The Process of Mechanical Adaptation in Trabecular Bone

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ISBN 90-9010773-8
Uitgegeven door Ponsen & Looijen, Wageningen

This publication was financially supported by grants from:

Sulzer Orthopedics Ltd., Research Department, Switzerland
D.H. Heijne Stichting - Basko health care, Amsterdam, Nederland
Howmedica Nederland, Haarlem, Nederland
Dr. h. c. **R**obert **M**athys **S**tiftung (Foundation), Bettlach, Switzerland
WEST Meditec, Bilthoven, Nederland
MERCK Biomaterial, Amsterdam, Nederland
Turnschool TURNACE Gymnastics Amsterdam

THE PROCESS OF MECHANICAL ADAPTATION IN TRABECULAR BONE

een wetenschappelijke proeve op het gebied van de
Medische Wetenschappen

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Katholieke Universiteit Nijmegen,
volgens besluit van het College van Decanen in het
openbaar te verdedigen op dinsdag 11 november 1997
des namiddags om 1.30 uur precies

door

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geboren op 19 augustus 1965
te Amstelveen

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1 INTRODUCTION

Trabecular bone, also called cancellous or spongeous bone, is one of two macroscopically distinct types It consists of a complex three-dimensional lattice of struts and plates, which occupies about five to fifty percent of the bone volume The remaining part contains marrow and blood vessels The other type is cortical (dense or compact) bone This is solid bone, except for blood vessels and cells embedded inside the matrix These two types form the skeleton, in which cortical bone is mainly found in the shafts of long bones, and trabecular bone is primarily located near joint surfaces, at the end of long bones and in the vertebrae, but also between cortical shells, such as inside the pelvic bone Bone is characterized by its calcified extracellular matrix, which provides its high stiffness This property points at the major function of bone, which is to sustain and transfer mechanical loads Other functions are storage of calcium and other matrix constituents and the production of blood cells in the bone marrow

Although bone appears to be a very stable and inert material it is, in fact, a vital and dynamic tissue Bone is constantly being broken down and rebuilt in a process called remodeling, which continues throughout life The primary advantage of this process is maintenance of mechanical integrity and adaptation to mechanical loads Bone is remodeled in a highly coordinated way by specialized cells, osteoclasts and osteoblasts, bone resorbing and bone forming cells, respectively In this process, osteoclasts first excavate a resorption pit, which osteoblasts refill with osteoid, which mineralizes slowly to form new bone During bone formation, some osteoblasts are embedded inside the bone matrix and differentiate into osteocytes The trabecular bone surface is covered with a layer of lining cells These cells are also thought to represent a final stage of osteoblastic differentiation The osteocytes are stellate shaped, with long thin processes that are connected to those from other osteocytes and with lining cells and osteoblasts at the bone surface Together they form a network throughout the bone matrix (Fig 1)

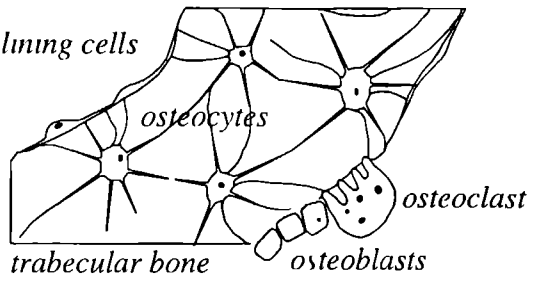

Figure 1
A schematic picture of bone tissue with the various types of bone cells

Humans gain bone mass during growth until a maximum is reached at the age of about 20 years, approximate-ly between the ages of 25 and 30, bone mass starts to decrease gradually In women, this loss is accelerated during the menopause as a result of a reduction in estrogen production Age related bone loss can be considered as normal, however, about one third of the population in developed countries develops osteoporosis (Wasnich, 1996, Ross, 1996) This condition is characterized by low bone mass and a deteriorated bone architecture (conference report, 1993) It presents itself through bone fractures, after minimal trauma The most common fracture sites in osteoporosis are the femoral neck, the vertebrae, and the distal forearm, where trabecular bone would normally attribute substantially to the mechanical strength The problem of osteoporosis is extensive For instance, the prevalence of vertebral fractures in elderly people ranges from 10-45% (O'Neill et al , 1996, Jones et al , 1996, Davies et al , 1996, Ross, et al , 1995) In the U S A , the total number of osteoporotic fractures annually is roughly 1 5 million, associated with costs of health care of $5-$10 billion (Zohman and Lieberman, 1995, Riggs and Melton, 1995) With continued aging of the population, it is only to be expected that the incidence of osteoporotic fractures will increase even further

Due to the complexity of the regulatory processes involved, it is presently unclear what causes the malfunction of bone maintenance in osteoporosis However, the world wide problem of osteoporosis impels researchers to unravel the mechanisms responsible for the control of bone architecture Many agents, such as growth factors and hormones, are involved in the regulation of bone remodeling, of which very little is currently known It is generally assumed that, besides chemical agents, mechanical factors play an important role Rodan (1996) summarized the regulatory process as *bone homeostasis is controlled by mechanical factors in a hormonal environment* Disturbances in this finely tuned process may lead to perturbation of the remodeling balance and result in bone loss, or occasionally, an increase of bone mass, but may perhaps also lead to inappropriate bone architectures

The competence of bone is determined by its mechanical properties The relationship between trabecular bone architecture and its mechanical function has been recognized for several centuries (Treharne 1981) In 1892 Wolff described how the directions of trabeculae coincide with the directions of the stress trajectories as can be calculated in a mathematical model Ever since, Wolff's law has been referred to as the phenomenon that bone architecture adapts to mechanical loads Nevertheless, the first one to speculate on the mechanism of bone adaptation, i e the process controlling the distribution of bone mass, was Roux (1881) He suggested a process analogous to Darwin's evolution theory, only with cells as the individuals Cells that are appropriately stimulated will prosper and form more matrix, whereas other cells, that do not receive the appropriate stimulus, will disappear Translated to bone tissue, Roux proposed that bone will be formed at locations where the local stresses are relatively high and bone will vanish where stresses are low Although this general theory has become more or less the prevalent one, its consequences for the behavior of the remodeling process have never been fully resolved

That changes in mechanical loading affect bone is clear from many experimental studies Whereas overloading stimulates bone formation (Rubin and Lanyon, 1984), disuse induces bone loss (Carter et al , 1981, Schaffler and Pan, 1992, Thomas et al , 1996, Biewener et al , 1996, Li et al, 1990, Palle et al , 1992, Jaworski and Uhthoff, 1986, Kannus et al , 1996) The difficulty to investigate the exact role of mechanical factors is partly due to the complexity of measuring and controlling mechanical variables in bone tissue In addition, it is difficult to assess the mechanical and morphological properties of the complex three dimensional trabecular bone architecture The techniques for making three dimensional reconstructions of bone recently developed (Odgaard et al , 1994, Ruegsegger et al , 1996) and large scale finite element models of bone architecture (van Rietbergen et al , 1994) have provided new prospects The reconstruction of bone architectures in the computer offers the possibility to accurately analyze its three dimensional morphological characteristics (Odgaard, 1994) which, until this moment, had to be inferred from two dimensional measurements of bone surfaces (Parfitt et al , 1983, 1987) Moreover, with the recently developed finite element methods these reconstructions can be mechanically analyzed Using this method, it is now possible to simulate mechanical tests and even to fully characterize the mechanical properties of trabecular architectures (Van Rietbergen et al , 1994, 1996)

It has been only recently that researchers have started to investigate the precise mechanisms by which bone tissue is able to detect mechanical loads and by which pathways mechanical signals may affect bone remodeling Presently the leading theory is

that the osteocyte network functions as a mechano-sensory system Some evidence for this theory was found In organ cultures, it was shown that osteocytes respond metabolically to mechanical loading of the bones (Pead et al , 1988 Skerry et al, 1989 Lean et al 1996) Isolated osteocytes were found to be extremely sensitive to mechanical stimulation by fluid flow (Klein-Nulend et al , 1995), which has been proposed to be the mechanism by which these cells are also stimulated in vivo (Reich et al 1990, Reich and Frangos 1991, Weinbaum et al , 1994, Harrigan and Hamilton, 1993)

The prediction that a local control mechanism, such as proposed by Roux, produces the typical trabecular structures that resist mechanical loads effectively with relatively little material is far from trivial Nevertheless, indications that this is indeed the case were given by the work of Weinans et al (1992) With the use of computer models they simulated adaptation of bone to mechanical loads in a locally regulated process They discovered that such a process induces the formation of patterns In a recent theoretical analysis of this type of process, Weinans and Prendergast (1996) explained that the nonlinear nature of this process is the reason for pattern formation The positive feed-back control loop and discrete sensor sites are the ingredients that are responsible for the dynamic behavior of the process The process passes through bifurcations and each resulting morphology is metastable, prone to jump to a different metastable morphology after perturbation of the system

The findings of Weinans et al (1992) and the experimental information have lead us to hypothesize that the morphology of trabecular bone is the result of a process of self organization, controlled by osteocytes, which in turn are stimulated by local mechanical signals Whereas Weinans and Prendergast (1996) conclude that in order to simulate the adaptive behavior of bone, this non linear behavior (caused by the positive feed-back loop and discrete sensor sites) should be incorporated in analytical models used we think that first it is necessary to investigate if the assumption that adaptive bone remodeling is such a non linear process is indeed a realistic one The principal questions that are addressed in this thesis are whether Roux s hypothesis is feasible (chapters 2 and 3) and, if this is the case, whether this hypothesis can be substantiated by measurements of parameters in the proposed process, specifically the density of sensor cells and the trabecular morphology (chapter 4) In addition, we have tested if the osteocytes, which we have assumed to be the mechano-sensors, are also the most suitable candidates for this role (chapter 5) Finally we have studied the question whether osteoporosis might be explained by differences in the parameters involved in the regulatory process (chapters 6 8)

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2

A Physiological Approach to the Simulation of Bone Remodeling as a Self Organizational Control Process

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Journal of Biomechanics 27 1389-1394, 1994

Abstract

Although the capacity of bone to adapt to functional mechanical requirements has been known for more than a century, it is still unclear how the bone adaption processes are regulated We hypothesize that osteocytes are sensitive to mechanical loading and control the regulation of bone mass in their environment Recently simulation models were developed of such a process It was discovered that these models produce discontinuous structures, not unlike trabecular bone However, it was also found that severe discontinuities violate the continuum assumption underlying the finite element method and that the solutions were element mesh dependent

We have developed a more physiologically and mechanically consistent simulation model which maintains the self-organizational characteristics but does not produce severe discontinuities This was accomplished by separating the sensor density and range of action from the element mesh

The results clearly show that predicted trabecular morphology, i e sizes and branching of struts, depend on the actual relationship between local load, sensor density and range of influence We believe that the model is suitable to study the relationship between trabecular morphology and load and can also explain transformations of morphology, in the sense of 'Wolff's Law'

INTRODUCTION

It is generally accepted that bone adapts to mechanical loading A decrease in mechanical load causes resorption of bone, whereas an increase leads to bone formation This adaptive process is usually referred to as bone adaptive modeling or remodeling Although this adaptive capacity of bone tissue has been known for more than a century, most of the processes involved in this behavior are still poorly understood Specialized cells, the osteoclasts and osteoblasts respectively, are responsible for resorption and formation, but the mechanisms involved in the regulation of these actor cells are still unclear Some way of measuring mechanical loading in bone must exist, in order for bone to adapt to the mechanical requirements It has been hypothesized that bone contains cells which are sensitive to mechanical signals and which in turn control the actor cells Cowin *et al* (1991) suggested that osteocytes, because of their favorable position and architecture, may function as mechanoreceptors It has indeed been shown that bone cells are sensitive to mechanical stimuli (Rodan *et al*, 1975, Harrell *et al*, 1977, Somjen *et al*, 1980, 1982, Binderman *et al*, 1984) More specifically, El Haj *et al* (1990) showed that mechanical loading affects the cellular metabolism of osteocytes in cancellous bone Recently these studies were reviewed by Burger and Veldhuizen (1993) Osteocytes are regularly distributed throughout the bone, interconnected and connected with bone lining and actor cells at the bone surface (Menton *et al*, 1984) This makes them very
suitable candidates for the role of mechanical sensor The hypothesis that bone contains mechanoreceptors implies that the regulation of bone mass by actor cells is governed locally by sensor cells In other words, it is assumed that bone mass regulation occurs at a local level, which is typical for a self-organizational control process (Yates, 1987) It is noteworthy that as early as 1881 Roux suggested remodeling to be governed by such a 'quantitative self-regulating mechanism' (Roesler, 1987)

Recently, simulation models, using finite element (FE) analysis, were developed in which bone remodeling is mathematically described as such a self organizational biological control process (Huiskes *et al*, 1989, Weinans *et al*, 1989, 1990, 1992, Beaupré *et al*, 1990) The sensor cells are assumed to 'measure' a mechanical signal and stimulate the actor cells (the osteoblasts and osteoclasts) in their vicinity to adapt bone mass accordingly Weinans *et al* (1990, 1992) discovered that when applied to a simple model of local trabecular bone, this process produced a discontinuous patchwork not unlike trabecular bone itself They found that this behavior was caused by a negative feedback loop in the regulation model For a realistic set of parameter values the only stable

solution is one in which elements are either empty or saturated to the maximal permissible density In addition, they found that the process displayed chaotic behavior, due to its dependence on a large set of non linear equations (one per element) Although Weinans *et al* (1992) emphasized the assets of the control scheme as an explanatory model for the emergence of trabecular morphology, its predictive quality was not without problems First of all, the discontinuous element patchwork that emerges violates the continuum assumptions on which the finite element method is based Hence, the stress calculation in the eventual mesh is highly inaccurate Secondly although the trends of the solution remain intact when the element mesh is refined, the solution is in fact mesh dependent

This work generated considerable interest from other groups working in the same area Jacobs and Beaupre (1992) reanalyzed the same local FE model with their bone remodeling simulation procedure (Beaupre *et al*, 1990) and found the same effects Harrigan and Hamilton (1992) developed analytical and numerical formulations of the problem They confirmed the instability of the continuous solution, depending on the values of the parameters in the solution process They stressed that the discontinuous solution, obtained for realistic parameters, was in fact impermissible Jacobs *et al* (1992) suggested to repair the process formulation by using quadratic instead of bilinear elements to attenuate the discontinuous behavior of the finite element model They also suggested to averaging the densities calculated in the nodes over the surrounding elements, whereby the development of discontinuities is prevented

We believe that the only problem of the self-organizational regulation models hitherto presented are the artifacts introduced inadvertently in the finite element formulation In these models, each element is assumed to have one sensor cell which regulates bone mass in that same element Hence, the region of sensor effect is limited by the boundaries of the elements, which are artefacts because they do not represent a physical reality As a result, the solution is influenced by the element mesh Or, in other words, the mesh begs the question

In this paper a more physiologically and mechanic-ally consistent approach to the self-organizational bone regulation process is presented It is hypothesized that osteocytes act as sensors by appraising a mechanical signal (Fig 1) Each sensor then produces a stimulus for bone mass regulation in its environment, the effect of which diminishes exponentially, away from the sensor's location So each actor cell is stimulated by the sensor cells, depending on their remoteness from the sensor location This approach introduces the concept of sensor (osteocyte) density, independent from the FE mesh, and of

sensor influence range (Cowin *et al*, 1991) as a model for the numerous interconnections of osteocytes and actor cells

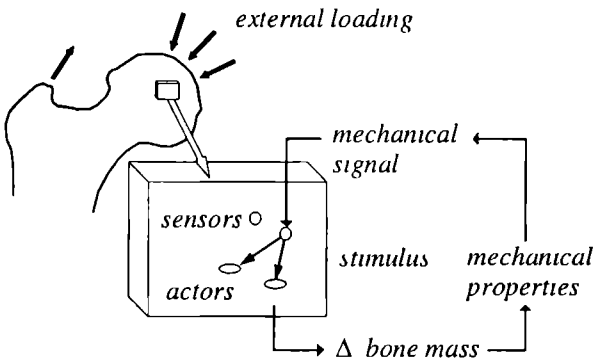

Figure 1
The basic assumptions of the biological control mechanism of adaptive bone remodeling are shown in this scheme a local mechanical signal is appraised by a sensor (osteocyte), which produces a stimulus for bone mass regulation to actors in its environment Changes of bone mass are trans lated in changes of mechanical properties, thus also of mechanical signals

The purpose of this project was to investigate whether this hypothesis produces mechanically permissible solutions consistent with the trabecular morphology of bone. In addition, the effects of the parameter values in the model - sensor influence range, sensor density - the FE mesh and the external load on the predicted morphology were studied

METHODS

The bone considered is assumed to have N sensor cells, arbitrarily distributed over its volume An arbitrary sensor i measures a signal S_i, the strain energy per unit of mass, at its location calculated from (Weinans *et al* 1989, 1992)

$$S_i = \frac{U_i}{\rho_i}, \tag{1}$$

where U_i is the strain-energy-density and ρ_i the density at the location of the sensor The density $\rho(\boldsymbol{x})$ at location $\boldsymbol{x}$ is regulated by the stimulus value $\Phi(\boldsymbol{x})$, to which all sensor cells contribute, relative to their distance from $\boldsymbol{x}$, hence

$$\Phi(\boldsymbol{x}) = \sum_{i=1}^{N} f_i(\boldsymbol{x})(S_i - k), \tag{2}$$

where k is a reference signal and $f_i(x)$ is a spatial influence function Here, the function

$$f_i(x) = e^{-d_i(x)/D} \tag{3}$$

was used, with $d_i(x)$ the distance between sensor i and location x This function is illustrated in Fig 2, the rate of the spatial influence reduction is given by the parameter D D represents the distance from a sensor at which location its effect has reduced to e^{-1}, i e 36 8 percent

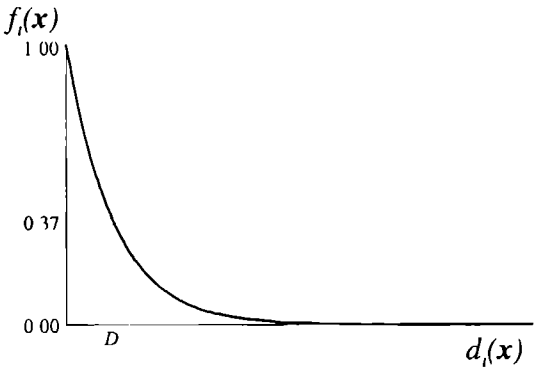

Figure 2
The spatial influence of sensor i, expressed as fi(x) is a function of di(x), the distance between sensor i and location x

The density $\rho(x)$ is now governed by the rate

$$\frac{d\rho(x)}{dt} = \tau\ \Phi(x), \qquad with\ \ 0 < \rho(x) \leq \rho_{cb}, \tag{4}$$

where ρ_{cb} is the (maximal) density of cortical bone, and τ is a time constant regulating the rate of the process

The elastic modulus at location x is calculated from the density according to (Rice *et al* 1988, Currey, 1988)

$$E(x) = C\rho(x)^{\gamma} \tag{5}$$

where C and γ are constants

In the FE model used, the stress components σ and the strain components ε are determined at the integration points of each element, and interpolated per element to give their values in the sensor points (σ_i and ε_i). The strain energy density is calculated from the tensor product $U_i = \frac{1}{2}\, \sigma_i\, \varepsilon_i$, where i refers to the sensor number. Using equation (1) the signal per sensor point S_i is then determined. The stimulus Φ_j is evaluated in the center of each element j, using equation (2), and a new density value ρ_j is calculated in element j, in accordance with equation (4), from

$$\rho_j(t+\Delta t) = \rho_j(t) + \Delta t\, \tau\, \Phi_j(t), \tag{6}$$

where Δt is the time step in the iteration process. The iteration is continued until no more significant changes in the density distribution occur.

This method was tested in a 2-dimensional plate model, as applied earlier by Weinans *et al.* (1992). The plate is loaded by a compressive stress distribution, decreasing linearly over the top edge (Fig. 3). In the calculations, a uniform initial density distribution of $\rho = 0.8$ g/cm^3 was used. The bone tissue is assumed to be isotropic. The reference signal value $k = 0.25$ J/g, the maximal density $\rho_{cb} = 1.74$ g/cm^3, $\tau = 1$ (g/cm^3)^2/(MPa time-unit), $C = 100$ MPa/(g/cm^3)^2 and γ is 2.0, as in the calculations of Weinans *et al.* (1992). The plate was meshed with 40x40 four-node elements. The sensor distribution was uniform and its density was assumed to be 1600/mm^2 (N = 1600), associated with a sensor influence parameter of $D = 0.025$ mm. The behavior of the model was studied for variations of the mesh density, the sensor density (N), the sensor range of influence D and the external load.

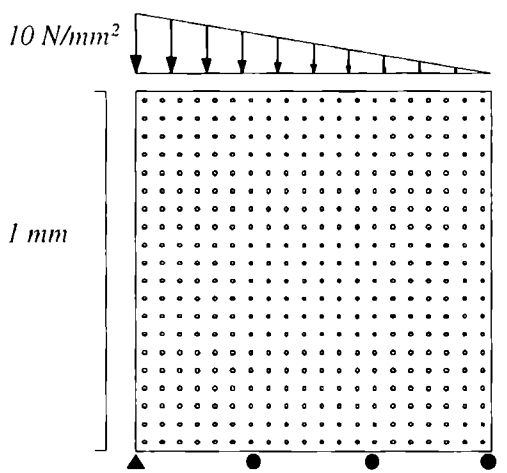

Figure 3
Two dimensional plate model of bone tissue with sensor cells (dots) subjected to a compressive load as indicated.

RESULTS

First the model was tested relative to the earlier one used by Weinans *et al.* (1992), by reproducing its conditions. For that purpose, the distance-influence parameter was diminished to D = 0.001 mm. The results shown in Fig. 4 are very similar for both models, indicating that indeed the conditions of earlier models can be reproduced. For a larger range of influence of the sensors, such that the stimuli generated by the sensors overlap, the model behaved as expected, producing trabecular-like structures without the checker board patterns seen as a result of earlier models (Fig. 5; D = 0.025 mm). The solution has been shown to be independent of mesh refinement, as shown in Figs. 5a and b, where the results for 80x80 and 40x40 meshes are compared. Although the morphology is not the same in both cases due to the differences in the density-pattern representations, the solutions are structurally similar.

As the sensor grid was separated from the FE-mesh, the number of sensors could be varied independently. A saturation effect occurred when the number of sensors was increased: increasing the sensor density beyond a particular value (N about 1000) did not further influence the results. The value of this 'cut-off' density number also depended on the influence parameter D; for a larger value of D, saturation occurred at a lower sensor density.

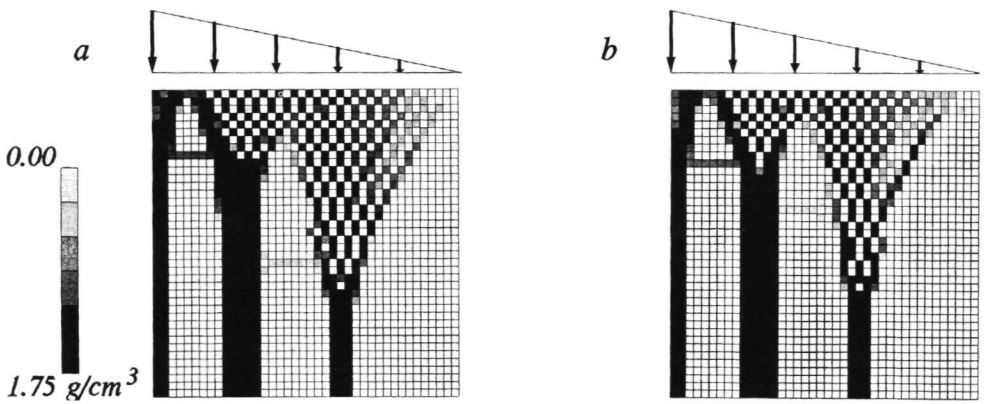

Figure 4
Comparison of the density distributions as predicted by the earlier model (a), used by Weinans et al. (1992), and the model described here, where the distance-influencing parameter is diminished to D = 0.001 mm (b). In both models the number of sensors is equal to the number of elements: N = 1600.

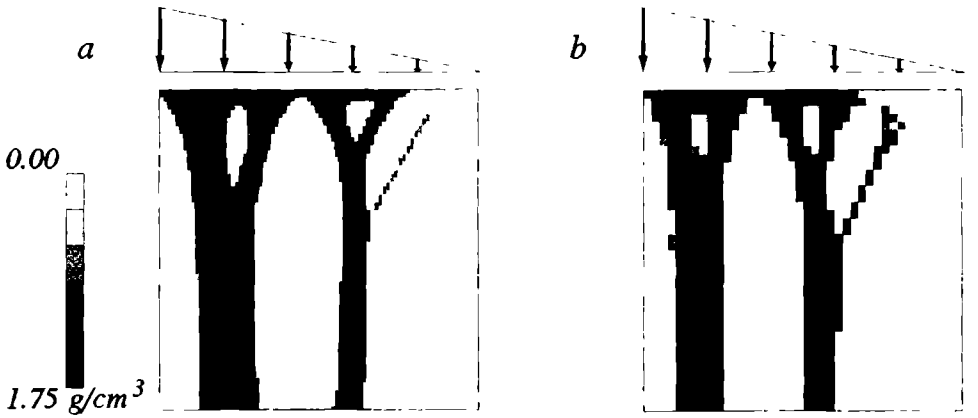

Figure 5
Density distribution resulting from the bone remodeling simulations using a 80 × 80 FE mesh (a) and a 40 × 40 FE mesh (b). The number of sensors N = 1600 and the influencing parameter D = 0.025 mm.

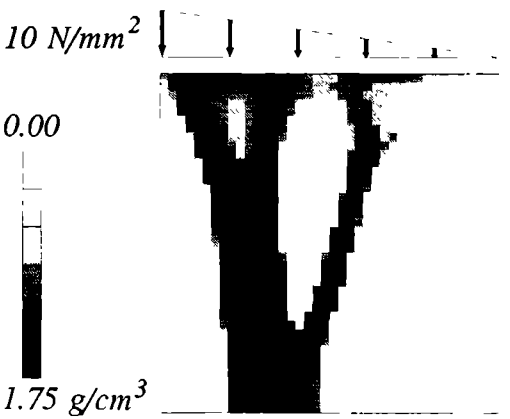

Figure 6
Density distribution as predicted by the bone remodeling simulation, where the influencing parameter D is increased to 0.05 mm (N = 1600).

Changing the range of action of the sensors D, for a constant sensor density, has a distinct influence on the morphology (Fig. 6). If D is small relative to the plate size, finer struts are formed and more branching can be seen than for a larger D. A form of 'self similarity' is evident when comparing Figs. 5 and 6. Similar results were obtained when the 'scale' of the problem was varied, i.e. by enlarging the plate dimensions and range of influence D by the same ratio.

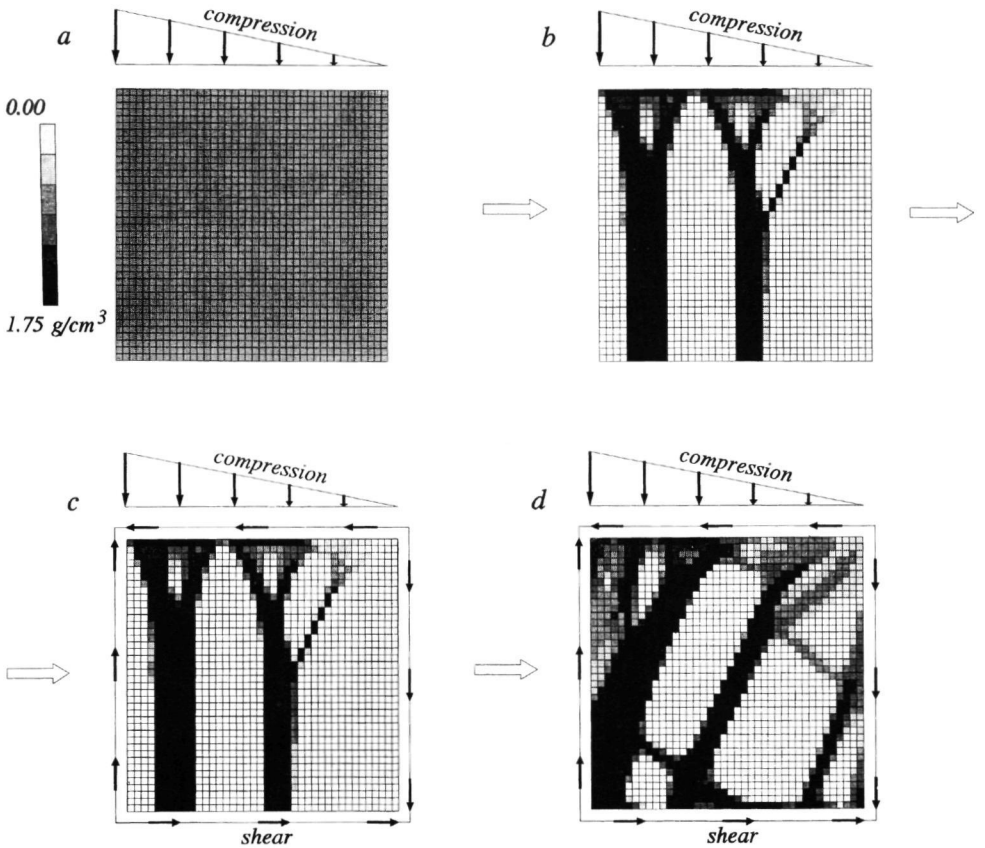

Figure 7
Bone remodeling is simulated starting with a uniform density distribution and using a compressive ramp load (a). After remodeling, a trabecular morphology has emerged (b). When equilibrium is reached an additional shear load (3.5 Nmm^{-2}) is applied (c). The simulation predicts that the morphology adapts to the new loading requirements and a new equilibrium is reached (d).

