average osteocyte SED of 2.9 Nm^{-2} (Fig. 8). Reducing horizontal loads by 75%, while vertical loads were preserved, led to a strongly anisotropic structure with trabecular alignment mainly in the vertical direction (Fig. 7). Vertical trabeculae maintained their thickness (Tr.Th = 171 *mm*). The average SED value in this situation was $2.8 \cdot 10^5$ Nm^{-2}.

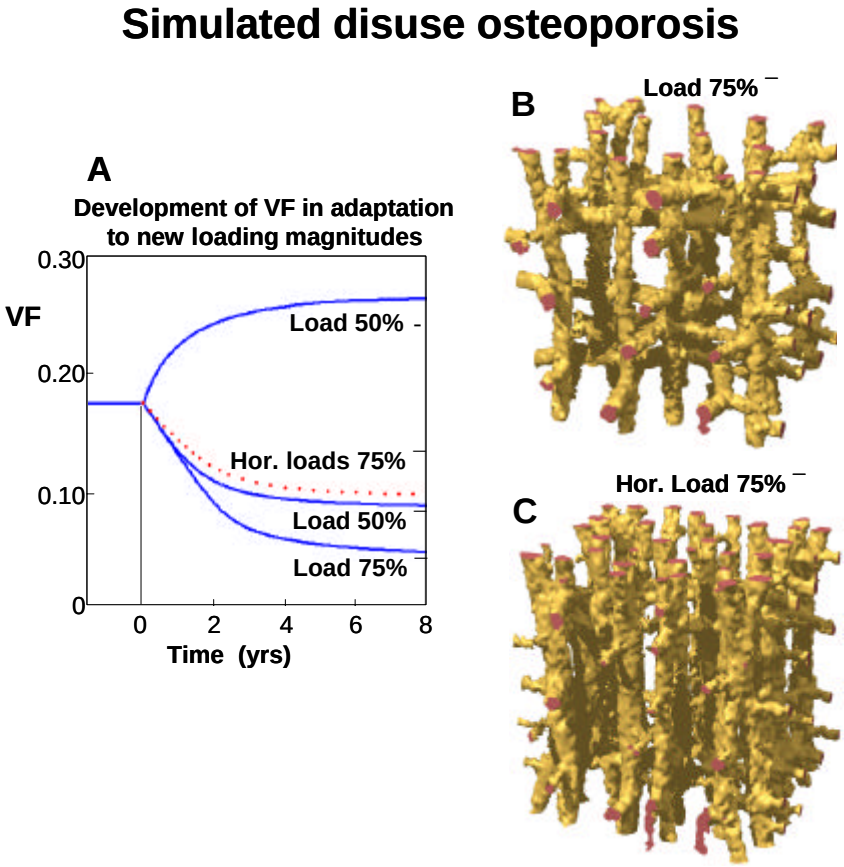

Fig 7: *(A) Increased loads result in net bone formation while reduced loads result in bone loss. Largest changes occur shortly after the new conditions set in. (B) Reduced loads result in loss of bone and trabecular connectivity. (C) Reducing horizontal loads only results in a strongly anisotropic configuration mainly oriented in the direction of the highest loads.*

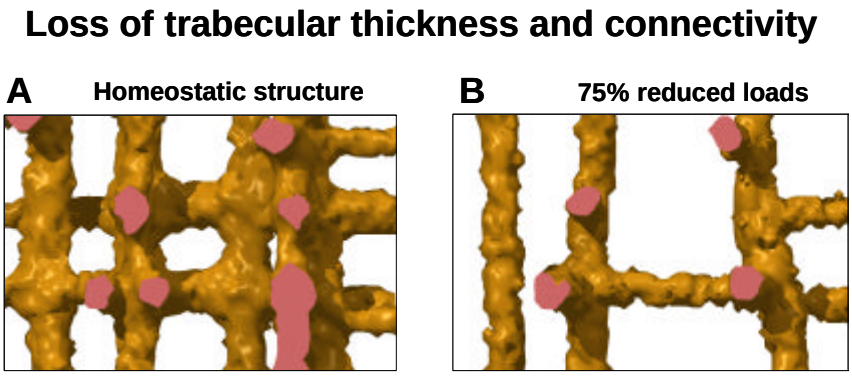

Fig 8: *These enlarged details of the homeostatic configuration (A) and the same piece of tissue after adapting to a reduction in the loading magnitude (B) clearly show trabecular thinning and loss of complete trabeculae.*

In addition to adaptation to variations in loading magnitudes, the regulation mechanism also predicted adaptation of trabecular directionality to alternative load orientations. Starting from the homeostatic morphology obtained earlier (Fig. 4F), the external loads were rotated by 20 degrees. In the course of approximately 12 simulated years, the trabecular structure re-aligned completely to the new forces (Fig. 9), with trabeculae oriented in the new force directions. The SED distribution within the tissue increased substantially (average $6.0 \cdot 10^5$ Nm^{-2}), with a large variation (Fig. 10B), immediately after rotation of the forces and the volume fraction increased rapidly to a maximal value of 0.200 after 1.7 simulated years (Fig. 10A). Eventually, after 12 simulated years, the structure was adapted again to the new loads, at a VF of 0.182 (Fig. 10A), the average SED restored to an average value of 2.6 Nm^{-2} and its variation reduced again (Fig. 10C).

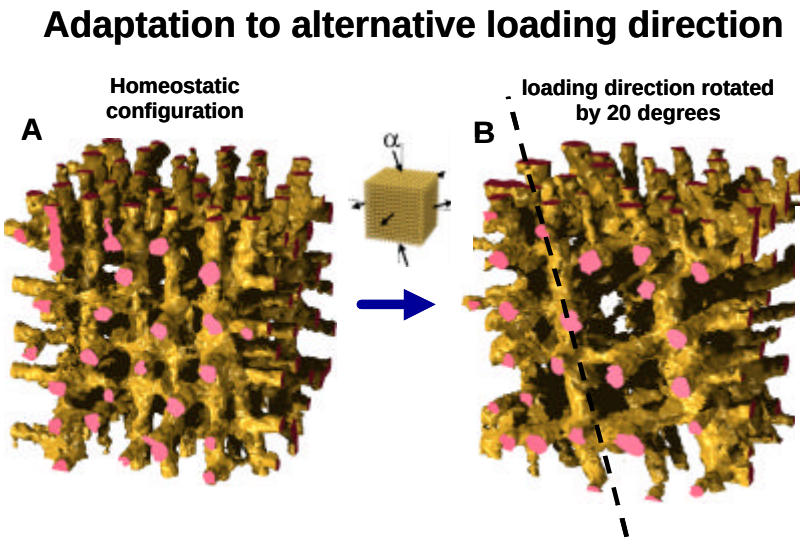

Fig 9: *Imposing the load in an alternative direction results in adaptation of the homeostatic structure (A). Trabeculae realign to the new loading direction (B).*

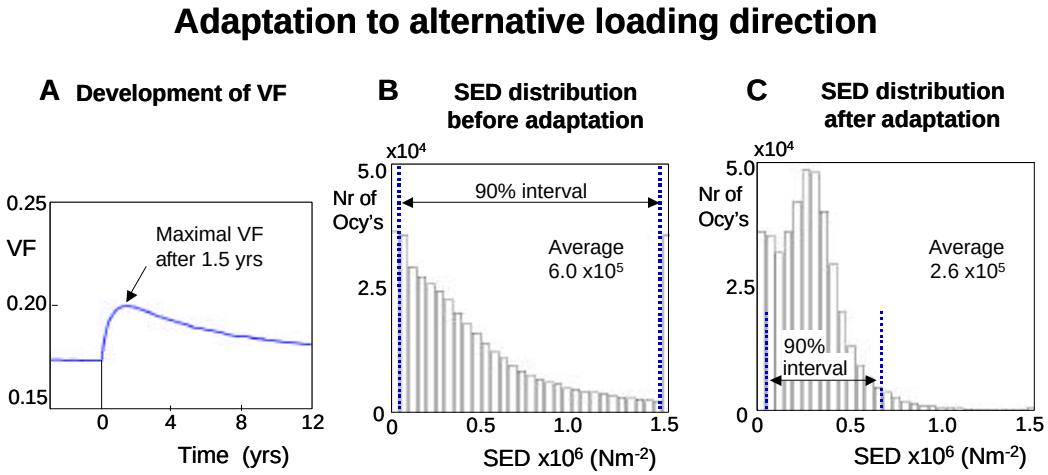

Fig 10: *(A) Development of volume fraction during adaptation to a new loading direction. Before adaptation the average mechanical signal and the 90% interval are high (B). After adapting to the new conditions the average value and 90% interval reduce to similar levels (C).*

In the third series we simulated post-menopausal osteoporosis by increasing the osteoclast activation frequency, which is a known effect of estrogen deficiency (Vaananen and Harkonen, 1996). Increases by 100 and 200 percent (Kanis, 1997; Garnero et al., 1996) resulted in proportional, gradual bone loss, until a new dynamic equilibrium was reached (Fig. 11). Implicitly, inherent to the formulation of the theory on which the simulation model is based, the osteoblast recruitment frequency increased as well. Eventually, after about 4 simulated years, a new homeostatic state was predicted, characterized by increased remodeling rates at a reduced VF, due to loss of trabecular thickness (Tr.Th = 157 *mm*) and connectivity (Tr.N = 0.771 mm^{-1}) (Fig 11). The average SED in the new homeostatic state was increased considerably to $5.7 \cdot 10^5$ Nm^{-2}, which implies a seriously increased fracture risk.

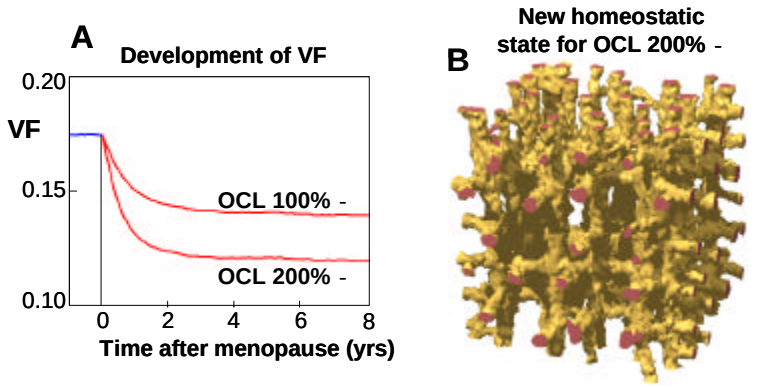

Fig 11: *(A) Development of volume fraction towards a new homeostatic state in simulated postmenopausal osteoporosis by increasing the osteoclast origination frequency by 100 respectively 200 percent. (B) The new homeostatic configuration after 200 percent increase.*

Discussion

Several computational models exist that describe bone adaptation as an optimization procedure (Huiskes et al., 1987; Beaupré et al., 1990; Weinans et al., 1992; Mullender and Huiskes, 1995; Langton et al., 1998; Adachi et al., 2001). The novelty of our theory is that it explains the effects of mechanical forces on trabecular-bone morphogenesis, maintenance and adaptation by relating local mechanical stimuli in the bone matrix to assumed expressions of the cells actually involved in bone metabolism. We assumed osteoclasts to be attracted by effects of local microcracks or damage, and osteoblast recruitment to be based on osteocyte mechano-sensation and signal transduction to the trabecular surfaces. As witnessed by the contemporary literature in this area (Burger and Klein-Nulend, 1999; Martin, 2000), these assumptions are not really controversial, although whether they are true remains to be seen. We added to these notions the stress-concentration effects of resorption cavities – 'notches' in mechanics – as coupling mechanisms for osteoclasts and osteoblasts in remodeling. Whether this is true we do not know either, but it does allow for the assumption that osteoblasts are recruited for both modeling and remodeling along the same signaling pathways, which at least makes sense.

More uncertainty is footed in the actual mathematical expressions we needed to allow for computer simulation with FEA. We made these as simple as possible, using lumped parameter representations where necessary. The model couples dynamic loading variables at the timescale of seconds, to the adaptive processes at the scale of months. The osteoblast-formation equations followed more-or-less directly from the theoretical basis itself.

We assumed that resorption is initiated by microdamage. The location of microdamage is hard to predict. First of all, the bone tissue is not completely homogeneous in structure and density, and local fatigue resistance can vary considerably (Currey, 2002). Secondly, as the structure is assumed to be mechanically optimal, there will not be a large difference in local stress values for normal loading conditions. Thirdly, extreme dynamic ("error") loads are likely to dominate the initiation of microdamage (Homminga et al., 2004). The orientation of these loads can vary considerably, hence there is a large variability in the locations where peak stresses occur. These issues make the occurrence of microdamage hard to predict. The large variability in locations where peak stresses occur, however, also provides the background for using spatially random resorption for selecting surface sites per time-iteration step in the computations. The study of Fazzalari et al. (2002) confirms this assumption. They found a relatively uniform distribution of microdamage in cancellous bone of the proximal femur.

As for the questions we asked for this work, the theory predicted a more-or-less realistic trabecular-morphology development in growth (Figs. 2 and 4), relative to density, trabecular directionality, thickness (Tanck et al., 2001), remodeling rate (Han et al., 1997) and resorption space (Liberman et al., 1995; Kanis, 1997). It also became a relatively effective structure in the sense of 'homogeneous trabecular stressing'. The comparison with reality also showed, however, that trabecular crossings were sharper – less rounded off - than in reality. We believe that to be the result of pure vertical and horizontal loading, while in reality loads vary to some extent in directionality in daily life. The overshot of the VF in initial development is also found in reality, as an effect of architectural adaptation lagging behind bone-mass production (Tanck et al., 2001). Initially, bone is added relatively fast as an effect of high mechanical signals, while resorption of poorly loaded tissue lags behind.

Increasing the external loads increased trabecular thickness – hence volume fraction as well. Trabecular number and separation remained unaffected (Jee and Li, 1990). Reduced loads caused the opposite: Decreased trabecular thickness and volume fraction, but also loss of some trabeculae. Severe reduction of loads caused tremendous loss of trabecular connectivity. Once disconnected, these trabeculae were removed completely by osteoclast resorption (Mosekilde, 1990). If only horizontal loads were reduced, the vertical ones remained intact, while some of the horizontal ones disconnected and resorbed, which is often seen in association with bone loss in aging (Mosekilde, 1988; Mosekilde et al., 1987). After restoring the original loading magnitudes, trabeculae lost did not come back, but those still present increased in thickness (Li et al., 2003). The average SED values after adaptation to alternative loads remained on a similar level. This was confirmed by analysis of osteoporotic versus normal trabecular morphologies in the proximal femur and vertebral bodies of females, all far beyond menopause (van Rietbergen et al., 2003; Homminga et al., 2004). The

osteoporotic suffered severe loss of trabecular connectivity but were equally well adapted to mechanical loads as the normal ones, be it for reduced loads, representing simplified loading tasks.

After rotating the external loads by 20 degrees, trabecular directionality eventually rotated by the same amount, through osteoclast and osteoblast modeling (Fig. 9). This is analogous to developments in growing bone (Tanck et al, 2001), after fracture healing (Wolff, 1892) and in relation with implant incorporation (Guldberg et al., 1997). However, according to Bertram and Swartz (1991) it was never found to occur after redirection of loads in healthy, mature bone. One explanation for this controversy may be that - according to our predictions - it takes a relatively long time (Fig. 10): After 12 simulated years the modeling process still continued. To our knowledge animal experiments on this question were never extended so long.

We invoked post-menopausal osteoporosis in the FEA model by increasing the frequency of osteoclast resorption per temporal increment. This is a known effect of estrogen deficiency (Vaananen and Harkonen, 1996). Because formation, in our theory, is assumed to be coupled to resorption through mechanical pathways, the osteoblast repair frequency increased as well. These results suggest that estrogen does not necessarily have a direct, separate effect on osteoblasts as well (Tobias and Compston, 1999), because osteoclast stimulation combined with mechanical osteoblast coupling may explain the morphological effects of postmenopausal osteoporosis equally well.

For the present FEA analyses the bone cubes analyzed were relatively small, due to computational restrictions. Hopefully whole bones can be studied at a similar resolution sometimes in the future.

In conclusion, our theory provides a *framework* for investigative computation, suitable to study relationships between mechanical loads on trabecular bone and their metabolic effects. Although the effects are obviously expressed through biochemical signaling pathways, these are only *implicitly* assumed in the theory. This is both its weakness and its strength. Strength, because it captures the relationship between form and function ("Wolff's Law") in a mechanistic sense. Weakness because bone diseases often find their origins in the biochemistry of bone physiology, which is not *explicitly* included. All the assumptions we made taken together allow for the similarities of our predictions to biological reality, discussed above, to be 'circumstantial evidence' to the validation of the theory. Only further research can teach us to what extent this is the case.

