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Citation for published version (APA):

Ruimerman, R., Rietbergen, van, B., Hilbers, P. A. J., & Huiskes, R. (2005). The effects of trabecular-bone loading variables on the surface signaling potential for bone remodeling and adaptation. *Annals of Biomedical Engineering*, 33(1), 71-78. <https://doi.org/10.1007/s10439-005-8964-9>

DOI:

[10.1007/s10439-005-8964-9](https://doi.org/10.1007/s10439-005-8964-9)

Document status and date:

Published: 01/01/2005

Document Version:

Publisher's PDF, also known as Version of Record (includes final page, issue and volume numbers)

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
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The Effects of Trabecular-Bone Loading Variables on the Surface Signaling Potential for Bone Remodeling and Adaptation

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(Received 28 January 2004; accepted 16 August 2004)

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

Keywords—Trabecular bone, Modeling, Remodeling, Mechanical adaptation, Computer simulation.

INTRODUCTION

Trabecular bone is continuously renewed by bone-resorbing osteoclasts and bone-forming osteoblasts. In growth or adaptation they change the shape of trabecular morphology and adapt it to external loading direction and

magnitude^{7,41,43} in a process called ‘modeling’.¹² In adulthood the tissue is continuously renewed by the same cells in a process called ‘remodeling’.¹³ In Huiskes *et al.*¹⁶ we proposed a theory for modeling and remodeling of trabecular structures as governed by external forces. It is based on the assumption that osteocytes within the bone tissue translate the mechanical signals they experience into biochemical messengers that are transduced through the canalicular network to the trabecular surface.^{4,8,20,21,24,27,29,37} At the bone surface, osteoblasts are recruited from the environment or by reactivation of lining cells^{6,10} in order to form bone relative to the total amount of stimulus they receive. Osteoclasts are assumed to be recruited by osteocyte apoptosis due to microdamage or cracks.^{3,30,31,39} We assumed that, due to daily loading conditions, microcracks and damage occur at spatially random locations, e.g., they can occur anywhere at any time. The theory is summarized in the regulation scheme depicted in Fig. 1. Our theory describes the coupling between formation and resorption as an effect of mechanical stress transfer, without specification of the regulatory biochemical factors involved, although these are implicitly assumed. This is illustrated in Fig 2. Applying 2D computer simulation methods we showed that this theory provides a qualitative explanation for both modeling and remodeling.^{16,34} We later studied the theory using a 3D finite element analysis (FEA) computational model and showed that it explains growth and maintenance of 3D trabecular-like architectures with realistic physiological and morphological characteristics. Also the adaptation of these structures to alternative loading conditions, and the morphological effects of osteoporosis and medical intervention, could be explained.^{35,36}

A basic assumption in our theory concerns the mechano-sensory and signaling functions assigned to osteocytes.^{4,8} The precise nature of the mechanisms by which osteocytes translate mechanical signals into bone-formation stimuli is unknown. Previously, we used the strain-energy-density (SED) rate as the relevant osteocyte signal. For the present study, we investigated whether other stress and strain-related signals are likely to produce realistic results as well. That alternative choices of the osteocyte stimulus signal

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FIGURE 1. The assumed metabolic regulatory mechanisms: (i) Osteoclasts resorb bone on trabecular surface locations where microdamage occurs, attracted by apoptotic osteocytes; in the computations the sites are randomly selected, to a specified percentage per iteration (month). (ii) Osteocytes locally sense a mechanical signal due to external load transfer through the architecture; the signal is transferred to the trabecular surface, where locally (iii) osteoblasts are recruited from bone tissue.^{16,34–36}

may affect the results considerably is obvious as the precise distribution of the mechanical signals throughout the structure can differ significantly (Fig. 3).

The fundamental issue behind the choice of the stimulus signal is whether (re)modeling signals originate in the osteocytes themselves or whether they are an effect of increased

FIGURE 3. A conceptual scheme showing the different effects of mechanical variables and their gradients, specified for SED. SED produces a local load. VS-gradient produces an incentive for flow.

flow through the osteocyte network. If the signals originate in the osteocyte lacunar stress and strain concentrations, which can be 15 times higher than the tissue (continuum) level ones.^{2,29} In this case the time-derivatives of (SED), *maximal principal strain* (MPS) or *volumetric strain* (VS) could be relevant controlling variables.