Solutions hitherto produced were based on a uniform density as the initial condition That the model can also explain transformations of morphology in the sense of Wolff's Law, is demonstrated in Fig 7 The morphology that emerged from the uniform distribution as stimulated by the compressive ramp load (Fig 7b) is provided with additional boundary shear stresses As a result, the morphology transforms to a new equilibrium (Fig 7d)

DISCUSSION

We have developed a simulation model of the self organizational biological control process of bone remodeling, suitable to study the relationship between trabecular morphology and load and the hypotheses comprised in Wolff's Law It should be clear that for the purpose of studying trabecular morphology the element mesh should be fine enough to adequately describe the trabeculae In order to compare the results with the results from previous models some simplifications have been made in this study We used a two-dimensional square plate and a uniform initial density distribution to investigate the model behavior This is not a realistic assumption for bone remodeling, but as shown, the model also performs well from a nonuniform starting configuration Further, a uniform sensor distribution was assumed, but this is no principle limitation of the model

In this model, the sensor points are defined independently of the FE-mesh In other words, the sensor cells are modeled separately from the actors which are represented by the elements in the model, enabling us to vary sensor density and sensor influence The model results reported by Weinans et al (1992) can be reproduced if the influence domain of the sensors is smaller than the element size The difference between the figures shown here and the figures from Weinans et al are due to a difference in the time increments used Weinans et al used larger time steps which resulted in a different remodeling pathway and slightly different results The behavior of the model confirmed our assumption that the checker board type density patterns found in earlier models were caused by a discontinuous stimulus distribution due to the artificial limitation of sensor action to the bounded region of one element

The sensors are considered to control the remodeling process by comparing the mechanical signal with a reference signal Further we presumed that their influence decreases exponentially with increasing distance Obviously, as it is still unknown if the remodeling process is controlled by the sensor cells, as opposed to the actor cells for instance, and how these cells influence actor cells, no evidence exists to support these

assumptions But, if we assume that the osteocytes, located in the bone matrix, are sensitive to mechanical stimuli as was shown by El Haj *et al* (1990) and that they activate the actor cells by using chemical pathways (Binderman *et al* 1984, Rodan, 1975), these propositions are quite realistic The distance over which the sensor cell can influence actor cells is also speculative The existence of a connective network of osteocytes is widely accepted and osteoblasts and bone lining cells are also believed to take part in this network (Menton *et al*, 1984, Cowin *et al*, 1991) Thus, it seems reasonable to assume that the area which is influenced by a sensor is limited, yet, that this area is large enough, such that at each arbitrary point in the bone tissue a significant stimulus can be received We assumed that the influence domain of the osteocytes has a magnitude in the order of the trabecular thickness (about 0 15 mm) Using this more realistic environmental-sensor-influence model, trabecular like structures are formed without the formation of alternating density patterns near the load application surface Hence, the problem of 'checker board' type density patterns can be solved by using a more realistic model without the addition of the averaging procedures proposed by Jacobs *et al* (1992), provided that the element size is small enough in proportion to the sensor influence range (i e the element size should be in the range of the influence parameter D or smaller)

Due to the separation of FE mesh and sensor grid, the FE-mesh can be refined without changing the essential model characteristics The similarity between the solutions for different mesh refinements confirm that the solution is no longer FE-mesh dependent

The model can be used to study the relationship between sensor distribution (e g osteocyte density) and trabecular morphology The results obtained suggest that the effects of sensor cell density are subject to a saturation criterion It appears that the solution is independent of the number of sensors, provided that the distribution of the mechanical signal is 'adequately' measured by the sensors in the structure A reduced number of sensors relative to the 'saturation' amount results in a coarser structure Note that reducing the number of sensors while maintaining a constant range of influence will ultimately lead to a discontinuous stimulus distribution and hence an alternating density pattern Accordingly, the sensor influence parameter D affects the minimum number of sensors necessary to obtain a continuous stimulus distribution For a larger D the critical number of sensors is smaller The critical sensor density was about 1,000/mm^2 where D is 0 025 mm This value lies in the same order of magnitude as the values of osteocyte lacunae numbers found in spongy bone of several different species, which ranged between 1,000/mm^2 and 3,000/mm^2 (Marotti et al , 1990) However, these figures should be

considered carefully Marotti et al only measured the number of osteocyte lacunae per area of bone tissue The number of osteocytes per volume of bone is still unclear In order to make a valid comparison between experimental data and the model results it is necessary to establish the number of osteocytes per volume of bone tissue

It was also shown that D has a distinct effect on the eventual morphology of the structure The results indicate that the formation of trabecular-like structures is characteristic of the model behavior, as has been suggested earlier by Weinans *et al* (1992) and Harrigan and Hamilton (1992), but that the thickness of the struts and the degree of branching is determined by the range of action of the sensors, whereby a smaller range results in finer struts and more branching This effect may be explained by a less homogeneous stimulus distribution, i e larger stimulus gradients, in the case of a smaller range of action of the sensors It was found that the thickness of the trabeculae produced by the model was similar to the magnitude of the sensor influence domain This was in agreement with our assumption stated earlier

The results presented here clearly show that predicted trabecular morphology, i e sizes and branching of struts, is dependent on the actual relationship between local load, sensor density and range of influence Our hypothesis is that differences in trabecular morphology in various species can be explained by variations in these parameters We believe that trabecular morphology can be explained as a result of a load-dependent local self-organizational biological control process We conclude that the method described here is suitable to study the effect of the various parameters presumably controlling this process and may be used to estimate physiological parameters

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3

PROPOSAL FOR THE REGULATORY MECHANISM OF WOLFF'S LAW

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Journal of Orthopaedic Research **13** *503-512, 1995*

ABSTRACT

It is currently believed that the trabecular structure in bone is the result of a dynamic remodeling process controlled by mechanical loads We propose a regulatory mechanism based on the hypothesis that osteocytes located within the bone sense mechanical signals and that these cells mediate osteoclasts and osteoblasts in their vicinity to adapt bone mass A computer-simulation model based on these assumptions was used to investigate if the adaptation of bone in the sense of Wolff's Law and remodeling phenomena as observed in reality, can be explained by such a local control process The model produced structures resembling actual trabecular architectures The architecture transformed after the external loads were changed, aligning the trabeculae with the actual principal stress orientation in accordance with Wolff's trajectorial hypothesis As in reality, the relative apparent density of the structure depended on the magnitude of the applied stresses Osteocyte density influenced the remodeling rate, which is also consistent with experimental findings Furthermore the results indicated that the domain of influence of the osteocytes affects the refinement of the structure as represented by the separation and thicknessof the struts We concluded that the trabecular adaptation to mechanical load as described by Wolff can be explained by a relatively simple regulatory model The model is useful for investigating the effects of physiological parameters on the development maintenance and adaptation of bone

INTRODUCTION

More than a century ago, Wolff (1892) put forward his trajectorial hypothesis, which implied that the internal structure of bone is adapted to mechanical demands, such that the trabecular patterns coincide with stress trajectories Although the hypothesis that the shape and the internal structure of bone adapt to functional or mechanical requirements generally has become known as Wolff's law, the present idea that remodeling of bone is a continuous dynamic control process originated from Roux (1881) He suggested that the adaptive processes in bone are regulated by cells influenced by the local state of stress

Only recently have scientists first begun experimenting with mathematical control models of mechanical bone-mass regulation (Pauwels, 1965, Frost, 1964, Cowin, 1976) The model of Cowin and Hegedus (1976) - in particular, the theory of adaptive elasticity - provided the mathematical background for future developments It assumed a continuous feed-back loop between the maintenance of bone mass and local strain values in the tissues, enabling mathematical predictions of local bone regulation based on external loads Others later proposed similar mathematical remodeling rules, albeit introducing different kinds of mechanical signals to control the feed-back loop to maintenance of mass (Hart et al , 1984, Huiskes et al , 1987, 1989 Carter et al 1989, Beaupre et al , 1990) These authors used finite element methods to link external loads to local mechanical signals, thereby enabling computer simulations of bone-mass regulation in complex geometrical structures, such as whole bones It was shown in validation studies, that these computer simulations could accurately predict long term bone formation and resorption around orthopaedic implants in animals and humans (van Rietbergen et al , 1993, Weinans et al , 1993, Huiskes, 1993) Nevertheless, these are empirical models, not physiological ones They are useful to estimate the gross outcome of a remodeling process, but do not explain anything about the remodeling process itself In addition, these models regulate only bone mass, and ignore the trabecular structure

Weinans et al (1992) found that these kinds of computer models are likely to produce noncontinuous patchworks when used to simulate remodeling of a continuous, uniform material, after application of an external load It was established that this phenomenon was based on instable behavior of the finite element solution procedure in conjunction with a positive feed-back loop Since every element in the model acts as a more or less independent strain sensor and mass regulator, it acts in competition with its neighboring elements Each element tends to fill up to its maximum capacity or,

alternatively, to fade out (Weinans et al , 1992) The results of these analyses were inconsistent with their underlying theory of continuum mechanics and hence impermissible However, they inspired us to re-examine the hypothesis of bone as a self-optimizing structure, as proposed by Roux (1881), which resulted in our proposal for a physiologically-based mathematical control model of local bone-mass regulation

We hypothesize osteocytes as sensors of a mechanical signal or "mechanoreceptors" (Cowin et al , 1991, Marotti et al , 1990, Lanyon, 1993) and regulators of bone mass by mediating the actor cells - the osteoblasts and osteoclasts (Fig 1) The mathematical model proposed to simulate this control process uses the strain energy density as the mechanical signal that the osteocytes appraise (Huiskes et al , 1987) The osteocytes, distributed through the bone in a particular density pattern, emit a stimulus in their environments equivalent to the difference between the local strain energy density and a constant reference value The actor cells regulate bone density in their area between zero and maximal density, dependent on the total stimulus they receive from the osteocytes, whereby the influence of an individual osteocyte stimulus diminishes exponentially according to its distance from the actor cell concerned

It was shown earlier that such a simulation model, when used together with the finite element method, produces trabecular patterns in an initial domain of uniform

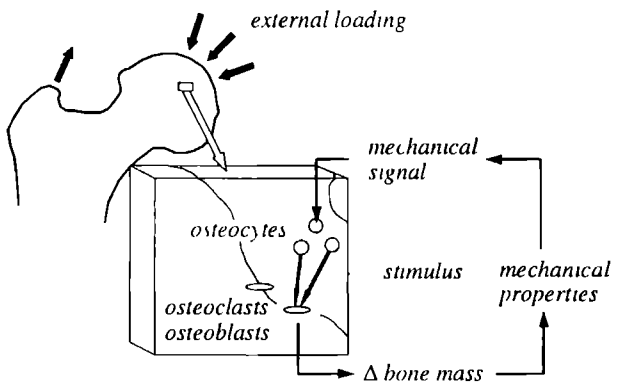

Figure 1

A schematic representation of the hypothetical regulatory mechanism Bone remodeling is assumed to be controled by an adaptive local feed back loop The osteocytes in the bone sense a local mechanical signal and in turn stimulate the actor cells the osteoblasts and osteoclasts - in their vicinity The actor cells adapt the local bone mass in accordance with the magnitude of the received stimulus This results in a change of the local mechanical properties which again affects the local mechanical signal

density, after it is externally loaded (Mullender et al , 1994) Furthermore, in contrast to other models (Weinans et al , 1992, Harrigan and Hamilton, 1992), the solution is spatially stable and mesh- independent, provided that the mesh is adequately refined (Mullender et al , 1994)

The purpose of this study was to investigate whether this proposed control model is indeed a feasible candidate for the cell based bone-mass regulation process suggested by Roux For that purpose, three questions had to be answered First, if the parameters of the model (initial density pattern, external load, reference strain energy density, osteocyte density, and maximal bone elastic modulus) are given realistic values, does the model produce trabecular patterns of realistic morphology? Second, can the model confirm the trajectorial hypothesis of Wolff? And third, can the model reproduce adaptive remodeling phenomena found in reality? These questions are addressed in this paper In addition, the effects of the physiological parameters in the model are investigated

METHODS

The bone tissue is assumed to contain n osteocytes per mm^3 located in the mineralized matrix, with a total of N in the domain considered Each osteocyte i measures a mechanical signal $S_i(t)$ (MPa), the strain energy density in its location In turn, the osteocyte stimulates actor cells (osteoclasts and osteoblasts) to adapt the bone mass depending on the difference between the measured signal ($S_i(t)$) and a reference signal k (Fig 1) The influence of an osteocyte on its environment is assumed to decrease exponentially with increasing distance to the actor cells The influence of osteocyte i on the actor cells at location $\boldsymbol{x}$ is described by the spatial influence function

$$f_i(\boldsymbol{x}) = e^{-d(\boldsymbol{x})/D} \tag{1}$$

where $d_i(\boldsymbol{x})$ (mm) is the distance between osteocyte i and location $\boldsymbol{x}$ The parameter D represents the distance (mm) from an osteocyte at which location its effect has reduced to e^{-1}, i e , 36 8 %

The relative density at location $\boldsymbol{x}$ is regulated by the stimulus value $F(\boldsymbol{x},t)$, to which all osteocytes contribute, relative to their distance from $\boldsymbol{x}$, hence

$$F(\boldsymbol{x},t) = \sum_{i=1}^{N} f_i(\boldsymbol{x})\,(S_i(t) - k) \tag{2}$$

The regulation of the relative density $m(x,t)$ in location x is governed by the rate

$$\frac{dm(x,t)}{dt} = \tau F(x,t) \quad with \ 0 < m(x,t) \leq 1 \tag{3}$$

where τ ($MPa^{-1}\ s^{-1}$) is a constant regulating the rate of the process It is assumed that the osteocytes disappear at locations where the density approaches zero, hence these sensors are disconnected in the model during the process The local elastic properties were calculated from the local relative density with use of a cubic power relationship in accordance with experimental data from Currey (1988) Hence, the elastic modulus at location x is calculated from

$$E(x,t) = Cm(x,t)^{\gamma} \tag{4}$$

where C (MPa) and γ are constants

The model was applied to a square domain of 2x2 mm, with a thickness of 0 02 mm (20 μm) The domain was loaded at each face with uniform tensile or compressive, and shear stress distributions, such that a particular principal stress state was mimicked, albeit with variable orientation relative to the domain Hence, the external load is characterized by principal stress values σ_1 and σ_2, associated with a principal stress orientation φ

Finite element analysis was used to calculate the mechanical variables inside the bone for externally applied loads The FE model was meshed with 80x80 four-node elements Stresses and strains in the locations of the osteocytes were calculated via linear interpolation from the stresses and strains in the nodal points The mechanical signal per osteocyte was then calculated according to

$$S_t = \frac{1}{2}\sigma_t\ \varepsilon_t \tag{5}$$

where σ_t and ε_t are the stress and strain tensors in the location of osteocyte t The local bone density was regulated per element by the total stimulus received from the osteocytes (equation (3))

Several of the model parameters were set from the beginning The maximal elastic modulus of the trabecular tissue was taken as E_{max} = 5,000 MPa (Choi et al , 1990, Rho et al , 1993, van Rietbergen et al , 1995) Using an exponent of γ = 3 (Currey, 1988) in

equation (4), this implies that C = 5,000 MPa. The rate constant was arbitrarily set at τ = 1 MPa^{-1} s^{-1}. This means that the velocity of the simulation process is measured in simulation time, unrelated to real time. The (external) principal stress values were taken as $\sigma_1 = \sigma$ and $\sigma_2 = -\sigma$, with σ = 4 MPa, which is considered to be a value in a realistic range for human trabecular bone in the proximal femur (Brown and DiGioia, 1984). However, σ was also varied to study its effects.

The osteocyte density was taken as n = 1,600/mm^2, uniformly distributed over the domain. This number was based on reports by Marotti et al. (1990), who found 500-3,000 lacunae per square milimeter in 20-30 μm histological slices from several species. However, to study its effect, osteocyte density was also varied in the analyses.

The reference strain energy density is not known, but estimates can be derived from investigations of Rubin (1984) and Rubin and Lanyon (1987). They found in several species that peak values of strains in bone during normal, "physiological" activities vary between 2,000 and 3,000 μstrain. With a tissue modulus of 5,000 MPa, and if uniaxial strain state is assumed, this implies normal peak strain energy density values between 0.01 and 0.0225 MPa, which was used as the range of variation of k in the analyses.

No information is available on the regional osteocyte influencing function represented by the influence parameter D (equation (1)). Its effect on the model behavior was studied by varying this parameter. It was shown earlier that the size of the element should not be smaller than the range of influence of the sensors (Mullender et al., 1994). Hence, a minimum value of D = 0.025 mm was assumed, as limited by the size of the elements.

Two kinds of initial conditions were considered. First a uniform relative density distribution of m = 0.8, and second, an arbitrary initial pattern of a regular lattice. This second pattern was based on the fact that in the process of bone morphogenesis, the initial configuration is always trabecular, due to the patterns of perichondral mineralization inherent to endochondral ossification (Aray, 1965; Schaffler et al., 1993).

RESULTS

When the reference parameter values that have been described were used, the density distributions converged to trabecular patterns of struts of maximal density surrounding empty pores after application of load. This occurred regardless of the initial distribution of density or the principal orientation of stress (Fig. 2). The morphological qualities of the resulting architectures can be characterized by three independent global

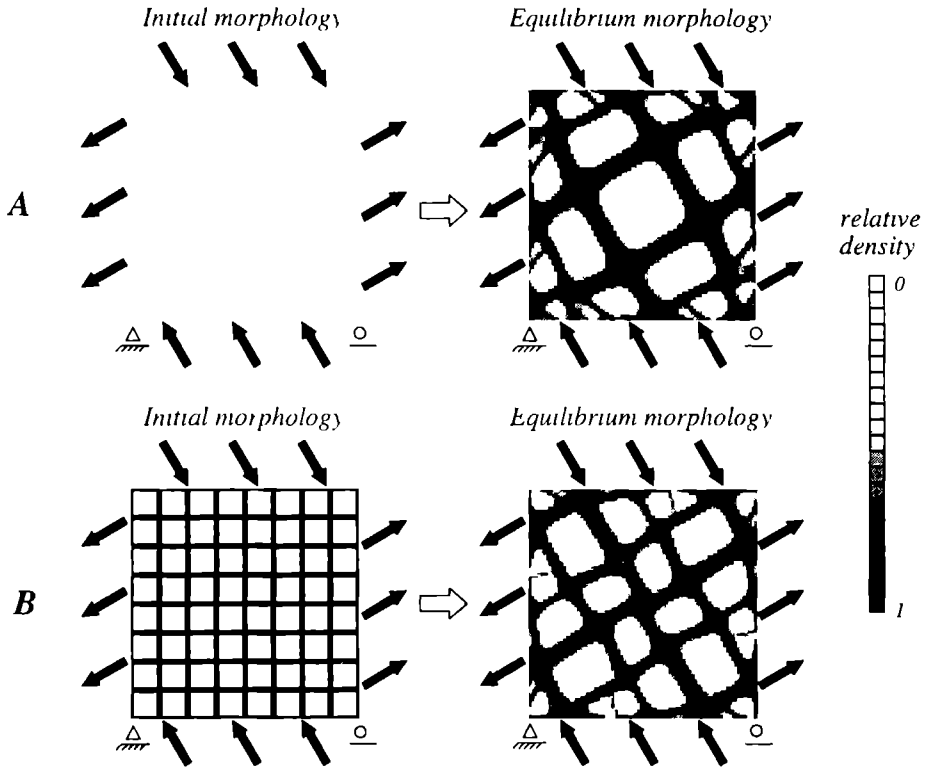

Figure 2

Trabecular-like patterns are formed by the simulated remodeling process starting from a uniform mineral distribution (A) or a lattice structure (B) The eventual directions of the trabeculae match the external principal stress orientations The osteocyte influence parameter D = 0 05 mm, the reference strain energy density k = 0 02 MPa The orientation of the principal stress φ = 30°

parameters the relative apparent density (or analogously, the bone area fraction), the average trabecular orientation, and the perimeter/area ratio (corresponding to the three-dimensional surface/volume ratio (Parfitt et al , 1987)).

The relative apparent density in the equilibrium morphology depends predominantly on the magnitude of the external load in relation to the value of the reference strain energy density k For k = 0 02 MPa and the chosen principal stress magnitude of 4 MPa, the resulting relative apparent density was about 0 5, associated with a bone area fraction also of 0 5 The osteocyte influence parameter D had an effect as well, however, twice the magnitude of D - from 0 05 to 0 10 mm - resulted in only a 10% increase in the relative apparent density The effect of the initial morphology on the relative apparent density was marginal. This is remarkable, because its effect on the final architecture was considerable (Fig 2).

Trabecular orientation was always directly related to the external principal stress orientation This implies that when, for a particular equilibrium morphology (e g , Fig 2b), the principal stress orientation is rotated, the architecture "transforms" and the trabeculae are realigned to the principal stress orientation (Figs 3 and 4) During this process, bone formation occurs at the surfaces of the trabeculae Evidently, although the overall equilibrium orientation of the trabeculae is in accordance with the principal stress orientation, not every single trabecula is aligned similarly (Figs 3 and 4)

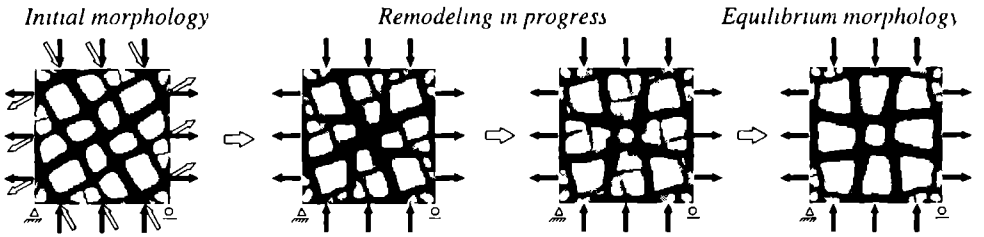

Figure 3
One particular equilibrium configuration (Fig 2B) was used as the initial morphology After the orientation of the applied stresses was changed from φ = 30° to φ = 0°, the architecture adapted to align with the new stress orientation The trabeculae that were unloaded gradually disappeared while other overloaded trabeculae adapted by realigning and thickening The parameter values D = 0 050 mm and k = 0 02 MPa

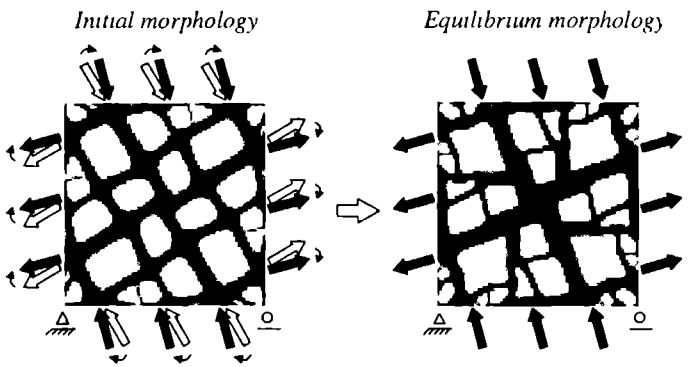

Figure 4
With use of the same initial morphology as in Fig 3 the principal stresses were rotated by 15° from φ = 30° to φ = 15° In this case, all existing trabeculae remained but they adapted to the new loading situation All trabeculae realigned some trabeculae thickened and others became thinner The parameter values D = 0 05 mm and k = 0 02 MPa

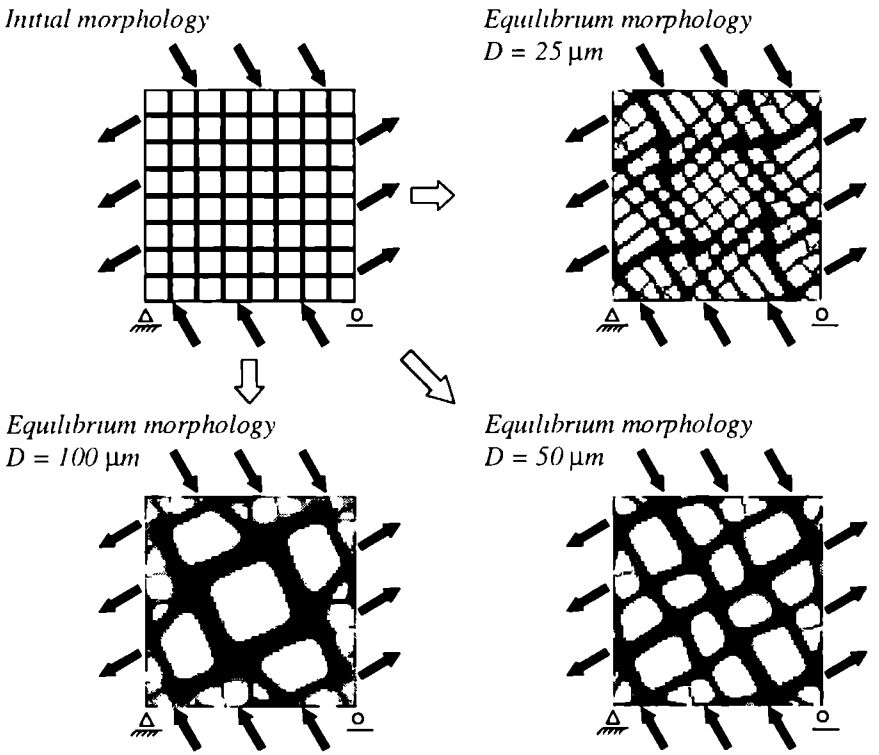

Figure 5

The effect of the influence parameter D on the end-configuration is shown With use of the lattice structure as the starting configuration, the osteocyte influence parameter D was varied D affects the dimensions of the formed structure Increasing values of D result in a lower pore density and less and thicker struts

The perimeter/area ratio depended mostly on the osteocyte regional influence range, as represented by the influence parameter D (Fig 5) For a smaller D, the architecture became more refined, with more, smaller pores and thinner trabeculae For given values of the external stress and reference strain energy density k, trabecular thickness seems to be directly related to the value of D However, the perimeter/area ratio also depended on the initial morphology, as illustrated in Fig 3, where the bone perimeter decreases after rotation of the principal stress orientation

The osteocyte density, for osteocyte densities higher than a certain threshold value, had little effect on the quality of the solution The threshold osteocyte density is the density where the distance between osteocytes is about equal to the influence parameter D of the osteocytes. Lower osteocyte densities resulted in large gradients in the stimulus, which

caused spatial differences in the remodeling rate When bone was resorbed, this occurred most rapidly in the location of the osteocyte itself, such that the osteocyte disappeared while the surrounding bone remained.

The remodeling process can be characterized by changes in the mineral distribution and changes of the relative apparent density in time, as represented by the rate of remodeling A general pattern could be observed in the change of mineral distribution. Bone formation always occurred at the surfaces of overloaded trabeculae while underloaded trabeculae gradually disappeared, thinned or became porous. This implies that the newly formed architecture is based on the initial configuration, such that trabeculae can only form at locations where bone already exists. The process also depended on the external load in relation to the initial configuration. When the principal stress orientation was rotated slightly relative to the directions of the trabeculae, the trabeculae realigned (Fig. 4) However, when the principal stress orientation was rotated considerably relative to the directions of the trabeculae, some trabeculae became totally unloaded and disappeared, while other trabeculae, which were overloaded, realigned and thickened at the same time (Fig 3). The effect of overloading and unloading on trabecular adaptation is also demonstrated in Fig 6, where a trabecula was artificially disconnected while the same externally applied load was maintained. The disconnected and therefore unloaded trabecula disappeared, while the neighboring, overloaded trabeculae thickened.