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Chapter 4

The effects of trabecular-bone loading variables on the surface signaling potential for bone remodeling and adaptation

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Abstract

It is widely believed that mechanical forces affect trabecular bone structure and orientation. The cellular mechanisms involved in this relationship, however, are poorly understood. In earlier work we developed a theoretical, computational framework, coupling bone-cell metabolic expressions to the local mechanical effects of external bone loading. This theory is based on the assumption that osteocytes within the bone tissue control the recruitment of bone-resorbing osteoclasts and bone-forming osteoblasts, by sending strain-energy-density (SED) induced signals to trabecular surfaces through the osteocytic, canalicular network. The theory explains the known morphological effects of external bone-loading variations in magnitude and frequency. It also explains the development of osteoporosis, as an effect of increased osteoclast resorption due to estrogen deficiency in postmenopausal women, and to reduced physical activity levels in general. However, the theory uses lumped variables to represent the mechanisms of osteocyte mechano-sensing and signaling. The question is whether these mechanisms could not be specified in a more realistic way. On the one hand, anabolic osteocyte signals might be triggered by the local mechanical loading variables they experience directly, as we assumed in our original theory. On the other hand, osteocyte signals might be triggered by fluid flow in the osteocytic network at large, as was suggested by others. For that purpose we compared the effects of *SED*, *maximal principal strain* and *volumetric strain* as representing local loading variables, to their spatial gradients on the morphological predictions of our computational model. We found that, in concept, they all produced reasonable trabecular structures. However, the predicted trabecular morphologies based on *SED* as the triggering variable were more realistic in dimensions and relevant metabolic parameters.

Accepted for publication in: Annals of Biomedical Engineering

Introduction

Trabecular bone is continuously renewed by bone-resorbing osteoclasts and bone-forming osteoblasts. In growth or adaptation they change the shape of trabecular morphology and adapt it to external loading direction and magnitude (Courteix et al., 1998; Wolff, 1892; Zerwerkh et al., 1998) in a process called 'modeling' (Frost, 1990a). In adulthood the tissue is continuously renewed by the same cells in a process called 'remodeling' (Frost, 1990b). In Huiskes et al. (2000) we proposed a theory for modeling and remodeling of trabecular structures as governed by external forces. It is based on the assumption that osteocytes within the bone tissue translate the mechanical signals they experience into biochemical messengers that are transducted through the canalicular network to the trabecular surface (Skerry et al., 1989; Cowin et al., 1991, Klein-Nulend et al., 1995; Mullender and Huiskes, 1995; Burger and Klein-Nulend, 1999; Martin, 2000; Knothe Tate and Schaffler, 2002; Nicollella and Lankford, 2002). At the bone surface, osteoblasts are recruited from the environment or by reactivation of lining cells (Dobnig and Turner, 1995; Chow et al., 1998) in order to form bone relative to the total amount of stimulus they receive. Osteoclasts are assumed to be recruited by osteocyte apoptosis due to micro-damage or cracks (Parfitt et al., 1983; Bronckers et al., 1996; Verborgt et al., 2000). We assumed that, due to daily loading conditions, micro-cracks and damage occur at spatially random locations, e.g. they can occur anywhere at any time. The theory is summarized in the regulation scheme depicted in Fig. 1.

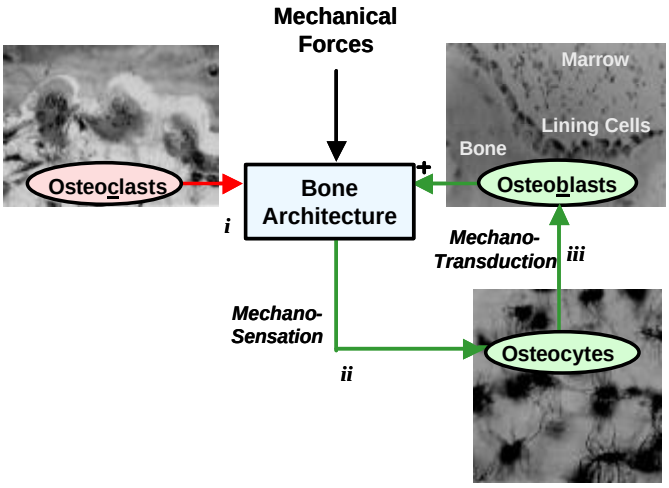

Fig. 1: *The assumed metabolic regulatory mechanisms: (i) Osteoclasts resorb bone on trabecular surface locations where microdamage occurs, attracted by apoptotic osteocytes; in the computations the sites are randomly selected, to a specified percentage per iteration (month). (ii) Osteocytes locally sense a mechanical signal due to external load transfer through the architecture; the signal is transferred to the trabecular surface, where locally (iii) osteoblasts are recruited from bone tissue (Huiskes et al., 2000; Ruimerman et al., 2001; 2003; 2004).*

Our theory describes the coupling between formation and resorption as an effect of mechanical stress transfer, without specification of the regulatory biochemical factors involved, although these are implicitly assumed. This is illustrated in Fig 2.

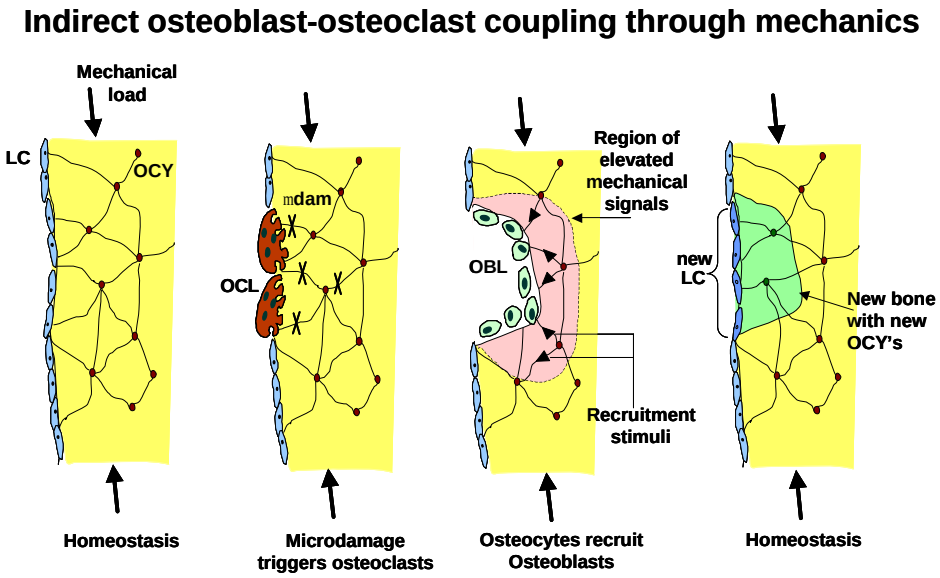

Fig 2: *During remodeling osteoclasts are attracted towards the trabecular surface due to microdamage. They produce a resorption lacunae resulting in local stress and strain concentrations surrounding the resorption cavity due to mechanical load transfer. Consequently osteocytes sense higher mechanical signals and recruit osteoblasts that form bone until the resorption cavity is filled. Some of the osteoblasts are encapsulated in the tissue matrix and differentiate to osteocytes. Other osteoblasts become inactive lining cells that cover the bone surface. Finally the homeostatic configuration is restored.*

Applying 2-D computer simulation methods we showed that this theory provides a qualitative explanation for both modeling and remodeling (Huiskes et al., 2000; Ruimerman et al., 2001). We later studied the theory using a 3-dimensional Finite Element Analysis (FEA) computational model and showed that it explains growth and maintenance of 3-dimensional trabecular-like architectures with realistic physiological and morphological characteristics. Also the adaptation of these structures to alternative loading conditions, and the morphological effects of osteoporosis and medical intervention, could be explained (Ruimerman et al., 2003, 2004).

A basic assumption in our theory concerns the mechano-sensory and signaling functions assigned to osteocytes (Cowin et al., 1991; Burger and Klein-Nulend, 1999). The precise nature of the mechanisms by which osteocytes translate mechanical signals into bone-formation stimuli is unknown. Previously, we used the strain-energy-density (SED) rate as the relevant osteocyte signal. For the present study we investigated whether

other stress and strain-related signals are likely to produce realistic results as well. That alternative choices of the osteocyte stimulus signal may affect the results considerably is obvious as the precise distribution of the mechanical signals throughout the structure can differ significantly (Fig. 3).

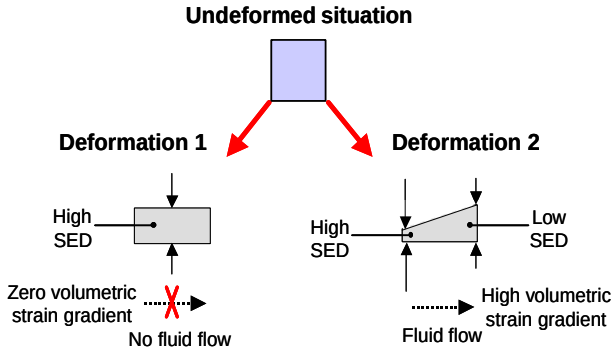

Fig. 3: *A conceptual scheme showing the different effects of mechanical variables and their gradients, specified for strain-energy density (SED). SED produces a local load, Volumetric strain gradient produces an incentive for flow.*

The fundamental issue behind the choice of the stimulus signal is whether (re)modeling signals originate in the osteocytes themselves or whether they are an effect of increased flow through the osteocyte network. If the signals originate in the osteocytes themselves, they could be triggered by the osteocyte lacunar stress and strain concentrations, which can be 15 times higher than the tissue (continuum) level ones (Brand, 2001; Nicollella and Lankford, 2002). In this case the time-derivatives of *strain energy density* (SED), *maximal principal strain* (MPS) or *volumetric strain* (VS) could be relevant controlling variables.

However, it was also found that osteocytes are sensitive to fluid flow (Klein-Nulend et al., 1995), so it was proposed that flow through the canalicular network is the actual trigger for metabolism (Burger and Klein-Nulend, 1999). If fluid flow in the osteocytic network is assumed to cause anabolic signaling, the time-derivatives of the spatial *gradients* of SED, MPS and VS could be more realistic candidates because tissue deformation causes fluid pressures within the lacunae, and differences, or gradients, in pressure will induce flow of fluid (Kufahl and Saha, 1990).

Methods

A summary of our theory is illustrated in the regulation scheme depicted in Fig. 1. Mathematical equations were introduced to quantify relationships (Huiskes et al., 2000; Ruimerman et al., 2001). The change in bone mass at a particular trabecular surface location *x* at time *t* is determined by

$$\frac{dm_{tot}(x,t)}{dt} = \frac{dm_{bl}(x,t)}{dt} - \frac{dm_{cl}(x,t)}{dt}, \qquad (1)$$

with osteoblast bone formation $\frac{dm_{bl}(x,t)}{dt}$ and osteoclast bone resorption $\frac{dm_{cl}(x,t)}{dt}$.

Osteoblast activity at the trabecular surface is controlled by osteocyte bone-formation stimuli. For a total stimulus P [mol·mm^{-2}·day^{-1}] that exceeds a certain threshold value k_{tr} [mol·mm^{-2}·day^{-1}] we assume osteoblast tissue formation according to

$$\frac{dm_{bl}(x,t)}{dt} = t\,(P(x,t) - k_{tr}), \qquad (2)$$

where t [mm^{5}·mol^{-1}] is a proportionality factor that regulates the formation rate relative to the formation stimulus. All osteocytes N within the influence region contribute to the bone formation stimulus P on trabecular surface location x, depending on their mechanosensitivity m_i, their distance d to surface location x and the signal R (SED rate in our original theory) the osteocytes sense in their location x_i, hence

$$P(x,t) = \sum_{i=1}^{N} f(x,x_i)\, m_i R(x_i,t), \quad \text{with} \quad f(x,x_i) = e^{-d(x,x_i)/D} \qquad (3)$$

where $f(x, x_i)$ is an exponential function that describes the signal intensity relative to distance d and a decay parameter D.

Osteoclast bone resorption is described by

$$\frac{dm_{cl}(x,t)}{dt} = -r_{cl}, \qquad (4)$$

where r_{cl} [mm^{3}·day^{-1}] represents a stochastic function that describes that portions of tissue are removed from the surface at spatially random locations.

To determine the stress-strain distribution in the structures we used Finite Element Analysis (FEA) for a domain divided in cubic voxels. The bone tissue within a voxel was assumed isotropic and linear elastic, with material properties depending on its density value m, according to (Currey, 1988; Mullender and Huiskes, 1995)

$$E = E_{\max} \cdot m^{g}. \qquad (5)$$

where the maximal elastic modulus E_{max} [MPa] and exponent g are constants.

For force-induced remodeling the signal R represented the time-derivatives of either the SED = ½ s :e, the MPS = max(e_{ii}), or the VS = $e_1 + e_2 + e_3$. These values were determined in the center of each voxel. For the simulations that consider flow-induced remodeling, R represented the time-derivatives of the *gradients* of these signals. The VS-*gradient* y_i in voxel i was determined by

$$y_i = \sqrt{e_{i,x}^2 + e_{i,y}^2 + e_{i,z}^2}\,, \tag{6}$$

where e is the VS and $e_{i,x}$, $e_{i,y}$ and $e_{i,z}$ are its gradients in voxel i in x, y and z directions, respectively, according to

$$e_{i,x} = \frac{\partial e_i}{\partial x} = \frac{e_{i+1} - e_{i-1}}{2h} \qquad \text{for voxels } 1 < i < N \tag{7a}$$

$$e_{1,x} = \frac{\partial e_1}{\partial x} = \frac{-3e_1 + 4e_2 - e_3}{2h} \tag{7b}$$

$$e_{N,x} = \frac{\partial e_N}{\partial x} = \frac{e_{N-2} - 4e_{N-1} + 3e_N}{2h} \tag{7c}$$

with h the spatial resolution and N the number of voxels in x-direction. Gradients in y and z directions are determined similarly. The SED-gradients and MPS-gradients were computed using the same procedure. In those cases e represents the SED and MPS values respectively.

The computer simulation models were applied to a cubic domain of 2×2×2 mm^3, divided in 60×60×60 cubic voxels. In each computation, the osteocytes were assumed to respond to the relevant mechanical variable. They were assigned the calculated values in the center of the voxel in which they were located. The mechanosensitivity factor m_i was set to the value relevant for the mechanical variable concerned (Table 1). Formation stimuli at the trabecular surface are all in the same range for the chosen values for the different mechanical variables. Changes in these values have the same effect as changes in the loading magnitudes.

Osteocytes susceptible to	Sensitivity m	Unit
SED	1.0	$nmol \cdot mm^{-2} \cdot day^{-1} \cdot (J^{-1} \cdot mm^3 \cdot s)$
SED gradient	$4.00\ 10^{-5}$	$nmol \cdot mm^{-2} \cdot day^{-1} \cdot (J^{-1} \cdot mm^4 \cdot s)$
Maximal principal strain	$2.55\ 10^{7}$	$nmol \cdot mm^{-2} \cdot day^{-1} \cdot s$
Maximal principal strain gradient	$0.64\ 10^{3}$	$nmol \cdot mm^{-2} \cdot day^{-1} \cdot mm \cdot s$
Volumetric strain	$2.55\ 10^{7}$	$nmol \cdot mm^{-2} \cdot day^{-1} \cdot s$
Volumetric strain gradient	$1.27\ 10^{3}$	$nmol \cdot mm^{-2} \cdot day^{-1} \cdot mm \cdot s$

Table 1: *Adjusted mechano-sensitivity factors in the regulatory formulas for the tested variables on which the computational analysis are based.*

Other parameter settings were similar to those used in earlier simulations (Ruimerman et al., 2004). Osteocyte density was set to 44.000 mm^{-3}, as was found by Mullender et al. (1998) in pigs, randomly distributed within the tissue, corresponding to approximately 6 osteocytes along the diameter of a trabecula with a thickness of 150 mm. The osteocyte influence distance D = 100 mm. The threshold for osteoblast bone

formation was $k_{tr} = 5.0 \cdot 10^{6}$ [nmol·mm^{-2}·day^{-1}], and the proportionality factor $t = 1.4 \cdot 10^{-10}$ [mm^{5}·nmol^{-1}]. Osteoclast recruitment frequency on the surface was set to $7.1 \cdot 10^{-2}$ [percent·voxel^{-1}·day^{-1}] and the amount of bone tissue per cavity was 1.5 voxels, i.e. $5.6 \cdot 10^{-5}$ [mm^{3}] (Eriksen and Kassem, 1992). The iteration time step was set to 1 month. The maximal elastic modulus per voxel was set to $E_{max} = 5.0$ *GPa*, the Poison ratio $n = 0.3$, and the exponent $g = 3$ (Currey, 1988). To impose external forces, plates with a thickness of one voxel were added at the surfaces of the cubic domain, disconnected at the ribs of the cube in order to avoid stress shielding (Ruimerman et al., 2001). The plates were given material properties as fully mineralized bone tissue. They do not participate in the remodeling process, i.e. they do not adapt. For the lower plate we repressed 6 degrees of freedom. The x-, y- and z-coordinate for one corner in the origin, the x- and y-coordinate for the second corner on the x-axis, and the z-coordinate of the second corner on the y-axis. As a mechanical load we imposed a distributed harmonic cyclic stress with 1 [Hz] and an amplitude of 2.0 [MPa]. This load was compressive in the vertical direction and tensile in the horizontal directions. We assumed that osteocytes respond to the maximal rate of change of the proposed mechanical signals during one loading cycle. We neglected inertia so that these values could be determined using static FE analysis (Ruimerman et al., 2001).