However, it was also found that osteocytes are sensitive to fluid flow,²⁰ so it was proposed that flow through the canicular network is the actual trigger for metabolism.⁴ If fluid flow in the osteocytic network is assumed to cause anabolic signaling, the time-derivatives of the spatial gradients of SED, MPS, and VS could be more realistic candidates

Indirect osteoblast-osteoclast coupling through mechanics

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

because tissue deformation causes fluid pressures within the lacunae, and differences, or gradients, in pressure will induce flow of fluid.²²

METHODS

A summary of our theory is illustrated in the regulation scheme depicted in Fig. 1. Mathematical equations were introduced to quantify relationships.^{16,34} The change in bone mass at a particular trabecular surface location x at time t is determined by

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

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

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

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

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

$$P(x, t) = \sum_{i=1}^N f(x, x_i) \mu_i R(x_i, t), \text{ with} \quad (3)$$

$$f(x, x_i) = e^{-d(x, x_i)/D}$$

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

Osteoclast bone resorption is described by

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

where r_{cl} ($\text{mm}^3 \text{ day}^{-1}$) represents a stochastic function that describes that portions of tissue are removed from the surface at spatially random locations.

To determine the stress-strain distribution in the structures we used (FEA) for a domain divided in cubic voxels. The bone tissue within a voxel was assumed isotropic and linear elastic, with material properties depending on its density value m , according to^{9,27}

$$E = E_{\text{max}} m^\gamma. \quad (5)$$

where the maximal elastic modulus E_{max} MPa and exponent γ are constants.

For force-induced remodeling the signal R represented the time-derivatives of either the $\text{SED} = 1/2\sigma:\varepsilon$, the $\text{MPS} = \max(\varepsilon_i)$, or the $\text{VS} = \varepsilon_1 + \varepsilon_2 + \varepsilon_3$. These values were determined in the center of each voxel. For the simulations that consider flow-induced remodeling, R represented the time-derivatives of the *gradients* of these signals. The VS-gradient ψ_i in voxel i was determined by

$$\psi_i = \sqrt{e_{i,x}^2 + e_{i,y}^2 + e_{i,z}^2}, \quad (6)$$

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

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

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

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

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

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

Other parameter settings were similar to those used in earlier simulations.³⁶ Osteocyte density was set to $44,000 \text{ mm}^{-3}$, as was found by Mullender *et al.* in pigs,²⁸ randomly distributed within the tissue, corresponding to approximately 6 osteocytes along the diameter of a trabecula with a thickness of $150 \mu\text{m}$. The osteocyte influence distance $D = 100 \mu\text{m}$. The threshold for osteoblast bone

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

Osteocytes susceptible to	Sensitivity μ
SED	1.0 [$\text{nmol mm}^{-2} \text{ day}^{-1} (\text{J}^{-1} \text{ mm}^3 \text{ s})$]
SED gradient	4.00×10^{-5} [$\text{nmol mm}^{-2} \text{ day}^{-1} (\text{J}^{-1} \text{ mm}^4 \text{ s})$]
MPS	2.55×10^7 ($\text{nmol} \cdot \text{mm}^{-2} \cdot \text{day}^{-1} \cdot \text{s}$)
MPS-gradient	0.64×10^3 ($\text{nmol} \cdot \text{mm}^{-2} \cdot \text{day}^{-1} \cdot \text{mm} \cdot \text{s}$)
VS	2.55×10^7 ($\text{nmol} \cdot \text{mm}^{-2} \cdot \text{day}^{-1} \cdot \text{s}$)
VS-gradient	1.27×10^3 ($\text{nmol} \cdot \text{mm}^{-2} \cdot \text{day}^{-1} \cdot \text{mm} \cdot \text{s}$)