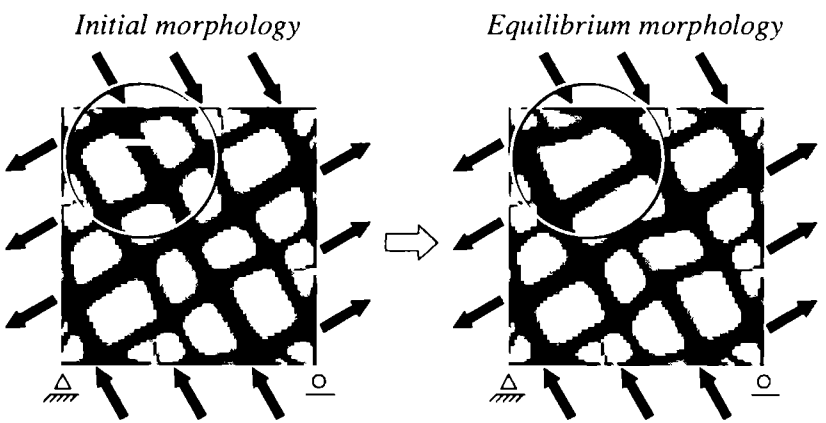

Figure 6

One strut in the equilibrium architecture is artificially discon-nected, while the external stress is maintained After remodelling the existing architecture is again adapted to the applied stresses by removal of the unloaded trabecula and thickening of the overloaded trabeculae. Note that the trabecula under the sectioned one, aligned with it, also disappears

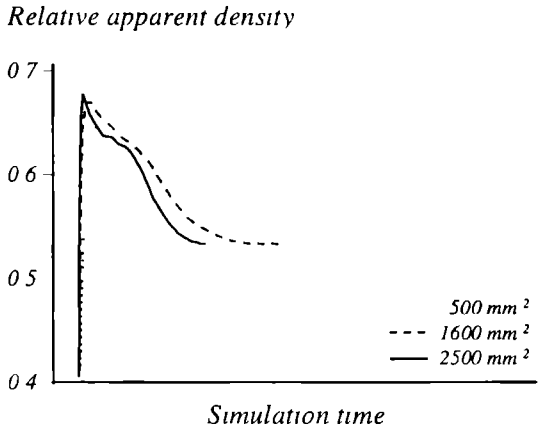

Figure 7
The relative apparent density of the structure as a function of remodeling time for three different osteocyte densities (n = 500 mm² n = 1600 mm² and n = 2500 mm²) Osteocyte density clearly influences the remodeling rate For higher osteocyte densities the remodeling rate increases The relative apparent density in the end-configuration is independent of osteocyte density

The rate of remodeling was affected by both the parameter *D* and the osteocyte density as both parameters influence the magnitude of the stimulus received in the bone tissue The remodeling rate increased for larger values of *D* and higher osteocyte densities (Fig 7)

The mechanical quality of the resulting architectures can be characterized by its principal strain, principal stress and strain energy-density distributions These distributions were determined primarily by the value of the reference signal k, but the influence parameter *D* also had a small effect When *D* = 0 050 mm, the principal strain in the structure averaged 4,200 mstrain, with principal strain values of 1,000 to 7,000 mstrain in more than 90% of the bone tissue The principal stress averaged 7 7 MPa, with a variation of 0-20 MPa in more than 90% of the area of bone The strain energy density, measured in the locations of the osteocytes, averaged 0 02 MPa, which equals the reference value k and varied between 0 and 0 045 MPa High stress or strain values were found at the boundary of the plate and can be considered as boundary artefacts

DISCUSSION

Presently, no consensus exists about the mechanisms controlling functional adaptation of bone It was suggested that osteocytes are primary candidates for the role of mechanical sensors because of their favorable architecture and distribution (Cowin et al ,

1991, Lanyon, 1993) It has frequently been demonstrated that the precursors of the osteocytes, the osteoblasts, are sensitive to mechanical stimuli (see Burger and Veldhuijzen (1993) for a recent review) but knowledge about the response of osteocytes to mechanical stimuli is scarce Skerry et al (1989) and Dodds et al (1993) found that osteocytes responded rapidly to dynamical loading of bone Recently, Klein-Nulend et al (1995) found that isolated osteocytes were more sensitive to fluid shear stress than osteoblasts Marotti et al (1990) suggested that osteocyte death precludes bone turnover These findings support the assumption that osteocytes are sensors of the local mechanical load The results of Dunstan et al (1990), who found that patients with hip fractures who had extensive osteocyte death in the femoral head had little microfracture callus compared to osteoporotic patients who had prominently viable bone, also indicate that bone remodeling and microfracture repair are related to the presence of sufficient viable osteocytes in bone

The mechanism by which the osteocyte within the bone may sense a mechanical signal still is subject to speculation It is believed that osteocytes are stimulated by the interstitial fluid flow caused by mechanical loading, either indirectly by the detection of streaming potentials (Harrigan and Hamilton, 1993) or directly by detection of shear stresses at the cell surface (Weinbaum et al , 1994) In addition, very little is known about the pathway by which the local mechanical stimuli are transduced into the activation of osteoblasts and osteoclasts A coupling between the activity of these cell types has been established (Parfitt, 1982) and units of combined resorptive and formative cell populations are referred to as basic multicellular units (Frost, 1964) Nevertheless, the regulation of these units still is obscure It has been hypothesized that the osteocytes communicate directly with adjacent cells through the osteocytic processes and that a signal propagates by way of the osteocytic network towards the osteoblasts and bone lining cells at the bone surface (Weinbaum et al , 1994, Harrigan and Hamilton, 1993, Marotti et al , 1990) Support for this assumption has been supplied by Jeansonne et al (1979), who demonstrated electrical coupling and molecular transport between osteoblasts, and Doty (1981) and Palumbo et al (1990) who showed that gap junctions between osteocytes and osteoblasts exist The lining cells and osteoblasts, in turn, are thought to influence the proliferation and activity of osteoclasts (Martin, 1983, Eriksen and Kassem, 1992) Furthermore, bone cells are involved in paracrine and possibly autocrine effects (Rodan 1993) It has been shown that osteoblastic cells do produce local factors and the sensitivity

of osteoclasts and osteoblasts to several mediators has also been established (Burger and Nijweide, 1991, Eriksen and Kassem, 1992)

In this study, we used a mathematical model to investigate if a local control mechanism, based on the hypothesis that osteocytes are mechanoreceptors and regulators of bone mass, actually can predict remodeling of trabecular bone as we would expect according to Wolff's law In the model we used quite simple relationships for (a) the signal sensing function of the osteocytes, (b) the influence of the osteocytes on the actor cells, and (c) the relationship between the stimulus received and the change in local bone density We assumed that the actual signal measured by the osteocytes is related to stress and strain at its location The strain energy density was used as the mechanical signal, and only the amplitudes of the strain energy density were taken into account, hence, the influence of strain rate was neglected Only net changes in bone mass were modeled, and only the net effects of the basic cellular units were considered Thus, the model cannot be used to investigate changes in osteoclast or osteoblast activity Implicitly, the material of a trabecula is modeled as being homogeneous and isotropic This is also a simplification of reality Finite element analysis was used to calculate the mechanical variables inside the bone specimen The solution process was introduced earlier by Mullender et al (1994), who showed that the results were independent of the finite element mesh, as long as the elements were smaller than the influencing parameter D and small enough to adequately describe the resulting trabecular structure from a continuum mechanics point of view

The location of the sensors within the mineralized matrix has consequences for the remodeling behavior The stimulus for remodeling always originates from within the mineralized matrix Due to the decay of the stimulus with increasing distance, the model predicts that new bone is formed at the boundaries of existing trabeculae, as it is in reality However, resorption of bone is not restricted to the boundaries of trabeculae In the model, in contrast to reality, it can take place at locations inside the bone matrix as well Although this happened only if the loading configuration was changed drastically, this behavior is not physiological

The most striking behavior of the proposed control mechanism is the formation of trabecular like patterns Weinans et al (1992) showed that positive feed-back loops in the regulating process cause spatial discontinuity It is interesting to note that, already in 1881, Roux described the regulation of bone remodeling as a positive feed-back loop, as he stated that parts of the bone that are stressed more than other parts will increase their strength, thereby unloading the other parts which then will eventually disappear, until a

structure has developed where bone is only present at the locations where the highest stresses occur (Roux, 1881) This phenomenon can also be observed in the model and results in the formation of a trabecular structure whereby the regional influence of the osteocytes prevents spatial discontinuity The development of the structure is such that the load is resisted by as few struts as possible but, the number and thickness of the struts are controlled by the parameter values in the model, particularly region of influence of the osteocyte, the reference strain energy, and the magnitude of the applied external load The outcome of the regulatory process depends principally on the applied loads It is noteworthy that the signal controlling the process - the strain energy density - is a scalar and independent of stress orientation Still, the results showed that the trabecular architecture is formed in accordance with the magnitude and the directions of the external principal stresses Hence, the osteocytes would not need information about the local strain orientation in order to form, in concert, an anisotropic structure

The adaptive behavior of the model was investigated by change of the orientation or the magnitude of the principal stresses and by artificial disconnection of one strut in an equilibrium architecture In all three cases, the behavior of the model showed similarities with actual remodeling behavior observed in cancellous bone After the orientation of the principal stress was changed, the architecture transformed to resist the new pattern of stress In the newly formed structure, the orientations of the trabeculae approximate the new principal stress orientation These predictions are consistent with Wolff's trajectorial hypothesis When the level of load is changed, the model predicted that the architecture adapts by changing the thickness of the struts while maintaining the same number of struts This is consistent with the results from Jee and Li (1990), who found that, in the overloaded limb of a rat, the trabecular number and separation remained unchanged while the trabecular thickness increased significantly Mosekilde (1990) showed that, once disconnected, trabeculae are removed by resorption and suggested this was due to mechanical adaptation This behavior also was reproduced by the model An important observation is that, again, the regional influence of the osteocyte is essential to the remodeling behavior of the model In order to form new bone the osteocytes' stimulus must reach outside the area of mineralized bone

The model is particularly suited for investigation of the dependence of the remodeling behavior on the physiological parameters in the remodeling process Variation of the osteocyte density within a certain range influenced only the remodeling rate in the model This result is in agreement with the finding that osteocyte lacunae are

larger and more numerous in bone regions with a higher bone turnover than in regions whith a lower turnover (Cané et al , 1982) However, lack of experimental data prevents further verification of this finding For extremely low osteocyte densities, remodeling rates depend predominantly on the distance from the osteocytes whereby resorption especially occurs most rapidly at the location of the osteocyte itself The result is that in some areas the osteocytes disappear, while the surrounding bone remains, in the end leaving very few osteocytes Although this model behavior is not compatible with reality, it implies that a certain minimal number of osteocytes is necessary for adequate functional adaptation

The function $f_i(x)$ with the influencing parameter D represents the relationship between distance and the osteocyte's influence on its environment, where an increase of D results in a larger influencing domain of the osteocyte If it is assumed that osteocytes communicate through the osteocyte network and by way of the release of local mediators, the relationship depends on the extent of the osteocytic network, its connectivity and the diffusion rate of the local mediators Actual information about these factors is far from complete It was shown here that the effect of distance not only is essential to the formation of trabecular patterns and to the adaptive capacity of the model, but that it also has important effects on the structure formed The parameter D affects the refinement of the architecture as represented by the perimeter to area ratio, dependent on trabecular separation and thickness Trabecular thickness is about twice the magnitude of the parameter D This indicates that the domain of influence of an osteocyte has indeed the same range as the extent of the connected osteocytic network Smaller values of D also resulted in a slightly lower total mass, which caused higher resultant strains and stresses in the trabeculae This implies that the existence of a network in the bone, by which a local mechanical stimulus can affect the local area within a certain distance, is useful for the regulation of the maximal local load

If, for instance, we compare the results from the model with the experimental finding that the trabecular thickness of the iliac cancellous bone in normal humans is 100-200 µm and the trabecular plate separation, 400-600 µm (Parfitt et al , 1983), we can estimate that the influence parameter D should be 50-100 µm Nevertheless, although the predicted morphologies show a general resemblance with actual trabecular morphologies, the trabecular structure is essentially a three-dimensional structure and a three-dimensional model is needed in order to compare the predicted morphology with actual trabecular bone

The distribution of mechanical variables, principal stress, principal strain and strain energy density, was non-uniform Fyhrie et al (1992, 1993) and van Rietbergen et al (1995) showed that values for stress, strain and strain energy density varied widely in a piece of trabecular bone loaded by uniaxial displacement Although these authors did not use physiological loads, it seems likely that there are always parts in the bone that are stressed more than other parts This assumption is consistent with our results

In conclusion, it was demonstrated that the genesis of trabecular morphology, its transformation induced by changes in the loading pattern and the alignment of trabeculae with the principal orientations of the stress patterns - in accordance with Wolff's hypothesis - can be explained as the result of a local biological control process It was shown that many features of bone remodeling can be explained by assuming a relatively simple mechanical regulatory process The behavior of the model corresponds very well with actual remodeling behavior observed in trabecular bone This mathematical model can be useful for the investigation of the effects of physiological parameters, such as density of osteocytes, domain of their influence, degree of mineralization, and distribution of stress Further validation of the hypothetical regulatory mechanism is currently ongoing

Our results do not prove that the regulation model proposed is correct They prove, however, that Roux's hypothesis was realistic bone morphogenesis, maintenance, and adaptation can be explained by a (surprisingly simple) local, cell-based control process

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4

OSTEOCYTE DENSITY AND HISTOMORPHOMETRIC PARAMETERS IN CANCELLOUS BONE OF THE PROXIMAL FEMUR IN FIVE MAMMALIAN SPECIES

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Journal of Orthopaedic Research **14** 972-979, 1996

ABSTRACT

The species-specific relationships between trabecular morphology and osteocyte density were investigated in the femoral heads of thirty adult animals of five mammalian species (rat rabbit, Rhesus monkey, pig and cow) Our hypothesis is that osteocytes are mechanosensory cells and are involved in the regulation of bone remodeling According to the predictions from a simulation model, this hypothesis implies that the influencing distance of osteocytes, together with the magnitude of the mechanical loads, determines the thickness of trabeculae and that the number of osteocytes primarily affects the rate of bone remodeling The number of osteocytes per bone volume ranged from 93,200 mm ^{3} in rat to 31,900 mm ^{3} in bovine cancellous bone Osteocyte density was inversely related to the size of the species Since basal metabolic output is related to body mass, we speculate that osteocyte density may be related to metabolic rates Trabecular thickness was larger in the cow than in the other species, but the range of variation between species was relatively small This agrees with the hypothesis that trabecular thickness is limited by the domain that can be regulated by an osteocyte and that this domain is of similar size regardless of the species Only in the rat was trabecular thickness considerably smaller than in the other species This is probably due to the presence of the cartilaginous growth plate in the femoral head of the rat The relationships with species are different for osteocyte density than for morphometric parameters Hence, our data support our hypothesis that osteocyte density is not directly associated with the macroscopic trabecular architecture

INTRODUCTION

Cancellous bone is thought to be designed to optimally fulfill its mechanical functions Wolff (1892) proposed that the orientations of the trabeculae are adapted to directions of the principal stresses acting on the bony structure As early as 1881, Roux suggested that the capacity of bone to adapt its architecture to mechanical loading is the result of local regulation by cells that are influenced by mechanical stimuli More than a century has passed, but the mechanisms by which the adaptive behavior of bone is regulated are still not understood Recently, in an attempt to clarify these relationships between adaptation of trabecular bone and mechanical load, a regulatory mechanism was proposed (Mullender et al , 1994, Mullender and Huiskes, 1995, van Rietbergen et al , 1995) It was based on the hypothesis that osteocytes regulate the local adaptation of bone mass, driven by mechanical stimuli Although, in fact, it is not certain what mechanism is responsible for sensing the mechanical loading in bone, the involvement of osteocytes has been suggested by several authors (Marotti et al , 1990, Cowin et al , 1991, Lanyon, 1993, Aarden et al, 1994) The simulation model could explain the morphogenesis of typical trabecular morphologies with plates or rods, depending on the external loading pattern (van Rietbergen et al , 1995), and could also explain the adaptation of the trabecular pattern to the directions of the applied loads (Mullender and Huiskes, 1995)

In addition, it has been shown that particular parameters in the regulatory process had distinct effects on the predictions of the model In the model, it was assumed that the amplitude of the stimulus produced by each individual osteocyte decreases exponentially with increasing distance from the osteocyte The decay of the stimulus with increasing distance is characterized by one parameter called the osteocyte-influencing distance, i e , the distance over which the osteocytes can effectively influence the activity of actor cells The model predicted that osteocyte-influencing distance typically affected the dimensions of the trabecular morphology an increase of the osteocyte-influencing distance caused a decrease of the number of trabeculae, while their thickness and separation increased (Mullender and Huiskes, 1995) In other words, the influencing distance of the osteocytes determined the scale of the predicted trabecular patterns The magnitude of the applied loads affected the relative volume of the bone by modulating trabecular thickness In contrast, the number of osteocytes per bone volume had no effect on the morphometric parameters of the predicted architecture but affected only the rate of the remodeling process

The actual values of osteocyte density and the domain of influence of osteocytes are unknown It is still unclear how osteocytes signal other cells If they produce chemical messengers (Klein-Nulend et al , 1995), their effect will depend on diffusion Diffusion of signaling molecules is determined by the same physical laws in all animals and is likely to be independent of cell packing On the other hand, osteocytes may signal other cells directly by means of gap junctions (Doty, 1981, Palumbo, 1990) and electrical coupling (Jeansonne et al , 1979) In that case, their range of influence may depend on cell density but may also depend on the density of their processes and other parameters Hence, the relationships between species, osteocyte density and the influencing distance of individual cells are not entirely clear yet

The predictions of the model have led us to hypothesize that trabecular thickness is determined primarily by the influencing distance of the regulating cells, which we assume to be the osteocytes, and that trabecular thickness is not directly related to osteocyte density If this is true, trabecular thickness can be used as an indicator of the range of influence of the osteocyte In addition, we hypothesize that the rate of bone modeling and remodeling is closely related to the number of osteocytes Since it is known that basal metabolic rate is inversely related to body size (Schmidt-Nielsen, 1984, Spaargaren, 1994, Couture and Hulbert, 1995), we speculate that turnover rates may also vary between mammals of divergent sizes To test these hypotheses, we have investigated wether histomorphometric parameters and osteocyte density depend on species size in trabecular bone of five different mammals of various sizes

METHODS

ANIMALS

Femoral heads were obtained from 30 healthy full grown mammals of five different species six Wistar rats (7 ± 0 months old (±SD)), six New Zealand White rabbits (18 ± 0 months old), seven Rhesus monkeys (5 6 ± 1 1 years old), six domestic pigs (3 0 ± 0 4 years old), and five domestic cows (6 4 ± 3 2 years old) The diameters of the femoral heads were measured The femoral heads were fixed with a 4% phosphate buffered (0 1 *M*) formalin solution, pH 7 2 Right femoral heads were used for morphometric measurements, and left femoral heads were used for measurements of osteocyte density

HISTOLOGY

The undecalcified right femoral heads were embedded in polymethylmethacrylate Sections of 20-40 μm thickness were made with use of a Leitz sawing microtome (Leitz, Rockleigh, NJ, U S A) fitted with a diamond sintered blade The surfaces of the sections were stained using a modified Von Kossa method, which stained the bone surface black The left femoral heads were decalcified with 20% EDTA and embedded in polymethylmethacrylate Before embedding, the femoral heads of pigs and cows were divided into eight blocks Serial sections (7 μm) were made using a Leitz microtome and stained with hematoxylin and eosin Both the left and right femoral heads were sectioned in the frontal plane

HISTOMORPHOMETRY

Morphometric parameters were measured using a microscope in conjunction with a digital camera (Videk Megaplus Camera, Kodak, NY, USA) Digital analysis soft-ware (TIM, Difa Measuring Systems B V and TEA, The Netherlands) was used to analyze the images The digital images were thresholded and manually corrected for artifacts The number of bone pixels divided by the total number of pixels (P_P) in the area of analysis and the number of intersections between bone and none-bone pixels per unit test line length (P_L) were determined P_L was taken as the average value measured in two perpendicular directions From these measure-ments a number of morphometric parameters were calculated according to the parallel plate model (Parfitt et al , 1987) bone volume fraction (BV/TV) = P_P , bone surface per bone volume (BS/BV) = $2P_L / P_P$ (mm^{-1}), mean trabecular plate thickness (Tb Th) = 2 / (BS/BV) = P_P / P_L (mm), mean trabecular plate separation (Tb Sp) = $(1 - P_P) / P_L$ (mm), trabecular number (Tb N) = P_L (mm^{-1})

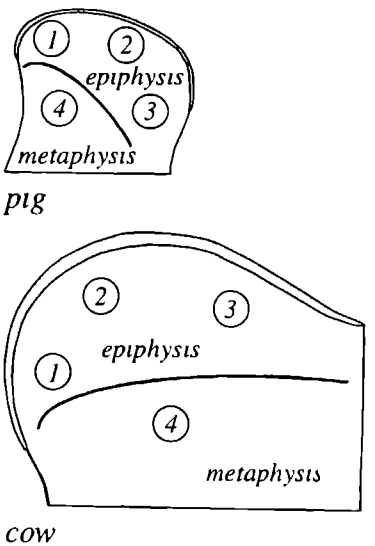

Figure 1
A schematic representation of the sites of measurement in the femoral heads of pigs and cows

Measurements were taken from eight sections from the midfrontal plane in each animal In the rat, rabbit, and monkey, the total trabecular area in each section was analyzed Due to the large size of the femoral heads of pigs and cows, only a part of the total trabecular area was analyzed per section, i e four fields of 16 mm^2 in four specific sites (Fig 1)

Osteocyte density was measured using a microscope (Leitz) in conjunction with a digitizing system (Videoplan, Kontron Bildanalyse, Eching-Munchen, Germany) The number of osteocyte nuclei per bone area (N Ot Nc/B Ar) was measured in a total of 32 randomly selected fields (0 16 mm^2) in 16 nonconsecutive sections per animal In total, a bone area of 1 5-3 5 mm^2 was analyzed per animal The method described by Gundersen (1986), by which osteocytes are identified in two successive sections, was used to measure a percentage of double counts, defined as

$$\%\ double\ counts = \frac{the\ number\ of\ osteocyte\ nuclei\ identified\ in\ both\ sections}{the\ total\ number\ of\ osteocyte\ nuclei\ in\ the\ first\ section} \times 100\% \quad (1)$$

The percentage of double counts was measured in two animals per species in two randomly selected fields of eight section pairs The mean value per species was then used to estimate the number of osteocyte nuclei per bone volume according to

$$N\ Ot\ Nc / BV = \frac{(100\% - \%\ double\ counts)}{100\%} \times \frac{N\ Ot\ Nc / B\ Ar}{t} \quad (2)$$

with t (mm) representing the section thickness

Averages and standard deviations of all measured parameters were determined per group Differences between groups were tested using analysis of variance

RESULTS

The trabecular morphologies of animals of the same species had very similar appearances This is also evident from the small variation among morphometric data of animals of the same species (Fig 2) In one rabbit trabecular only were thickness and separation distinctly larger than in the other rabbits (Fig 2) From a qualitative examination of the trabecular morphologies of the different species, it appeared that the dimensions of the trabecular structures are quite similar in all species, except for the rat (Fig 3) In the rat, trabecular thickness and trabecular separation were significantly smaller and trabecular number and bone surface per bone volume were significantly larger

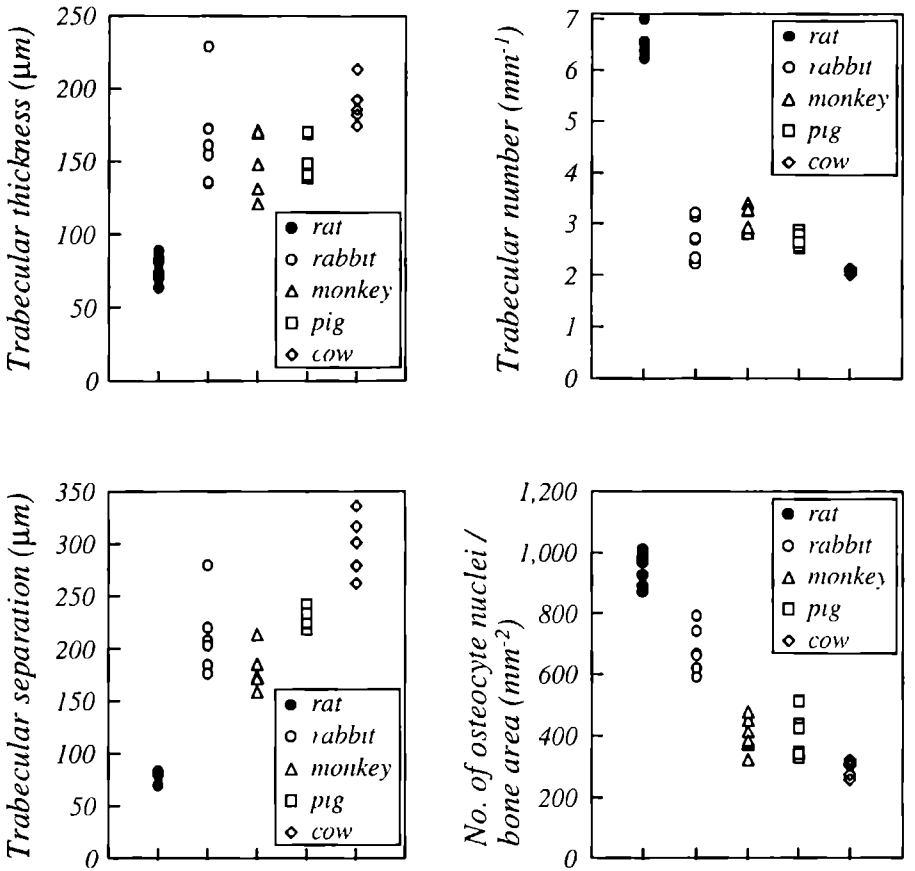

Figure 2

The individual data points of three structural parameters - trabecular thickness, trabecular separation, and trabecular number - are given for each animal In addition, the individual data points of the number of osteocyte nuclei per bone area are presented

than in the other species The measurements indicated, however, that several morphometric parameters in the cow also differ from those in the other species (Table 1). In the cow, trabecular number was significantly lower and trabecular thickness and separation were higher than in the other species, although the difference between the trabecular thickness in the cow and that in the rabbit was not significant The morphometric parameters in the rabbit, monkey and pig were not significantly different, with the exception of trabecular separation, which was significantly larger in the pig than in the monkey (Table 1).

Table 1 *Histomorphometric parameters (average ± SD) of trabecular bone in the femoral heads of five mammalian species.*

	Rat (n = 6)	Rabbit (n = 6)	Monkey (n = 7)	Pig (n = 6)	Cow (n = 5)
Femoral head (cm)	0 3 ± 0 0^{a}	1 0 ± 0 0^{b}	1 4 ± 0 1^{c}	3 6 ± 0 2^{d}	5 5 ± 0 4^{e}
Relative bone volume	0 50 ± 0 04^{a}	0 44 ± 0 05^{a,b}	0.45 ± 0 05^{a,b}	0 40 ± 0 02^{b}	0 40 ± 0 04^{b}
Trabecular number (mm^{-1})	6 54 ± 0 24^{a}	2 72 ± 0 37^{b}	3 08 ± 0 21^{b}	2 70 ± 0 12^{b}	2 07 ± 0 05^{c}
Bone surface per bone volume (mm^{-1})	26 8 ± 2 9^{a}	12 6 ± 2 1^{b}	14 0 ± 2 0^{b}	13 6 ± 1 2^{b}	10 8 ± 0 7^{b}
Trabecular thickness (µm)	77 ± 9^{a}	165 ± 32^{b,c}	147 ± 21^{b}	151 ± 13^{b}	190 ± 13^{c}
Trabecular separation (µm)	77 ± 5^{a}	212 ± 34^{b,c}	180 ± 16^{b}	229 ± 8^{c}	299 ± 26^{d}
No of osteocyte nuclei per bone area (mm^{-2})	942 8 ± 49 5^{a}	679 2 ± 68 5^{b}	400 1 ± 47 9^{c}	399 5 ± 65 4^{c}	294 8 ± 24 4^{d}
Double counts (%)	30 8 ± 0 6 (n=2)	36 6 ± 1 6 (n=2)	34 7 ± 0 4 (n=2)	24 5 ± 0 8 (n=2)	24 4 ± 2 4 (n=2)
No. of osteocyte nuclei per bone volume (mm^{-3}) ×10^{3}	93 2 ± 5 4^{a}	61 5 ± 6 8^{b}	37 3 ± 4 8^{c,d}	43 1 ± 7 6^{c}	31 9 ± 2 9^{d}

The parameter values are compared between species
^{a,b,c,d,e} Unequal characters indicate a significant difference with $p < 0.01$.