Several computational series were performed: (1) simulated growth (modeling) from an initial fine, porous structure towards a mature homeostatic 3-D trabecular-like structure. Once this homeostatic structure is obtained no more gross architectural changes occurred, but remodeling continued. (2) The mature structure was subjected to 50 percent reduced loads and to 50 percent increased loads to investigate the effect of alternative loading magnitudes. (3) The structure was also subjected to a 20 degrees rotated load to investigate whether the mature structure realigns accordingly. During the simulations we monitored the developments in morphology, volume fraction (VF) and the distribution of the signals sensed by osteocytes. For the homeostatic configurations we determined trabecular thickness (Tr.Th), trabecular spacing (Tr.Sp) and trabecular number (Tr.N) according to the parallel plate model (Parfitt et al., 1987). In addition we determined the remodeling rates (net full turnover). The ‘remodeling space’ – the total volume of unfilled resorption cavities – was determined by prolonging the simulation with osteoclast resorption completely inhibited. The morphologic and metabolic effects of increased and reduced external loads were also studied.

Results

An example of the adaptive effects in a simulation, from the initial, refined morphology with thin trabeculae (Fig. 4A) to maturity, is demonstrated in Fig. 4B, relative to VS-gradient as the mechanical feed-back variable. With proceeding time the

structure became coarser until finally homeostasis was reached, with less, but thicker, trabeculae, aligned with the external loads. Initially volume fraction (VF) rose sharply in *modeling* and later stabilized to a final, mature value of 0.28 (Fig. 5A). The overshoot in volume fraction that was observed in morphological studies of bone development in growing pigs (Tanck et al., 2001) did not occur, however. From that stage on formation and resorption were balanced (*remodeling*). Trabecular thickness was on the order of 300 *mm* and the remodeling rate was such that the net total bone mass was replaced in 5.7 years. The remodeling space was 21%. The average VS-gradient sensed by osteocytes was large initially (200 [m^{-1}]), but reduced to 110 [m^{-1}] in the homeostatic configuration, illustrating the mechanical 'shape optimization' that had taken place.

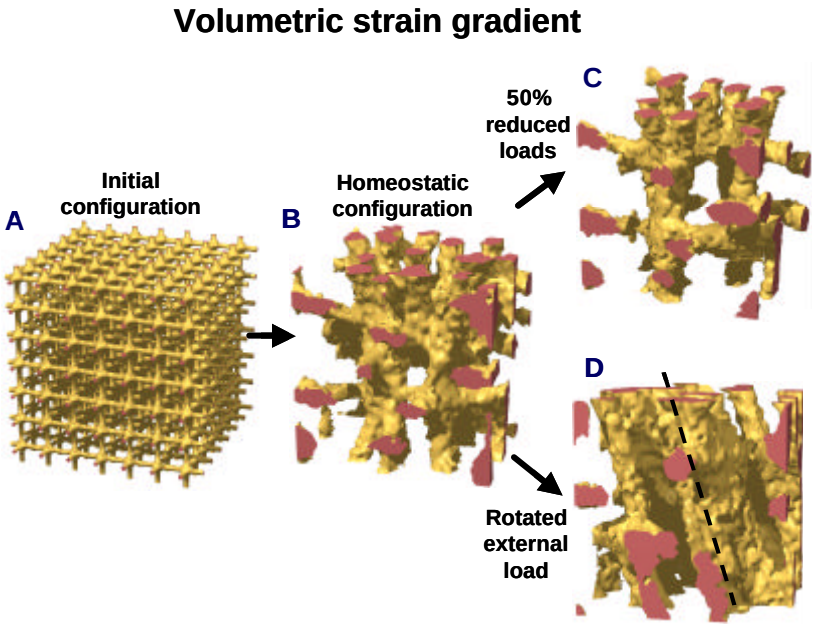

Fig. 4: *Morphological results of the simulation using volumetric strain gradient as the controlling variable. Starting from a refined initial configuration, representing bone in the post mineralized fetal stage (A) the structure developed a mature structure in approximately 10 simulated years in modeling, with trabecuale aligned to the load orientations. (B) Henceforth the structure was maintained; no more large architectural changes occurred, although osteoclast resorption and osteoblast repair continued in remodeling. (C) The structure adapted to 50 percent reduced loads by trabecular thinning and loss of complete trabeculae. (D) Rotating the external load led to reorientation of the trabeculae to the new loading direction, completed after about 12 simulated years. (The trabecular surface was smoothed for clarity of the images.)*

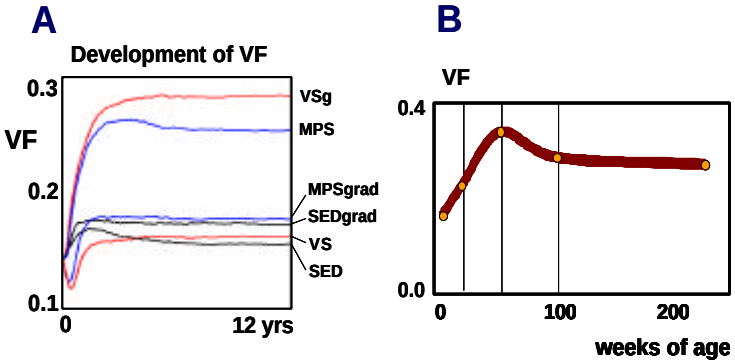

Fig. 5: *(A) Development of volume fraction (VF) during the simulation for the different controlling variables. Only the simulations using SED and maximal principal strain as controlling variables reproduced a clear overshoot in bone mass (VF) as it occurs around puberty. (B) Development of volume fraction as found in a study of bone growth in pigs (Tanck et al., 2001).*

We also investigated the effects of alternative loads on the homeostatic configuration. A load reduction to 50%, in the VS-gradient based simulation, caused loss of bone tissue by trabecular thinning and loss of connectivity towards a VF of 0.15 (Fig. 4C). *Increasing* the loading magnitude by 50% increased VF to 0.39, but no new trabeculae were formed. Besides adaptation to alternative loading magnitudes, the simulations predicted that the structure realigns to alternative loading directions. Rotating the external load by 20 degrees resulted in a sharp rise in VF that gradually decreased until after 20 simulated years (Fig. 4D).

The simulations based on the other mechanical loading variables (SED, SED-gradient, MPS, MPS-gradient, and VS), produced largely similar mature morphologies. In all cases 3-D trabecular-like architectures were formed (Fig. 6), with trabeculae aligned to the external loading orientations. Volume fractions (VF) were between 0.16 and 0.28 (Table 2). Average trabecular numbers (Tr.N) varied between 0.64 and 1.51 [mm^{-1}], trabecular spacing (Tr.Sp) between 500 and 1300 [µm] and average trabecular thickness (Tr.Th) between 133 to 300 [µm] (Table 2). Remodeling rate, expressed in years of net full turnover varied from 3.8 to 5.7 [yrs] and remodeling space was between 0.08 and 0.24 (Table 2). Bone mass reduced by 44 to 50% (Table 2) – by trabecular thinning and loss of connectivity – after reducing the external loads by 50%. Increasing the loads by 50% produced bone-mass increases between 33 and 56%, by expansion of trabecular thickness (Table 2).

The values of the mechanical variables in each simulation converged to similar spatial frequency distributions in the homeostatic (mature) state. The coefficients of variation (COV), i.e. standard deviation to mean ratio, is a measure for stability as low values relate to uniform loading distributions whereas high values relate to inhomogeneous loading. COV values varied between 1.17 and 1.63 (Table 2).

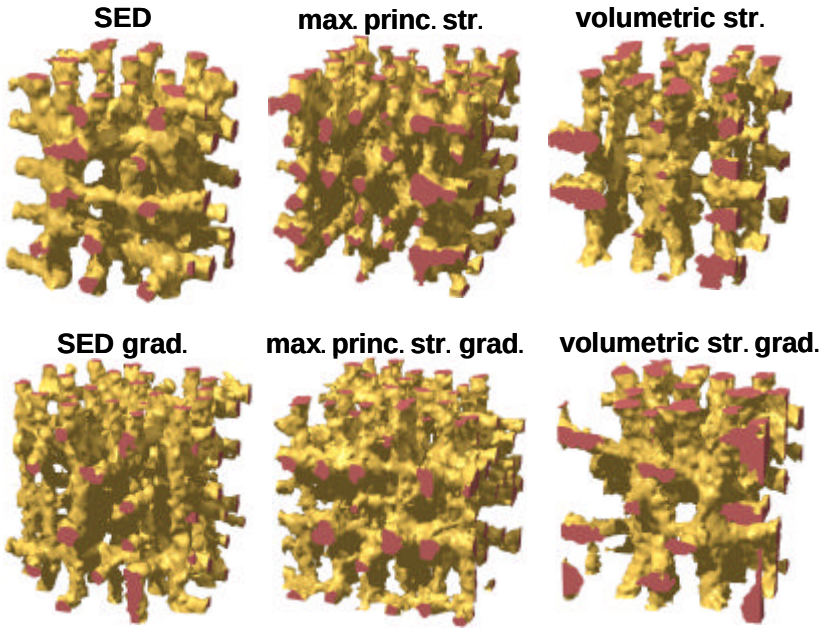

Fig. 6: *Homeostatic trabecular morphologies as a result of the different controlling mechanical variables tested.*

Osteocytes susceptible to	VF	Tr.N (mm^{-1})	Tr.Sp (mm)	Tr.Th (mm)	Resorption rate net full turnover (yrs)	Resorption space	signal COV	F 50% –	F 50% -
SED	0.16	0.96	880	166	4.2	0.09	1.23	- 50 %	+ 56 %
SED gradient	0.18	1.35	610	133	3.8	0.08	1.17	- 50 %	+ 39 %
MPS	0.25	1.51	500	166	4.2	0.24	1.50	- 44 %	+ 36 %
MPS grad.	0.18	1.08	760	166	4.1	0.13	1.36	- 44 %	+ 33 %
VS	0.17	0.64	1.300	266	4.8	0.22	1.63	- 47 %	+ 47 %
VS grad	0.28	0.93	770	300	5.7	0.21	1.50	- 46 %	+ 43 %
Literature	0.15-0.25	0.70-2.00	400-1100	100-200	3-5	0.03-0.05	-	-	-

Table 2: *Morphological results of the computational analyses with the 6 mechanical variables tested: volume fraction (VF), trabecular number (Tr.N), average trabecular spacing (Tr.Sp), average trabecular thickness (Tr.Th), resorption rate (the number of years required for net full turnover), the remodeling space and the coefficients of variation of the mechanical signal sensed, all in the homeostatic configurations. Additionally, the loss/gain of bone mass in response to 50 percent reduced, respectively increased loads, both relative to the homeostatic morphology. Data from literature (Han et al., 1997; Jee 2001; Kanis, 1997; Parfitt et al., 1983) are added in the last row for comparison.*

Simulations using SED and its *gradient* had the lowest coefficients of variation. This indicates that osteoclast-resorption cavities were repaired more rapidly. This fits with the observation that the remodeling space for SED-based simulations was smaller (Table 2). Development of total bone mass (VF) during the simulation to maturity over time was very similar as well in most simulations. Only the simulations using SED and MPS as mechanical variables reproduced the typical overshoot in VF at around puberty (Fig. 5B), as it was found in morphological bone modeling – growth – studies in pigs (Tanck et al., 2001).

Discussion

We developed a computational framework for strain-related trabecular bone remodeling and adaptation (Huiskes et al., 2000; Ruimerman et al., 2001). We already showed how it can be applied in the search for relationships suitable to explain trabecular deterioration in disused or post-menopausal osteoporotic bone (Ruimerman et al., 2003, 2004). For this study we asked the question whether mechanical variables other than SED-rate could be more realistic or effective in simulating remodeling and adaptation. In particular, we were interested in comparing the effects of the mechanical variables themselves – as variables representing the actual loads on the osteocyte 'sensors' – to the effects of their *gradients*, as related to the mechanical effects on fluid flow in the canalicular network. We found that all six loading variables studied produced reasonable, trabecular-like morphologies. Moreover, for all variables, adaptive behavior persisted, despite changes in loading magnitude and direction. Elevated external loads increased trabecular thickness (Jee and Li., 1990; Li et al., 2003), and reduced loads caused trabecular thinning and loss of connectivity (Mosekilde, 1990). Alternative loading directions caused trabeculae to realign accordingly. This is analogous to what is observed in growing bone (Tanck et al., 2001), after fracture healing (Wolff, 1892) and in relation with implant incorporation (Guldberg et al., 1997). However, according to Bertram and Swartz (1991) it was never found to occur after redirection of loads in healthy, mature bone. One explanation for this controversy may be that - according to our predictions - it takes a relatively long time for the structure to adapt, whereas animal experiments on this question were never extended so long.

The question is now how these alternative signal formulations could be discriminated in their effects. For that purpose we first compared actual morphological data predicted to literature data of the volume fraction, trabecular number, trabecular spacing, trabecular thickness, net full turnover rate and remodeling space. Comparing these values with the results of the computations (Table 2) eliminates all but SED, SED-gradient and MPS-gradient as suitable variables, although each of them had remodeling-space values up from about two times higher than what is normal (Kanis, 1997).

Another criterion for realistic behavior is the overshoot in VF in puberty, found in morphological studies in pigs (Fig. 5) (Tanck et al., 2001). This was only reproduced for SED and MPS as regulating variables. Furthermore, we compared the efficacy of the different variables: which produced the least bone mass (VF) for a given loading set. SED appears most effective to that criterion. SED and its gradient were also the variables producing the most homogenous distributions of mechanical signals in their homeostatic structures, with coefficients of variation (COV) at 1.23 and 1.17 - compared to the others at 1.36 to 1.63, which makes them the most effective ones.

Safety against fracture is also an evolutionary issue of importance. Short 'net full turnover' (Table 2) is an asset in this respect, as it implies swift repair of micro-cracks, and so is a low 'remodeling space', as it reduces the amount of notches at any time, and hence the probability of fatigue failure. Again SED and SED-gradient come out as the most advantageous relative to these criteria.

Finally, *adaptability* to external loading is an evolutionary issue. It is metabolically advantageous when a temporary reduction in external loads has a mild response in bone loss and, conversely, increases in load swiftly add bone. It appears that our theory is most sensitive to SED as loading variable, although the differences with other variables are not so large in this respect.

Compared to these criteria, with 'closeness to reality' as the most prominent asset, and 'efficacy' as the second, SED appears the most attractive variable. Certainly VS and its gradient seem less attractive, according to these criteria.

Hence, it seems reasonable to employ SED as the loading variable for further investigative computations concerning trabecular bone (re)modeling. However, the results do not prove that osteocytes respond to local loads in a direct, mechanical sense, as opposed to being sensitive to fluid flow, generated by stress on the osteocytic canalicular network as a whole. The reason for this is basically that the theory employed is extremely robust. This robustness is caused by the negative feedback between tissue loading and bone formation. We saw that whatever the mechanical signal, the theory produces reasonable trabecular structures. Although we did not investigate this, it might be that equally realistic morphologies and metabolic parameters could be generated by all signals investigated, by variation of the process parameters for which a realistic value is not known – like osteocyte sensitivity, distance decay parameter, proportionality factor or others. More information could only be obtained if more realistic, and refined – 'mechanistic' – models (Kufahl and Saha, 1990; Weinbaum et al., 1994; Klein-Nulend et al., 1995; You et al., 2001) for signal transduction would be included in the theory. This would also imply the use of a more realistic description for the dynamic external forces. Computational restrictions defy that, at the moment.

Although the theory is restricted by the use of lumped, conceptual variables and parameters, it does include both mechanical and metabolic descriptions of the relevant

processes, however crudely the latter are represented. This makes the model a suitable tool to investigate relationships between mechanical forces, its metabolic effects and bone architecture. The results show, again, that coupling of osteoclast resorption to osteoblast bone formation, through the effects of stress concentrations (‘notching’) around resorption lacunae (Rodan, 1991; Chambers, 1998; Huiskes et al., 2000; McNamara et al., 2002) is a viable hypothesis. And finally, the predictions of the theory are quite insensitive to the refinement of its parametric and signal representations, as shown here once more – hence, attractive in an evolutionary sense.

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Chapter 5

Estrogen deficiency has an indirect stimulatory effect on bone-formation, through coupling with resorption.