formation was $k_{tr} = 5.0 \times 10^6$ (nmol mm⁻² day⁻¹), and the proportionality factor $\tau = 1.4 \times 10^{-10}$ (mm⁵ nmol⁻¹). Osteoclast recruitment frequency on the surface was set to 7.1×10^{-2} (percent voxel⁻¹ day⁻¹) and the amount of bone tissue per cavity was 1.5 voxels, i.e. 5.6×10^{-5} (mm³).¹¹ The iteration time step was set to 1 month. The maximal elastic modulus per voxel was set to $E_{max} = 5.0$ GPa, the Poisson ratio $\nu = 0.3$, and the exponent $\gamma = 3$.⁹ To impose external forces, plates with a thickness of one voxel were added at the surfaces of the cubic domain, disconnected at the ribs of the cube in order to avoid stress shielding.³⁴ The plates were given material properties as fully mineralized bone tissue. They do not participate in the remodeling process, i.e. they do not adapt. For the lower plate we repressed 6 degrees of freedom. The x -, y -, and z -coordinate for one corner in the origin, the x - and y -coordinate for the second corner on the x -axis, and the z -coordinate of the second corner on the y -axis. As a mechanical load we imposed a distributed harmonic cyclic stress with 1 (Hz) and an amplitude of 2.0 (MPa). This load was compressive in the vertical direction and tensile in the horizontal directions. We assumed that osteocytes respond to the maximal rate of change of the proposed mechanical signals during one loading cycle. We neglected inertia so that these values could be determined using static FE analysis.³⁴

Several computational series were performed: (1) Simulated growth (modeling) from an initial fine, porous

structure toward a mature homeostatic 3D trabecular-like structure. Once this homeostatic structure is obtained no more-gross architectural changes occurred, but remodeling continued. (2) The mature structure was subjected to 50% reduced loads and to 50% increased loads to investigate the effect of alternative loading magnitudes. (3) The structure was also subjected to a 20° rotated load to investigate whether the mature structure realigns accordingly. During the simulations, we monitored the developments in morphology, volume fraction (VF) and the distribution of the signals sensed by osteocytes. For the homeostatic configurations, we determined trabecular thickness (Tr.Th), trabecular spacing (Tr.Sp), and trabecular number (Tr.N) according to the parallel plate model.³² In addition to we determined the remodeling rates (net-full turnover). The 'remodeling space'—the total volume of unfilled resorption cavities—was determined by prolonging the simulation with osteoclast-resorption completely inhibited. The morphologic and metabolic effects of increased and reduced external loads were also studied.

RESULTS

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

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

FIGURE 5. (A) Development of volume fraction (VF) during the simulation for the different controlling variables. Only the simulations using *SED* and *MPS* as controlling variables reproduced a clear overshoot in bone mass (VF) as it occurs around puberty. (B) Development of volume fraction as found in a study of bone growth in pigs.³⁸

proceeding time the structure became coarser until finally homeostasis was reached, with less, but thicker, trabeculae, aligned with the external loads. Initially VF rose sharply in *modeling* and later stabilized to a final, mature value of 0.28 [Fig. 5(A)]. The overshoot in VF that was observed in morphological studies of bone development in growing pigs³⁸ did not occur, however. From that stage on formation and resorption were balanced (*remodeling*). Tr.Th was on the order of 300 μm and the remodeling rate was such that the net total bone mass was replaced in 5.7 years. The remodeling space was 21%. The average VS-gradient sensed by osteocytes was large initially (200 m^{-1}), but reduced to 110 m^{-1} in the homeostatic configuration, illustrating the mechanical ‘shape optimization’ that had taken place.

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

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

The values of the mechanical variables in each simulation converged to similar spatial frequency distributions in the homeostatic (mature) state. The coefficients of variation (COV), i.e., standard deviation to mean ratio, is a measure

FIGURE 6. Homeostatic trabecular morphologies as a result of the different controlling mechanical variables tested.

TABLE 2. Morphological results of the computational analyses with the 6 mechanical variables tested: VF, Tr.N, average Tr.Sp, average Tr.Th, resorption rate (the number of years required for net full turnover), the remodeling space and the COV of the mechanical signal sensed, all in the homeostatic configurations. Additionally, the loss/gain of bone mass in response to 50% reduced, respectively increased loads, both relative to the homeostatic morphology. Data from literature^{15,18,19,31} are added in the last row for comparison.