The number of osteocyte nuclei per bone area was similar for animals of the same species, but showed large variation between species (Fig. 2). The mean values differed significantly between all species, except between the pig and the monkey (Table 1). The number of osteocytes per bone area decreased with increasing size of the animal. The percentage of double counts is an indirect measure of the average size of the osteocyte nuclei. This percentage ranged from 24 and 37 % and was lowest in the pig and the cow and highest in the rabbit and the monkey. Due to these differences, the relative differences between the calculated number of osteocyte nuclei per bone volume were smaller than the relative differences between the measured number of osteocyte nuclei per bone area. The number of osteocyte nuclei per bone volume differed significantly between all species except between the pig and the monkey and between the cow and the monkey (Table 1).

Table 2 *Histomorphometric parameters in four specific sites within the femoral heads of pigs and cows. Average values ± standard deviation*

	Relative bone volume	Trabecular number (mm^{-1})	Bone surface/bone volume (mm^{-1})	Trabecular thickness (μm)	Trabecular separation (μm)	No. of osteocyte nuclei/bone area (mm^{-2})
Pig (n=6)						
Epiphysis						
1	0.41 ±0.02^{a}	2.94 ±0.20^{a}	14.3 ±0.8^{a}	142 ±8^{a}	201 ±16^{a}	440.8 ±97.8^{a}
2	0.43 ±0.02^{a}	2.88 ±0.24^{a}	13.6 ±1.2^{a}	149 ±14^{a}	202 ±19^{a}	388.2 ±82.9^{a}
3	0.40 ±0.05^{a}	2.84 ±0.22^{a}	14.4 ±2.6^{a}	143 ±24^{a}	211 ±16^{a}	397.9 ±62.6^{a}
Metaphysis						
4	0.37 ±0.04^{a}	2.15 ±0.16^{b}	12.0 ±1.6^{a}	171 ±21^{a}	298 ±33^{b}	376.6 ±68.8^{a}
Cow (n=5)						
Epiphysis						
1	0.37 ±0.04^{c,e}	2.16 ±0.12^{c}	11.8 ±0.8^{c}	172 ±13^{c}	294 ±34^{c}	306.1 ±29.0^{c}
2	0.49 ±0.06^{d}	2.24 ±0.07^{c}	9.3 ±1.3^{d}	220 ±28^{d}	229 ±23^{d}	288.9 ±30.4^{c}
3	0.41 ±0.04^{c,d}	2.14 ±0.08^{c}	10.5 ±1.0^{c,d}	194 ±19^{c,d}	275 ±23^{c,d}	274.6 ±47.1^{c}
Metaphysis						
4	0.31 ±0.03^{e}	1.76 ±0.11^{d}	11.7 ±1.0^{c}	174 ±15^{c}	398 ±42^{e}	312.1 ±33.4^{c}

The parameter values are compared between the different sites within the same species
$^{a\,b\,c\,d\,e}$ Unequal characters indicate a significant difference with $p < 0.01$

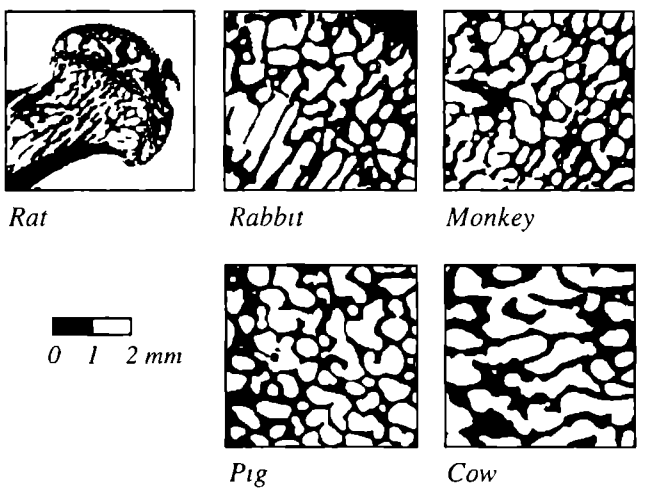

Figure 3
Examples of digital images of trabecular morphologies of each species are given. Note that in the rat the growth plate is still present.

Consistent differences could be found between the morphometric parameter values measured at the different sites in the femoral heads of pigs and cows The average parameter values per site are presented in Table 2 The relative bone volume was consistently lower in the metaphysial area than in the epiphysial area, due to a significantly smaller trabecular number and larger trabecular separation in the metaphysial area In both species, the trabecular bone was densest in site 2, i e the most central location of the epiphysis This difference was significant only in the cow No significant differences in osteocyte numbers were observed between the different locations

DISCUSSION

We have assumed that osteocytes regulate the local bone turnover, influenced by mechanical stimuli Previous results suggested that trabecular thickness and separation might be independent of osteocyte density (provided that a certain minimum number of osteocytes is present) but dependent on the distance over which the regulatory signals can affect the actor cells (Mullender et al , 1994, Mullender and Huiskes, 1995), and that osteocyte density affects the rate of bone remodeling In the light of these results two questions were investigated in this study First, are the values of morphometric parameters in trabecular bone limited within a certain range or do they differ between species of various sizes, and second, how is bone cellularity related to bone structure and species?

Significant differences in morphometric parameters between sites of measurement were found The differences between sites in cows and pigs consisted of a smaller bone volume in the metaphysis compared with the epiphysis due to a significantly smaller trabecular number In the most central area of the epiphysis, the relative bone volume was highest, this was associated with larger trabecular thickness and smaller separation These differences within the epiphysis may be explained by differences in loading, assuming that the central part of the epiphysis transfers the largest part of the load to the underlying bone However, the differences in morphometry were significant only in bovine epiphysial bone

When the morphometric parameters for the different species are compared, it appears that trabecular thickness and separation are smallest in the smallest species (rat) and largest in the largest species (cow) The opposite holds for trabecular number These results indicate that trabecular thickness and separation tend to increase and trabecular number tends to decrease with increasing size of the species However, the absolute range of variation between morphometric parameters, especially trabecular thickness, in the

rabbit, monkey, pig and cow was actually remarkably small (trabecular thickness ranged from 147 to 190 μm). The femoral diameter increases as much as 5.5-fold from the rabbit to the cow, whereas trabecular thickness showed at most a 1.2-fold increase (between monkey and cow). Although few allometric studies of trabecular bone structure are available, histomorphometric parameters are regularly being used to give an indication of the quality of cancellous bone structure. In Table 3, an arbitrary selection of values is given for trabecular thickness in animals of several mammalian species, which served as controls in a wide range of experimental studies. These data indicate that trabecular thickness is indeed of the same order of magnitude in all of these species and that no relationship between the size of the species and trabecular thickness seems to exist. Nevertheless, the values reported for trabecular thickness in the rat are invariably lower than those reported in the other species. Within this group of species, the rat is not only the smallest but also the only one in which the growth plates do not usually close. Hence, the smaller mean trabecular width in the rat might be due partly to the presence of primary trabeculae and relatively new secondary trabeculae, which usually are more numerous and thinner than relatively older secondary trabeculae (Schaffler et al., 1993). Furthermore, the growth plate provides a more even distribution of the mechanical load, which causes a very different mechanical environment compared with the other species.

Table 3 *Trabecular thickness measured in cancellous bone of several mammalian species.*

Source	Species	Age	Location	Trabecular thickness (μm)
Jee and Li, 1990	Sprague-Dawley rats	9 mos	Prox. tibia metaphysis	40-45
Vico et al., 1993	Wistar rats	15 wks	Prox. tibia metaphysis	37
Vico et al , 1993	Wistar rats	15 wks	Prox tibia epiphysis	71
Vico et al , 1993	Wistar rats	15 wks	L2	53
Vico et al., 1993	Wistar rats	15 wks	Femoral fossa trochanteri	78
Wu et al., 1990	New Zealand White rabbits	Mature	Femoral head	146
Wu et al , 1990	New Zealand White rabbits	Mature	L3	111
Fettman et al , 1992	Cats	Adult	Iliac crest	184
Norrdin et al , 1993	Cats	13-18 mos	Iliac crest	105
Kuhn et al , 1990	Mongrel dogs	Mature	Distal femur	170-210
Mosekilde et al , 1993	Minipigs	16 mos	L4	110
Kragstrup et al , 1984	Pigs	14 mos	L4	134
Bourrin et al , 1992	Rhesus monkeys	Adult (9 yrs)	Iliac crest	103
Lundon et al., 1994	Macaques	Adult (9 5 yrs)	L2	125
Schnitzler et al , 1993	Baboons	Adult	Iliac crest	91
Parfitt et al , 1983	Humans	Adults ≤ 50 yrs	Iliac crest	145
Odgaard, personal communication, 1995	Sperm whale (11 m, n=1) (*Physeter catodon*)	?	Vertebra	180

The differences between morphometric parameters found between species suggest that in these species the remodeling process is regulated within a specific spatial range. However, the absolute differences between parameter values are quite small, which indicates that the local domain of regulation is of similar size in the mammalian species that we used, except in the rat. This is consistent with the hypothesis that the regulatory domain of osteocytes is similar regardless of species. Only in the rat is the trabecular thickness clearly smaller; this may be due to the different mechanical environment in the femoral heads of rats but also to a smaller spatial regulatory domain. Possibly the regulation in the femoral head of the rat is modified by the presence of the cartilageous growth plate and the presence of chondrocytes within the primary trabeculae.

The percentage of double counts is an indirect measure of the size of the cell nucleus, because a larger cell nucleus has a greater chance of being hit by sectioning. The percentage of double counts was measured in only two animals per species; however, the results from the animals of the same species were always very similar (which can be seen from the small standard deviation). It was noteworthy that the percentage of double counts was lowest in the cow and the pig, indicating that these animals have smaller osteocyte nuclei than the other species. The percentage of double counts was not related to the number of osteocyte nuclei per bone area.

The results show that the number of osteocyte nuclei per bone volume is specific for each species and seems to be inversely related to species size, although osteocyte density was similar in the pig and the Rhesus monkey. The number of osteocyte nuclei per bone volume ranged from 93,200 mm^{-3} in the rat to 61,500 mm^{-3} in the rabbit to 31,900 mm^{-3} in bovine cancellous bone. Data of osteocyte density in various species are scarce. Mullender et al. (1995) found that osteocyte density in cancellous bone of the iliac crest in humans (30-55 years old) varied around 13,000 mm^{-3}, which is lower than the values measured in the animals. They also reported that both osteocyte and lacunar density decreases with increasing age. Li et al. (1991) measured osteocyte density in 5 μm sections of cortical bone in several regions of the tibia and the second metatarsal in the rat. They found that osteocyte density varied between 810 and 1,060 per mm^2. They also found that osteocyte density could be affected by exercise. Although their data agree very well with the values that we obtained for the rat, these values are actually not comparable with ours, because they counted osteocytes (not osteocyte nuclei) and used thinner sections. Norrdin et al. (1993) counted osteocyte nuclei in trabecular bone of the iliac crest in cats. They reported an osteocyte density of 156 per mm^2 bone. However, they measured osteocyte

density in 1 μm sections, which explains the small number compared to our values If it is assumed that osteocyte nuclei in cats are similar in size to those in the species that we used, the number of osteocytes per bone volume estimated from their data is close to the values that we found for monkeys and pigs Marotti et al (1990) and Hobdell and Howe (1971) have been, to our knowledge, the only authors to compare osteocyte densities in various species Contrary to our findings, the data from Marotti et al (1990) suggests no relationship with animal size, therefore they stated that neither osteocyte density or lacunar volume seem to be related to animal species Since they found osteocyte density to be much higher in woven bone than in lamellar bone, they argued that osteocyte density, distribution, and shape are strictly related to collagen fiber texture Most of the data of Hobdell and Howe (1971) were collected in primary bone They found only small differences in osteocyte density in primary bone between several mammalian species (24,000-33,000 per mm^3) For example, they found no difference in osteocyte density between a rat and an elephant From these findings, it can be seen that the relationship between osteocyte density and species is not straightforward and depends on the type of bone. In addition, age and loading may affect osteocyte density Hence, more data are needed to draw definite conclusions about the relationships between osteocyte density and other variables

Still, we speculate that osteocyte density may be related to turnover rates It is well known that body size, basal metabolic rate, life span and growth rate are all interrelated according to physiological scaling laws (Schmidt-Nielsen, 1984) Hence, it can be expected that turnover rates also are related to the size of the species, and our finding that in cancellous bone osteocyte density decreases with the size of the species fits well with this concept Furthermore, as the rate of turnover is much higher in woven bone than in lamellar bone it is also consistent with the greater osteocyte densities in the former type of bone than in the latter In addition, Cané et al (1982) found that, in dogs, the number and size of osteocytes are larger in locations within the bones with a higher turnover rate than in locations where turnover is lower

In conclusion, it was found that morphometric parameters and osteocyte density both are related to species size The range of variation of trabecular thickness between species, however, is relatively small This finding agrees with the hypothesis that the thickness of trabeculae is limited by the size of the domain that can be regulated by osteocytes Osteocyte density in trabecular bone, however, varies widely between species and is inversely related to species size The relationships with species are different for

osteocyte density than for the morphometric parameters. Hence, the data support our hypothesis that osteocyte density is not directly related to the macroscopic trabecular architecture.

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5 Osteocytes and Bone Lining Cells — Which Are The Best Candidates for Mechano-Sensors in Cancellous Bone?

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Bone 6: 527-532, 1997

Abstract

Previously, we have investigated the possible role of osteocytes as mechano-sensors, and mediators of bone turnover. It was found that the proposed regulatory mechanism produced morphologies of trabecular bone, under particular loading conditions, which were consistent with morphogenesis and adaptation as seen in reality. The main objective of this study was to discern whether lining cells or osteoblasts could possibly play a similar role as effectively with regard to their capacity for self-optimization of the trabecular architecture, in terms of a low apparent mass to stiffness ratio. For that purpose the earlier analyses with osteocytes as mechano-sensors, distributed throughout the bone, were repeated for mechano-sensors located at bone surfaces only. Compared to the osteocyte model, the surface cell remodeling algorithm was reluctant to change its architecture, which implies that it is less sensitive to changes in the loading pattern. This resulted in less efficient bone adaptation, which was reflected by a considerably higher relative mass for a similar apparent stiffness in the loading direction. In other words, more mass is needed to obtain an equally stiff structure, at the apparent level, with respect to the externally applied loads. Furthermore, stresses and strains at the tissue level vary across a much wider range, relative to the osteocyte model, where the higher incidence of elevated strains indicates an increased failure risk. Therefore, we conclude that mechanical information at the bone surface may not be sufficient to adequately regulate functional bone adaptation.

INTRODUCTION

Although it is generally accepted that bone tissue adapts to mechanical demands, the regulatory mechanisms responsible for this process are not understood For one thing, the mechanism by which the bone tissue senses mechanical stimuli has not been established Neither the cells responsible for transduction of mechanical into chemical signals nor the means by which the actual bone remodeling processes are subsequently regulated are identified

Osteocytes, located within the bone matrix, bone lining cells, covering the bone surface, and osteoblasts have all been proposed to play important roles as mechano-sensors in the regulatory process In vitro studies showed that the activity of both lining cells and osteocytes increased after loading (Skerry et al , 1989, El-Haj et al , 1990) Osteocytes were suggested as the most suitable candidates for the role of mechano-receptors, because of their location and the interconnections by which they communicate with each other and with cells at the bone surface (Marotti et al , 1990, Cowin et al , 1991, Lanyon, 1993) It was proposed that these cells are stimulated by fluid flow in the caniculi, due to mechanical loading of the tissue (Weinbaum et al , 1993, Harrigan and Hamilton, 1993) In fact, it was shown experimentally that osteocytes are very sensitive to fluid flow across their cell membranes (Klein-Nulend et al , 1995)

The hypothesis that osteocytes sense mechanical signals and regulate the local adaptation of bone mass was recently investigated for its feasibility, using a computer simulation model (Mullender and Huiskes, 1995) In this regulatory model, bone density was adapted at any location within the tissue, according to a stimulus received from the osteocytes in the vicinity It was shown that the proposed regulatory mechanism could explain the genesis and adaptation of trabecular patterns in accordance with the external loads, indicating that such a hypothesis is realistic (Mullender et al , 1994, Mullender and Huiskes, 1995, van Rietbergen et al , 1995a, van Rietbergen et al , 1996a) Nevertheless, in reality trabecular bone turnover occurs only at surfaces and not within the tissue Furthermore, the question remains, if sensors located at the bone surface (lining cells and osteoblasts) could regulate bone remodeling equally well It was shown in many studies that osteoblasts and osteoblast-like cells are very sensitive to mechanical loads as well (see Burger and Veldhuijzen (1993) for a review) In addition, Miller et al (1989) and Parfitt (1984) suggested that bone lining cells probably play important roles in bone remodeling by mediating the activation of the bone remodeling sequence

We have investigated two questions in this paper First, do the results of the regulatory model based on osteocytes as mechano-sensors differ from those previously described, if, as in reality, remodeling is allowed only at trabecular surfaces? Second, can a simulation model, based on the assumption that lining cells and osteoblasts are mechano-sensors and regulators of

bone turnover, explain mechanical adaptation of trabecular bone equally well? To investigate the latter question, a model was developed based on the alternative hypothesis that sensor cells are located only on the trabecular surfaces. The behavior of this regulatory model was compared to the regulatory models in which the sensors are located within the bone matrix.

METHODS

Two hypothetical regulatory mechanisms for trabecular bone (re)modeling were compared. In both regulation schemes local mechanical signals are appraised by sensor cells which subsequently stimulate populations of osteoclasts and osteoblasts (basic multicellular units, BMUs) to adapt the local bone mass (Fig. 1). In the first regulation model, osteocytes are assumed to be the sensor cells (osteocyte model), while in the second regulatory mechanism it is presumed that only cells covering the trabecular surface act as sensors (surface cell model). For the osteocyte model two possibilities were compared. The first is "overall remodeling", which implies that bone density can be adapted at any location in the tissue. This model was described earlier by Mullender et al. (1994) and Mullender and Huiskes (1995). The second is "surface remodeling", where the bone density is allowed to change only at the bone-marrow interface. In the surface cell model, surface remodeling was investigated only

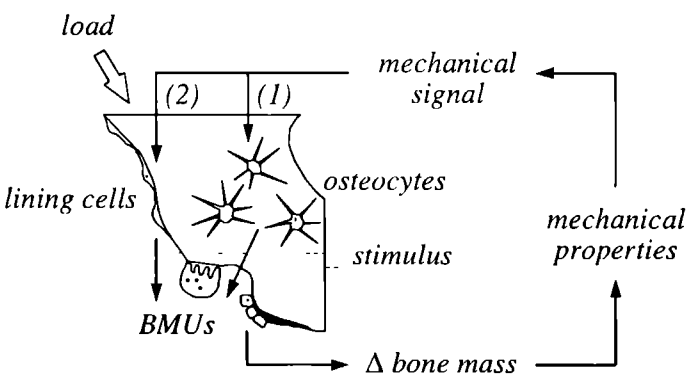

Figure 1

Two alternative hypothetical regulatory schemes are compared. Bone remodeling is assumed to be controlled by local feedback. The first hypothesis is that osteocytes appraise mechanical signals and stimulate BMUs to adapt bone mass (1) The second hypothesis states that bone surface cells (lining cells and osteoblasts) are the mechano-sensors and that these cells stimulate bone turnover by BMUs (2). In both schemes, this results in a change of local mechanical properties, which again affects local mechanical signals.

MATHEMATICAL FORMULATION OF THE REMODELING HYPOTHESES

The mathematical foundation of the models was analogous to Mullender and Huiskes (1995) The strain energy density (SED) was taken as the mechanical signal $S_i(t)$ (MPa) measured by a sensor cell i The strain energy density is in fact the local elastic (strain) energy stored per volume of bone tissue According to the difference between the actual signal and a reference signal k, the sensor produces a stimulus The amount of stimulus received by the BMUs depends on the distance between the sensor cell i and the location x of the actor cells The local stimulus value $F(x,t)$ at location x at time t is the sum of the stimuli received from all sensors

$$F(x,t) = \sum_{i=1}^{N} f_i(x)\,(S_i(t) - k), \qquad (1)$$

with N the number of sensors and the spatial influence function

$$f_i(x) = e^{\frac{-d_i(x)}{D}}, \qquad (2)$$

describing the decrease in stimulus with increasing distance $d_i(x)$ (mm) between location x and sensor i The parameter D determines the decay of the influence function

The change in the relative density $m(x,t)$ in location x is governed by the local stimulus value $F(x,t)$ Hence, in case of overall remodeling

$$\frac{dm(x,t)}{dt} = \tau\, F(x,t) \qquad with\ 0 < m(x,t) \le 1, \qquad (3)$$

and in case of surface remodeling

$$\begin{cases} \dfrac{dm(x,t)}{dt} = \tau\, F(x,t) & x \in trabecular\ surface \\ \dfrac{dm(x,t)}{dt} = 0 & x \notin trabecular\ surface \end{cases} \qquad with\ 0 < m(x,t) \le 1 \qquad (4)$$

where τ ($MPa^{-1}s^{-1}$) is a constant regulating the rate of the process The local elastic properties were calculated from the local relative density using a cubic power relationship in accordance

with experimental data (Currey, 1988) Hence, the elastic modulus at location x was calculated from

$$E(x,t) = C\ m^3(x,t), \qquad (5)$$

with C (MPa) a constant

NUMERICAL FORMULATION

The regulatory models were applied to a volume of bone tissue Input to the regulation models was given by the magnitudes and directions of the mechanical loads In turn, the model predicted the distribution of bone mass in time, i e the development of architecture, for given parameter values The bone tissue was modeled by finite elements, which allowed the calculation of the mechanical variables inside the tissue for externally applied loads The development of bone architecture in time was simulated numerically, i e equations *(3)* and *(4)* were solved recursively, using a numerical integration technique (forward Euler) to find the new values for the relative density per element The procedure was continued until the changes in architecture were virtually zero A variable time step was used that was calculated from the maximal stimulus and a prescribed maximal change in relative density at any location according to van Rietbergen et al (1996a)

For surface remodeling, it was necessary to define a bone-marrow interface within the finite element model since changes in density are only allowed at this location Bone surfaces were modeled by elements of intermediate density, representing partial volumes of bone and marrow These elements are located between elements with minimal density (marrow) and elements with maximal density (bone) (Fig 2)

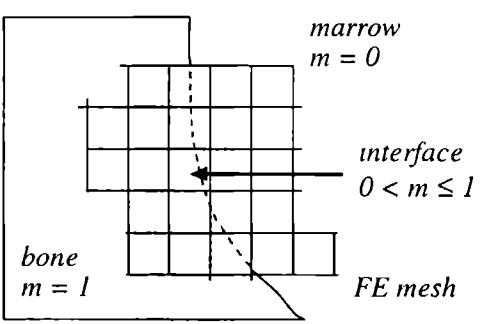

Figure 2
The jagged boundaries of the elements prevent an accurate representation of a smooth bone surface This problem has been solved by allowing boundary elements to have intermediate densities representing partial volumes of bone

The difference between the osteocyte model and the surface cell model was the location of the sensors In the osteocyte model the sensors were uniformly distributed over the mineralized bone matrix In the surface cell model, the sensors were located in the centers of the elements representing the bone surface Stresses and strains in the locations of the sensors were calculated via extrapolation of the values in the integration points to the nodal points of each element, and subsequent-ly linear interpolation to the location in which the sensor was situated The mechanical signal per sensor was calculated according to

$$S_i = \frac{1}{2}\sigma_i \varepsilon_i , \qquad (6)$$

where σ_i and ε_i are the stress and strain tensors in the location of sensor i

APPLICATION OF THE REMODELING HYPOTHESES

The regulation models were applied to a plate of 2×2mm (thickness 0 02 mm), meshed with 80×80 four-node elements The initial architecture was an arbitrary trabecular structure resembling a lattice (Fig 3) The physiological parameters in the models were the reference energy k, the sensor density n [equal to their number N divided by the area of bone tissue (osteocyte model) or divided by the length of the bone perimeter (surface cell model)], the exponential osteocyte-influence function (characterized by the distance parameter D), and the constants τ and C (equal to the maximal elastic modulus) n was taken as 1600 mm^{-2} in the osteocyte model and 40 mm^{-1} in the surface cell model Osteocyte density was chosen within a physiological order of magnitude, which was estimated form measurements by Marotti et al (1990) and Mullender et al (1996a, 1996b) The value for lining cell density was based on an average length of the cells of 25 μm However, no actual data of lining cell density were available A value of 0 02 MPa was used for k and D was 100 μm (Mullender and Huiskes, 1995) C was taken as 6 GPa (van Rietbergen et al , 1995b) and the rate constant was arbitrarily set at τ = 1 MPa^{-1}s^{-1}

The plate was loaded at each side with uniform stress distributions The stress magnitudes σ_1 and σ_2 were 5 MPa and -2 5 MPa respectively After 200 increments (after which stable configurations were reached) the loading configuration was altered by changing the applied stress orientations from 20° to 0° relative to the plate Hereafter, the simulations were continued for another 200 increments

EVALUATION OF THE RESULTS

The resulting architectures were evaluated for their apparent mechanical properties and the relative apparent density. To assess the mechanical properties of the architectures produced by the models, the global stiffness matrices were determined for each equilibrium architecture from the structure morphology and the element stiffness matrices according to van Rietbergen et al. (1996b). From the global stiffness matrix, the axes of orthotropy and the principal Young's moduli associated with these axes were determined (Rietbergen et al., 1996b). To appraise the differences between architectures at the tissue level, the stress, strain and SED distributions in the structures produced were determined, by calculation of the maximum principal stress and strain values and the SED values in each element.

RESULTS

All models converged towards equilibrium solutions which resembled trabecular-like structures (Figs. 3a-c). The number of increments necessary to reach a stable solution was smaller for "overall remodeling" than for "surface remodeling". In addition, the remodeling rate (although given in arbitrary units) was about two times higher for "overall remodeling" as compared to "surface remodeling". After convergence, the signals (SED) in the sensors averaged 0.02 MPa, which is equal to the value of the reference signal k, in all models.

The architectures produced by the osteocyte model described earlier and the osteocyte model, in which remodeling was only allowed at the bone-marrow interface, were not identical but very similar. The apparent properties of the equilibrium architectures were very similar as well. In these architectures, the axes of orthotropy were identical to the external loading directions. Furthermore, the proportion between the principal Young's moduli, E_{xx} and E_{yy} was between 1.7 and 2.0 (Table 1), which approximates the proportion between the load magnitudes σ_1 and σ_2. In other words, the anisotropy of these structures matched the externally applied loads. Both the relative apparent densities and the maximal principal Young's moduli were somewhat higher (2-6%) in the structures produced by surface remodeling than those produced by overall remodeling (Table 1).

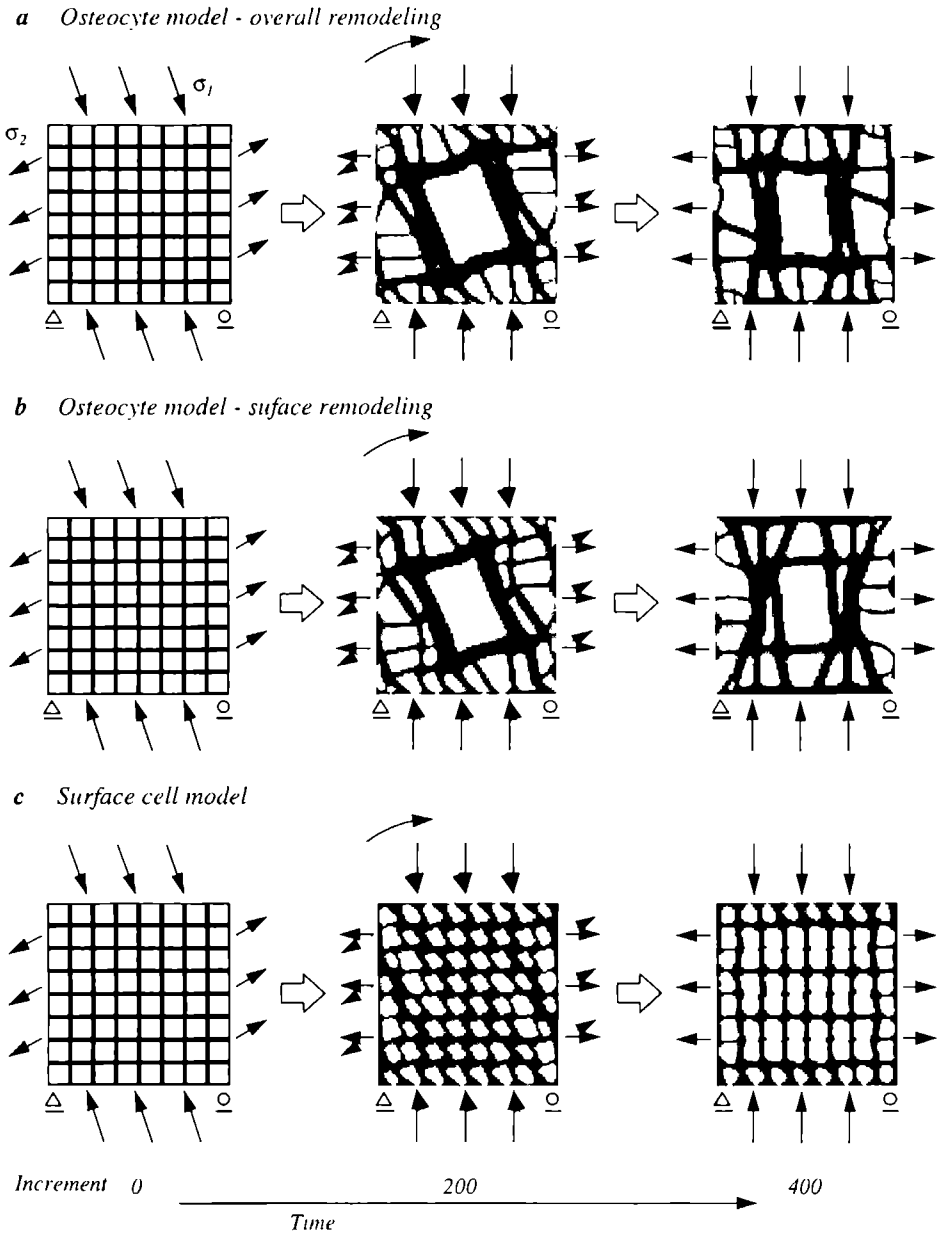

Figure 3

The equilibrium architectures are presented for the osteocyte models using overall remodeling (a), and surface remodeling (b), and for the surface cell model (c). The architectures produced by both osteocyte models show large resemblance. Note that the architectures produced by the surface cell model are closer to the initial architecture.