R Ruimerman, P Hilbers, B van Rietbergen, R Huiskes

Abstract

Post-menopausal osteoporosis in women is caused by estrogen deficiency. It leads to increased remodeling spaces and remodeling rates, and bone loss due to reduced connectivity of the trabecular structure. It was reported that the thickness of trabeculae is preserved after menopause, and even increased after ovariectomy. It is not known how estrogen deficiency produces these characteristic changes in architecture. Estrogen has an inhibitory effect on bone resorbing osteoclasts, but whether it has a direct effect on osteoblasts is still debated. In this study we investigated the pathways by which estrogen deficiency lead to the characteristic changes in architecture and metabolic expressions, seen after menopause. We investigated two different hypotheses: I) Estrogen has a direct inhibitory effect on osteoclasts only. II) Estrogen has a direct inhibitory effect on both osteoclasts and osteoblasts. The hypotheses were tested with a computational bone modeling and remodeling theory.

The theory assumes osteocytes as mechanosensitive cells, controlling osteoclast and osteoblast activities at the trabecular surface. The effects of postmenopausal osteoporosis could be explained when estrogen was assumed to inhibit only osteoclast activity. Simulated estrogen deficiency caused loss of connectivity until eventually osteoblast bone formation was enhanced as a secondary effect, due to ‘mechanical’ coupling of formation to resorption. It was also found that increased osteoclast activation frequencies, as well as increased osteoclast resorption depths, both caused connectivity losses. However, the former caused remaining trabeculae to become slightly thinner, while the latter made them slightly thicker. When estrogen was assumed to have the same inhibitory effect on osteoblasts as it has on osteoclasts, estrogen deficiency did not produce osteoporotic bone loss.

Introduction

The total bone mass in the human body increases up to the age of approximately thirty years. After reaching 'peak bone mass', bone formation and resorption are unbalanced in favor of resorption, causing loss of bone mass and increasing fracture risk. Women undergo an *accelerated phase* of bone loss, early after menopause (Fig 1). This makes them more likely to become osteoporotic than men (Riggs and Melton, 1983). The accelerated phase of bone loss is caused by estrogen deficiency. It leads to increased remodeling spaces and increased remodeling rates, i.e. both osteoclast and osteoblast activities are enhanced (Chambers, 1998). The acceleration of bone loss tends to stop after about 4-8 years (Riggs et al., 2002). During that period bone is lost mainly due to loss of entire trabecular elements rather than due to generalized thinning of trabeculae (Aaron et al., 1987; Dempster, 1995; Barger-Lux and Recker, 2002). It was even reported that trabecular thickness increases due to estrogen deficiency after ovariectomy (Waarsing et al., 2004). This mechanism of bone loss is in sharp contrast to the overall thinning of trabeculae that occurs in aging men (Aaron et al., 1987; Seeman, 1999), and due to mechanical disuse (Gardner et al., 2001). When the accelerated phase of bone loss in women stops, remodeling rates remain high (Garnero et al., 1996).

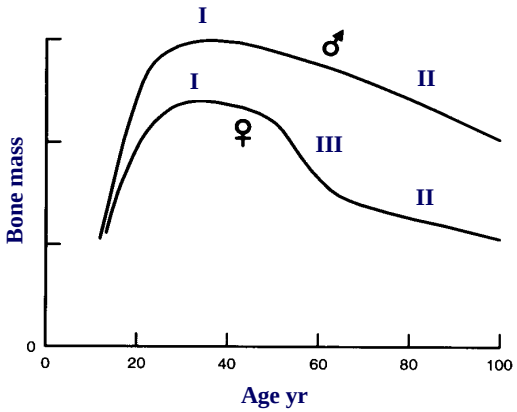

Fig 1: *Total bone mass as a function of age. Peak bone mass (I) is reached around the age of thirty. Thereafter bone is slowly lost (II). Women lose additional bone in the years early after the menopause (III). Adapted from Riggs and Melton, Am. J. Med. 75, pp 899-901, 1983.*

The physiological pathways by which estrogen deficiency lead to these specific metabolic and architectural changes in women are not well understood. The effects of estrogen deficiency on osteoclasts are well known, however. It increases number and size of osteoclasts (Vaananen and Harkonen, 1996; Jilka et al., 1992; Riggs et al., 2002), enhances their survival due to reduced apoptosis (Hughes et al., 1996; Kameda et al., 1997) and increases resorption depths (Eriksen et al., 1999). Whether estrogen directly

enhances osteoblast bone formation *in vivo*, or whether this is a secondary effect due to coupling phenomena of formation to resorption is still uncertain (Vaananen and Harkonen, 1996; Rickard et al., 1999; Riggs et al., 2002). Revealing the effects of estrogen deficiency on bone architecture requires an understanding of the regulatory mechanisms that control the remodeling processes. It is now generally accepted that in addition to biochemical factors, also mechanobiological factors are involved (Rodan, 1996). We believe that answers to the questions formulated above can be found in the mechanobiological factors involved in remodeling.

In previous studies we developed a theory for bone modeling and remodeling, that relates mechanical forces to metabolic expressions of osteoclasts and osteoblasts (Huiskes et al., 2000; Ruimerman et al., 2001; 2004). It assumes that mechanosensitive osteocytes in the bone tissue matrix control osteoclast and osteoblast activities at the bone surface. Through computer simulations we demonstrated that this theory explains the effects of mechanical forces on morphogenesis, maintenance and adaptation of trabecular bone structures (Ruimerman et al., 2004).

For the present study we applied the computational theory to investigate the pathways through which estrogen deficiency may cause the typical architectural and metabolic changes observed in postmenopausal women, i.e. loss of trabecular connectivity, preserved trabecular thickness, increased remodeling spaces and increased remodeling rates. For that purpose we tested two hypotheses:

(I) Estrogen deficiency has a direct stimulatory effect on osteoclast resorption only. In this case we assume that bone formation is enhanced as a secondary effect only, through coupling of formation to resorption.

(II) Estrogen deficiency has a direct stimulatory effect on both osteoclast resorption and osteoblast formation

We tested two different hypothetical cases for the increase in osteoclast activity (1) by increasing osteoclast activation frequency, i.e. increasing the probability for osteoclast resorption to occur, and (2) by increasing osteoclast resorption depth.

Methods

The (re)modeling theory applied (Huiskes et al., 2000; Ruimerman et al., 2001; 2004) is based on the assumptions that (a) osteocytes are mechanosensitive cells, capable of translating local mechanical signals to biochemical messengers. These signals are transferred through the canalicular network to the trabecular bone surface. (b) At the trabecular surface osteoblasts form bone if the total amount of osteocyte signal exceeds a certain threshold. (c) Osteoclast resorption is initiated by osteocyte apoptosis due to microcracks or damage. This is assumed to occur at spatially random locations throughout the tissue, i.e. it can occur anywhere at any time. For the mathematical formulation of the theory we refer to Appendix A.

To investigate our research hypotheses, we applied the theory in an FEA model, representing a cubic domain of 3.3×3.3×3.3 mm^3, divided in 100×100×100 cubic voxels. To impose external forces, plates with a thickness of one voxel were added at the surface of the cubic domain. These plates did not participate in the remodeling process and were given the material properties of fully mineralized bone. Harmonic loads were imposed that were compressive in vertical and tensile in horizontal directions (frequency 1 *Hz*, magnitude 2 *MPa*).

As an initial structure for the simulation we used one that was obtained in an earlier study (Fig. 2A, Ruimerman et al., 2004). This structure was the result of a simulation started from a fine and porous configuration towards a mature homeostatic steady state. This means that it is fully adapted to the imposed mechanical load. Its remodeling rate, remodeling space and morphology were in a realistic range for actual trabecular bone (Table 2, row 1). Parameter values used to obtain this homeostatic structure were as specified in Table 1. The iteration-time step for this simulation was 2 weeks. These parameter settings were also applied for the simulations in this study. During the simulations we monitored the morphological developments of volume fraction VF, the SED values in the structure and the remodeling rates.

The 'remodeling space' – the total volume of unfilled resorption cavities - in the homeostatic configurations were determined by prolonging the simulation with osteoclast resorption completely inhibited.

With simulation series 1 we tested the hypothesis that estrogen deficiency after menopause has a direct effect on *osteoclasts only*. We assumed that osteoclast activity after menopause increases with 100% (Riggs et al, 2002). We performed two studies for a simulated period of eight years, starting with the homeostatic structure. In the first study we mimicked the effects of reduced estrogen levels by doubling the osteoclast *activation frequency* f_{ocl}, i.e. the probability for osteoclast resorption cavities to occur. In the second run we doubled the *resorption cavity size* V_r, i.e. the amount of tissue that is removed in one resorption cavity.

In simulation series 2 we investigated the morphological effects of estrogen deficiency under the assumption that estrogen has a direct inhibitory effect on *both osteoclasts and osteoblasts*. Again we performed two analyses of eight simulated years. In the first we mimicked the effects of estrogen deficiency by doubling the osteoclast activation frequency. Concurrently we doubled the osteoblast *bone formation rate t* relative to the osteocyte stimulus. In the second simulation we doubled the resorption cavity size as well as the osteoblast bone formation rate.

variable	symbol	unit	value
Osteocyte density	n	mm^{-3}	44.000
Osteocyte mechanosensitivity	m	$nmol \cdot mm \cdot J^{-1} \cdot s \cdot day^{-1}$	1.0
Osteocyte influence distance	D	mm	100
Formation threshold	k_{tr}	$nmol \cdot mm^{-2} \cdot day^{-1}$	$5.0 \cdot 10^{6}$
Bone formation rate	t	$mm^{5} \cdot nmol^{-1}$	$1.4 \cdot 10^{-10}$
Resorption amount per cavity	V_r	mm^{3}	$5.6 \cdot 10^{-5}$
Osteoclast recruitment frequency	f_{ocl}	$voxel^{-1} \cdot day^{-1}$	$7.1 \cdot 10^{-4}$
Maximal elastic modulus	E_{max}	GPa	5.0
Poison ratio	n	-	0.3
Exponent gamma	g	-	3.0
Loading frequency	f	Hz	1
Loading magnitude	A	MPa	2.0

Table 1: *Parameter settings for the reference simulations (Ruimerman et al., 2004).*

Results

We started the simulations from a mature trabecular bone structure in a situation of homeostasis (Fig. 2A). This structure had a volume fraction (VF) of 0.175. Trabecular thickness (Tr.Th) was 172 *mm* and trabecular number (Tr.N) was 1.01 mm^{-1}. Tissue loading (measured by average SED), as supposed to be sensed by the osteocytes within the structure, was $2.6 \cdot 10^{5}$ Nm^{-2}. The remodeling space was 8%, the remodeling rate 21% per year.

Increasing the *osteoclast activation frequency* by 100 percent resulted in a gradual decrease of bone mass. After about 4 simulated years a new dynamic equilibrium was reached and further bone loss did not occur (Fig. 2C) (Riggs et al., 2002). The VF stabilized at about 0.140. Bone loss could be attributed to loss of trabecular thickness as well as to loss of trabecular connectivity (Table 2, row 3). Average SED values in the new steady state were increased considerably. As a result osteoblast bone formation was enhanced as well, resulting in a new balance for bone mass. The remodeling space and the remodeling rate in the new steady state were increased (Garnero et al., 1996).

The morphological effects were different when the *osteoclast-resorption cavity size* was increased by 100% (Fig. 2B). Bone loss was less severe, in this case, and could be attributed completely to loss of connectivity, while trabecular thickness even slightly increased. Again the average SED values increased until a new equilibrium between formation and resorption was established. In the new steady state, the remodeling space and the remodeling rate were considerably increased (Table 2, row 4).

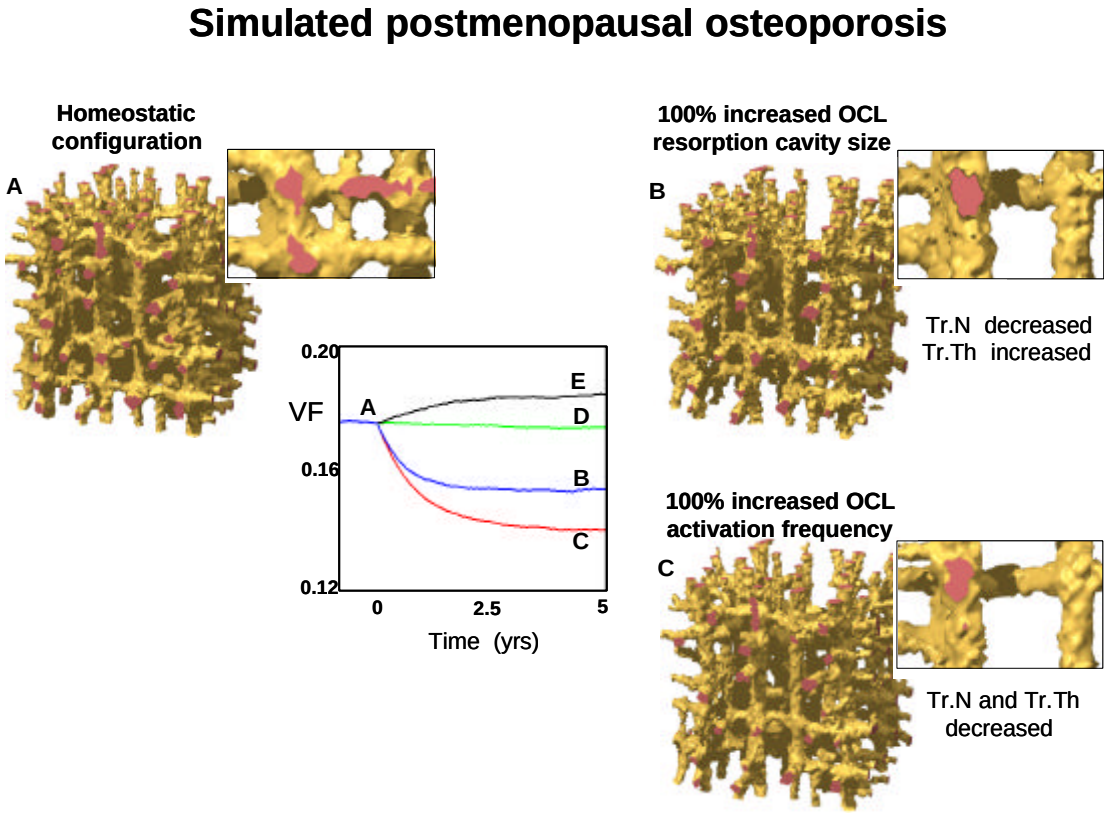

Fig 2: *Morphological consequences for the homeostatic configuration (A) after simulated menopause if estrogen deficiency is assumed to (B) increase osteoclast resorption depths and (C) increase osteoclast activation frequencies. The enlarged details of the same area show that enhanced resorption depths leads to slightly more loss of trabecular connectivity, yet thicker trabeculae. Bone loss did not occur when estrogen deficiency was assumed to affect both osteoclast and osteoblast activity: (D) increased osteoclast activation frequencies and bone formation rate. (E) Increased resorption depths and osteoblast bone formation rate.*

There was no osteoporotic bone loss when estrogen deficiency was assumed to affect *both osteoblasts and osteoclasts*. When the osteoblast bone formation rate, as well as the osteoclast activation frequency, were doubled, VF was hardly affected, even after eight simulated years. Bone mass even increased slightly when formation rate and resorption cavity size were doubled (Fig 2D,E). Remodeling rates in the new steady state were doubled. The remodeling space was not affected.

Simulation	VF -	Tr.Th *mm*	Tr.N mm^{-1}	Remodeling space %	Remodeling rate %/yr	average mechanical signal ($x10^5$) Nm^{-2}
Homeostatic mature configuration	0.175	172	1.01	8	21	2.6
OCL activation frequency	0.140	165	0.85	12	46	4.2
OCL resorption depth	0.153	184	0.83	12	49	3.9
OCL activation frequency and OBL formation rate	0.173	176	0.98	8	41	2.6
OCL resorption depth and OBL formation rate	0.183	183	1.00	8	47	2.5

Table 2: *Morphological and physiological characteristics of the simulated bone structures in the homeostatic configuration and the effects of estrogen deficiency on the new steady state.*

Discussion

In this study we investigated the pathways by which estrogen deficiency leads to postmenopausal, osteoporotic bone loss. For that purpose we applied a computational bone modeling and remodeling theory (Huiskes et al., 2000, Ruimerman et al., 2001, 2004). In concept, this theory describes a negative feedback mechanism, explaining bone adaptation towards a homeostatic steady state. Coupling between resorption and formation is established through feedback of mechanical signals, as was also proposed by others (Rodan, 1991). The resorption cavities cause stress concentrations in their surrounding tissue. This leads to elevated mechanical strains, initiating signals from the osteocytic network that stimulate bone formation locally.

That mechanical factors are involved in the coupling process can be concluded from the observation that unloaded trabeculae are rapidly resorbed by osteoclasts (Mosekilde, 1990), but also by the observation that increased bone formation after ovariectomy is reduced by underloading (Lin et al., 1994). Mechanical coupling also provides an explanation for increased resorption preceding enhanced formation, as observed in actual trabecular bone (Dempster et al., 1995; Sims et al., 1996). It appears that the structure must deteriorate, before osteocytes are stimulated sufficiently to enhance bone formation by osteoblasts.