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

for stability as low values relate to uniform loading distributions whereas high values relate to inhomogeneous loading. COV values varied between 1.17 and 1.63 (Table 2). Simulations using SED and its *gradient* had the lowest (COV). This indicates that osteoclast-resorption cavities were repaired more rapidly. This fits with the observation that the remodeling space for SED-based simulations was smaller (Table 2). Development of total bone mass (VF) during the simulation to maturity over time was very similar as well in most simulations. Only the simulations using SED and MPS as mechanical variables reproduced the typical overshoot in VF at around puberty [Fig. 5(B)], as it was found in morphological bone modeling—growth—studies in pigs.³³

DISCUSSION

We developed a computational framework for strain-related trabecular bone remodeling and adaptation.^{16,34} We already showed how it can be applied in the search for relationships suitable to explain trabecular deterioration in disused or postmenopausal osteoporotic bone.^{35,36} For this study, we asked the question whether mechanical variables other than SED-rate could be more realistic or effective in simulating remodeling and adaptation. In particular, we were interested in comparing the effects of the mechanical variables themselves—as variables representing the actual loads on the osteocyte ‘sensors’—to the effects of their *gradients*, as related to the mechanical effects on fluid flow in the canalicular network. We found that all six loading variables studied produced reasonable, trabecular-like morphologies. Moreover, for all variables, adaptive behavior persisted, despite changes in loading magnitude and direction. Elevated external loads increased Tr.Th,^{17,23} and reduced loads caused trabecular thinning and loss of connectivity.²⁶ Alternative loading directions caused trabeculae to realign accordingly. This is analogous to what is observed in growing bone,³⁸ after fracture healing⁴¹ and in relation with implant incorporation.¹⁴ However, according to Bertram and Swartz¹ it was never found to occur after

redirection of loads in healthy, mature bone. One explanation for this controversy may be that—according to our predictions—it takes a relatively long time for the structure to adapt, whereas animal experiments on this question were never extended so long.

The question is now how these alternative signal formulations could be discriminated in their effects. For that purpose, we first compared actual morphological data predicted to literature data of the VF, Tr.N, Tr.Sp, Tr.Th, net full turnover rate, and remodeling space. Comparing these values with the results of the computations (Table 2) eliminates all but SED, SED-gradient, and MPS-gradient as suitable variables; although each of them had remodeling-space values up from about two times higher than what is normal.¹⁹

Another criterion for realistic behavior is the overshoot in VF in puberty, found in morphological studies in pigs (Fig. 5).³⁸ This was only reproduced for SED and MPS as regulating variables. Furthermore, we compared the efficacy of the different variables: which produced the least-bone mass (VF) for a given loading set. SED appears most effective to that criterion. SED and its gradient were also the variables producing the most homogenous distributions of mechanical signals in their homeostatic structures, with COV at 1.23 and 1.17—compared to the others at 1.36–1.63, which makes them the most effective ones.

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

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

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

Hence, it seems reasonable to employ SED as the loading variable for further investigative computations concerning trabecular bone (re)modeling. However, the results do not prove that osteocytes respond to local loads in a direct, mechanical sense, as opposed to being sensitive to fluid flow, generated by stress on the osteocytic canalicular network as a whole. The reason for this is basically that the theory employed is extremely robust. This robustness is caused by the negative feedback between tissue loading and bone formation. We saw that whatever the mechanical signal, the theory produces reasonable trabecular structures. Although we did not investigate this, it might be that equally realistic morphologies and metabolic parameters could be generated by all signals investigated, by variation of the process parameters for which a realistic value is not known—like osteocyte sensitivity, distance decay parameter, proportionality factor, or others. More information could only be obtained if more realistic, and refined—'mechanistic'—models^{20,22,40,42} for signal transduction would be included in the theory. This would also imply the use of a more realistic description for the dynamic external forces. Computational restrictions defy that, at the moment.

Although the theory is restricted by the use of lumped, conceptual variables, and parameters, it does include both mechanical and metabolic descriptions of the relevant processes, however, crudely the latter are represented. This makes the model a suitable tool to investigate relationships between mechanical forces, its metabolic effects and bone architecture. The results show, again, that coupling of osteoclast resorption to osteoblast bone formation, through the effects of stress concentrations ('notching') around resorption lacunae^{5,16,25,33} is a viable hypothesis. And finally, the predictions of the theory are quite insensitive to the refinement of its parametric and signal representations, as shown here once more—hence, attractive in an evolutionary sense.

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