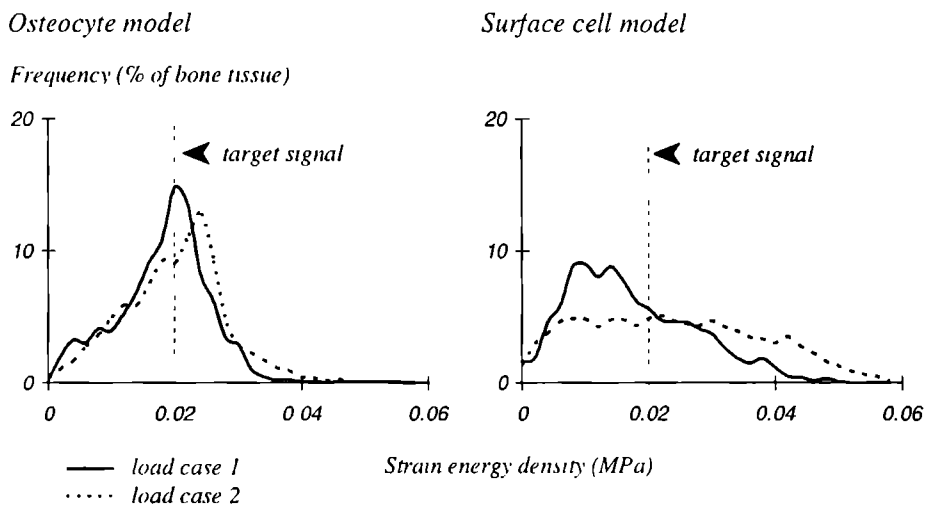

Figure 4

The distributions are shown of strain energy density in the tissue of the equilibrium architectures produced by the osteocyte model (surface remodeling) and the surface cell model. The distributions for the osteocyte model are very similar for both load cases and peak at the value of the target signal. In contrast, no clear optimum is present in the distributions for the surface cell model.

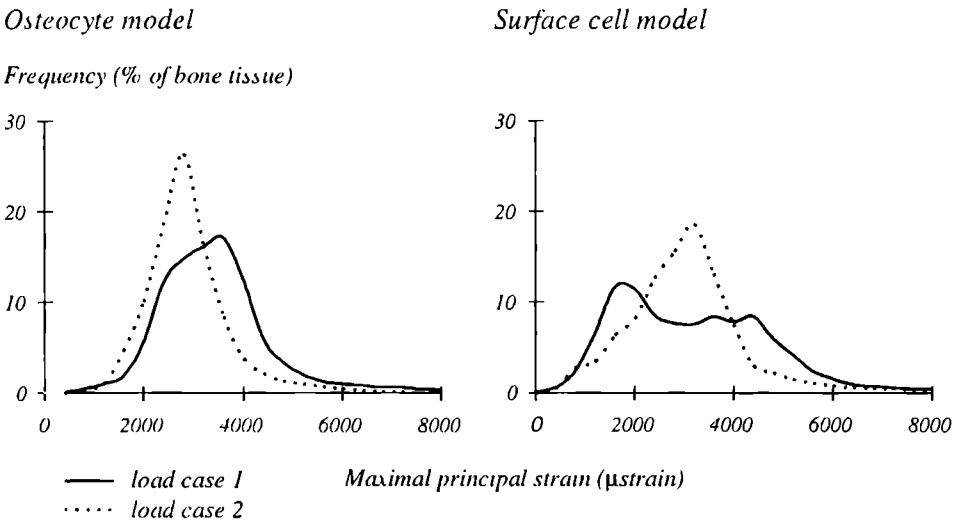

Figure 5

The maximal principal strain values vary around 3000 mstrain in the architectures produced by the osteocyte models The maximal principal strain distributions for the surface cell model are different for each load case In particular, the architecture produced by the first load case shows a much wider distribution, indicating that it is less well adapted to the applied loads.

The surface cell model produced very different architectures compared to the osteocyte models (Fig 3c) The equilibrium architecture after 400 increments shows clearly that, using this remodeling algorithm, the initial architecture is more persistent in the eventual result Local adaptation occurs, but the equilibrium architecture is closer to the initial one than those produced by the osteocyte models The equilibrium architecture for the first load case was not orthotropic, which implies that the principal Young's moduli cannot be determined Instead, the Young's moduli in the directions of the applied loads were determined The apparent density in this architecture was about 11% higher than for the osteocyte model, whereas the apparent Young's modulus in the main loading direction was only 2% higher The stiffnesses in all other directions were considerably higher than those in the osteocyte models After the direction of loading was changed, the trabeculae re-orientated In this second architecture, which was rather similar to the initial configuration, both the apparent density and the apparent stiffness values were lower in the surface cell model than in the osteocyte models

Table 1 *Apparent properties of the produced architectures for each model*

Model		Apparent properties			
			Principal Young's moduli in orthotropic directions		
Algorithm	Load case	Relative density	Exx (Gpa)	Eyy (Gpa)	Gxy (Gpa)
Osteocyte model					
Overall remodeling	1	0 52	1 79	1 04	0 23
	2	0 52	1 85	1 08	0 17
Osteocyte model					
Surface remodeling	1	0 55	1 89	1 03	0 22
	2	0 54	2 00	1 05	0 23
Surface cell model	1	0 61	1 92[1]	1 50[1]	[2]
	2	0 50	1 77	1 07	0 12

[1] Young's modulus in direction of applied load
[2] Architecture was not orthotropic

The evaluation of the mechanical variables at the tissue level revealed that, for the architectures produced by the osteocyte models, the SED values always varied around 0.02 MPa, which is equal to the target signal. In contrast, the SED distributions for the surface cell model have no clear optimum value (Fig. 4). With osteocytes as the sensors, the maximum principal stress and strain distributions were similar for the two load cases as well as for both the overall and surface remodeling algorithms. The narrow shape of the maximum principal strain distributions show that the greater part of the tissue was strained at a similar level (Fig. 5). Compared to the osteocyte models, these distributions were much wider for the surface cell model and also varied more between the two equilibrium architectures.

DISCUSSION

Previously, we have considered a strain adaptive bone-remodeling theory for which it was assumed that osteocytes appraise mechanical signals and regulate bone adaptation (Mullender and Huiskes, 1995). Adaptation of bone density could take place at any location within the tissue represented in the model. It is known, however, that bone remodeling in trabecular bone occurs only at surfaces. A more realistic description of the process, based on surface remodeling, was compared with the previous model. Both remodeling algorithms produced qualitatively similar architectures. This is not surprising, because although the previous model allowed remodeling to take place throughout the tissue, it was in fact limited predominantly to the surfaces of existing trabeculae (Mullender and Huiskes, 1995). Nevertheless, it was found that remodeling occurs much faster in overall remodeling as compared to surface remodeling. This is caused by the restricted volume available for simultaneous transformation in the latter case. We conclude, however, that the results from the surface remodeling algorithm are very similar to the ones from the overall remodeling algorithm reported earlier (Mullender and Huiskes, 1995).

The second goal of this project was to establish whether bone surface cells, such as lining cells and osteoblasts, could potentially regulate bone remodeling by themselves, without mechano-sensory stimuli from osteocytes. For this purpose, two regulation models were compared. Some limitations of these models need to be discussed. First, trabecular bone tissue was represented in two dimensions. This limits comparison with actual bone, but presumably has no consequences for the comparison between the regulatory schemes, because van Rietbergen et al. (1995a) showed that the osteocyte-based regulation process

behaves similarly in a three-dimensional model Second, the use of square elements limits the representation of trabecular geometry This is partly resolved by allowing intermediate bone densities in boundary elements (Fig 2) More importantly however, digital models introduce solution errors at boundary elements It has been shown that these errors can be significant and that further refinement of the element mesh does not necessarily reduce these boundary artifacts (Hollister et al , 1992) The use of smooth boundary models does not solve this problem, for these were shown to produce similar errors (Guldberg and Hollister, 1994) To minimize boundary artifacts, the sensors in the surface cell model were chosen at the centers of the boundary elements (as opposed to the element nodes) Nevertheless, errors are inevitable when calculating signals in boundary elements and no solution for this problem is yet available (Guldberg and Hollister, 1994) However, the effect of these errors on the remodeling results should be limited, because the pattern of boundary errors is oscillatory (Guldberg and Hollister, 1994) and therefore, errors are averaged out by the sensor influence function, which "filters" the erroneous oscillations and reduces their effect on the stimulus distribution Another limitation is that only one specific load case was considered, whereas in reality bone is loaded by a variety of loading patterns, changing both in amplitude and direction However, this probably does not affect the quality of the differences found between the models and it facilitates the assessment of effectiveness of the resulting architectures considerably Furthermore, only net bone loss or gain was considered Hence, the models do not account for separate effects of osteoblasts and osteoclasts and effects associated with the remodeling sequence Finally, strain energy density was chosen as the mechanical signal, because the models used here are too coarse to obtain a more precise measure of the mechanical signals that osteocytes or lining cells perceive when the tissue is loaded Strain energy density is a measure of the energy stored as a result of deformation of the tissue, and may be considered as a reasonable indication of the mechanical deformation which the cells experience Although in fact, it is still unclear how bone cells sense mechanical signals, it is also uncertain how a more accurate representation of the mechanical signals might affect the results

It was found that bone remodeling regulated by osteocytes is very effective indeed The bone mass is distributed such that the apparent stiffness of the architecture is very well adapted to the externally applied loads, i e the equilibrium architecture has properties of (near) minimal mass for a certain average strain energy density value As a result, the total mass of the structure produced is relatively independent of the loading direction However, with surface cells as sensors, remodeling is less sensitive to the external loads,

which is manifested by less change in the architecture When bone remodeling is regulated by osteocytes, it leads primarily to adaptation of the architecture, but if it is regulated by surface cells, it mainly causes adaptation of strut thickness in the existing architecture, which leads to changes in the total mass Apparently, with osteocytes as sensors, the remodeling process drifts more easily towards a significantly different morphology Bone adaptation is less effective for changes of the loading directions with surface cells as sensors This is evident from the frequency distribution plots, which indicate that parts of the structure receive relatively little loading, whereas other parts carry relatively high loads It might be argued, however, that in reality loading patterns are relatively constant For changes in load magnitudes only, surface cells should be equally capable to regulate adaptation as osteocytes, since changes in the load magnitudes require changes in mass rather than in architecture It should be noted that adding surface cells to the osteocyte model makes no difference for the output of the model This is due to the choice of the surface cell location in the boundary elements, which are also included in the tissue area where osteocytes are located

Computational models, according to which strain derived signals are evaluated at the tissue surface and where bone mass is adapted accordingly, as in the present surface cell model, have been introduced by Luo et al (1995) and Siffert et al (1996) They found changes in strut thickness after changing the load magnitudes in a unit cell model (Siffert et al , 1996), but also shape changes when applying loads to idealized structures (Luo et al , 1995) However, their results can not be easily extrapolated to larger, more complex structures, which inhibits comparison with our results

The present results indicate that osteocytes would be more efficient sensors than bone surface cells, in the sense that they produce architectures with a more appropriate mass distribution relative to the applied loads It has long been suggested (Wolff, 1892) or even implicitly assumed that bone is an optimal structure, however, it is uncertain if this is in fact the case Therefore, the superior performance of osteocytes as sensors gives no direct evidence that they actually fulfill this role

In conclusion, the incorporation of surface remodeling into the remodeling algorithm had no essential effects on the architectures produced or their properties The regulation of functional bone adaptation by mechano-sensitive osteocytes would be the most effective modality This indicates that mechanical information at the bone surface may not be sufficient to adequately regulate functional bone adaptation

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6

OSTEOCYTE DENSITY CHANGES IN AGING AND OSTEOPOROSIS

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Bone **18** *109-113, 1996*

ABSTRACT

Recently, it was suggested that osteocytes are involved in the regulation of bone remodeling We have examined human trabecular bone of the iliac crest of fracture patients and control subjects to determine if osteoporosis is associated with changes in osteocyte density or osteocyte death The relationships of these parameters with age was also investigated It was found that osteocyte death was not related to age, nor was it increased in osteoporosis compared with the controls In healthy adults ranging from 30 to 91 years, lacunar number per bone area decreases with advancing age, from about 210/mm^2 to 150/mm^2 Significantly higher lacunar and osteocyte numbers per bone tissue volume were found in osteoporotics than in controls (17,100 lacunae/mm^3 and 13,300 osteocytes/mm^3 vs 12,900 lacunae/mm^3 and 10,500 osteocytes/mm^3 respectively), whereas lacunar area was significantly reduced in osteoporotics (from 44 1 μm^2 to 39 1 μm^2) These findings are compatible with the hypothesis that in osteoporosis osteoblasts produce less bone per cell This can in turn explain the reduced wall thickness, which has previously been described as characteristic for osteoporosis

INTRODUCTION

Bone is a dynamic tissue undergoing continuous renewal. The mechanical integrity of bone is ensured by removal of bone and subsequent replacement by new bone. After the age of 25-30 years, a slightly negative balance between bone resorption and formation may cause progressive bone loss. Usually, mechanical integrity is maintained. However, in osteoporosis, excessive bone loss and loss of structural elements can lead to mechanical failure (Parfitt et al., 1983). As turnover is highest in cancellous bone, osteoporosis becomes manifest particularly in regions where the trabecular architecture is of great structural importance such as, for instance, in the vertebral body and the femoral trochanter. The cause of osteoporosis is sought in a disturbed regulation of the bone remodeling process (Eriksen et al., 1994). It is well established that mechanical usage is essential for the maintenance of bone. Hence, several investigators suggested that osteoporosis is possibly caused by an inadequate appraisal of the mechanical load in bone, because the changes in bone occurring in osteoporosis are similar to changes resulting from disuse (Frost, 1988; Rodan, 1991). Yet, the normal regulation of bone remodeling has still to be unraveled. The regulation of the remodeling sequence and the coupling mechanism between the activity of osteoclasts and the activation of osteoblasts are still unknown.

Recently, it was suggested that osteocytes play a role in the regulation of bone remodeling. It was hypothesized that osteocytes regulate the recruitment of basic multicellular units (BMUs) in response to mechanical stimuli (Marotti et al., 1990; Cowin et al, 1991; Lanyon, 1993; Aarden et al., 1994) and that they play a role in the modulation of osteoblast activity and the recruitment of osteoblasts which differentiate into osteocytes (Marotti et al., 1992). A number of experiments (in vivo and in vitro) showed that osteocytes respond to mechanical loading with an increased production of factors which are known to affect bone turnover (Lanyon, 1993; Klein-Nulend et al., 1995). Mullender et al. (1994) and Mullender and Huiskes (1995), using a computer simulation model, have shown that trabecular modeling patterns due to mechanical stimuli can be explained quantitatively by assuming osteocytes to act as strain-sensing cells in a regulatory process. They showed that osteocyte density and range of influence, i.e. the distance from which they can affect BMU activity, may have distinct effects on the trabecular morphology. They also showed that a reduced sensitivity of osteocytes to mechanical load caused bone loss in a similar way as did disuse.

The above hypotheses and findings have led us to the hypothesis that a disturbance in the regulation of bone remodeling in osteoporosis may be associated with a lack of osteocytes or inefficacy of function To investigate this hypothesis, we have determined the number of osteocytes per bone area and volume and the fraction of empty lacunae, as an indicator of osteocyte death, in osteoporotic patients and control subjects

MATERIALS AND METHODS

SUBJECTS

The osteoporosis group consisted of 14 patients [4 men and 10 women, age 65 2 ± 7 2 years (mean ± SD)] with each at least one vertebral crush fracture (collapsed vertebra), and 23 patients (9 men and 14 women, age 73 6 ± 11 3) with hip fractures Transiliac biopsies were taken from all patients Iliac crest bone samples from twenty-five autopsy subjects (sudden death in previously healthy persons) and four patients who received cosmetic or orthopaedic surgery unrelated to bone disease were obtained for the control group (24 men and 5 females, age 57 0 ± 18 5 years) None of the control group had a history of any disease known to predispose to osteoporosis and neither control subjects or patients received drugs with known effects on bone

HISTOLOGY

The undecalcified biopsies were embedded in methylmetacrylate, sectioned (5 μm) and stained with Goldner's trichrome Histomorphometry was performed on the trabecular bone of two sections per biopsy Microscopic fields were sampled in equally spaced rows by moving the specimens in equally sized steps such that the total specimen area was covered A Zeiss integrating eyepiece was used for the measurement of trabecular bone volume (BV/TV) by counting the number of hits and the number of intersections More extensive histomorphometric data of both groups were published in a different study (Uitewaal et al , 1987)

In addition, the number of lacunae occupied by osteocytes and the number of empty lacunae per bone area were measured in trabecular bone Osteocytes are colored red and are readily visible in the bone matrix Empty lacunae were defined as lacunae without any visible remnant of a cell inside These measurements were performed using a Zeiss microscope in conjunction with a digital image analysis system (Videoplan) Twenty fields were sampled in two sections per subject (×25 objective) by moving the specimen in equally sized steps in x and y directions In this way, a total bone area of approximately 1 mm^2 per

subject was measured to determine lacunar and osteocyte numbers The investigator had no knowledge about the origin of the sections From these measurements the following parameters were deducted the fraction of empty lacunae (number of empty lacunae / total number of lacunae), the total number of lacunae per bone area (N Lc/B Ar) and the number of lacunae occupied by osteocytes per bone area (N Ot/B Ar)

The measured number of osteocyte lacunae per area depends on both the number of lacunae per volume and the average lacunar size As we wanted to determine if differences in lacunar number per bone area between controls and osteoporotics are due to differences in lacunar size or to differences in lacunar number per bone volume, we also measured lacunar area (Lc Ar) as an indicator of lacunar size These measurements were made by outlining at least 75 lacunae per section in randomly selected fields, totaling at least 150 lacunae per subject (×100 oil immersion objective)

The measured parameters N Ot/B Ar, N Lc/B Ar and Lc Ar were used to estimate the number of osteocytes per bone volume (N Ot/BV) and the number of lacunae per bone volume (N Lc/BV) First, the measured lacunar area was used to calculate an average "osteocyte radius' R Assuming that osteocytes have a spherical shape, the measured Lc Ar is equal to Lc Ar = $^2/_3 \pi R^2$, for infinitely thin sections If the equation is corrected for the section thickness (t) and if it is further assumed that k is the thickness of the smallest part of a cell which must be included in the section for its identification, the equation modifies to

$$Lc\ Ar = \pi\left(R^2 - \frac{(R - k)^3}{3(R - k + \frac{1}{2}t)}\right) \quad (1)$$

As this equation is not easily inverted in order to calculate R, it was estimated by fitting R to obtain the measured Lc Ar with a maximal error of 0 005 μm^2 This is only a rough estimate of R, because osteocytes in fact have an elliptical shape

The number of osteocytes and lacunae per volume were calculated according to

$$N\ Ot/BV = \frac{N\ Ot/B\ Ar}{2R + t - 2k} \quad (2)$$

$$N\ Lc/BV = \frac{N\ Lc/B\ Ar}{2R + t - 2k} \quad (3)$$

where k and t are the same factors as above (Sissons and O'Connor, 1977). A section thickness $t = 5.0$ μm and a value of $k = 0.2$ μm was used.

STATISTICAL ANALYSIS

Averages and standard deviations of all measured parameters were determined per group. The reproducibility of the method for measuring lacunar density and size was assessed using the coefficient of variation. These parameters were measured four times in six sections by one observer and were again measured by a second investigator. To increase reproducibility, all measurements were conducted by the same investigator. Correlations between parameters and age were examined by linear regression analyses. Differences between groups were tested using the two-tailed Student's t-test. To exclude effects of age differences, only subjects older than 55 years were used for the comparison between the control group and the osteoporosis group. However, it should be noted that the group of hip fracture patients was still older than the control group.

RESULTS

The values for reproducibility are given in Table 1. The mean values and standard deviations of all measured parameters are given in Table 2. No differences in parameter values were found between males and females within each group. BV/TV was significantly decreased in the osteoporotic patients relative to the control group older than 55 years. The BV/TV of two control subjects could not be reliably measured because the specimen sizes were too small. The N.Lc/B.Ar and also the N.Ot/B.Ar were significantly higher in younger controls than in older controls. In addition, the N.Lc/B.Ar and N.Ot/B.Ar were

Table 1
Coefficient of variation (%) for repeated measurements[a].

	Intraobserver	Interobserver
Lacunae per bone area	4.5	3.5
Osteocytes per bone area	4.8	5.3
Percentage of empty lacunae	3.9	7.6
Lacunar area	14.6	23.1

[a]Reproducibility of lacunar numbers, osteocyte numbers and fraction of empty lacunae is good, but measurements of osteocyte size show large variation.

Table 2
Subject data and histomorphometric parameters in control subjects and osteoporotic patients (average values and standard deviations).

	Controls			Osteoporotic patients >55 ys.		
	≤55 ys.	>55 ys.	combined	hip fract.	vert. fract.	combined
Total	12	17	29	22	12	34
Male	12	12	24	8	2	10
Female	—	5	5	14	10	24
Age (ys)	39.0 ± 7.7[b]	69.7 ± 12.0	57.0 ± 18.5	79.7 ± 9.3[a]	67.6 ± 4.7	75.4 ± 9.9
BV/TV (%)	18.0 ± 6.3	16.8 ± 5.9[c]	17.4 ± 6.1	12.5 ± 4.1[a]	8.4 ± 4.2[b]	11.0 ± 4.5[b]
N.Lc/B.Ar (mm^2)	206.5 ± 29.2[a]	165.7 ± 38.3	182.6 ± 39.9	203.0 ± 29.7[a]	228.9 ± 28.2[b]	212.1 ± 31.4[b]
N.Ot/B.Ar (mm^2)	172.8 ± 34.9[a]	135.1 ± 38.0	150.7 ± 40.7	158.3 ± 23.6[a]	176.0 ± 21.6[a]	164.5 ± 24.2[a]
Fract. empty lacunae	0.17 ± 0.08	0.19 ± 0.10	0.18 ± 0.09	0.22 ± 0.05	0.22 ± 0.06	0.22 ± 0.05
Lc.Ar (μm^2)	47.3 ± 5.8	44.1 ± 7.3	45.5 ± 6.8	38.5 ± 4.1[b]	40.0 ± 6.2	39.1 ± 4.9[b]
N.Lc/BV (10^3 mm^{-3})	15.6 ± 2.0[a]	12.9 ± 3.2	14.0 ± 3.0	16.5 ± 2.5[b]	18.4 ± 2.2	17.1 ± 2.5[b]
N.Ot/BV (10^3 mm^{-3})	13.1 ± 2.4	10.5 ± 3.0	11.6 ± 3.0	12.8 ± 1.9[b]	13.7 ± 2.2[a]	13.3 ± 2.0[b]

[a] significantly different from the control group >55 years ($p < 0.05$)
[b] significantly different from the control group >55 years ($p < 0.01$)
[c] measurements of two subjects discarded ($n = 15$).

significantly higher in the hip fracture and vertebral fracture groups relative to the older control group (Fig. 1). Lc.Ar was significantly smaller in hip fracture patients and in the combined osteoporosis group than in the older control subjects (Fig. 2). As the lacunar size was smaller in the osteoporosis group relative to the older control group, the differences in number of lacunae and osteocytes per bone volume were even more pronounced than the differences between numbers per bone area. The N.Lc/BV and N.Ot/BV were also significantly higher in the younger controls in comparison with the older controls. The fraction of empty lacunae did not differ significantly between groups.

The relationships between several parameters and age are presented in Table 3. In the control group, the number of lacunae and osteocytes per bone area and per bone volume declined significantly with advancing age (Figs. 1a and 1b). The fraction of empty lacunae and lacunar area were not significantly related to age. No significant relationships between age and other parameters were found in the osteoporosis group.