Proper understanding of the negative feedback mechanisms in the remodeling process is obviously important, as it may provide answers to the question why most non-mechanical agents have only limited, transient effects on bone mass and architecture. The idea that negative feedback is important for the ability of bone to adapt is not new. Many

computational models developed to study bone adaptation have negative feedback as their basis (Carter et al., 1987; Huiskes et al., 1987; Beaupre et al., 1990; Weinans et al., 1992; Mullender and Huiskes, 1995; Adachi et al., 2001). These models were inspired by Frosts' Mechanostat Theory (Frost, 1987, 2003). This theory assumes that bone adapts to a reference state, or mechanical set-point. It was proposed that osteoporosis is caused by an elevation of the set-point. This would lead to degeneration of trabecular structure, until eventually the bone is adapted to the new reference state, and further bone loss is depressed (Frost, 1987, Mullender et al., 1998; Turner, 1999). The physiological background of the mechanical set-point - where and what it is - was not specified.

According to our theory, the homeostatic steady state is not a predefined one. The theory features parameters, specifying the behavior of the actual cells involved, i.e. osteocytes, osteoclasts and osteoblasts. If the behavior of one category of cells changes, then bone is lost or gained, until eventually a new homeostatic steady state is established, due to negative feedback of the mechanical load. In our theory, this is not a 'preprogrammed' state, i.e. there is no specific set-point to which the bone adapts.

The research question we investigated in this study was how estrogen deficiency could cause increased bone turnover, increased remodeling spaces and loss of complete trabeculae, whereas the thickness of remaining trabeculae is preserved. We predicted that osteoporotic bone loss will not occur when estrogen deficiency has the same stimulatory effect on bone formation as it has on bone resorption. This is not what happens in reality. However, all the phenomena found in real bone could be explained if estrogen deficiency was assumed to affect osteoclasts only. We demonstrated that increased resorption causes degeneration of the trabecular structure, because resorption prevails over formation. This causes stress-shielding effects and subsequent loss of complete trabeculae. As an effect, remaining trabeculae become more highly stressed and undergo compensatory thickening, because more osteoblasts are recruited by the osteocytes. As an effect of the feedback of mechanical signals, the theory explains that the thickness of remaining trabeculae increase slightly after menopause if osteoclast activation frequencies are increased (Dempster, 1995; Barger-Lux and Recker, 2002). The same feedback mechanism explains that trabecular thickness may even increase (Waarsing et al. 2004) if estrogen deficiency increases the resorption depths. In both cases, a new homeostatic steady state is eventually attained.

The results of this study suggest that the direct effects of estrogen deficiency on osteoblasts are relatively small, compared to the direct effects on osteoclasts. It appears that increased osteoblast activity, as it occurs after the menopause, can equally well be explained as a secondary effect due to 'mechanical' coupling of formation to resorption.

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Appendix A

The main mathematical equations to quantify the bone (re)modeling theory are as follows (see Huiskes et al., 2000; Ruimerman et al., 2001, 2004 for more details). The change in bone mass at a particular trabecular surface location x at time t is determined by

$$\frac{dm_{tot}(x,t)}{dt} = \frac{dm_{bl}(x,t)}{dt} - \frac{dm_{cl}(x,t)}{dt}, \qquad (1)$$

with osteoblast bone formation $\frac{dm_{bl}(x,t)}{dt}$ and osteoclast bone resorption $\frac{dm_{cl}(x,t)}{dt}$.

Osteoblast bone formation at a location on the trabecular surface is determined by the total amount of osteocyte signal P [mol·mm^{-2}·day^{-1}] that is received on that location, hence

$$\frac{dm_{bl}(x,t)}{dt} = t\,(P(x,t) - k_{tr}). \qquad (2)$$

Here k_{tr} [mol·mm^{-2}·day^{-1}] is the threshold value for bone formation to occur, and t [mm^{5}·mol^{-1}] is the bone formation rate relative to the osteocyte signal.

Harmonic loads are imposed on the structure (frequency f and amplitude A). Osteocytes are susceptible to the maximal SED-rate during one loading cycle. This value is calculated using a substitute static stress (Ruimerman et al., 2001). The total formation stimulus P that is received on trabecular surface location x depends on osteocyte density n, the influence distance, their mechanosensitivity m_i [mol·mm·J^{-1}·s·day^{-1}], their distance d [μm] to surface location x, and the SED-rate R [J·mm^{-3}·s^{-1}] they sense in their location x_i, hence

$$P(x,t) = \sum_{i=1}^{N} f(x,x_i)\, m_i R(x_i,t), \qquad \text{with} \quad f(x,x_i) = e^{-d(x,x_i)/D} \qquad (3)$$

where $f(x, x_i)$ describes the decay in signal intensity relative to distance d [μm] and decay parameter D [μm].

Osteoclast bone resorption is described by

$$\frac{dm_{cl}(x,t)}{dt} = r_{cl}, \qquad (4)$$

where r_{cl} [mm^3/day] represents a stochastic function that determines the chance for resorption at the bone surface. Per resorption cavity, a constant amount of tissue V_r is removed.

The material properties in one voxel are assumed isotropic, linear elastic with elastic modulus E [MPa] and poison ration n depending on the density value m, according to (Currey, 1988)

$$E = E_{\max} \cdot m^{g}, \qquad (5)$$

where the maximal elastic modulus E_{max} [MPa] and exponent g are constants.

Chapter 6

Mechanically induced osteocyte signals explain osteoclast resorption direction and coupling of formation to resorption in Basic Multi-cellular Units

R Ruimerman, R van Oers, P Hilbers, E Tanck, R Huiskes

Abstract

The bone remodeling process is conducted by osteoclasts and osteoblasts, collaborating in Basic Multi-cellular Units (BMU's). In cortical BMUs, osteoclasts excavate cylindrical tunnels in the predominant loading direction of the bone. They are followed by osteoblasts, filling the tunnel, creating secondary osteons of renewed tissue. Trabecular bone remodeling is mainly a surface event, in which osteoclasts dig a trench rather than a tunnel and also here they are followed by bone forming osteoblasts creating hemi-osteons. That osteoblasts follow osteoclasts in such a coordinated manner indicates that a coupling mechanism must exist. Its precise nature, however, is currently uncertain. Another unresolved issue in bone mechanobiology is the mechanism that guides osteoclast resorption in its directionality.

It was suggested that BMU activity is controlled by osteocytes in the bone matrix, serving as mechanosensors, sending signals through the osteocytic canalicular network to the BMU cells. In our present study we tested whether this hypothesis can explain the osteoclast resorption direction and coupling of formation to resorption. For that purpose we refined our bone (re)modeling theory that earlier explained growth, maintenance and adaptation of trabecular bone structures. We assumed that the mechanically induced osteocyte signals inhibit osteoclast bone resorption and stimulate osteoblast bone formation. Applying computer simulations, we showed that these assumptions explain osteoclast-resorption orientation as well as coupling of osteoblast bone formation to resorption, in the individual BMU's of both cortical and trabecular bone. We also found that this regulatory mechanism is effective in terms of bone repair as it targets dead bone, i.e. bone without any viable osteocytes.

Chapter 6

Introduction

The mechanical competence of cortical and trabecular bone is maintained throughout life by Basic Multi-cellular Units (BMU's) that continuously remodel the tissue (Frost, 1964; Frost, 1990a). In *cortical bone* the BMU's are cylindrical structure up to 2 *mm* long and 200-250 *mm* wide. They gradually burrow through the bone, forming (secondary) osteons of renewed bone (Schenk and Willenegger, 1964; Lee & Einhorn, 2001). In the tip, or cutting cone, about ten osteoclasts dig a circular tunnel. They are closely followed by the closing cone where several thousands of bone forming osteoblasts fill the tunnel (Parfitt, 1994). That osteoblast formation follows osteoclast resorption in such a coordinated fashion indicates the existence of a coupling mechanism (Frost, 1964). Its precise nature, however, is currently uncertain. Another unresolved issue in mechanobiology is the mechanism that guides osteoclast resorption in its directionality. The alignment of osteons along the dominant loading direction indicates that mechanical forces are involved (Lanyon and Bourn, 1979; Petryl et al, 1996). Smit and Burger (2000) showed that normal loading conditions result in reduced strains at the tip, where osteoclasts excavate bone. Elevated strain levels, conversely, appear in the tunnel wall behind the cutting cone, where osteoblasts are active. Burger and Klein-Nulend (1999) suggested that BMU activity is controlled by osteocytes in the bone matrix, serving as mechanosensors, sending signals through the osteocytic canalicular network to the BMU cells. These assumptions may relate mechanical forces to osteoclast and osteoblast activities (Burger et al., 2003). *Trabecular bone* experiences the same remodeling cycle of activation, resorption and formation as cortical bone, suggesting that similar cellular communication pathways are involved (Frost 1986, Eriksen et al, 1986, Parfitt, 1994). However, some differences exist. In trabecular bone, remodeling is mainly a surface event where osteoclasts travel across the surface digging a trench rather than a tunnel with a depth of 40-60 *mm* (Eriksen and Kassem, 1992). Like in cortical bone they are followed by bone forming osteoblasts. Active remodeling sites in trabecular bone cover areas of varying sizes from as small as 50×20 *mm* up to 1000×1000 *mm*, often elongated in the direction of the trabecula (Mosekilde, 1990). The resulting structure that is formed is called a trabecular osteon or hemi-osteon (Frost, 1986; Eriksen and Kassem, 1992).

Osteoclasts and osteoblasts are, in addition to bone maintenance (or 'remodeling'), also responsible for development of the bone structure in growth and its adaptation to alternative loading conditions, processes called 'modeling' (Frost, 1990b). How osteoclast and osteoblast activities are controlled to form the complex 3D trabecular structures is not known. We developed a conceptual, mechanobiological theory for trabecular bone modeling and remodeling to explain how mechanical forces are translated to trabecular morphology (Huiskes et al., 2000; Ruimerman et al., 2001). Its basic assumptions are that (a) osteocytes are mechanosensors that send signals to the

bone surface, through the canalicular network, attracting osteoblasts for bone formation, that (b) a lack of osteocyte signals at the bone surface attracts osteoclasts for bone resorption, that (c) such a lack of osteocyte signals can be due to mechanical disuse or to micro-cracks, and that (d) micro-cracks occur spatially random at the trabecular surfaces. These assumptions, expressed in formulae, produced an apparent coupling mechanism between osteoclast resorption and osteoblast formation in trabecular remodeling, owing to the stress concentrations that the resorption cavity produced, leading to increased osteocyte signalling (Huiskes et al., 2000). Applied in 3D computer-simulations of bone modeling and remodeling this theory predicted the development and maintenance of trabecular bone structures with realistic characteristics in terms of morphology and metabolic activity (Ruimerman et al., 2004).

For the present study we asked the question whether we could extend our trabecular computational theory to explain cortical bone remodeling as well. This implied adapting the scale of the problem in terms of spatial and temporal dimensions. Where in our trabecular model the many surface-BMU's were represented as immediate local disturbances, the cortical-BMU requires treatment as a more extended biological process. Once this new formulation for cortical BMU's was developed as an adaptation of spatial and temporal dimensions, we could apply it in the same way to the trabecular BMU, the hemi-osteon, thereby unifying the theories for cortical and trabecular remodeling.

We quantified our theory using mathematical equations in which the proposed regulatory mechanisms are expressed. We then incorporated it in a computer-simulation model to test whether it relates external forces to osteoclast resorption direction and whether it explains coupling of formation to resorption as it occurs in cortical and trabecular bone. We then investigated the robustness of the theory by exposing the simulated piece of bone tissue to alternative loading conditions. Finally we tested the effectiveness of the regulatory mechanism in terms of bone repair.

Methods

The theory was quantified with mathematical equations largely similar to our earlier theory (Huiskes et al., 2000; Ruimerman et al., 2001, 2004). It assumes that the bone mass at a particular bone surface location x can be increased by osteoblasts or reduced by osteoclasts. The behavior of these cells on the surface is determined by the total amount of biochemical messengers P they receive from all N osteocytes within the influence region. The contribution of osteocyte i in location x_i depends on the mechanical signal R [J·mm^{-3}·s^{-1}] it experiences in a recent loading history, its mechanosensitivity m_i [mol·mm·J^{-1}·s·day^{-1}], and its distance d [mm] to the surface, hence

$$P(x,t) = \sum_{i=1}^{N} f(x,x_i) m_i R(x_i,t), \quad \text{with} \quad f(x,x_i) = e^{-d(x,x_i)/D} \tag{1}$$

where $f(x, x_i)$ describes the decay in signal intensity relative to distance d and decay parameter D [mm].

Osteoclasts are assumed to migrate and attach to bone that receives low amounts of osteocyte signal. How strongly they attach to the bone surface depends on the (lack of) osteocyte signal. If attached to bone, they resorb it, preferably at locations where the osteocyte signal is low. Conversely, they retract from surfaces that receive high amounts of signal. The number of osteoclasts in the cutting cone is small. Their dimensions (diameter ± 60 μm) are on the order of the osteonal tunnel width and the local mechanical environment may vary considerably within that range (Smit et al., 2000). We therefore took account of osteoclast numbers, size, form and position relative to the internal bone surface for which we applied the Glazier & Graner model based on differential cell adhesion (Glazier & Graner, 1993). It assumes that the shape of a cell is determined by energy minimization. Hence, they are spherical if placed in a homogeneous medium, but another shape can be energetically more optimal if they are placed adjacent to a tissue surface. This is similar to why a droplet is spherical in the air but flattens if attached to a surface. The state of an osteoclast is characterized the amount of free (Hamiltonian) energy H_s [J]

$$H_s = H_{surf} + H_{vol}, \tag{2}$$

where the surface energy H_{surf} of an osteoclast is determined by its surrounding tissue (Fig. 1), hence

$$H_{surf} = \int_{surf} h(A)dA, \tag{3}$$

where $h(A)$ [J/mm^{-2}] is the contact surface energy between the osteoclast and its outer medium. We assumed that the contact surface energy to marrow (h_m) and to osteoclast neighbors (h_{ocl}) are constants. The contact-surface energy between an osteoclast and the bone matrix (h_b) depends on the osteocyte signal P that arrives on that surface, according to

$$h_b(P) = k(P - P_{ocl}), \tag{4}$$

where P_{ocl} [mol·mm^{-2}·day^{-1}] is a constant. The value k [J·mol^{-1}·day] determines osteoclast-matrix adhesion relative to the osteocyte signal. It is energetically favorable for an osteoclast to attach to bone if $h_b(P) < h_m$.

The harmonic potential term H_{vol} ensures that the osteoclast volume remains close to its target volume V_0 [mm^3], as

$$H_{vol} = l(V - V_0)^2, \tag{5}$$

where V is the current osteoclast volume and l [J·mm^{-6}] the inelasticity of the osteoclast. Osteoclasts are nearly incompressible for high values l.

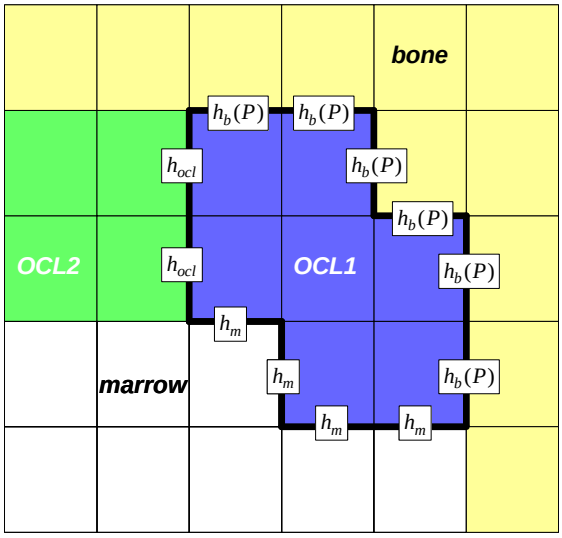


Fig 1: *Sketch of the Glazier & Graner formalism as applied on a 2D grid. The contact surface energy of an osteoclast (OCL1) to marrow h_m and to other osteoclasts h_{ocl} are constants. The contact surface energy of an osteoclast to bone tissue $h_b(P)$ depends on the osteocyte signal P.*

Osteoclast migration and attachment to the bone is mimicked using equations 2-5. We define the form of the osteoclast on a discrete grid and change its shape by adding or removing a voxel at its surface to its volume. For each step we evaluate the free energy change *DH* for each possible shape change and choose one option stochastically, weighted relative to $w = e^{-a\Delta H}$ but assumed that osteoclasts cannot push other osteoclasts or osteoblasts aside and that they cannot split up. If we repeat this procedure iteratively, osteoclasts assume compact forms, preferably attached to bone surfaces where low amounts of osteocyte signal are received. The positive value a [J^{-1}] relates the change in free energy *DH* to the chance for the corresponding step to be carried out.