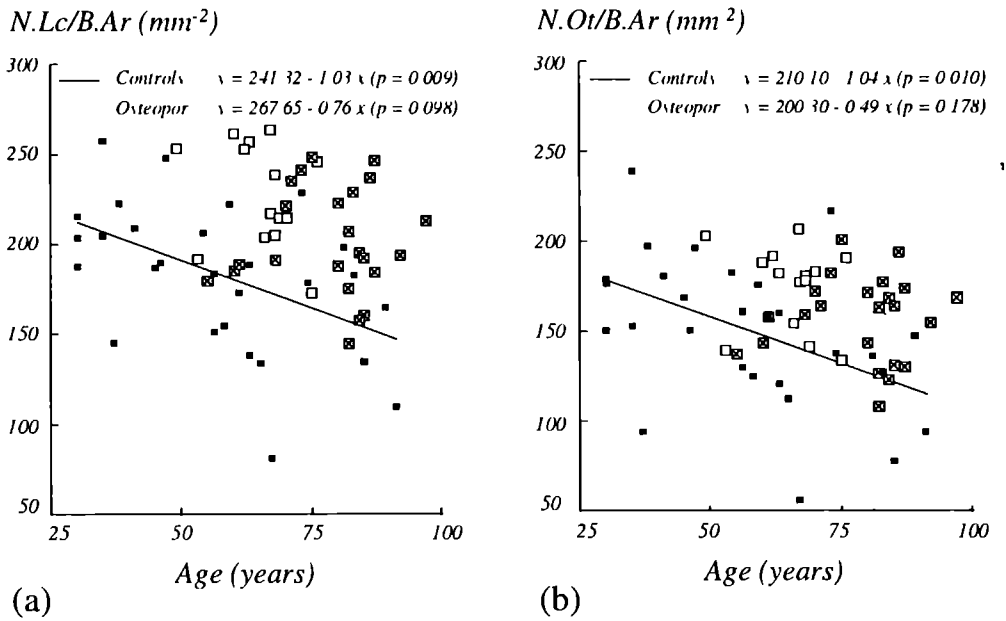

Figure 1

The relationships between age and the number of lacunae per bone area (a) and the number of osteocytes per bone area (b) are presented. In the control group (■), the number of lacunae as well as the number of osteocytes per bone area decrease with age. In both groups of fracture patients (vert. fract. □; hip fract. ⊠), the numbers of lacunae and osteocytes per bone area are significantly increased compared with controls of similar ages

Table 3

Linear regression equations and correlation coefficients of several parameters in relation to age

x	*y*	Group	*n*	Regression equation	Correlation coefficient
Age (years)	N Lc/BAr (mm ^{2})	Controls	29	$y = 241.32 - 1\ 03\ x$ [b]	-0.48 [b]
		Osteoporotics	37	$y = 267.65 - 0.76\ x$	-0.27
Age (years)	N Ot/BAr (mm^{-2})	Controls	29	$y = 210.10 - 1.04\ x$ [b]	-0.47 [b]
		Osteoporotics	37	$y = 200.30 - 0.49\ x$	-0 23
Age (years)	N Lc/BV (10^{3} mm ^{3})	Controls	29	$y = 18.00 - 0.07\ x$ [a]	-0.42 [a]
		Osteoporotics	37	$y = 21\ 16 - 0.06\ x$	-0.25
Age (years)	N.Ot/BV (10^{3} mm ^{3})	Controls	29	$y = 15\ 66 - 0.07\ x$ [a]	-0.44 [a]
		Osteoporotics	37	$y = 15\ 83 - 0.04\ x$	-0.20

[a] $p < 0.05$; [b] $p < 0.01$

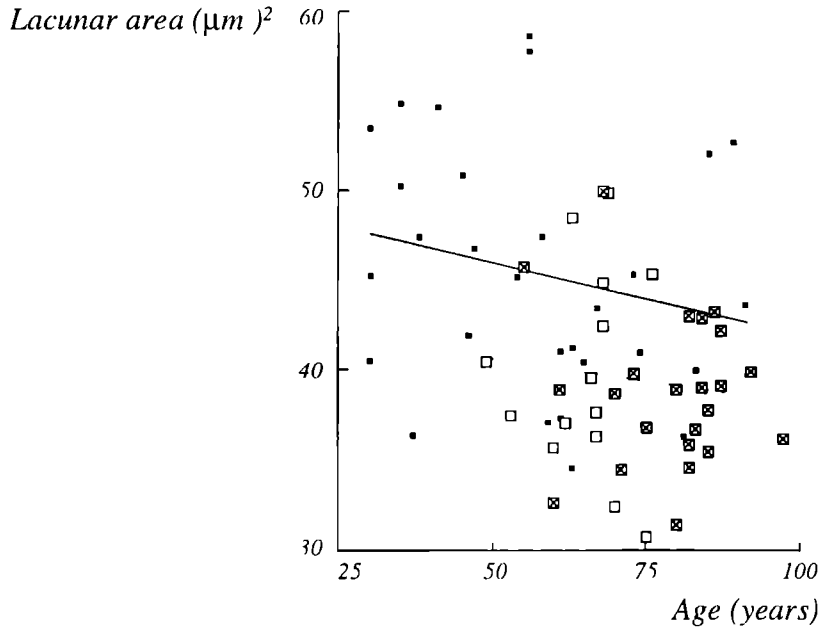

Figure 2
The lacunar area is given as a function of age Although the regressions are not significant they illustrate that lacunar area is reduced in osteoporosis compared to the control group (control ■, vert fract □, hip fract ⊠) Regressions (—) Controls y = 49 96 - 0 08 x (p = 0 26) and ( ) Osteoporotics y = 43 11 - 0 05 x (p = 0 46)

DISCUSSION

Recently, it was suggested that osteocytes are involved in the regulation of bone remodeling (Marotti et al , 1990, 1992, Cowin et al , 1991, Lanyon, 1993, Aarden et al , 1994, Mullender et al , 1994, Mullender and Huiskes, 1995) Furthermore, it was proposed that the incorporation of osteoblasts into the matrix (i e the inclusion of osteocytes) is a highly regulated process in which the pre-osteocytes themselves and osteocytes already incorporated are actively involved (Nefussi et al 1991, Palumbo et al 1990a, 1990b, Marotti et al , 1992) Therefore, it is plausible that changes or disturbances in (pre-) osteocyte function also affect osteocyte morphology and osteocyte number If we assume that osteocytes do indeed play a central role in the regulation of bone turnover, a disturbance of this regulatory process may be caused by disturbances in osteocyte presence or viability The questions investigated in this study were Does osteocyte number and size differ between osteoporotic patients compared with controls, and does excessive osteocyte

death occur in osteoporosis? In addition, we have investigated whether osteocyte death, osteocyte density and lacunar density are related to age

Few investigators have actually measured osteocyte density Hobdell and Howe (1971) found that the average volume of bone matrix associated with one osteocyte lacunae was 0 000077 mm^3 in human adult lamellar bone This is equivalent to 13,000 mm^3 lacunae per bone volume Sissons and O Connor (1977) report values for N Lc/BV between 13,900 and 19,400 mm^3 in human cortical bone, depending on the type of sections used The most accurate method to determine numbers of cells per volume is known as the disector method (Sterio, 1984, Gundersen, 1986) However, this method requires serial sectioning of the specimen As our specimens were already processed for the purpose of histomorphometric analysis it was not possible to use the disector method Instead we measured lacunar area, which is a direct estimate of lacunar volume and the number of osteocytes per bone area, which is a method generally accepted for other cell types such as osteoblasts or osteoclasts Although our calculated figures for osteocyte and lacunar numbers per bone volume only give an estimate of the actual figures, our figures (N Lc/BV ranging from 12,900 to 18,400 mm^3) agree very well with the values reported earlier

Significant differences were found in osteocyte density and lacunar density between osteoporotics and controls (>55 years) Lacunar and osteocyte number per bone area were significantly increased in osteoporotic patients relative to controls A higher number of osteocyte lacunae per bone area may be due to a higher number of lacunae per bone volume and/or to enlarged lacunar sizes (Sissons and O'Connor, 1977, Sterio, 1984) Enlarged osteocyte lacunae have been reported in osteoporotic patients (Wright et al , 1978) and in calcium deficient rats (Sissons et al , 1990, 1984) However, in this study we found that the lacunar area was smaller in the osteoporosis group compared with controls Hence, the differences in numbers of lacunae and osteocytes per bone volume were even more pronounced than these differences in numbers per bone area These results suggest that in osteoporosis less bone volume was produced per osteocyte There are three possible explanations for this phenomenon (1) a higher percentage of the bone forming osteoblasts is embedded as osteocytes, whereas the average activity or longevity is unchanged, (2) the bone forming activity of osteoblasts is reduced, and (3) the average life span of osteoblasts is shorter The latter two explanations seem to be the most likely ones, because they are compatible with the findings that bone formation and mean wall thickness are decreased in osteoporotic patients compared with normals (Eriksen et al , 1990, Darby and Meunier,

1981). Eriksen and Kassem (1992) even state that the most marked difference between osteoporotic and normal women is a considerable reduction of mean wall thickness in osteoporotics. While Eriksen and Kassem (1992) suggested this to be due to a reduced osteoblastic vigor, Lips et al. (1978) explained the decrease in mean wall thickness by a decreased longevity of the osteoblasts. To distinguish between these two hypotheses, Shih et al. (1993) investigated the relationship between bone formation rate and osteoblast surface. They found that although bone formation rate is reduced in women with osteoporosis compared to normal women, the relationship was similar in both groups. This implies that in osteoporotics either fewer osteoblasts are recruited or that active osteoblasts are transformed quicker into less active ones. Our results suggest that the bone forming capacity per cell is reduced, which is compatible with the latter explanation of Shih et al. (1993). For, a decreased longevity of osteoblasts may explain that a higher number is incorporated as osteocytes per bone volume. The reduced lacunar size may also indicate a history of reduced activity of these cells.

The relationship between age and osteocyte density in humans has (to our knowledge) not yet been reported. A significant decrease of osteocyte and lacunar density associated with a (not significant) decrease in lacunar size was found with increasing age in healthy adults from 30-91 years. This decrease was also observed in the osteoporosis group, but it was not significant due to the smaller age range. Some contradiction exists in these results. As Lips et al. (1978) showed that mean wall thickness in trabecular bone decreases with aging, it would be expected that osteocyte number increases with increasing age and that this increase is more pronounced in osteoporosis. However, it was found that osteocyte density decreases with age. Hence, it seems that two different phenomena occur at the same time. In order to explain these phenomena, it is necessary to investigate the relationship between osteocyte number and remodeling activity more closely.

The percentage empty lacunae was used as an indicator of osteocyte death. Nonviable osteocytes can stain normally up to 16 weeks (Kenzora, 1978), but a gradual loss of osteocytes will be reflected by an increase of empty lacunae. It is possible that lacunae appear to be empty due to sectioning artifacts. However, this is very unlikely because the bone is undecalcified and the cellular processes of osteocytes are integrated within the bone matrix. Further, if artifacts occur, it is assumed that they occur equally in both control and osteoporosis groups such that differences in the number of empty lacunae between the two groups will still be detected. The percentage of empty lacunae ranged from 5 to 40% in all subjects. No significant correlation was found between the percentage

empty lacunae and age in both the control and the osteoporosis groups Similar results were found by Baud and Auil (1971), who looked at bone from the mandible In contrast, Wong et al (1985, 1987) observed a loss of viable osteocytes in the femoral head with increasing age and Frost (1960) also found that the percentage empty lacunae increased with age Dunstan et al (1993) showed that the occurrence of osteocyte death with age depends strongly on the location of measurement They found that osteocyte death did increase with age in the femoral head but did not increase in the second lumbar vertebrae They suggested that bone with a constant high viability is remodeled at a higher rate, and thus bone is replaced before the osteocytes have a chance to die

The fraction empty lacunae was not significantly elevated in osteoporosis compared with controls Hence, it seems that osteoporosis is not associated with increased osteocyte death, which challenges the hypothesis that mechanical load is appraised inadequately due to increased osteocyte death However, the hypothesis that osteocytes are less sensitive to mechanical stimuli still needs to be investigated

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7

Differences in Osteocyte Density and Histomorphometry between Men and Women and Between Healthy and Osteoporotic Subjects

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submitted

Abstract

Recently, the hypothesis that osteocytes sense mechanical stimuli and are involved in the regulation of bone remodeling has gained support It was suggested that osteoporosis is associated with a reduction in osteocyte mechano-sensitivity However, little is known about osteocytes and their function in healthy or in diseased bone In this study the relationships between osteocyte density, bone remodeling parameters, gender and osteoporosis were investigated The numbers and sizes of osteocytes were measured, in addition to conventional histomorphometric parameters of trabecular bone, in healthy postmenopausal women and healthy men of similar ages and in men and women with vertebral fractures Females were found to have markedly more osteocytes and higher total lacunar area per bone area than males, independent of the disease Furthermore, patients with vertebral fractures had reduced osteocyte numbers and reduced total lacunar area Histomorphometric parameters revealed no differences between parameters of bone architecture, bone formation and resorption between men and women In vertebral fracture patients, bone mass, trabecular number and thickness, and bone turnover were significantly reduced while eroded surface was increased relative to healthy subjects These results are consistent with impaired osteoblast function in patients Moreover, the differences in osteocyte numbers present evidence that alterations occur within the whole population of cells of the osteoblastic lineage Further research is needed to elucidate the interplay between the role of osteocytes, mechanical load, hormones and other factors

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INTRODUCTION

Both men and women lose bone with advancing age During menopause the rate of bone loss is increased, which leads to an increased risk of bone fracture in postmenopausal women compared to men of the same age However, the incidence of fractures is not only related to bone mass, but also to bone architecture It is thought that, in osteoporosis, the quality of the bone architecture is reduced as well as bone mass (Kleerekoper et al , 1985, Parfitt, 1984) Loss of bone mass is the result of increased osteoclastic activity, reduced osteoblastic activity or both Several authors have reported that the mechanism of bone loss differs between men and women Whereas women lose bone mass mainly by the loss of whole trabeculae (Parfitt et al , 1983, Recker et al , 1988, Steiniche et al , 1994, Moore et al , 1992), in men the trabecular architecture remains intact but trabecular thickness reduces (Aaron et al , 1987, Scane et al , 1993) The precise mechanisms and causes of changes in remodeling activity in osteoporosis, and differences between gender, are still unclear In fact, the regulation of normal bone turnover has not yet been unraveled

During the last decade, the hypothesis that osteocytes play an important role in the regulation of bone remodeling has gained support It is assumed that they mediate other cells to initiate remodeling activity in response to mechanical stimuli (Marotti, 1990, Cowin et al , 1991, Lanyon, 1993, Mullender and Huiskes, 1995, Aarden et al , 1996, Parfitt, 1996) It has been shown that osteocytes are mechano-sensitive (Pead et al , 1988, Skerry et al, 1989, Klein-Nulend et al , 1995, Lean et al , 1996) In addition, it was shown in computer simulation studies that osteocytes are extremely well suited for the regulation of functional mechanical adaptation in bone (Mullender et al , 1994, Mullender and Huiskes, 1995) Rodan (1997) suggested that the coupling between osteoclastic and osteoblastic activity may be mechanically regulated In addition, it has been hypothesized that in osteoporosis mechanical signals are poorly measured, mimicking a situation of disuse (Frost, 1988, Rodan, 1991) It is well known that accelerated loss of bone in women during menopause is associated with reduced levels of estrogen The finding that estrogen receptors are abundantly present in osteocytes (Braidman et al , 1995) and relatively sparsely in other cells of the osteoblast lineage (Oursler et al , 1996) suggests that osteocytes are likely to be involved in the regulation of bone remodeling Moreover, the incorporation of osteoblasts into the bone matrix is a highly regulated process in which osteocytes, osteoblasts and pre-osteoblasts act in close cooperation (Nefussi et al 1991, Palumbo et al 1990a, 1990b, Marotti et al , 1992) Hence, knowledge of the osteocyte

population may be important for normal bone biology and for the assessment of bone diseases

Previously we have found that osteocyte density in trabecular bone from the ilium decreases significantly with increasing age and that osteocyte density differed between normal and osteoporotic subjects (Mullender et al , 1996) As osteocyte density reflects the result of the remodeling process and osteocytes may in turn affect remodeling activity, these results suggest that differences exist between normal and osteoporotic subjects In this study we aimed to investigate the relationships between osteocyte density, bone remodeling parameters, gender and osteoporosis For this purpose histomorphometric parameters were measured in cancellous bone in healthy postmenopausal women and healthy men of similar ages and in women and men with vertebral crush fractures

MATERIALS AND METHODS

SUBJECTS

In this study four subgroups of subjects were compared a control group and an osteoporotic group, each subdivided in males and females The control subjects were all healthy volunteers, who gave their informed consent for the tests performed The male control group consisted of 21 men over 60 years of age (mean age 67 ± 6 (SD) years), the female control group consisted of 13 postmenopausal women (mean age 64 ± 5 (SD) years) All control subjects were healthy and none of them were taking any medication known to affect bone density or calcium and bone metabolism The osteoporotic groups were male and female subjects with untreated osteoporosis They each had at least one non traumatic vertebral crush fracture, but no other bone disease These groups consisted of 15 men (mean age 60 ± 11 (SD) years) and 40 women (mean age 70 ± 11 (SD) years) None of the patients was taking drugs with any known effect on bone metabolism

MATERIALS

Transiliac bone biopsies, were obtained with a Bordier trephine of 8 mm in diameter from all subjects The site of biopsy was 2cm inferior from the iliac crest and 2 cm posterior from the anteriosuperior iliac spine The biopsies were processed according to the methods described by Chappard et al (1983a) All control subjects and 35 female and 11 male osteoporotic subjects had received two demethyl chlortetracycline labels separated by an interval of 12 days 7 µm sections were cut (K Jung microtome) Four unstained sections were used for the assessment of fluorescent labels For the measurements of

structural indices and osteocyte density non-serial sections were used, and stained with Mallory staining or Goldner staining (eight each) In 31 control and 30 osteoporotic subjects osteoclast numbers and surfaces were measured For these measurements, six sections were stained for the osteoclastic tartrate resistant acid phosphatase (TRAP), and counterstained with phosphomolybic analine blue (Chappard et al , 1983b)

Measurements of trabecular bone volume (BV/TV) and cancellous bone surface (BS) were performed with an automatic image analyzer (Leitz TAS +) at a 25× magnification From these parameters architectural parameters were calculated (Parfitt et al , 1987) In addition, a number of parameters associated with bone formation were measured the distance between double fluorescent labels in order to calculate the mineral apposition rate (MAR), relative osteoid volume (OV/BV), surface (OS/BS) and thickness (O Th) Measurements associated with bone resorption were the relative trabecular surface covered by osteoclastic resorption lacunae (ES/BS), the number of osteoclasts per tissue area (N Oc/T Ar) and the trabecular surface covered by osteoclasts (Oc S/BS) Finally, the number of osteocytes per bone area (N Ot/B Ar) and the area of osteocyte lacunae (Lac Ar) were measured The numbers of osteocytes were counted in trabecular bone Osteocytes are colored red and are readily visible in the bone matrix Twenty fields were sampled in two sections per subject by moving the specimens in equally sized steps in x and y directions In this way, a total bone area of approximately 1 mm^2 per subject was measured to determine osteocyte numbers Lacunar area was measured by outlining at least 75 lacunae in the trabecular bone area per section in randomly selected fields (×100 oil immersion objective), totaling at least 150 lacunae per subject (Sissons et al , 1990, Mullender et al , 1996) For the latter two parameters the intra-observer reproducibility was determined by measuring the parameters six times in four different subjects

The parameters were measured with a semi-automatic system (a microscope and a digitizing tablet connected to a computer) using a magnification of ×100 and ×250 for mineral apposition rate, osteoid thickness, relative osteoclast surface and lacunar area All measured and calculated parameters are listed in Table 1

STATISTICS

To assess the reproducibility of the measured osteocyte number and lacunar area, the coefficients of variation were calculated for repeated measurements Comparison of means between groups was performed with two way analyses of variance (ANOVA) to analyze gender differences and differences between control subjects and osteoporotics and

Table 1 Measured and calculated parameters of bone histomorphometry

Parameter	Abbreviation	Unit	Equation
Bone architecture			
Bone volume	BV/TV	%	
Bone surface to volume ratio	BS/BV	mm^{-1}	
Trabecular thickness	Tb Th	µm	= 2 × BV/BS
Trabecular number	Tb N	mm^{-1}	= (BS/BV) × (BV/TV) / 2
Trabecular separation	Tb Sp	µm	= (1-BV/TV) / Tb N
Bone formation			
Relative osteoid volume	OV/BV	%	
Relative osteoid surface	OS/BS	%	
Osteoid thickness	O Th	µm	corrected by π/4
Mineral apposition rate	MAR	mm/d	corrected by π/4
Bone resorption			
Eroded surface	ES/BS	%	
Osteoclast numer per tissue area	N Oc/TAr	mm^{-2}	
Osteoclast numer per bone area	N Oc/BAr	mm^{-2}	= (N Oc/TAr) / (BV/TV)
Active resorption surface	Oc S/BS	%	
Osteocytes			
Number of osteocytes per bone area	N Ot/BAr	mm^{-2}	
Lacunar area	Lac Ar	$µm^2$	
Total lacunar area per bone area	Lac Ar/BAr	%	= 10^{-4} × N Ot/B Ar × Lac Ar

their interdependence Linear regressions and correlation coefficients between parameters were calculated using the method of least squares

RESULTS

All architectural parameters differed significantly between controls and patients Bone volume was lower and trabeculae were fewer and thinner in osteoporotic patients (Table 2) Trabecular number was more reduced in osteoporotic women, whereas trabecular thickness was more reduced in osteoporotic men However, the differences between control subjects and patients did not depend significantly on gender

Parameters related to bone formation differed significantly between control subjects and osteoporotic patients as well (Table 2) All osteoid indices and the mineral apposition rate were significantly smaller in osteoporotic patients compared to controls Furthermore, osteoid thickness was significantly smaller in men compared to women, independent of the condition

The eroded surface was significantly larger in osteoporotics compared to controls The number of osteoclasts per tissue area, however, was significantly smaller in the

osteoporotic groups (Table 2) This difference was larger in women than in men In addition, the trabecular bone surface covered by osteoclasts was smaller in osteoporotics The number of osteoclasts per bone area was not significantly different between groups

In two female and four male vertebral fracture patients, osteocyte number and lacunar size could not be measured due to the poor quality of the sections The intra-observer reproducibility of both parameters was satisfactory, the coefficient of variation was 6 6% for the number of osteocytes per bone area and 8 4% for lacunar area The number of osteocytes per bone area and total lacunar area per bone area was significantly reduced in osteoporotic patients compared to controls However, these parameters depended even more strongly on gender the number of osteocytes and total lacunar area per bone area were both larger in women compared to men (Table 2)

Between groups of parameters (bone architecture, bone formation, bone resorption and osteocytes) no consistent correlations were found

DISCUSSION

In this study, histomorphometric parameters were compared between postmenopausal women and men of similar ages and between untreated vertebral fracture patients and healthy volunteers In addition to the conventionally measured parameters, the numbers and sizes of osteocytes were measured The most intriguing observation was the marked difference in osteocyte numbers and total lacunar area per area of bone between male and female subjects Females were found to have about 15% more osteocytes than males, independent of the disease It is known that sex steroid hormones have gender-dependent effects on bone cells, bone growth and modeling (Ornoy et al , 1994a, Oursler et al , 1996). Therefore, the difference in osteocyte number might be related to gender-dependent regulation by sex hormones, either indirectly or directly, as estrogen receptors have been identified in osteocytes (Braidman et al , 1995) and in osteoblasts (Oursler et al , 1996) Furthermore, Cheng et al (1994, 1995) found that combined mechanical loading and estrogen administration had a synergistic effect on bone formation in rat ulnae, but only in bones from female rats (Cheng et al , 1995) Parathyroid hormone (PTH) has been shown to potentiate the response of bone cells to mechanical strain as well (Carvalho et al , 1994) PTH receptors are documented in osteoblasts and also in osteocytes and PTH was found to increase cAMP levels in both cell types (van der Plas et al , 1994) Furthermore, differences in PTH secretion profiles between men and women have been reported (Calvo et al , 1991) These findings suggest an interplay between

Table 2

Results of histomorphometric measurements for all groups and statistic comparison (two-way ANOVA) between groups

Variable	Healthy Females X ± SD	Males X ± SD	Osteoporotic Females X ± SD	Males X ± SD	Female vs Male P<	Healthy vs Osteoporosis P<
N[a]	13	21	40	15		
Age (years)	64 4 ± 5 4	67 3 ± 5 6	69 6 ± 11 1	59 9 ± 11 0		
Bone architecture						
Bone volume (%)	22 8 ± 5 9	19 7 ± 6 4	14 2 ± 6 2	12 7 ± 5 4	NS	0 001
Bone surface to volume ratio (%)	15 2 ± 3 3	15 6 ± 3 0	18 5 ± 5 7	20 6 ± 3 9	NS	0 001
Trabecular thickness (µm)	138 ± 32	133 ± 28	117 ± 31	101 ± 20	NS	0 001
Trabecular separation (µm)	479 ± 105	581 ± 182	790 ± 298	786 ± 318	NS	0 001
Trabecular number (mm^{-1})	1 66 ± 0 23	1 47 ± 0 30	1 19 ± 0 31	1 26 ± 0 41	NS	0 001
Bone formation						
Relative osteoid volume (%)	2 80 ± 1 75	2 93 ± 2 04	1 69 ± 1 21	1 97 ± 1 64	NS	0 01
Relative osteoid surface (%)	14 0 ± 7 3	15 0 ± 7 9	8 8 ± 5 1	8 8 ± 5 4	NS	0 001
Osteoid thickness (mm)	15 4 ± 4 0	11 7 ± 5 5	11 2 ± 4 9	8 1 ± 2 9	0 01	0 001
Mineral apposition rate mm/d	0 70 ± 0 08 (N=13)	0 66 ± 0 11 (N=21)	0 52 ± 0 21 (N=35)	0 63 ± 0 21 (N=11)	NS	0 05
Bone resorption						
Relative eroded surface (%)	4 37 ± 1 31	3 85 ± 1 38	5 69 ± 2 47	5 94 ± 2 33	NS	0 001
Osteoclast numer / tissue area (mm^2)	1 60 ± 0 54	1 02 ± 0 37	0 53 ± 0 30	0 59 ± 0 53	0 05	0 001
Osteoclast numer / bone area (mm^2)	7 54 ± 3 12	5 71 ± 2 53	4 03 ± 2 51	5 80 ± 7 26	NS	NS
Active resorption surface	2 53 ± 0 84 (N=12)	2 02 ± 0 89 (N=19)	1 42 ± 0 76 (N=20)	1 36 ± 1 29 (N=10)	NS	0 001
Osteocytes						
Osteocyte number / bone area (mm^2)	271 3 ± 28 2	223 2 ± 29 9	222 6 ± 55 7	198 9 ± 54 2	0 01	0 01
Lacunar area ($µm^2$)	44 8 ± 7 7	41 3 ± 5 2	42 1 ± 5 1	40 7 ± 5 2	NS	NS
Total lacunar area / bone area (%)	1 20 ± 0 18 (N=13)	0 91 ± 0 14 (N=21)	0 94 ± 0 29 (N=38)	0 81 ± 0 27 (N=11)	0 001	0 01

[a] parameters within brackets are parameters for which N's vary from the total N's

osteocyte function estrogen, PTH and mechanical load, which in turn depends on gender Other sex-related differences in bone biology have also been demonstrated such as differences in the distribution of osteocalcin in the extracellular matrix (Ingram et al 1994) and gender-dependent responses to vitamin D metabolites in rats (Ornoy et al , 1994b) However, in order to understand the interdependence of these sex differences further investigations are needed

It was also found that osteoporotic subjects have fewer osteocytes than control subjects This is in contrast to previous findings (Mullender et al , 1996) This discrepancy may be explained by a confounding effect of the mixing of sexes, since in the first study the groups were not matched for gender Moreover, the use of autopsy patients as controls in the previous study may also have affected the results, as it remains questionable if autopsy subjects can be regarded as "normal" (Recker et al , 1988) The meaning of reduced osteocyte density in vertebral fracture patients is unclear Since there is no evidence for osteocyte death in iliac cancellous bone (Parfitt, 1993, Mullender et al , 1996, Dunstan et al , 1993), the number of osteocytes present reflects the number of cells which are embedded into the matrix A reduction in this number may be related to a decreased number of osteoblasts available for embedding in the matrix, since it has been suggested that in aging and osteoporosis defective osteoblast recruitment is a major factor contributing to bone loss (Parfitt et al , 1995, Vernejoul 1989) Likewise, Roholl et al (1994) showed that the maturation of pre-osteoblasts into osteoblasts decreases with advancing age However, no relationships were found between osteocyte number or osteocyte lacunar area and parameters of bone formation Gohel et al (1995) found that the formation of osteocytes in fetal rat bone, i e osteocyte density, was stimulated by insulin-like growth factor I (IGF-I), which is associated with enhanced osteoblastic activity and bone formation In addition, mechanical loading has been reported to affect osteocyte density in rat bones (Li et al , 1991) It was further found that IGF-I mRNA was strongly expressed in osteocytes after mechanical loading of rat bone (Lean et al 1996) Combining these observations, it is conceivable that mechanical loading does not only affect bone mass, but that it influences bone cellularity as well On the other hand, the effects of mechanical strain in bone depend on the bone s capacity to detect it and perhaps also on the number of osteocytes present Moreover, the response of bone to mechanical strain depends on the hormonal environment It is established that PTH levels increase with advancing age Yet, the role of PTH in osteoporosis is still controversial Nevertheless, the indication that PTH enhances sensitivity to mechanical loads (Carvalho

et al., 1994) and the observation that PTH secretion and PTH response to a hypocalcemic stimulus are blunted in osteoporotic women (Silverberg et al., 1996) would favor the hypothesis that PTH protects against age related bone loss (Silverberg et al., 1996).

The values of the architectural parameters in healthy and osteoporotic subjects found in this study are consistent with the results of previous studies (Recker et al, 1988; Recker, 1993; Parfitt et al., 1983; Arlot et al., 1990). Although both in male and female patients trabecular number and thickness were significantly reduced compared to controls, our findings confirm the observations of earlier studies that bone loss in osteoporotic women is primarily due to the loss of whole trabeculae, while in osteoporotic men trabecular thickness is reduced (Scane et al., 1993; Parfitt, 1992; Aaron et al., 1987). The reduced values of bone formation parameters in the vertebral fracture patients indicate that bone formation is impaired in these patients. Reduced osteoid indices and mineral apposition rates in osteoporotics have been reported by other authors as well (Parfitt et al., 1995; Arlot et al, 1990; Moore et al., 1992). The values for osteoclast covered surface and osteoclast number are high in comparison with other studies (Recker et al., 1988), but this is probably due to the use of TRAP as an osteoclast marker (Parfitt, 1993). In contrast with most other studies (Arlot et al., 1990), we found osteoclast number per tissue area and osteoclast surface to be reduced in patients relative to controls. The eroded surface, however, was significantly larger in patients. These results indicate that in these patients overall turnover is reduced compared to controls. The increased extent of surface with erosions may indicate uncoupling between resorption and formation, which is consistent with impaired osteoblast function, or may be the result of a former period of increased osteoclastic activity.

In conclusion, presently there is strong evidence that osteocytes are involved in the mechanical regulation of bone turnover. Osteocytes are the most numerous cells in bone tissue and are located in the most advantageous site to sense mechanical signals. Our results show that the numbers of osteocytes which are embedded in the bone matrix depend strongly on gender and differ between healthy and osteoporotic people. Patients with vertebral fractures showed changes in bone architecture typical for osteoporosis and had reduced bone turnover. These differences may present evidence that alterations occur within the whole population of cells of the osteoblastic lineage. At present one can only speculate on the physiological meaning of this finding. Further research is needed to elucidate the interplay between the role of osteocytes, mechanical load, hormones and other factors.

ACKNOWLEDGMENT

The authors thank Dorien van der Meer for assisting with the measurements of osteocyte density.