Osteoclast bone resorption takes place on a much larger time scale than osteoclast migration and attachment. We therefore incorporated resorption as a separate feature. In one '*resorption step*' the osteoclast is assumed to resorb a specified amount of tissue, preferably where the osteocyte signal is low. We evaluate all surfaces at which the osteoclast attaches to the bone matrix and selected the volume beneath one of those surfaces, again stochastically and weighted relative to $w_{resorp} = e^{-a_r P}$. Here a_r [$mm^2 \cdot day \cdot mol^{-1}$] determines how sensitive osteoclasts are for variations in the osteocyte signal. After resorption, the density in the chosen voxel is set to zero and the voxel is added to the osteoclast volume.

Osteoblasts are responsible for bone formation. We assume that they are recruited to uncovered bone surfaces if the osteocyte signal P [mol·mm^{-2}·day^{-1}] exceeds a certain threshold value k_{tr} [mol·mm^{-2}·day^{-1}] for a period longer than the recruitment time $T_{recruit}$ [days]. Once attached osteoblasts form bone according to

$$\frac{dm_{obl}(x,t)}{dt} = t\,(P(x,t) - k_{tr})\,, \tag{6}$$

where t [mm^{5}·mol^{-1}] is the osteoblast bone formation rate relative to the osteocyte signal.

Material properties of the bone tissue are assumed isotropic and linear elastic, with elastic modulus E [GPa] depending on the density value m, according to

$$E = E_{\max} \cdot m^{g}\,, \tag{7}$$

where the maximal elastic modulus E_{max} [GPa] and exponent g are constants (Currey, 1988).

We implemented the mathematical equations in a 2D computer simulation model assuming plain strain. We imposed a harmonic load on the external boundary of the tissue and assumed that osteocytes are susceptible to the maximal SED-rate during one loading cycle. These values can be calculated using static finite element analysis, using a substitute static stress (Ruimerman et al., 2001).

In the simulation we iteratively perform the following procedure. We determine osteocyte sensation and evaluate the amount of osteocyte signal on the bone surface. Subsequently all osteoclasts performed 100 ‘energy minimization steps’, to migrate to positions that receive low amounts of osteocyte signal, and two ‘resorption steps’. Finally we determine where osteoblasts form bone. The end result of this procedure is the new configuration for the next iteration.

Parameter settings for the simulations were as specified in Table 1. Actual values for the contact surface energies of osteoclasts to other media are unknown and were therefore taken in an arbitrary range. However, osteoclast behavior is determined by ratios of contact surface energies and not by their absolute values. The minimal contact surface energy $h_{b,min}$ corresponds to the maximal adhesive strength of an osteoclast to bone. It has a negative value, which means that the osteoclast is actively attracted. In one resorption step the osteoclast resorbs one bone voxel. The resolution of the model was set to 12.5 *mm*, the time step to four hours.

Cortical bone remodeling

Several simulations were performed in order to investigate whether the proposed regulatory mechanisms are capable of translating the strain fields to direct osteoclast resorption in the main loading direction and whether osteoblasts follow to fill the tunnel. The simulations considered a small 2×2 mm^2 piece of cortical bone tissue. In the first simulation three osteoclasts were placed in the tip of an initial cutting cone (Fig. 2A). The tissue was mechanically loaded with a 1 *Hz*, 10 *MPa* compressive load which corresponds to a substitute static stress of 20 *MPa* (Ruimerman et al., 2001). This simulation was repeated with 30° and 45° rotated loads in order to test mesh independency. We also gradually rotated the mechanical loading orientation from 0° to 30° after 30 simulated days in order to investigate whether the BMU would realign its course. The effects of alternative loading magnitudes were investigated by repeating the simulations with a 20% reduced load and with a 20% increased load, and in the absence of mechanical loading. The robustness of the regulatory mechanism was investigated by repeating the above simulations with four osteoclasts placed in the tip of the cutting cone. Finally we investigated whether the proposed theory is an effective repair mechanism. We therefore artificially 'killed' some osteocytes in one region of the tissue to simulate the effect of a microcrack (Fig. 3G), and investigated whether the BMU changes its course to replace this damaged tissue.

Trabecular bone remodeling

In a second series of simulations we investigated whether the theory also explains the osteoclast resorption direction and subsequent coupling of osteoblast bone formation in the trabecular hemi-osteon. We mimicked the remodeling process on a single trabecula with a length of 1 mm and a thickness of 200 mm. An initial resorption cavity with an osteoclast was placed at the surface (Fig. 4A). The trabecula was loaded with a 1 Hz, 10 MPa cyclic pressure. All parameter values where taken similar to the values used in the cortical-bone remodeling simulation, except for the threshold for bone formation. We increased this formation threshold with 50% in order to prevent that osteoblasts were immediately recruited to the whole surface of the trabecula. That bone formation characteristics must be altered is not unreasonable as, in contrast to the cortical BMU, the external surfaces in trabecular bone are covered with lining cells. Finally, we investigated the effect of alternative loading magnitudes by reducing, respectively increasing, the load by 20% and by removing the load completely.

Osteocyte variables	**symbol**	**unit**	**value**
Osteocyte density	n	mm^{-2}	1.600 [1]
Osteocyte mechanosensitivity	m	$nmol \cdot mm \cdot J^{-1} \cdot s \cdot day^{-1}$	1.0
Osteocyte influence distance	D	mm	100 [2]
Osteoblast variables			
Formation threshold	k_{tr}	$nmol \cdot mm^{-2} \cdot day^{-1}$	$1.0 \cdot 10^{5}$
Proportionality factor	t	$mm^{5} \cdot nmol^{-1}$	$4.0 \cdot 10^{-11}$
Osteoblast recruitment time	$T_{recruit}$	$days$	4.0
Osteoclast variables			
Target volume	V_0	$voxels$	18 [3]
Reversal osteocyte signal	P_{ocl}	$nmol \cdot mm^{-2} \cdot day^{-1}$	$4.0 \cdot 10^{-5}$
Ratio between osteocyte signal and contact surface energy to bone	k	$J \times nmol^{-1} \times day$	$2.0 \cdot 10^{-6}$
Minimal contact surface energy to bone	$h_{b,min}$	$J \times mm^{-2}$	- 0.5
Contact surface energy to marrow	h_m	$J \times mm^{-2}$	1.0
Contact surface energy to osteoclasts	h_{ocl}	$J \times mm^{-2}$	1.0
Motility	a	J^{-1}	$1.0 \cdot 10^{5}$
Resorption sensitivity to osteocyte signal	a_r	$mm^{2} \cdot day \cdot nmol^{-1}$	$2.0 \cdot 10^{-6}$
Osteoclast inelasticity	λ	$J \cdot mm^{-6}$	$1.0 \cdot 10^{4}$
Resorption amount	r	$voxel$	1
Other variables			
Maximal elastic modulus	E_{max}	GPa	15.0 [4]
Poison ratio	n	-	0.3 [4]
Exponent gamma	g	-	3.0 [5]
Loading frequency	f	Hz	1
Loading magnitude	A	MPa	10.0

Table 1: *Parameter settings for the simulations. [1] Mullender et al. (1996), [2] Huiskes et al. (1995), [3] this corresponds to an osteoclast diameter of 60 mm (Eriksen & Kassem, 1992; Ross & Teitelbaum, 2001), [4] Reilly & Burstein, (1975), and [5] Currey (1988).*

Results

Cortical bone remodeling

When the cortical bone remodeling simulation was started, the three osteoclasts in the initial cutting cone (Fig 2A) immediately grew to their target volume and remained close to that volume thereafter. They resorbed bone in the direction of the external load, producing a tunnel of 225 *mm* wide (Fig. 2) (Lee & Einhorn, 2001).

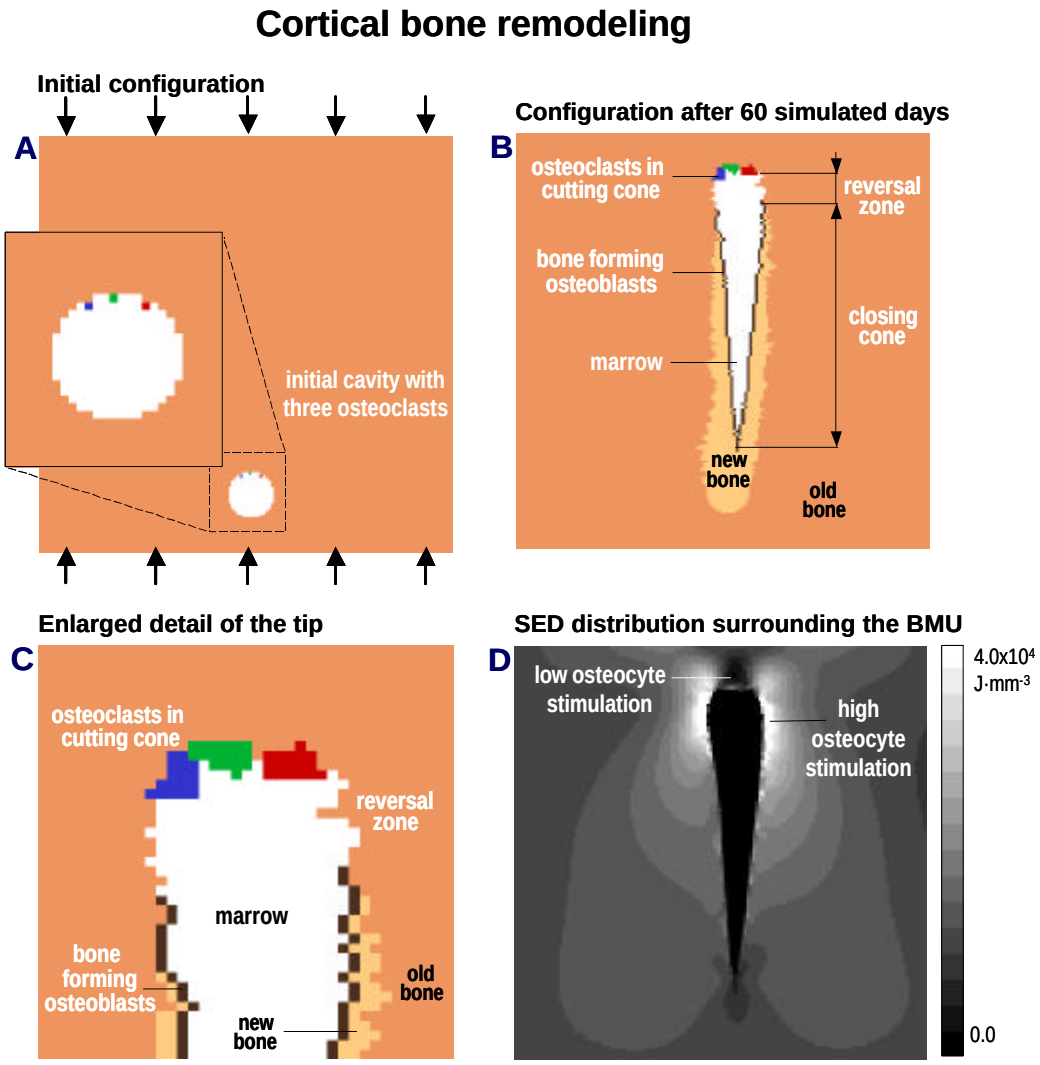


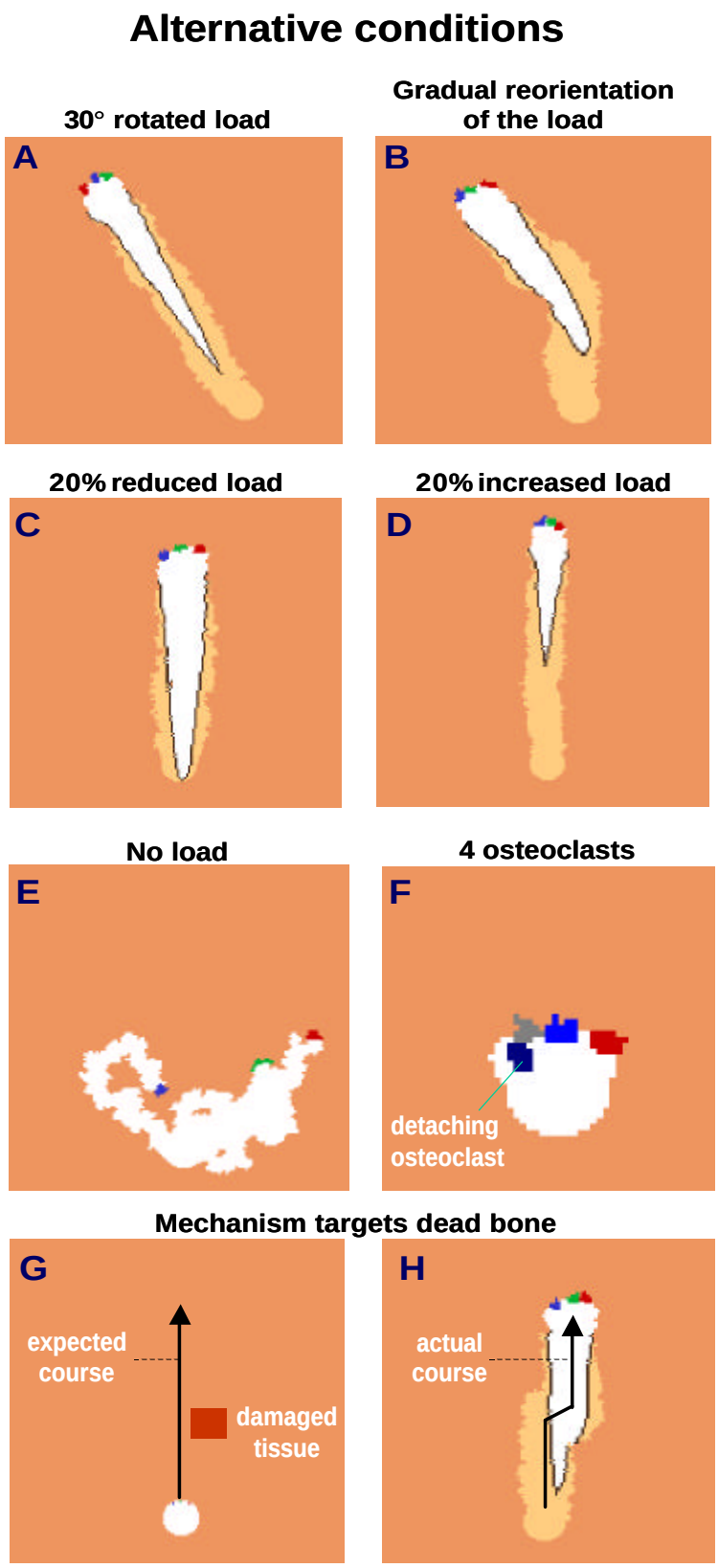
Fig 2: *A) The initial configuration consists of a 2´2 mm^2 piece of cortical bone tissue with three osteoclasts in the initial cutting cone. B) After 60 simulated days osteoclasts excavated a tunnel in the main loading direction. They are followed by bone forming osteoblasts that form a secondary osteon of renewed tissue. C) Enlarged detail of the cutting cone. D) Distribution of the strain energy density (SED) in the tissue surrounding the BMU.*

The cutting cone moved gradually through the bone at a realistic velocity of 25 $mm \cdot day^{-1}$ (Parfitt, 1994). Osteoblasts closely followed the cutting cone, forming an osteon of renewed bone tissue (Fig. 2). The length of the BMU was 1250 *mm* (Parfitt, 1994). The spatial organization of the BMU remained intact during the simulation, indicating that the regulatory mechanism is quite robust. Osteoclasts and osteoblasts behavior in the BMU is easily understood if we consider the mechanical environment surrounding the BMU. Mechanical signals were reduced under the tip of the cutting cone where osteoclasts resorb bone, and high at the side of the tunnel wall where osteoclasts retract and osteoblasts are recruited to form bone (Fig. 2D).

The results were similar when the *loading direction* was initially rotated by 30° and 45° (Fig. 3A). Again osteoclasts resorbed bone in the main loading direction and bone formation closely followed. A gradual reorientation of the external load, during the simulation process, caused the course of the cutting cone to bend and reorient accordingly (Fig. 3B).

Alternative loading magnitudes had a considerable effect on the morphology of the BMU. Twenty percent *reduced loads* caused the osteoclasts to excavate a tunnel with a larger diameter (275 *mm*). The traveling speed of the BMU reduced (21 $mm \cdot day^{-1}$) because osteoclasts were assumed to resorb a constant amount of tissue per increment. Bone formation followed at a reduced rate, causing the BMU to become elongated (Fig. 3C). Eventually, the tunnel was filled. Twenty percent *increased loads* caused the opposite effects. The osteoclasts burrowed faster through the bone (30 $mm \cdot day^{-1}$) creating a tunnel with a smaller diameter (188 *mm*). In this case bone formation followed more rapidly. The length of the BMU reduced to about 900 *mm* (Fig. 3D). Larger variations of the mechanical load caused the BMU to become unstable, i.e. not all osteoclasts stayed in the tip. Complete *unloading* of the structure resulted in undirected, stochastic resorption, not followed by formation (Fig. 3E).