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8 THE EFFECT OF THE MECHANICAL SET POINT OF BONE CELLS ON THE MECHANICAL CONTROL OF TRABECULAR BONE ARCHITECTURE

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Bone (in press)

ABSTRACT

The architecture of trabecular bone is thought to be controlled by mechano-sensitive bone cells, where hormones provide a background for their responses to mechanical signals. It has been suggested that in osteoporosis this response is hampered by changed hormonal levels, thereby reducing the mechano-sensitivity of the cells or, in other words, increasing their mechanical set point, which would lead to bone loss. We have investigated if a temporary increase of the mechanical set point causes deterioration of trabecular bone architecture such as seen in osteoporosis. Furthermore, the effects of a changed loading pattern were investigated for the same reason. For this purpose, we used a computer simulation model, which was based on the regulation of bone architecture by mechano-sensitive osteocytes. It was found that a temporary shift of the mechanical set point causes no lasting changes in architecture. Although an increase of the mechanical set point induces bone loss, the mechanism of bone loss (trabecular thinning) differs from what is observed in osteoporosis (loss of whole trabeculae). Hence, a change of the mechanical set point alone cannot explain bone loss as seen in osteoporosis. On the other hand, the removal of load components in a particular direction resulted in irreversible loss of whole trabeculae. These results indicate that such temporary changes in loading patterns could be important risk factors for osteoporosis.

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INTRODUCTION

The architecture of trabecular bone is thought to be related to the mechanical loads to which it is subjected This balance between bone architecture and mechanical loading is a dynamic one, where bone is constantly being resorbed and new bone is formed In osteoporosis, however, bone is inadequately maintained, leading to inferior quality of bone architecture and osteopenia (Kleerekoper et al , 1985, Parfitt, 1984) Because the normal regulation of bone turnover is complex, with many factors involved, the causes for bone deterioration are unclear Rodan (1996) summarized its essence as a process which is controlled by mechanical loading in an hormonal environment Yet, the precise role of mechanical factors in the process of bone remodeling is unclear

In previous studies, we investigated the effects of mechanical factors on trabecular bone architecture, using computer simulation models (Mullender et al , 1994, Mullender and Huiskes, 1995, Van Rietbergen et al , 1996a) We assumed that bone turnover is controlled locally by mechano-sensitive osteocytes In these simulations, a piece of bone tissue was modeled with finite elements to determine local mechanical quantities The specimen was loaded at the boundaries, and the mechanical adaptation process was simulated until a balance between loading and bone morphology was reached The assumed process could explain the genesis and adaptation of trabecular patterns These patterns resembled typical trabecular architectures, including plates and struts (Van Rietbergen et al , 1996a), which aligned with the external load orientations (Mullender and Huiskes, 1995) This model demonstrated that the hypothesis of bone remodeling as a locally regulated process, governed by mechanical signals, sensed by osteocytes, is feasible

In this study, the question is addressed whether temporary alterations in mechanical loads or deficiencies in the metabolic response to mechanical loads could be responsible for the deterioration of bone architecture Several authors proposed that osteoporosis is associated with alterations in the response of bone cells to mechanical loads (Frost, 1987, 1992, Kimmel, 1993, Rodan, 1996), in other words, a change in the mechano-sensitivity (set point) of sensor cells We tested this hypothesis, using the osteocyte-regulated bone-remodeling theory In addition, the effects of temporary changes of mechanical loads on trabecular bone architecture were investigated To permit comparison of the results with phenomena as seen in real trabecular bone, the theory was applied to a three-dimensional finite element model of a reconstructed trabecular bone specimen from a vertebral body

Within the computer simulation model, the mechanical set point of the osteocytes and mechanical loads were varied, and their effects on the trabecular architecture evaluated

METHODS

MATERIAL

A core of trabecular bone was excised from the 4th lumbar vertebra of a 37 year old male autopsy patient The subject had no history of bone disease The specimen was digitized using a micro-CT scanner (Ruegsegger et al , 1996) with a resolution of 28 μm The CT-image was processed to obtain the trabecular morphology of the specimen (Ruegsegger et al , 1996) From the total reconstruction a 3 9 mm cubic specimen was selected for use in the model The axes of the cube were oriented along the anatomical axes The digital reconstruction was reduced to a courser grid by grouping 7x7x7 voxels to one new voxel, which measured 98 μm on each side A density value was assigned to each voxel, varying between 0 003 and 1, depending on the number of original "bone voxels" per new voxel The bone tissue was modeled by finite elements (FE) for the calculation of the local signal distribution from the external loads The model was generated by directly converting each voxel in the reconstruction into one element with material properties calculated from the relative density (eq 4) In order to analyze the relatively large three-dimensional model, a special purpose FE-code was used, which was specially developed to solve large scale FE problems (van Rietbergen et al , 1995a, 1996c)

MODEL FOR MECHANICAL REGULATION OF BONE REMODELING

The regulatory process according to Mullender and Huiskes (1995) was applied to the FE model of the reconstructed bone specimen (Fig 1) It assumes that osteocytes are distributed in a network throughout the bone tissue The external mechanical load is transmitted through the tissue in the architecture, whereby each osteocyte senses a particular mechanical signal After evaluation of the signal, the osteocyte stimulates populations of osteoblasts and osteoclasts, the basic cellular units (BMUs), to regulate net bone turnover It is further assumed that the effect of each osteocyte on a BMU depends on the distance from the osteocyte At each location in the bone tissue, the total stimulus value is evaluated and bone mass is adapted according to its magnitude This process is continued until a balance between the external loads and bone architecture is reached

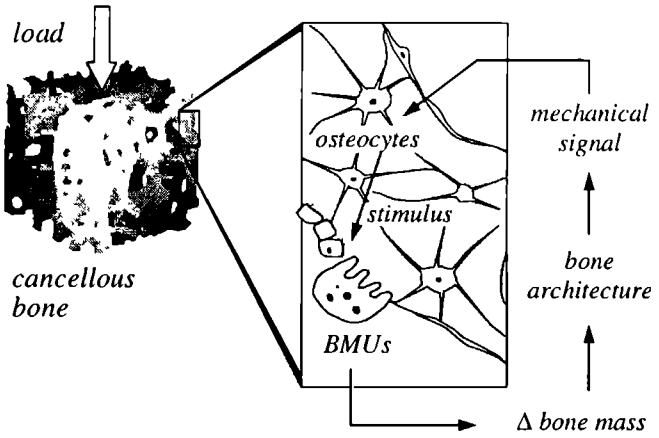

Figure 1
The assumed feed-back control process for bone adaptation. The osteocytes sense mechanical signals. Consequently, they stimulate populations of osteoclasts and osteoblasts (bone multi-cellular units) to add or remove bone. The changed architecture affects the local mechanical loading of the structure, which in turn affects the signal sensed by the osteocytes.

MATHEMATICAL FORMULATION

For the mechanical signal S, sensed by each osteocyte, we take the strain energy density (J/mm^3) (Huiskes, 1997). According to the difference between the actual signal and a reference signal k, the osteocyte produces a stimulus. The amount of stimulus received by a BMU depends on the distance between the osteocyte and the location of the actor cells. Hence, the stimulus value $F(\boldsymbol{x},t)$ at location $\boldsymbol{x}$ at time t is the sum of the stimuli received from all osteocytes:

$$F(\boldsymbol{x},t) = \sum_{i=1}^{N} f_i(\boldsymbol{x})\,(S_i(t) - k), \qquad (1)$$

with N the number of osteocytes and the spatial influence function

$$f_i(\boldsymbol{x}) = e^{\frac{-d_i(\boldsymbol{x})}{D}}, \qquad (2)$$

which describes the reduction of stimulus with increasing distance $d_i(\boldsymbol{x})$ (mm) between osteocyte i and location $\boldsymbol{x}$. The parameter D determines the gradient of the decline.

The change in the relative density $m(\boldsymbol{x},t)$ in location $\boldsymbol{x}$ is governed by the local stimulus value $F(\boldsymbol{x},t)$. Hence,

$$\frac{dm(\boldsymbol{x},t)}{dt} = \tau\, F(\boldsymbol{x},t) \quad with\ 0 < m(\boldsymbol{x},t) \leq 1, \qquad (3)$$

where τ (MPa^{-1}s^{-1}) is a constant regulating the rate of the process The local elastic properties of the tissue $E(x,t)$ were calculated from the local relative density using a cubic power law

$$E(x,t) = C\, m^{3}(x,t), \qquad (4)$$

with C (MPa) a constant (Currey, 1988)

The development of bone architecture in time was simulated numerically (Fig 2). A variable time step (Δt_i) was used, which was calculated from a maximal change in density in all elements (Δm_{max}) (van Rietbergen et al , 1996a), with the restriction that $\Delta t_i \leq 2\Delta t_{i-1}$

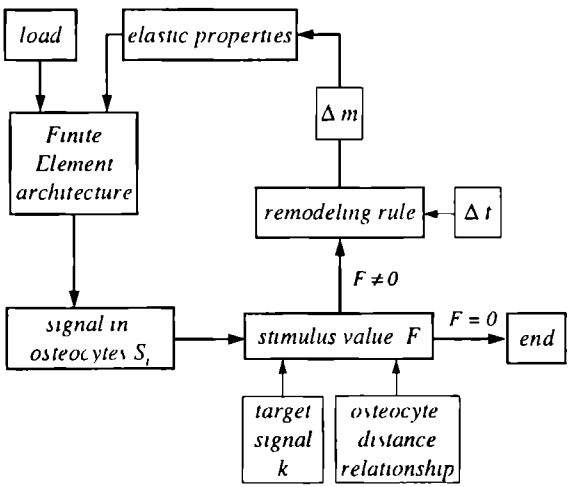

Figure 2
Schematic representation of the computer simulation model

REMODELING SIMULATIONS

The physiological parameters in the models are the reference signal k, the sensor density n, the exponential osteocyte-influence function (characterized by the distance parameter D), the rate constant τ, and the constant C, which is equal to the maximal elastic modulus of bone tissue The reference signal k is the mechanical "set point" of the osteocytes, an increase of its value results in a decrease of the stimulus produced by the osteocytes, for the same mechanical load The osteocyte density n was chosen within a

physiological range as 10,625 mm^{-3} (Mullender et al., 1996) and D was 117 μm (Mullender and Huiskes, 1995). C was taken as 10 GPa (Rho et al., 1993) and the rate constant was arbitrarily set at $\tau = 0.1$ $mm^3/J \cdot s$. The maximal change in density allowed per time step Δm_{max} was 0.1.

The axes of orthotropy of the architecture were determined according to van Rietbergen et al., (1996b). As these axes coincided with the anatomical axes, the specimen was loaded in these orientations, as compressive stresses distributed over all 6 faces. The loads in the antero-posterior (x) and medio-lateral (y) directions were both 1 MPa, the load in the cranio-caudal (z) direction was 2 Mpa (Fig. 3, load case A). The set point k was assigned a value of 0.02 J/mm^3 and the process of bone remodeling was simulated for 50 increments, at which time changes in architecture were minimal. The new architecture was used as the starting configuration for a number of variations. The effects of a change in the mechanical set point for the osteocytes was assessed for two variations of k: first, k was increased from 0.02 to 0.03 J/mm^3 and then k was increased from 0.02 to 0.20 J/mm^3, after which its value was restored to 0.02 J/mm^3. The effects of changes in load were investigated for two cases as well. First, the load was reduced by removing compressive forces in the transverse directions, leaving only the load of 2 MPa in the vertical direction (Fig. 3, load case B), and then the process was restarted after restoring the original loading situation (load case A). Second, the load was changed by rotating the loading configuration by 45° around the x-axis, relative to the specimen (Fig. 3, load case C). The remodeling simulation for each of these variations was also continued for 50 increments. The resulting architectures were evaluated by their morphologies and by characterization of their apparent mechanical properties, according to Van Rietbergen et al. (1996b).

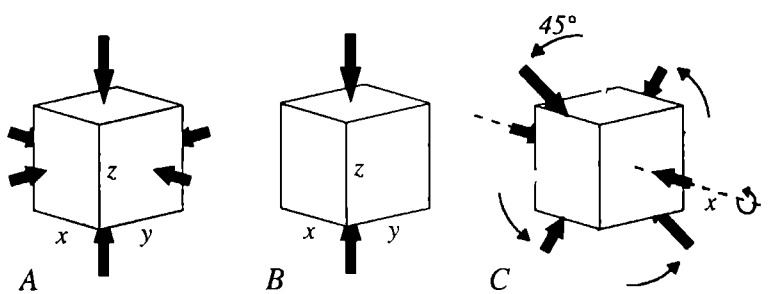

Figure 3
The directions of the applied loads are illustrated for the three load cases (A, B, C).

RESULTS

During simulations the remodeling process maintained a trabecular architecture. The relative apparent density and apparent stiffness values (in the orthotropic main directions) for all architectures are given in Table 1. The initial morphology adapted to the applied loads by increasing the overall density (Table 1), after which a balance between the loads and the relative apparent density was obtained. This was accomplished by thickening of the existing trabeculae and by the formation of thin plates at the surfaces of the cube (Fig. 4). The overall stiffness of the specimen increased by approximately 150%, without changing the anisotropy of the specimen (Table 1). Increasing the mechanical set point, i.e. the reference signal k, to 0.03 J/mm^3, reversed this, resulting in loss of bone mass to almost that of the original architecture (Table 1). Again, the change in bone mass was mainly the result of a modulation of trabecular thickness without significant changes in architecture (Fig. 4). Even a ten-fold increase of k to 0.20 J/mm^3 did not change the integrity of the architecture significantly. Although the relative apparent density reduced considerably (Table 1), due to extreme thinning of the trabeculae, few trabeculae were actually lost (Fig. 4). Nevertheless, the stiffness of the specimen was reduced dramatically (Fig. 5). Restoration of bone mass, architecture and apparent mechanical properties were almost complete after reparation of k to 0.02 J/mm^3 (Fig. 4, Table 1).

Table 1
The relative apparent density values and the orthotropic elastic moduli for the reconstructed specimen and for the architectures produced by the model.

	Relative apparent density (%)	**Apparent Young's moduli** Exx (GPa)	Eyy (GPa)	Ezz (GPa)
Architecture				
Original specimen	13	0.15	0.12	0.34
After initial remodeling	20	0.24	0.21	0.49
Variation of *k*				
k = 0.03 J/mm^3	16	0.17	0.16	0.38
k = 0.20 J/mm^3	9	0.04	0.04	0.09
reversal of k to 0.02 J/mm^3	19	0.23	0.23	0.49
Variation of loading				
load case B	14	0.14	0.15	0.69
reversal to initial load case A	20	0.28	0.29	0.58
load case C	19	0.23	0.19	0.39

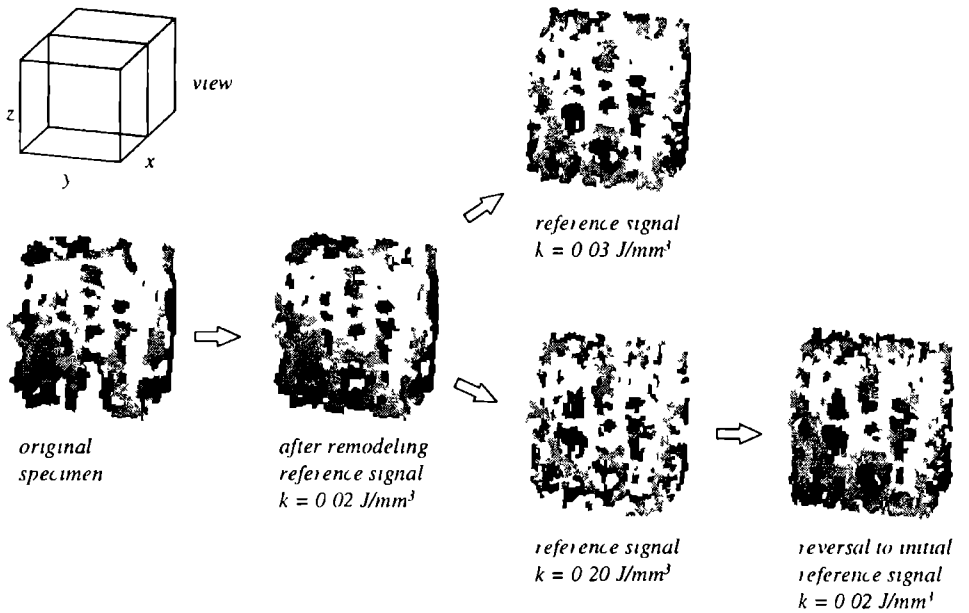

Figure 4

The initial architecture and the architectures after remodeling are shown for the variations of the mechanical reference signal k To reveal the internal structure, only half of the model is shown and the top layer is omitted

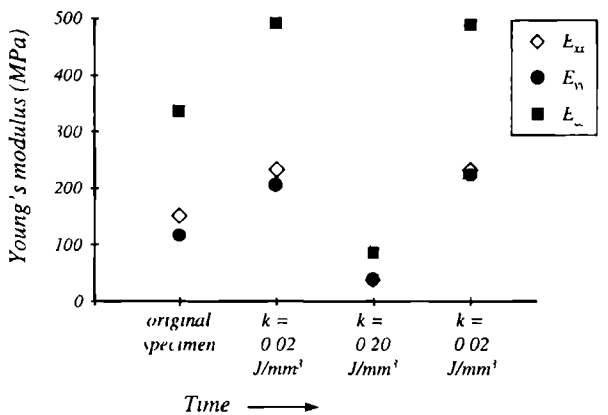

Figure 5

The changes in the orthotropic Young's moduli are shown for the subsequent variations of the reference signal Initially the apparent stiffness of the architecture increases After increasing the value of k to 0 20 J/mm³ the stiffness reduces significantly, however, reparation of k to its initial value restores the apparent stiffness completely

In contrast, removing the loads in the transverse plane led to removal of almost all horizontal struts and caused better alignment of the remaining trabeculae with the vertical axis In addition, a few plate-like structures that were present transformed into vertical columns, i e strut-like structures (Fig 6) This resulted in a highly anisotropic architecture with increased stiffness in the vertical direction (Table 1) The relative apparent density was reduced from 20% to 14% (Table 1) After reapplying the loads in the transverse plane, no new horizontal trabeculae were formed The trabeculae that were still connected thickened and the plates at the top and bottom faces thickened as well (Fig 6) After an equilibrium situation was reached, the apparent relative density was returned approximately to the value before removal of the loads (Table 1) Nevertheless, the apparent stiffness in the three loading directions was about 20% higher than before elimination of the horizontal loads (Table 1)

Altering the loading direction resulted in transformation of the architecture and adjustment of the apparent material properties to the new loading directions During the transformation process, some trabeculae were lost, leaving a structure with fewer and slightly thicker trabeculae (Fig 6) Nevertheless, the new apparent mechanical properties

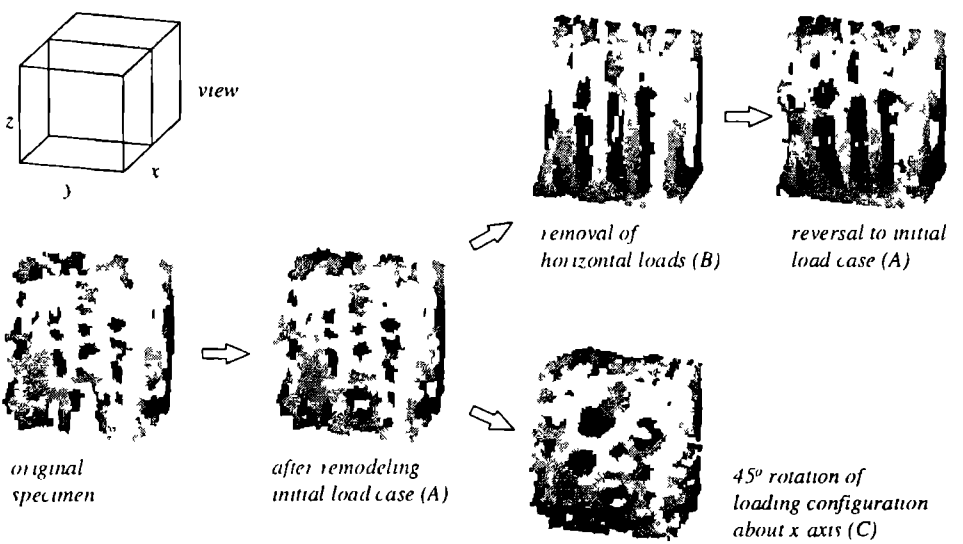

Figure 6

The trabecular architectures are presented for variations of the applied loads Trabecular elements that are lost after removal of transversal loads (B) are not restored after reversal of the loading configuration Rotation of the loads (C) resulted in re-alignment of trabeculae

of the architecture relative to the applied loads differ from those after initial remodeling, in the sense that the stiffness values in the orthotropic directions (which coincide with the directions of loading) are lower.

DISCUSSION

We have tested the hypothesis that osteoporosis is caused by a shift in the mechanical set point of osteocytes, which we assumed to be the mechano-sensors. In addition, we have looked at the effects of changed loading conditions on the trabecular architecture. For this purpose, a regulatory process was studied, using a computer simulation model in which the architecture is controlled by mechanical load only. Although, it is still unclear how bone cells sense mechanical signals, the hypothesis that osteocytes are the mechano-sensors in bone has gained support (Marotti, 1990; Cowin et al., 1991; Lanyon, 1993; Aarden et al., 1996; Parfitt, 1996). Furthermore, osteocytes have shown to metabolically respond to mechanical loading (Pead et al., 1988; Skerry et al, 1989; Klein-Nulend et al., 1995; Lean et al., 1996). It was shown earlier that this process produces patterns resembling typical architectures of cancellous bone (Mullender and Huiskes, 1995; Van Rietbergen et al., 1996a). With the use of true three dimensional trabecular architectures as input for the model, it is possible to investigate the effects of several parameters in the proposed regulation process. The results can be compared with genuine bone structures, because they can be assessed in the same way as real bone. The advantage is that mechanical variables can be completely controlled and that the three-dimensional morphological and mechanical properties of the architectures can be fully characterized.

It was indicated that such a nonlinear control process is able to generate many different morphologies, which are in fact metastable (Weinans and Prendergast, 1996). Perturbation of the system can cause it to converge to a significantly different morphology and may result in a degenerated state. Nevertheless, it was found that the original trabecular architecture was remarkably stable for the loading configuration applied. Except for changes at the boundaries and modulations of trabecular thickness, the architecture remained virtually unchanged. This can be interpreted in two ways. First, it indicates that in reality the vertebral body was loaded in a similar way as was simulated in the model. Secondly, it shows that an existing morphology is quite resistant to perturbations. Moreover, the convergence of the local control process to an architecture that is basically equivalent to the original one confirms the feasibility of the assumed control process.

Frost (1987) suggested that bone loss in osteoporosis is due to a shift of the mechanical set point to higher values. It was proposed that sensitivity of bone cells to mechanical loads is modulated by hormones, which in turn affects maintenance of bone mass (Kimmel, 1993). This assumption was further confirmed by findings that estrogen and parathyroid hormone, which are both associated with changes in bone mass, potentiate the response of bone cells to mechanical stimuli (Cheng, 1994; 1995; Carvalho et al., 1994). Increasing the target signal in the model indeed resulted in the expected loss of bone mass. However, the mechanism of bone loss differed fundamentally from that observed in osteoporosis. In osteoporosis, bone mass is lost primarily by complete removal of trabeculae (Parfitt et al., 1983; Recker et al., 1988; Moore et al., 1992; Steiniche et al., 1994), whereas changing the mechano-sensitivity in the model caused trabecular thinning predominantly. Furthermore, bone loss and reduction of mechanical stiffness were almost completely reversed after restoring the set point to the initial value. Yet, the current consensus is that in osteoporotic patients reversal of bone loss is highly unlikely, even after treatment or after physical exercise. Hence, these results do not substantiate the hypothesis that a change in the mechanical set point is the main cause of osteoporosis, as it cannot explain the mechanism of bone loss as observed in osteoporosis. A different mechanism, which explains the perforations and disconnections of trabeculae must be responsible for the loss of whole trabeculae. Of course, once a trabecula is disconnected, resorption of the remainder may be explained by mechanical adaptation (Mullender and Huiskes, 1995). Nevertheless, if the mechanical set point increases, the initial change in architecture should be trabecular thinning.

In contrast, elimination of the loads in the horizontal plane caused complete resorption of horizontal struts. This pattern of bone loss is similar to that observed in aging and osteoporosis (Mosekilde, 1990). Re-application of the horizontal loads did restore the apparent mechanical properties of the structure, but not the initial architecture. Instead, the remaining trabeculae and the "end plates" of the specimen thickened. Rotation of the loads caused re-alignment of the architecture and loss of some elements. However, many features of the previous architecture were preserved. Whether such changes in loading patterns play a role in the pathogenesis of osteoporosis is unknown, but degeneration of the intervertebral disk, atrophy of muscles and ligaments and relative immobility are probable causes for changed loading patterns and therefore may be important risk factors contributing to osteoporosis.