→ Fig 3: *A) Similar results are obtained for alternative loading directions. B) Reorientation of the loading direction during the simulation causes the BMU to change its course accordingly. C) Reduced loads cause formation to follow at a reduced rate. The resulting osteon has a larger diameter. D) Increased loads cause rapid filling of the tunnel and narrower osteons. E) Complete unloading causes uncontrolled and rather stochastic resorption that is not followed by formation. F) The number of osteoclasts that can be active in the tip is rather constant. When initially four instead of three osteoclasts are placed in the tip, one is left behind and three proceed "normally". G,H) The BMU changes its course to resorb and replace damaged tissue. Thus it appears to be an effective mechanism for bone repair (G: initial configuration, H: configuration after 60 simulated days).*


Alternative conditions
30° rotated load
A
Gradual reorientation of the load
B
20% reduced load
C
20% increased load
D
No load
E
4 osteoclasts
F
detaching osteoclast
Mechanism targets dead bone
G
expected course
damaged tissue
H
actual course

← Fig 3

The BMU became instable when initially four osteoclasts were placed in the tip. They competed to attach to the surface in the tip of the cutting cone. Eventually, one osteoclast was incapable of staying in the tip. It detached from the bone surface and was left behind. The other three osteoclasts proceeded 'normally' (Fig. 3F). When also the loading magnitude was reduced (by 20%), all four osteoclasts were capable of staying in the tip of the cutting cone. This suggests that the number of osteoclasts that can be active in the tip is influenced by the loading magnitude.

The proposed regulatory mechanisms appear to be effective as a repair mechanism. The BMU changed its course to replace tissue where osteocytes were artificially killed (Fig. 3H).

Trabecular bone remodeling

The same regulatory mechanisms also explained the remodeling process in trabecular bone. The osteoclast in the initial resorption cavity (Fig. 4A) did not perforate the trabecula, but resorbed bone in the mechanical loading direction, i.e. parallel to the surface. The traveling speed of 35 $mm \cdot day^{-1}$ was higher than in cortical bone. The resorption depth was 54 *mm* (Eriksen and Kassem, 1992). Bone forming osteoblasts followed resorption, creating a hemi-osteon of renewed tissue (Fig. 4B).

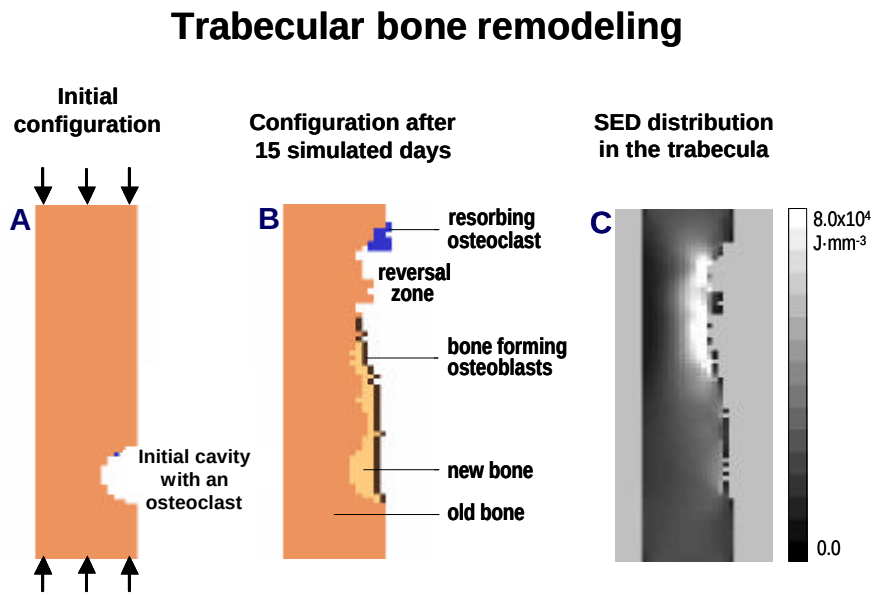


Fig 4: *A) The initial trabecula is 1 mm long and 200 mm thick. An initial resorption cavity with one osteoclast are placed at the surface. B) Configuration after 15 simulated days. The osteoclast resorbed bone along the surface in the loading direction. It is followed by bone forming osteoblasts. C) Osteoclast resorption can be explained by the strain energy densities in the trabecula. Low values are found in the resorption direction of the osteoclast. High values are found where the osteoclast retracts and formation is initiated.*

Alternative loads caused changes in the architecture, so we could speak of modeling, in this case. A 20% load reduction increased the resorption depth to 57 *mm* and led to incomplete filling due to reduced bone formation rates of the osteoblasts. A 20% increased load caused trabecular thickening, as formation was initiated on the whole surface of the trabecula. It also reduced the osteoclast resorption depth to 41 *mm*. Complete unloading caused the osteoclasts to resorb bone in an uncontrolled, stochastic manner. Eventually, after 10 simulated days, the trabecula was perforated and resorption was no longer followed by formation (Mosekilde, 1990).

Discussion

We developed a mechanobiological theory to relate mechanical forces to local osteoclast and osteoblast expressions in bone remodeling. The theory is largely based on unproven paradigms, but these are not really controversial. It is now generally believed that osteocytes are mechanosensitive cells, capable of sending signals to the bone surface (Skerry et al., 1989; Cowin et al., 1991; Klein-Nulend et al, 1995; Burger and Klein-Nulend, 1999; Smit and Burger, 2000). The assumed effects of these signals on osteoclasts and osteoblasts are less certain (Martin, 2000). Only few studies were done that concern (potential) relationships between osteocytes, on the one hand, and osteoblasts and osteoclasts on the other. The observation that osteoclasts form resorption pits if placed on bone slices that contain no viable osteocytes (Parikka et al., 2001) suggests that osteoclast resorption is inhibited in viable tissue. We assumed that osteoclast activity in physiological conditions is repressed by signals from the osteocytic network. The study of Heino et al. (2002) shows that this assumption is reasonable. They found that conditioned medium from an osteocyte-like cell line dramatically inhibited bone resorption by cultured rat osteoclasts. This conditioned osteocyte medium also stimulated mesenchymal stem cells to proliferate and differentiate to osteoblasts, suggesting that our assumed stimulatory effect of osteocytes on osteoblast bone formation is reasonable as well (Heino et al., 2004).

As for the questions we addressed in this study, our theory provides a cellular-level regulatory mechanism that relates external forces to local osteoclast and osteoblast activities, as observed in both cortical and trabecular bone remodeling. It explains that osteoclasts resorb bone in the main mechanical loading direction (Petryl et al., 1996) and it also explains coupling of bone formation to resorption (Frost, 1964). The regulatory mechanism proposed is robust and efficient in repair, as it targets dead bone, i.e. bone without viable osteocytes. The theory also relates osteon diameter, and the velocity of cone refilling with new bone, to loading intensity. Although several studies indicate that mechanical forces indeed affect osteon morphology (Su et al., 1999) such a relationship was never clearly demonstrated. Osteon diameters in actual cortical bone are fairly

constant (Lanyon, 1984). One explanation is that thinning/thickening of the cortex due to alternative loading magnitudes causes local tissue stresses to vary less than expected.

It is important to notice that a largely similar theory, although with a coarser description of the FEA mesh, explained modeling (growth and adaptation) and remodeling (maintenance) of 3-dimensional trabecular bone structures under influence of mechanical forces (Huiskes et al., 2000; Ruimerman et al., 2001; 2004). It is remarkable that such a limited set of parameters can explain modeling and remodeling of 3D trabecular bone structures at a time scale of months, as well as osteoclast and osteoblast activity in trabecular and cortical BMU's at a time scale of days.

The theory provides a computational framework that explains the remodeling activities of osteoclasts and osteoblasts at the BMU level – as shown in this article – as well as modeling and remodeling of trabecular bone structures (Huiskes et al., 2000; Ruimerman et al., 2001; 2004). However, the cascade of biochemical processes that are obviously involved are only implicitly assumed, but not explicitly accounted for. For example, we applied (and extended) the Glazier & Graner formalism to mimic bonding behavior of the osteoclasts. This was suitable to address the research questions of this study, as it mimics cell migration and attachment to the bone surface, as well as bone resorption, but it does not specify the underlying biochemical processes. These are extremely complex and involve active participation from the osteoclasts themselves, as actual resorption involves polarization of the cell due to re-organization of its cytoskeletal apparatus, the formation of a sealing zone to attach it to the bone matrix, and the formation of a ruffled border which is the actual resorbing organelle of the cell (Vaananen et al., 1995).

The assumed osteocyte-influence distance has a significant effect on the dynamical details of the processes. Larger values create a more homogeneous osteocyte signal at the internal bone surface and therefore less control over osteoclast resorption. This indicates that the osteocyte signal must have a local effect only, suggesting that the biochemical messengers involved must either diffuse poorly, or be instable and decay quickly. This fits with the theory from Burger et al. (2003) who proposed a role for nitric oxide NO, which is extremely instable.

Another issue concerns the question of how the process starts or how it ends. This is not explicitly considered in the present theory, which is focused on how the remodeling process progresses after having been initiated. As for our trabecular modeling and remodeling theory, we assume that the remodeling processes in the present formulation are triggered by the presence of microcracks (Burr et al., 1985; Mori and Burr, 1993). These are likely to cause osteocyte apoptosis which then initiates osteoclast resorption (Verborgt et al., 2000; Noble, 2003). The process of resorption is likely to end when osteoclasts reach an endosteal surface or another osteonal canal.

In conclusion, we developed a computational bone modeling and remodeling theory based on the assumption that mechanically induced osteocyte signals control osteoclast and osteoblast activities at the bone surface. We already showed that these assumptions could explain the effects of forces in the self-organizational processes of trabecular bone in growth, maintenance and adaptation (Huiskes et al., 2000; Ruimerman et al., 2001, 2004). In the present study we showed that similar cellular communication pathways also explain osteoclast resorption direction and coupling of formation in remodeling BMU's of both cortical and trabecular bone. The earlier computational theory for trabecular modeling and remodeling (Huiskes et al., 2000; Ruimerman et al., 2001, 2004), and the present one for local BMU-based remodeling in both trabecular and cortical bone are fully compatible, but different in the scale of topological refinement.

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Chapter 7

Discussion

Proper understanding of bone modeling and remodeling processes is of clinical importance since it may provide answers to the question of how we must deal with metabolic bone diseases and conditions, such as osteoporosis, osteopetrosis, osteogenesis imperfecta, and others. The modeling and remodeling processes are complex and currently poorly understood. Much work on the biology of bone formation, resorption, adaptation and osteoporosis is conducted relative to a biochemical background. There are many biochemical messengers involved, while only some were identified. However, from the biochemical angle the relationship with biomechanics is – while not denied – commonly ignored, or rather bypassed.

The objective of our research is to reveal the cellular regulatory mechanisms that control bone architecture. For that purpose we develop a coherent theory that could potentially explain the relationships between mechanical forces, metabolic activities and bone architecture. This involves integrating knowledge of different fields of research, like endocrinology, cell biology, orthopedics and mechanical engineering. This is the premise and asset of mechanobiology. However, it is also what makes it difficult.

In this research project we demonstrated that modeling and remodeling of bone tissue can be explained by a remarkably simple theory, relating effects of load transfer to cell expressions. Its basic assumption is that osteocytes are mechanosensitive cells, capable of controlling resorption and formation at bone surfaces. Applying computer simulations we showed that a control mechanism based on this assumption explains bone modeling in growth towards a mature trabecular structure with realistic characteristics in terms of morphology and physiology, as compared to actual trabecular bone. In addition we showed that the theory explains that eventually a homeostatic steady state is reached in which the bone structure is maintained by ongoing resorption and formation. The theory also mimics the known morphological effects of disuse and overloading, and it predicts how the structure adapts to alternative loading directions. Finally, we refined the scale of the problem in terms of spatial and temporal dimensions to demonstrate that the same cellular control mechanisms also explains osteoclast and osteoblast activities in single remodeling BMU’s of both cortical and trabecular bone, i.e. it directs osteoclast resorption in the main loading orientations and ensures that osteoblasts follow in a coordinated manner.

If we summarize these results, we conclude that our theory provides a cellular

level regulatory mechanism that explains bone adaptation according to Wolff's Law (Wolff, 1892). The theory also confirms the feasibility of Roux's hypothesis, implying that trabecular architecture is controlled by local regulatory mechanisms (Roux, 1881). That such a simple regulatory mechanism mimics so many different features of the (re)modeling process, is remarkable. This is suggestive for its correctness, but it does not actually proof it. Only future research may reveal if the theory is correct, or that alternative explanations exist for all these phenomena.

From a biochemical perspective, our model is empiric. We used lumped parameters, which are supposed to represent cascades of crucial biochemical reactions. This certainly does not mean that biochemical factors are believed less relevant. There is no doubt that biochemical factors play crucial roles in the coupling phenomena. We do not ignore them, but just made them subordinate to mechanobiological signaling. Or, in other words, we assumed that biochemical signaling cascades are initiated by mechanical signals. It was shown that growth factors, like TGF-β, are released during the resorption phase. These factors are potent stimulators of osteoblast bone formation and thus may couple formation to resorption (Bonewald & Mundy, 1990; Mundy, 1991). Another form of communication that was recently proven to exist is that cells of the osteoblast lineage, that express RANKL, are involved in maturation and activation of osteoclasts. Osteoclast precursors that express RANK, a receptor for RANKL, recognize RANKL through direct cell-to-cell interaction and then differentiate to osteoclasts (Udagawa et al., 1999; Katagiri & Takahashi, 2002). This indicates that cells of the osteoblast lineage have to be present before osteoclast resorption can take place. However, although this sets the conditions for resorption, it does not actually couple formation to it.

Direct biochemical communication pathways between osteoclasts and osteoblasts were not accounted for in our computational model, although it is possible in principle. We believe that coupling finds its origin in mechanical factors (Rodan, 1991). The observation that lack of mechanical loading uncouples formation from resorption makes such a hypothesis plausible (Mosekilde, 1990). In our theory resorption lacunae cause stress concentrations in their surrounding tissue. The elevated mechanical signals are likely to trigger osteocytic network response that stimulates bone formation. We showed that this form of mechanical feedback is sufficient to explain the coupling phenomenon. Again, we did not incorporate direct biochemical coupling in our models. However, we must bear in mind that the assumed communication between osteocytes and osteoblasts probably involves many complex biochemical messengers itself. The importance of unraveling the crucial biochemical reactions of the remodeling process is obvious, as it is reasonable to assume that actual pharmaceutical intervention has to interfere at that level. These crucial biochemical processes are largely unknown. Our theory might provide guidelines for where to find them.

Another simplification is that we considered the bone tissue as a homogeneous

material and that osteocytes were assumed to respond to the strain energy density (SED) rate. This is a serious simplification. Detailed models at the microscopic level are required to further unravel the process of mechanosensation by osteocytes. In such detailed models we must take local deformations of tissue matrix, fluid flow through the canalicular network and deformations of the osteocyte membrane into account. More detailed mechanobiological models of the osteocyte and its surrounding may also provide insight in how signals are transferred over a certain distance, i.e. how biochemical substances, like hormones and drugs, are transferred from the surface through the canaliculi to the osteocytes, and vice versa. This involves both diffusion and convection as effect of dynamic loading and perhaps also active transport mechanisms. Recently, detailed numerical models were developed to study the mechanical environment of the osteocytes (Weinbaum et al., 1995; You et al., 2001). Due to computational restrictions it is currently not feasible to incorporate this level of detail in our simulation models.

An important asset of our theory is that it relates cell activity to local trabecular structure. We can therefore use it to predict how the trabecular structure will adapt if the metabolic activity of osteoclasts, osteoblasts or osteocytes is altered. We showed two examples of this.

The first example is the study we performed to investigate the pathways by which estrogen deficiency after menopause causes the typical patterns of bone loss as they occur in postmenopausal osteoporotic women. We found that the assumption that estrogen deficiency enhances only osteoclast resorption is sufficient to explain the increased remodeling rates and the rapid, yet transient, loss of bone mass due to loss of complete trabeculae, preserving trabecular thickness. Osteoblast bone formation was enhanced as a secondary effect due to ‘mechanical’ coupling of formation to resorption. These simulation results indicate that estrogen does not necessarily have a direct, separate effect on osteoblasts as well, because osteoclast stimulation combined with mechanical osteoblast coupling may explain the morphological effects of postmenopausal osteoporosis equally well.

The second example of how our theory can be applied is the investigation of medical intervention effects on osteoporotic bone (Ruimerman et al., 2003). Osteoporotic patients are often treated with anti-resorptive drug administration to arrest the degeneration of the bone structure. The morphological consequences of such a treatment are poorly understood. We applied our theory to investigate these effects. Our theory predicted that *preventive* anti-resorptive drug administration reduces the rate of bone loss. Compared to *late* treatment it does not preserve more bone mass on the long term, but it does preserve trabecular connectivity, and thus fracture resistance (Mosekilde, 1987). It would be interesting to further investigate these findings in animal experiments, as it is obviously clinically relevant to know whether these predictions are true.

At this time, very little is known about where, precisely, micro-architectural

changes take place when biochemical or mechanical conditions are altered. The ongoing development of high-resolution CT and MRI-scanners holds great promises to obtain insight in this issue. It is only since very recently that *in vivo* reconstructions of trabecular bone structures can be made at reasonably small resolutions (Waarsing et al., 2003; David et al., 2003). This makes it possible to follow bone micro-architectural changes *in vivo*. Thus, it is feasible to measure morphological changes in one subject during growth, due to disuse or as an effect of estrogen deficiency due to ovariectomy (a model for postmenopausal osteoporosis). It also enables the investigation of morphological effects of pharmaceuticals. It would be interesting to compare information that can be obtained from these studies with the predictions of our theory. This would provide new ways for proper validation, and refinements, if necessary. A present complication is that the current simulations were performed on small bone cubes with artificial boundary conditions. Proper validation requires scaling of the model to larger domains, so that we can mimic the adaptive behavior in complete bones with (more) realistic mechanical boundary conditions. The application of efficient algorithms and parallel computing are inevitable in order to achieve this. When this is possible, it will also open the way for more practical applications. In concept, the theory can be used to predict the development of osteoporosis, its severity for fracture risk, and the viability of treatment modalities in whole human bones. Then it may also be applied to predict how the bone adapts to the altered mechanical environment after incorporation of orthopaedic joint prostheses, like in hips or knees. Hence, it has the potential to become a viable tool for pre-clinical testing of prostheses as we can optimize the prosthesis design with a limited amount of animal experiments.

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Summary

Bone tissue, the main material the skeleton consists of, has remarkable properties. Two macroscopically different types are distinguished. The first type is *cortical* or compact bone, which is a rather dense tissue. The second type is *trabecular* or cancellous bone. This is a porous tissue with a complex three-dimensional structure consisting of struts and plates, called trabeculae. Already in 1892 Wolff found that the orientation of these trabeculae coincides with the direction of the stress trajectories. He proposed that bone loading is somehow sensed and that bone adapts its structure accordingly. This principle of functional adaptation is generally known as 'Wolff's Law'. It occurs in conditions of disuse when bone is lost, and in intense physical usage when bone mass increases, but also during growth, after fracture healing and in relation with implant incorporation, when the orientation of trabeculae changes. The ability of the bone to adapt to mechanical loads is brought about by continuous bone resorption and bone formation. If these processes occur at different locations, the bone structure is altered. This is called *modeling*. In a homeostatic equilibrium resorption and formation are balanced. In that case old bone is continuously replaced by new tissue, ensuring the maintenance of mechanical integrity of bone tissue without any global changes in the architecture. This is called *remodeling*. The modeling and remodeling processes are conducted by specialized bone-resorbing and bone-forming cells, called osteoclasts and osteoblasts respectively. The pathways by which mechanical forces are expressed in osteoclast and osteoblast activity is currently one of the main unresolved issues in bone mechanobiology.

For this dissertation we studied the modeling and remodeling processes as modulated by mechanical forces. We demonstrated that many of the phenomena as they occur in the bone tissue can be explained by a remarkably simple theory, based on the assumptions that osteocytes, which reside in the bone tissue matrix, are mechanosensitive cells capable of sending biochemical messengers to the bone surface. These signals stimulate osteoblasts to form bone whereas they inhibit resorption by osteoclasts.

Applying computer simulations we demonstrated that this control mechanism explains how the bone is modeled in growth towards a mature 3D trabecular structure with realistic characteristics in terms of morphology and physiology, as compared to actual trabecular bone. Eventually a homeostatic steady state is reached in which the mechanical integrity of the bone structure is maintained by ongoing resorption and formation, precisely as it occurs in mature trabecular bone. We demonstrated that the theory also mimics how the structure adapts to alternative loading conditions. It describes how reduced loads lead to bone loss due to trabecular thinning and loss of trabecular connectivity, and how increased loads lead to trabecular thickening. In addition, it also predicts that new loading directions cause trabeculae to gradually realign accordingly.

An important asset of our theory is that it relates cell activity to local trabecular structure. We showed how this feature can be applied to investigate the effects of altered osteoclast, osteoblast and osteocyte activities. We investigated the pathways by which estrogen deficiency after menopause causes the typical patterns of bone loss as they occur in postmenopausal osteoporotic women. These pathways are largely unknown, but we found that the assumption that estrogen deficiency enhances only osteoclast resorption is sufficient to explain the increased remodeling rates and the rapid, yet transient, loss of bone mass due to loss of complete trabeculae, whereas the thickness of remaining trabeculae is preserved. The enhanced osteoblast bone formation could be explained as a secondary effect due to 'mechanical' coupling of formation to resorption, indicating that estrogen does not necessarily have a direct, separate effect on osteoblasts. In a second study we investigated the medical intervention effects on osteoporotic bone. Osteoporotic patients are often treated with anti-resorptive drug administration to arrest the degeneration of the bone structure. The morphological consequences of such a treatment are poorly understood. We applied our theory to investigate these effects. A clinically important result is that our theory predicts that *preventive* anti-resorptive drug administration reduces the rate of bone loss. Compared to *late* treatment it does not preserve more bone mass on the long term, but it does preserve trabecular connectivity, and thus fracture resistance.

If we consider the modeling and remodeling processes at the cellular level, then it appears that osteoclasts and osteoblasts closely collaborate in what is called a "Basic Multicellular Unit", or BMU. In cortical BMU's, osteoclasts excavate cylindrical tunnels in the predominant loading direction of the bone. They are followed by osteoblasts, filling the tunnel, creating secondary osteons of renewed tissue. Trabecular bone remodeling is mainly a surface event, in which osteoclasts dig a trench rather than a tunnel and also here they are followed by bone forming osteoblasts creating hemi-osteons. That osteoblasts follow osteoclasts in such a coordinated manner indicates that a coupling mechanism must exist. Its precise nature, however, is currently uncertain. Another unresolved issue in bone mechanobiology is the mechanism that guides osteoclast resorption in its directionality. We refined the scale of our computational theory in terms of spatial and temporal dimensions to address these unresolved issues and demonstrated that the cellular communication pathways that are proposed also provide an explanation for the osteoclast resorption direction and coupling of formation to resorption as observed in remodeling BMU's of both cortical and trabecular bone.

Summarizing these results, we conclude that bone adaptation according to Wolff's Law, but also the coordinated fashion by which osteoclasts and osteoblasts collaborate in the remodeling BMU's of cortical bone and trabecular bone, can all be explained by one coherent cellular level theory for bone modeling and remodeling.

Samenvatting

Botweefsel is een bijzonder materiaal. Er kunnen twee verschillende soorten worden onderscheidden. Het eerste soort is corticaal bot. Dit is een compact weefsel met een hoge dichtheid. Het tweede soort bot is trabeculair bot. Dit type heeft een complexe 3-dimensionale structuur bestaande uit balkjes en plaatjes. Al in 1892 vond Wolff dat de oriëntatie van deze zogenaamde trabekels in de mechanische belastingrichting ligt. Hij veronderstelde dat bot op de een of andere manier mechanische belastingen kan waarnemen, en dat het zijn interne structuur aan die belasting kan aanpassen. Dit principe van functionele adaptatie staat nu algemeen bekend als ‘De wet van Wolff’. Adaptatie kan onder verschillende omstandigheden plaatsvinden. Bij lage belastingen verdwijnt er bot terwijl de hoeveelheid bot toeneemt in geval van intensief gebruik. Adaptatie vindt ook plaats gedurende de groei, gedurende het helen van fracturen en in botweefsel rondom implantaten. Het vermogen om zich aan te passen wordt veroorzaakt door continue botresorptie en botformatie. Indien dit op verschillende locaties plaatsvindt, dan leidt dit tot veranderingen in de structuur en spreekt men van ‘modelleren’ van het weefsel. In een homeostatisch evenwicht zijn resorptie en formatie in balans. In dat geval wordt oud botweefsel voortdurend vervangen door nieuw weefsel zonder dat er veranderingen in de globale structuur plaatsvinden. In dat geval spreekt men van ‘remodelleren’.

Bot modelleren en remodelleren zijn processen die worden uitgevoerd door gespecialiseerde cellen, namelijk botresorberende osteoclasten en botvormende osteoblasten. Het is overduidelijk dat mechanische krachten de modellerings en remodellerings processen beïnvloeden. Eén van de belangrijkste onopgeloste vraagstukken in de botbiologie is hoe mechanische krachten worden vertaald naar locale activiteit van osteoclasten en osteoblasten.

In deze studie onderzochten we hoe de botmodellerings en remodellerings-processen worden beïnvloed door mechanische krachten. Het blijkt dat veel fenomenen zoals ze in botweefsel plaatsvinden kunnen worden verklaard met een relatief eenvoudige theorie. Deze theorie is gebaseerd op de aanname dat osteocyten in de botmatrix gevoelig zijn voor mechanische belasting. Relatief aan het mechanische signaal waaraan zij onderhevig zijn, sturen zij biochemische signalen naar het botoppervlak. Die signalen stimuleren oteoblasten tot botvorming terwijl ze resorptie door osteoclasten inhiberen.

Met behulp van computer simulaties tonen we aan dat dit regelmechanisme verklaart hoe 3-dimensionale botstructuren ontstaan gedurende het groeiproces. Vergeleken met echt bot hebben deze structuren realistische eigenschappen. De theorie verklaart ook hoe de structuur wordt onderhouden door voortdurende resorptie en formatie zonder dat de globale structuur verandert, en hoe de structuur zich aanpast aan veranderde belastingen. Het laat zien hoe lagere belastingen op twee manieren tot botverlies leiden: trabekels worden dunner en trabeculaire connectiviteit gaat verloren.

Het laat ook zien hoe trabekels dikker worden ten gevolge van verhoogde belastingen en hoe het bot zijn interne structuur aanpast aan nieuwe belastingrichtingen.

Een aantrekkelijkheid van onze theorie is dat ze de functionaliteit van cellen in het botweefsel relateert aan de botstructuur. Deze eigenschap kunnen we gebruiken om de invloed van veranderde activiteit van osteoclasten, osteoblasten en osteocyten te onderzoeken. We onderzoeken hoe oestrogeen deficiëntie na de menopauze leidt tot botverlies zoals dat plaatsvindt in post-menopauzale osteoporotische vrouwen. Het blijkt dat de aanname dat oestrogeen alleen de activiteit van osteoclasten inhibeert, verklaart dat de remodelleringssnelheid verhoogt, en dat er vrij snel een beperkte hoeveelheid bot verloren gaat door verlies van trabeculaire connectiviteit terwijl de dikte van overblijvende trabekels behouden blijft. Dit resultaat suggereert dat oestrogeen geen direct effect heeft op osteoblasten. Namelijk, de verhoogde activiteit van osteoblast kan evengoed worden verklaard door indirecte effecten ten gevolge van mechanische terugkoppeling. In een andere studie onderzoeken we de effecten van medisch ingrijpen op osteoporotisch bot. Osteoporose wordt vaak behandeld door medicijnen die botresorptie onderdrukken. De gevolgen voor de botstructuur worden op dit moment niet goed begrepen. Wij onderzochten die effecten m.b.v. onze theorie. Een belangrijke voorspelling van onze theorie is dat preventief toedienen van medicijnen de snelheid van botverlies vertraagt. De uiteindelijke hoeveelheid botmassa is echter hetzelfde als vergeleken met een behandeling die pas later wordt gestart. De weerstand tegen falen blijft daarentegen wel behouden doordat de trabeculaire connectiviteit behouden blijft.

Als we het remodelleringsproces in detail beschouwen, dan blijkt dat osteoclasten en osteoblasten nauwkeurig samenwerken in zogenaamde “Basic Multicellular Units”, of BMUs. In corticale BMUs resorberen osteoclasten tunnels, die in de richting van de mechanische belasting liggen. Ze worden gevolgd door osteoblasten die de tunnel weer opvullen met nieuw bot. Trabeculair botremodelleren vindt voornamelijk aan het botoppervlak plaats. Osteoclasten graven een soort goot i.p.v. een tunnel, en ook hier worden ze gevolgd door osteoblasten. Dat osteoblasten zo nauwkeurig volgen suggereert het bestaan van een koppelingsmechanisme. Hoe dit mechanisme werkt is onbekend. Hoe het komt dat osteoclasten in de belastingrichting resorberen, is ook onbekend. In het laatste hoofdstuk verfijnen we onze computer modellen in lengte en tijdschaal. Hierdoor konden we aantonen dat dezelfde theorie, die het modelleren en remodelleren van trabeculaire botstructuren verklaart, ook verklaart dat osteoclasten bot resorberen in de mechanische belastingrichting en dat botformatie door osteoblasten op een zo gecoördineerde wijze volgt.

Deze resultaten samenvattend concluderen we dat we botadaptatie volgens ‘De wet van Wolff’ kunnen verklaren. Tevens kunnen we verklaren hoe osteoclasten en osteoblasten samenwerken in zowel corticale als trabeculaire BMU’s. Dit alles met behulp van één theorie voor modelleren en remodelleren van bot.

Dankwoord

Het tot stand komen van dit proefschrift is niet uitsluitend aan mij te danken. Velen hebben hun bijdrage geleverd op verschillende wijzen. Als eerste wil ik Rik Huiskes bedanken. Dat ik samen met jou aan dit onderzoek heb mogen werken was mij een groot genoegen. Bedankt voor de leerzame inzichten in de filosofie van wetenschap en alle discussies over botbiologie. Verder ben ik je erg dankbaar dat je mij hebt leren kennismaken met de “bot”-wereld met zijn vele meetings en congressen.

Ook mijn andere “bot”-collega’s wil ik bedanken: Bert, Keita, Esther, Rene, Wouter, Eelco en Marlies, en ook de leden van het NWO-project “Bone Cell Mechanosensitivity, Estrogen Deficiency and Osteoporosis”, of kortweg het BEO-project. Bedankt voor de leerzame overleggen en interessante discussies. Ook mijn afstudeerders Rene en Tristan wil ik bedanken voor een, naast (hopelijk) voor jullie, in ieder geval voor mij, leerzame samenwerking.

Ook mijn overige “niet-bot” collega’s wil ik noemen. Anthony, Bart, Huub, Koen, Nico, Dragan, Luc en Peter, bedankt voor de gezellige sfeer, de vele schaakuitdagingen en de interessante en gevarieerde discussies over uiteenlopende onderwerpen.

Last, but not least, in mijn wetenschappelijke omgeving, wil ik Peter Hilbers bedanken. Voor de mogelijkheid die je mij gaf me te ontplooien op een manier die ik wenselijk achtte en voor het aanleren van een brede visie waarmee je op zoveel verschillende manieren tegen de zaken (al dan niet wetenschappelijke) aan kan kijken. Ik beschouw dat als een grote toegevoegde waarde.

Speciale aandacht verdienen mijn Ouders. Jullie hebben mij altijd weten te stimuleren mijn best te doen. Jullie hebben mij de mogelijkheid gegeven te studeren, en jullie stonden, ook de afgelopen jaren, altijd klaar. Super!

En kleine Sterre, als kleine 2,5-jarige onderzoeker die volop bezig is de wereld te ontdekken, heb ook jij een aandeel geleverd. In extra in- maar vooral ook ontspanning.

Mijn laatste woord van dank wil ik aan Falke richten. Dat we allebei in dezelfde periode ons proefschrift moesten afronden was niet gemakkelijk. Bedankt voor je steun en liefde. Samen hebben we het toch maar mooi voor elkaar gekregen.

Curriculum vitae

Ronald Ruimerman werd op 31 maart 1974 geboren in Apeldoorn. Na het behalen van het VWO diploma aan het Elshof College te Nijmegen begon hij in 1992 met de studie werktuigbouwkunde aan de Technische Universiteit Eindhoven. Na een jaar besloot hij Werktuigkundige Medische Technologie te gaan studeren aan diezelfde universiteit. Zijn afstudeerproject deed hij aan de Katholieke Universiteit Nijmegen in het Orthopaedic Research Lab van de afdeling Orthopedie. Daarna werkte hij een jaar in het bedrijfsleven. In 2000 begon hij als AIO aan de Technische Universiteit Eindhoven. Gedurende zijn promotie bestudeerde hij de groei en adaptatie processen in botweefsel. Op dit moment werkt hij als post-doc verder aan dit onderwerp op basis van een subsidie van het onderzoeksprogramma Computational Life Sciences van NWO.