The distinct patterns of bone loss for altering the mechano-sensitivity and for changing the loading configuration, may partly explain the differences found between several experimental disuse models In fact, increasing the mechanical set point in the model is analogous to a general reduction of the applied loads This can be seen directly from equation (1), in which an increase of the reference signal k is equivalent to a decrease of the mechanical signal values $S_i(t)$ Thinning of trabeculae after disuse was described by several authors (Schaffler and Pan, 1992, Thomas et al , 1995, 1996, Biewener et al , 1996), but loss of trabecular elements has also been reported (Li et al, 1990, Palle et al , 1992) Furthermore, Krolner and Toft (1983) reported that bone loss in the lumbar vertebrae after bed rest was almost completely restored after re ambulation, whereas other authors reported incomplete recovery of bone loss after a period of disuse (Jaworski and Uhthoff, 1986, Kannus et al 1996) Our results indicate that alterations of the loading patterns or selective disuse may lead to loss of trabeculae and that general disuse (as for instance after tenotomy, e g Biewener et al , 1996) or a change of the mechanical set point primarily causes trabecular thinning In other words, a change in the loading pattern can cause the system to converge to a different state with fewer trabecular elements, whereas a change of the mechanical set point does not perturb the system enough to change it to a different architecture

Some limitations of the model one should keep in mind when discussing the results First, the method is computationally costly, which restricts the size of the model that can be analyzed As a whole vertebra is too large for analysis, the boundaries and application of loads are artificial This results in artificial densifications at the specimen boundaries, but it also causes a relatively homogeneous distribution of the loads, which favors preservation of bone near the perimeter This phenomenon can be seen in real vertebrae as well, where trabeculae are also better preserved near the endplates, i e near the location of load introduction Although the architectures are affected by this limitation, it is unlikely that it affects the mechanisms of bone adaptation The second limitation is that only net bone loss or gain was considered Hence, the model does not account for separate effects of osteoblasts and osteoclasts and effects associated with the remodeling sequence Clearly, these effects are important For instance, the initial action of osteoclasts may be responsible for disconnection of trabeculae, for which the model did not account It is conceivable that thin trabeculae have a high chance of being perforated by osteoclasts, such that trabecular thinning will ultimately lead to loss of trabeculae as well In order to investigate the combined effects of mechanical control and the remodeling sequence, a

more detailed model is necessary Thirdly, no failure criteria were incorporated in the model This implies that the trabeculae in the model do not fracture Even when the deteriorated architectures are fully loaded, failure will not be predicted In reality, however, fracture would occur and this would in turn affect the remodeling process To prevent fractures, loading should be increased gradually Finally, the model is too coarse to obtain a precise measure of the mechanical signals that osteocytes perceive Therefore, strain energy density, which is a measure of the energy stored as a result of deformation of the tissue, was chosen as the mechanical signal (Huiskes, 1997) It may be considered as a reasonable indication of the mechanical deformation which the cells experience Moreover, it was shown that the concentrations of potassium and sodium ions in osteocytes assume a similar pattern as the distributions of strain energy density (McDonald and Yettram, 1995)

To conclude, the remodeling hypothesis can explain three-dimensional adaptation of trabecular bone The method offers the possibility to validate results of computer simulations relative to real bone architectures The morphology of the reconstructed vertebral bone specimen was roughly adapted to loads in vertical and horizontal directions The architecture is quite stable when loaded in its structural directions A shift of the mechanical set point per sé cannot explain the loss of trabecular elements as seen in osteoporosis, but may reflect bone loss after general disuse However, a change of the loading pattern, the removal of horizontal loads in particular, causes loss of whole trabeculae as seen in osteoporosis Hence, changes in the loading pattern may contribute to the pathogenesis of osteoporosis

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9 DISCUSSION

The process of trabecular bone remodeling is subject to extensive study in many fields of research The interest in this topic is the consequence of its prominent role in clinical problems such as osteoporosis, other metabolic bone diseases, and prosthetic loosening Whereas endocrinologists focus on the effects of hormones, drugs and many other local and systemic factors, with regard to metabolic bone diseases (Eriksen and Kassem, 1992), biologists investigate the cells involved in the process, by exposing their behavior, chemical pathways, and gen expressions under different circumstances (Rodan, 1992) Orthopaedic surgeons, on the other hand, are mainly interested in the outcome of the remodeling process (Rosenberg, 1989) while engineers translate it into mechanical terms, for instance, predicting changes in bone density after altered loading conditions with the use of computer models (Huiskes and Hollister, 1993, Huiskes, 1995a, 1995b, Huiskes and van Rietbergen, 1995) It is not easy to integrate information from all these research areas into a coherent theory of bone remodeling What is clear, however, is that it is a very complex process, which is influenced in every phase by many different factors

The more remarkable is it to find that a very simple control mechanism, as described in the previous chapters, can explain so many features of the bone remodeling process First of all, it explains the emergence of trabecular patterns, regardless of the initial morphology Although the actual patterns depend on the initial ones, when exposed to comparable mechanical environments, their properties are similar This could explain the resemblance of trabecular patterns of different individuals in the same anatomical location More importantly, it would provide a relatively consistent mechanical quality of the trabecular structure for certain loading conditions The advantage is, that this quality is not introduced in the process as a prerequisite, but as its outcome Furthermore, the typical structures that are found in bone, plates and struts, can be explained as a result of the type of loading (van Rietbergen et al , 1996) It was also found that the regulation scheme

produces densifications at boundaries where loads are applied, and ramifications towards the boundaries, these phenomena can also be observed in real trabecular bone (Currey, 1984) Finally, the observations described by Wolff (1892), that the trabecular orientation is closely related to the direction of load transfer, are reproduced by the proposed control process Our aim was to investigate the feasibility of the regulation scheme, first proposed by Roux (1881) In spite of the simple character of the regulation model, its dynamic, non-linear nature make its behavior complex and often unpredictable This feature in itself is reminiscent of the actual process, however, it complicates verification of its validity Still, the many similarities between the outcomes and trabecular bone itself allow the conclusion that the proposed control mechanism is indeed feasible Or perhaps more generally, that the trabecular architecture may well be the result of a dynamic process of self-organization

In this thesis we have focused on the macroscopic level of the trabecular architecture, which is also the most important limitation of the study We did not consider trabecular bone at the apparent level Only very small volumes of bone were studied This restricts especially the representation of realistic loading conditions, which in turn hampers validation of the models The size of the volume studied is limited by the amount of computer time associated with its analysis Since computer capacity and speed are growing continuously, this limitation will probably be solved in due time It will then be interesting to model, for example, a whole vertebra to investigate the effects of mechanical factors on its trabecular architecture The trabecular tissue itself was modeled as a continuum The sensor cells were represented in a simplified manner, and only net changes in bone mass were considered, whereby the separate activities of osteoclasts and osteoblasts are ignored In order to further unravel the regulation process, and to investigate the activity of bone cells, a more detailed model, at the microscopic level, is needed, thereby deserting the continuum assumption Following this avenue, it will become possible to include the role of other factors, besides mechanical ones, known to affect the cellular behavior, such as hormones and drugs Eventually, by studying the interplay between these factors, we could begin to understand the process of bone remodeling

However, before complicating matters, it is useful to see what can be learned from the simple model we introduced It showed, for instance, that the rate of remodeling was strongly related to the number of sensor cells The trabecular morphology, however, was insensitive to the density of the sensors (above a certain threshold) In a number of

different mammalian species, it was indeed found that, whereas the density of osteocytes varied over a wide range, the histomorphometric parameters varied relatively little Furthermore, it was discovered that the scale of the structure was determined by the distance over which the mechanical signal can affect the activity of the actor cells The average thickness of the trabeculae was found to be in the same order of magnitude as the domain of influence of the osteocytes These results suggest a significant role for the osteocyte network in bone Communication through this network over a certain distance might ensure the relatively constant dimensions of trabecular bone Regulation of these dimensions is important, because, both excessively thick and thin trabeculae are disadvantageous Thin trabeculae increase the probability of perforation by osteoclastic resorption and may therefore lead to loss of trabecuale In addition, the stiffness of the tissue itself is probably lower for very small dimensions of the trabeculae, which in turn causes a reduction of the apparent stiffness for a given bone volume fraction (Choi et al , 1990) On the other hand, thick trabeculae would increase the amount of material unnecessarily and would therefore be inefficient Unfortunately, not much is known about the communication between osteocytes and other bone cells The functions of the osteocyte network are unclear and it is still possible that it affects these cells However, if the domain of influence of osteocytes is considered as being relatively constant, the remaining parameters that influence the trabecular architecture are the initial architecture, i e its history, the mechano sensitivity of the osteocytes, and the mechanical loads Whereas the mechano sensitivity (or the mechanical set point) and the magnitudes of the loads predominantly determine the bone volume fraction, primarily by modulating trabecular thickness, the directions and proportions of the loads determine the anisotropy The effects of the initial architecture and the loading history were not thoroughly investigated in this thesis Nevertheless, it was shown that after a trabecular architecture was perturbed such that it remodeled to a different state, this was often attended with loss of elements Elements that are lost will never be regained This indicates that extensive remodeling activity, without changing the loading magnitude or mechanical set point, could intrinsically form a risk for deterioration of the architecture In this view, history might be a very important parameter for the properties of a trabecular architecture and deserves further attention

Disturbances of the bone remodeling process can cause bone disease It is the general assumption that osteoporosis is caused by such a disturbance Essential for treatment is knowledge about where the regulation process is disturbed However,

osteoporosis is probably the manifestation of a number of different disorders, each causing deterioration of the trabecular architecture and a reduction of bone mass Osteoporosis has been associated with increased activity of osteoclasts (Eriksen et al , 1990) and diminished recruitment or activity of osteoblasts (Parfitt et al , 1981) The underlying assumption of inherent changes in cellular activity would be that the bone tissue is continuously being overloaded, yet bone formation cannot keep up with the demand for more bone Another possible cause that has been suggested is that of a reduced sensitivity of bone cells to mechanical loads (Frost, 1987, 1992, Kimmel, 1993) In this case, the load is sensed inadequately, and the bone responds as if it is being disused, which induces bone loss Of course, reduced mechanical loading is of itself known to result in loss of bone mass (Jaworski and Uhthoff, 1986, LeBlanc et al , 1990) Our regulatory model suggests that additional possibilities for malfunction exist First, altered properties of the osteocyte network might cause alterations in the trabecular dimensions, resulting in a less efficient structure Secondly, perturbations that cause extensive remodeling, such as changes in the loading patterns or even elevated bone turnover, may by itself cause loss of trabeculae and deterioration of the trabecular architecture The feasibility of these possibilities needs to be investigated further

Measurements of osteocyte density and lacunar area (i e the area of osteocyte lacunae) indicated that differences do exist in the osteocyte network between healthy subjects and osteoporotic patients and between men and women Although it was first concluded that osteoporotic patients have more osteocytes than normal subjects, the opposite was found in a second study In the later study it was also found that a significant difference existed between men and women This dependency on gender probably confounded the results of the first study The precise meaning of these differences is not clear yet Again, in order to unravel this further it is necessary to study the regulation of bone remodeling at the microscopic level Osteocytes have only recently regained interest Because of their inaccessibility, not much is known about these cells The fact that their density depends strongly on gender and also differs between healthy subjects and subjects with vertebral fractures, suggests that it is worthwhile to further investigate their function

The central theme of this thesis is Roux's hypothesis, that the trabecular architecture is controlled by cells, where cells are sufficiently stimulated by mechanical loads, bone will form, where they are not stimulated enough, bone will disappear The process, as shown in this thesis, is likely to be one of self-organization, just as Roux suggested We have shown that it is a realistic hypothesis, but it will probably take many

years before we know if this hypothesis is in fact the "truth", if we can ever be sure of such a thing In the mean time, it can be used as a guideline, for understanding the behavior of a complex system, for investigating possible effects of certain parameters, and as a starting point for experiments, suggesting new questions And after all, this is what science is all about, is it not?

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SUMMARY

Trabecular bone tissue consists of a complex three-dimensional structure of plates and struts. It is essential for the mechanical integrity of bone, especially in the vertebrae and the extremities of long bones, and as such, it plays an important role in load bearing. Its architecture is closely related to the mechanical loading patterns, in the sense that the material directions correspond with the main loading directions.

Trabecular bone is continuously being remodeled by specialized bone cells; osteoclasts degrade small amounts of bone and osteoblasts refill the resorption cavities with new bone. With aging, bone mass is gradually lost and as a result the trabecular architecture becomes weaker. Although this is a normal process, in one third of the population, the architecture degenerates excessively, such that it becomes extremely fragile and may break after minimal trauma. This condition is known as osteoporosis.

In this thesis we have investigated the regulation of the process of bone remodeling with the emphasis on the role of mechanical factors. Very little is currently known of how bone architecture is controlled. Nevertheless, it is well known that it is affected by mechanical loading. In the previous century, Roux suggested that the trabecular architecture is the outcome of a process, in which bone mass is controlled locally by cells under the influence of mechanical stresses. Bone is removed at locations where stresses are too low and bone is formed or maintained where stresses are sufficiently high. In chapters 2 and 3, a computer simulation model was developed to investigate the feasibility of such a regulation process. The cells that control the adaptation process were assumed to be the osteocytes, i.e. bone cells that are embedded within the mineralized bone matrix. It was found that the model produced trabecular-like architectures, starting from any initial configuration. This formation of patterns was due to the non-linear dynamic behavior of the control process. The model could explain adaptation to changes of the applied loads; after the directions of the external loads were changed, the trabeculae aligned with the stress orientations. As in reality, the relative apparent density of the structure depended on the magnitude of the applied stresses. The number of osteocytes influenced the remodeling rate, and the domain of influence of the osteocytes affected the refinement of the structure. The distant control of actor cells by osteocytes was found to be fundamental to the genesis of trabecular-like structures, and to adaptation of the architecture to changes of the loads. Furthermore, it prevented the numerical inaccuracies, which were described in earlier studies.

The model results induced us to study the actual relationships between trabecular morphology and osteocyte density In chapter 4, these relationships were determined in cancellous bone of the proximal femur in five mammalian species of diverse sizes (rat, rabbit, Rhesus monkey, pig and cow) The measurements showed that osteocyte density varied considerably between species and was inversely related to the size of the species Differences were also found in histomorphometric parameters, but the range of variation between species was relatively small Hence, if the model predictions are accurate, these results indicate that the domain of influence of osteocytes is of similar size in all of these species The rat, however, proved to be an exception, trabecular thickness in rats was considerably smaller than in the other species Probably, this is due to the presence of the cartilaginous growth plate in the femoral head of the rat, which introduces a different mechanical and biological environment compared to the other species The relationships with species were found to be different for osteocyte density and morphometric parameters Hence, these data suggest no direct relationship between osteocyte density and the macroscopic trabecular architecture, which is consistent with the results of the computer model

It was assumed that osteocytes are the mechano-sensors in bone However, no proof exists of the mechanism for sensing mechanical signals in bone In chapter 5, we investigated if lining cells or osteoblasts could possibly play a similar role as effectively with regard to their capacity for self-optimization of the trabecular architecture, in terms of a low apparent mass to stiffness ratio The behavior of two models of bone remodeling were compared, first a regulation model with osteocytes as mechano-sensors and second with mechano-sensors located at bone surfaces It was found that the surface cell remodeling algorithm was less sensitive to changes in the loading pattern, which resulted in less efficient bone adaptation This was reflected by a considerably higher relative mass for a similar apparent stiffness in the loading direction, i e more mass was needed to obtain an equally stiff architecture, with respect to the externally applied loads Furthermore, stresses and strains at the tissue level varied across a much wider range, relative to the osteocyte model These results indicated that osteocytes would be more efficient sensors than lining cells and osteoblasts, although the superior performance of osteocytes as sensors gives no direct evidence that they actually fulfill this role.

The relationships between osteocyte density, histomorphometric parameters, osteoporosis, and gender and were investigated in chapters 6 and 7 Measurements were performed in trabecular bone from the iliac crest In the first study, it was found that

osteocyte density declines with advancing age Subjects with hip or vertebral fractures were found to have more osteocytes then normal subjects However, opposite results were obtained in the second study Patients with vertebral fractures had reduced osteocyte numbers and reduced total lacunar area Furthermore, females were found to have markedly more osteocytes and higher total lacunar area per bone area than males, independent of the disease The contradictory findings may partly be explained by the confounding of the first results by the mixing of sexes, as the groups were not matched for gender Moreover, the use of autopsy patients as controls in the first study might also have affected the results Histomorphometric parameters revealed no differences between men and women In vertebral fracture patients, bone mass, trabecular number and thickness, and bone turnover were significantly reduced while eroded surface was increased relative to healthy subjects These results are consistent with impaired osteoblast function in patients Moreover, the differences in osteocyte numbers present evidence that alterations occur within the whole population of cells of the osteoblastic lineage Yet, further research is needed to elucidate the interplay between the role of osteocytes, mechanical load, hormones and other factors

It has been suggested that in postmenopausal osteoporosis the response of bone cells to mechanical signals is hampered by changed hormonal levels Such an increase of the mechanical set point of the cells, would lead to bone loss In chapter 8 we have investigated if a temporary increase of the mechanical set point causes deterioration of trabecular bone architecture such as seen in osteoporosis Similarly, the effects of a temporary change of the loading pattern were studied In this study, a three dimensional computer simulation model, based on the regulation of bone architecture by mechano-sensitive osteocytes, was used It was found that a temporary shift of the mechanical set point causes no lasting changes in architecture Although an increase of the mechanical set point induced bone loss, the mechanism of bone loss (trabecular thinning) differed from what is usually observed in postmenopausal osteoporosis (loss of whole trabeculae) Hence, a change of the mechanical set point alone cannot explain bone loss as seen in osteoporosis On the other hand, the temporary removal of load components in a particular direction resulted in irreversible loss of whole trabeculae The results indicated that such temporary changes in loading patterns could be important risk factors for osteoporosis

In chapter 9 we concluded that with this thesis we have been able to show that Roux's hypothesis was indeed feasible What does that finding help us? It is still unclear whether the hypothesis is actually false or true However, it has provided a new way of

looking at the remodeling process It has offered surprising answers to unanswered questions and it has provided new questions that hitherto no one thought of asking

SAMENVATTING

Trabeculair, ofwel spongieus botweefsel bestaat uit een complexe driedimensionale structuur van botbalkjes en plaatjes Het draagt bij aan de mechanische sterkte, en speelt met name in de ruggewervels en de uiteinden van pijpbeenderen een belangrijke rol bij het dragen en doorleiden van mechanische belasting De architectuur is gerelateerd aan de belastingpatronen, doordat de richtingen van de trabekels in belangrijke mate overeenstemmen met die van de voornaamste krachten die op het bot worden uitgeoefend Bot wordt voortdurend geremodelleerd Dit gebeurt door gespecialiseerde botcellen, osteoclasten breken kleine hoeveelheden bot af, waarna osteoblasten nieuw bot vormen De totale botmassa neemt tijdens het ouder worden geleidelijk af Dit is een normaal verouderingsproces, echter, bij eenderde van de bevolking in ontwikkelde landen is er sprake van een overmatige degeneratie van de botstructuur In dat geval wordt het botweefsel zeer zwak en kan als gevolg van een minimaal trauma al breken Er is dan sprake van osteoporose

In dit proefschrift hebben wij het botremodelleringsproces onderzocht, met de nadruk op de rol van mechanische factoren Er is op dit moment weinig bekent over de regulatie van botremodellering Wel is duidelijk dat mechanische belasting het proces beinvloedt In de vorige eeuw heeft Roux de hypothese voorgesteld dat de trabeculaire architectuur het resultaat is van een proces waarin cellen lokaal de botmassa reguleren onder invloed van mechanische stimuli Bot wordt afgebroken op plaatsen waar het te weinig wordt belast, bot wordt gehandhaafd of gevormd op plaatsen waar de belasting groot genoeg is
In hoofdstuk 2 en 3 is een computersimulatiemodel ontwikkeld om te onderzoeken of een dergelijk regelmechanisme waarschijnlijk is Aangenomen werd dat osteocyten (cellen die zijn ingebed in de botmatrix) de mechanische signalen detecteren en het proces sturen Er werd gevonden dat het model vanuit een willekeurige initiele morfologie patronen genereert die lijken op trabeculair bot De patroonformatie was het gevolg van het niet lineaire dynamische gedrag van het regelmechanisme Het model kon adaptatie aan belastingveranderingen verklaren, wanneer de belastingrichtingen werden veranderd heroriënteerde de trabeculaire structuur zich conform de opgelegde belasting Overeenkomstig met de werkelijkheid, hing de globale dichtheid af van de grootte van de belasting Het aantal osteocyten bepaalde de remodelleringssnelheid en de invloedsafstand van de osteocyten bepaalde de fijnheid van de structuur De controle op afstand van de

osteocyten bleek wezenlijk te zijn voor het ontstaan van trabeculaire structuren en voor de mogelijkheid tot adaptatie Daarnaast werden hiermee numerieke problemen zoals beschreven in eerdere studies voorkomen

De modelresultaten waren voor ons aanleiding om de feitelijke relaties tussen trabeculaire morfologie en osteocytdichtheid te bestuderen In hoofdstuk 4 zijn deze relaties bepaald in bot uit de heupkop van vijf verschillende zoogdiersoorten, uiteenlopend in grootte (rat, konijn, resusaap, varken en koe) Osteocytdichtheid vertoonde een grote spreiding tussen de verschillende diersoorten en bleek af te nemen met toenemende afmeting van het soort dier Er werden ook verschillen gevonden in histomorfometrische parameters De trabekels waren bijvoorbeeld dikker in de koe dan in de andere diersoorten De verschillen waren echter relatief klein Wanneer de resultaten van het simulatiemodel juist zijn, betekent dit dat de invloedsafstand van osteocyten vergelijkbaar is in deze diersoorten Alleen de rat vormde een uitzondering, in de rat waren de trabekels aanzienlijk dunner Waarschijnlijk heeft dit te maken met de blijvende aanwezigheid van de groeischijf in de heupkop van ratten Deze groeischijf veroorzaakt een ander biologisch en mechanisch milieu ten opzichte van de andere diersoorten De relaties met diersoort verschilden voor osteocytdichtheid en morfometrische parameters Deze resultaten wijzen erop dat osteocytdichtheid niet direct gerelateerd is aan de macroscopische trabeculaire architectuur, wat overeenkomt met de modelresultaten

Aangenomen werd dat osteocyten de cellen zijn die mechanische belasting kunnen voelen en de massahuishouding reguleren Er is echter geen bewijs dat dit ook werkelijk het geval is In hoofdstuk 5 hebben we bekeken of osteoblasten en liningcellen deze rol op een even effectieve wijze zouden kunnen vervullen, met het oog op de verhouding tussen massa, stijfheid en belasting Twee modellen werden met elkaar vergeleken, waarbij de massahuishouding in het ene geval werd gereguleerd door osteocyten en in het andere geval door cellen op het botoppervlak Het laatste remodelleringsalgoritme bleek minder gevoelig te zijn voor veranderingen in het belastingpatroon Dit resulteerde in een aanzienlijk hogere massa van de gevormde architectuur met een vergelijkbare stijfheid in de belastingrichting De rek- en spanningswaarden in het botweefsel varieerden ook over een veel groter gebied dan in de architectuur geproduceerd door het osteocyt-regulatiemodel Deze hogere rekwaarden betekenen een hogere kans op falen van de structuur Er werd geconcludeerd dat osteocyten efficientere sensoren en regulatoren zijn

voor botremodellering. De resultaten zijn echter geen direct bewijs dat osteocyten ook werkelijk deze rol vervullen.

De relaties tussen osteocyt dichtheid, histomorfometrische parameters, osteoporose and geslacht werden onderzocht in hoofdstuk 6 en 7. Hiervoor werd trabeculair bot uit de bekkenrand van gezonde en osteoporotische mensen bekeken. In de eerste studie werd gevonden dat osteocytdichtheid afneemt met toenemende leeftijd. Het aantal osteocyten was hoger in patiënten met heup- of ruggewervelfracturen in vergelijking tot normale personen. De tweede studie gaf echter tegengestelde resultaten. Patiënten met ingezakte ruggewervels hadden een significant lagere osteocytdichtheid dan gezonde proefpersonen. Bovendien werd gevonden dat osteocytdichtheid in vrouwen hoger is dan in mannen. Deze tegengestelde resultaten kunnen deels worden verklaard doordat groepen in de eerste studie niet waren gematcht voor geslacht. Daarnaast kan het gebruik van autopsie materiaal als controle de resultaten in de eerste studie hebben beïnvloed. Er werden geen verschillen gevonden tussen mannen en vrouwen wat betreft de histomorfometrische parameters. In patiënten met ruggewervelfracturen waren bot massa, trabekel aantal en dikte, en botombouw significant lager en het resorptieoppervlak was significant hoger vergeleken met gezonde controles. Deze resultaten komen overeen met een verminderde functie van osteoblasten in osteoporotische patiënten. De verschillen in osteocytaantallen tonen aan dat er verschillen zijn in de gehele populatie cellen van de osteoblast-stamboom. Nader onderzoek is noodzakelijk om het verband tussen osteocyten, mechanische belasting, hormonen en andere factoren te ontrafelen.

Verschillende auteurs hebben gesuggereerd dat in vrouwen met postmenopausale osteoporose de respons van botcellen op mechanische stimuli verstoord is door veranderde hormoonspiegels. Deze verandering van het mechanische "set point" van de cellen zou dan leiden tot een verminderde botmassa. In hoofdstuk 8 hebben we onderzocht of een tijdelijke verandering van het mechanische set point inderdaad een verslechtering van de botstructuur te weeg brengt zoals wordt waargenomen in osteoporose. Dit zelfde werd bekeken voor een tijdelijke verandering van het belastingpatroon. In dit hoofdstuk werd een driedimensionaal computer simulatiemodel gebruikt. Er werd gevonden dat een tijdelijke verhoging van het set point geen blijvende veranderingen aan de botarchitectuur veroorzaakt. Hoewel verhoging van het set point leidde tot botverlies, was het mechanisme waarmee dit gebeurde (dunner worden van de trabekels) principieel anders dan dat wat

over het algemeen wordt waargenomen in osteoporosis (verdwijnen van hele trabekels) Dus, een verandering van het mechanische set point op zich kan het botverlies, zoals dat wordt waargenomen bij osteoporose, niet verklaren Anderzijds, resulteerde het tijdelijk wegnemen van belastingcomponenten in een onomkeerbaar verlies van trabekels Deze resultaten wijzen erop dat zulke tijdelijke belastingveranderingen belangrijke risicofactoren zouden kunnen zijn voor osteoporose

In hoofdstuk 9 concludeerden we dat we met dit proefschrift hebben laten zien dat Roux's hypothese realistisch is Wat betekent deze conclusie nu eigenlijk? Er is geen bewijs dat de hypothese ook werkelijk waar is Wat deze bevinding evenwel biedt is een nieuw denkraam, een nieuwe wijze van kijken naar het remodelleringsproces Vanuit dit denkraam werden verassende antwoorden gevonden op gestelde vragen en daarnaast leverde het nieuwe vragen op waar tot nog toe niemand aan dacht om ze te stellen

THANKS EVERYBODY

Toen ik solliciteerde naar de baan als AIO bij de sectie Biomechanica, wist ik niet waar ik aan begon Ik was nog nooit eerder in Nijmegen geweest en eigenlijk had ik nooit eerder van het Biomechanica Lab gehoord Achteraf besef ik dat ik het heel erg goed heb getroffen Het wetenschappelijk niveau van de groep is hoog, de begeleiding en samenwerking zijn goed, en er worden je alle kansen geboden om je wetenschappelijk te ontwikkelen en je nationaal en internationaal te profileren Ik kan dan ook zeggen dat ik het dankzij dit gunstige klimaat heb volgehouden om op en neer te reizen naar Nijmegen

Als eerste wil ik natuurlijk Rik Huiskes, mijn promotor, bedanken Met name jouw enthousiasme voor dit onderzoek, de milde aansporing om iets af te maken of weer verder te gaan, maar ook de vrijheid waarmee je mij mijn gang liet gaan waardeer ik zeer Ik heb ontzettend veel van je geleerd, en niet alleen dat, je hebt me ook de kans gegeven om van anderen te leren Je stimuleert iedereen om naar meetings en congressen te gaan, om met anderen over het onderzoek te praten, om bij te blijven Aan de andere kant verwacht je ook iets, een goed stuk werk, een goede presentatie, een goed artikel, je probeert het beste eruit te halen Al met al, ben ik blij dat jij mijn promotor bent Dankjewel Rik

Er zijn veel mensen die mij hebben geholpen en aan wie ik dank verschuldigd ben Een aantal wil ik hier persoonlijk noemen Bert, zonder jou had dit proefschrift hier niet gelegen Ik wil je bedanken voor al die keren dat je problemen hebt opgelost, ideeen naar voren bracht, met mij discussieerde over de resultaten, en niet te vergeten voor al die methoden die jij ontwikkeld hebt, die ik dan weer gebruiken kon Ik heb je niet kunnen bijhouden, maar met jouw hulp ben ik er toch gekomen Harrie, ik weet dat je wat frustraties hebt overgehouden aan mijn onderzoek Toch ben ik blij dat je in de buurt was Fantastisch is het hoe jij je kunt opwinden over het onderwerp van mijn proefschrift Ik heb genoten van al die discussies die ik met je heb gehad en ik denk dat er heel wat ideeen van jou in mijn werk zijn beland

Verder wil ik Diny Verleyen en Dorien van der Meer bedanken voor het vele werk dat zij voor mij hebben gedaan Diny, hartelijk dank dat jij bereid was om die eindeloze hoeveelheden botten te zagen en te kleuren Dorien, het tellen van cellen is zeker niet het leukste werk Ik ben je dan ook heel erg dankbaar dat jij er zo veel voor me geteld hebt

Paul Lips en Laurence Vico wil ik bedanken dat zij participeerden in een deel van mijn onderzoek en mij de gelegenheid gaven om metingen te doen aan botbiopten van patienten Merci bien Laurence!

Dan wil ik ook nog de meiden van mijn kamer bedanken Eigenlijk voornamelijk voor het feit dat onze kamer een gezellige meidenkamer is Heerlijk om te praten over wat er allemaal aan schort, over dochters of over sport, Dorethé, Esther, houden zo!
Ook alle anderen van ons lab ben ik erkentelijk voor hun hulp en de goede sfeer

Pa en ma, jullie wil ik ook bedanken, niet alleen voor de juiste genen, en eigenlijk ook niet alleen voor jullie steun, maar gewoon omdat jullie echte ouders zijn

En dan Fred en Lavinia Fred, jouw rol in de totstandkoming van dit boekje is een beetje dubbelzijdig Aan de ene kant had ik het zonder jou nooit volgehouden om op en neer te blijven pendelen, en als jij niet voor Lavinia zou zorgen, was het al totaal onmogelijk geweest Zonder jouw pep talks, had ik de moed al vele malen laten zakken Aan de andere kant heb je mijn aandacht ook aardig van het werk af weten te leiden Er was altijd wel iets dat "belangrijker" was Één ding is zeker, je hebt er voor gezorgd dat ik me nooit heb verveeld Tot slot, Lavinia, jij kunt dit nog niet lezen, maar ik wil toch even zeggen dat jij de allerbeste dochter bent die ik me maar kan wensen

CURRICULUM VITAE

Mijn naam is Margriet Gezina Mullender Ik ben geboren in Amstelveen op 19 augustus 1965 Wanneer ik voor het eerst naar school ging, dat ben ik vergeten De laatste schooldag van het VWO op de Christelijke Scholengemeenschap in Buitenveldert kan ik me echter nog goed herinneren, dat was in mei 1983 Ik behaalde toen mijn diploma Atheneum B Na twee jaar genoten te hebben van het vrije leven, besloot ik dat vanaf 1985 te combineren met een studie Bewegingswetenschappen aan de VU in Amsterdam Gedurende de laatste 2 à 3 jaar van mijn studie was ik tevens studentassistent bij (inmiddels) prof dr ir G J van Ingen Schenau Augustus 1991 studeerde ik af, met als hoofdvakken Functionele Anatomie en Inspanningsfysiologie Op zoek naar een baan, stuitte ik op een advertentie voor een AIO plaats bij de sectie Biomechanica aan de Universiteit van Nijmegen Aangezien ik mij al in die richting had georienteerd, heb ik hierop gesolliciteerd Nu werk ik hier inmiddels al weer ruim 5 jaar Op dit moment houd ik mij bezig met de biomechanica van cellen in een gezamenlijk project van de afdelingen Biomechanica en Biomaterialen

Moment Suprème

Het vervangen van een heup of knie is iets waar de meeste mensen tegenop zien. En dat is vervelend. De patiënt moet volledig op u kunnen vertrouwen. Net zoals u dat moet kunnen op uw mensen, uw apparatuur en de materialen die u gebruikt. Alles staat of valt nu eenmaal met de geleverde kwaliteit.

ORTOMED

Natuurlijk in beweging